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Mechanisms and Physiological Significance of the Cholinergic Control of Pancreatic β-Cell Function

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Acetylcholine (ACh), the major parasympathetic neurotransmitter, is released by intrapancreatic nerve endings during the preabsorptive and absorptive phases of feeding. In β-cells, ACh binds to muscarinic M_3 receptors and exerts complex effects, which culminate in an increase of glucose (nutrient)-induced insulin secretion. Activation of PLC generates diacylglycerol. Activation of PLA_2 produces arachidonic acid and lysophosphatidylcholine. These phospholipid-derived messengers, particularly diacylglycerol, activate PKC, thereby increasing the efficiency of free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) on exocytosis of insulin granules. IP3, also produced by PLC, causes a rapid elevation of $[Ca^{2+}]_c$ by mobilizing Ca^{2+} from the endoplasmic reticulum; the resulting fall in Ca^{2+} in the organelle produces a small capacitative Ca^{2+} entry. ACh also depolarizes the plasma membrane of β-cells by a Na^+-dependent mechanism. When the plasma membrane is already depolarized by secretagogues such as glucose, this additional depolarization induces a sustained increase in $[Ca^{2+}]_c$. Surprisingly, ACh can also inhibit voltage-dependent Ca^{2+} channels and stimulate Ca^{2+} efflux when $[Ca^{2+}]_c$ is elevated. However, under physiological conditions, the net effect of ACh on $[Ca^{2+}]_c$ is always positive. The insulinotropic effect of ACh results from two mechanisms: one involves a rise in $[Ca^{2+}]_c$ and the other involves a marked, PKC-mediated increase in the efficiency of Ca^{2+} on exocytosis. The paper also discusses the mechanisms explaining the glucose dependence of the effects of ACh on insulin release. (*Endocrine Reviews* 22: 565–604, 2001)

Abbreviations: α-cell, Glucagon-secreting cell; ACh, acetylcholine; ASCI-PLA_2, ATP-stimulatable, Ca^{2+}-independent PLA_2; β-cell, insulin-secreting cell; $[Ca^{2+}]_c$, free cytosolic Ca^{2+} concentration; CGRP, calcitonin gene-related peptide; CHO, Chinese hamster ovary; DAG, diacylglycerol; δ-cell, somatostatin-secreting cell; GIP, glucose-dependent insulin-releasing peptide or gastric inhibitory polypeptide; GRP, gastrin-releasing peptide; 5-HT, 5-hydroxytryptamine (or serotonin); 1 IP, inositol 1-phosphate; K^+-ATP channel, ATP-sensitive K^+ channel; LHA, lateral hypothalamic area; $[Na^+]_c$, free cytosolic Na^+ concentration; NO, nitric oxide; PA, phosphatidic acid; PACAP, pituitary-adenylate cyclase activating polypeptide; PC, phosphatidylcholine; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PMA, phorbol 12-myristate 13-acetate; PP-cell, pancreatic polypeptide-secreting cell; SP, substance P; SERCA pump, sarco-endoplasmic reticulum Ca^{2+}-ATPase; SOC, store-operated channel; VMH, ventromedial hypothalamic nuclei.

I. Introduction

DESPITE THE ALTERNATION of fasting and feeding periods, the concentration of plasma glucose is maintained within a narrow range by a finely tuned balance between insulin, the only hypoglycemic hormone, and glucagon, epinephrine, corticosteroids, and GH, the major hyperglycemic hormones. The secretion of insulin by β-cells of the endocrine pancreas is regulated by glucose and other circulating nutrients. It is also modulated by several hormones and neurotransmitters, among which acetylcholine (ACh) plays a prominent role.

The complex neural control of hormone secretion by the endocrine pancreas has been the subject of other reviews (1–3). It will be addressed only briefly in our contribution, which focuses on the cholinergic control of the β-cell function. After an overview of the *in vivo* data demonstrating the role of the parasympathetic system in the regulation of glycemia, we analyze and synthesize the *in vitro* experiments that have elucidated the cellular mechanisms by which ACh influences β-cells. Particular attention is paid to the effects of ACh on phospholipid metabolism, membrane potential, free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$), and insulin secretion. This article updates and extends other reviews on the subject (4–7).

II. The Innervation of the Endocrine Pancreas

A. General anatomical considerations

The endocrine pancreas is organized in small organs, the pancreatic islets or islets of Langerhans, that are dispersed in the exocrine parenchyma. The islets are composed of a few hundred to several thousands of cells, of which 65–80% are insulin-secreting β-cells. These cells are mainly located in the center of the islet and are surrounded by a mantel of three other cell types, *i.e.*, glucagon-secreting α-cells, somatostatin-secreting δ-cells, and pancreatic polypeptide-secreting cells (PP-cells).

The endocrine pancreas is richly innervated, but the abundance and organization of this innervation are highly variable between species (8). Most of the nerve fibers enter the pancreas along the arteries (9, 10). Unmyelinated nerve fibers are found in the neighborhood of all islet cell types at the periphery and within the islet. At some distance from the islets, glial Schwann cells often form a thin sheet around nerve fibers on their travel toward and within the islet. In the vicinity of islet cells, however, it is not rare to see some nerve fibers lacking this glial protection and coming close to or ending blindly 20–30 nm from the endocrine cells (8, 11–17). Well differentiated synapses with islet cells have rarely been observed (18–20). Interestingly, the innervation of the islet is very plastic, as suggested by the observation that islets transplanted in the portal vein of diabetic rats became reinnervated by hepatic nerves (21).

The autonomic innervation of the endocrine pancreas has several origins (for review, see Refs. 2 and 3). Classically, the autonomic nervous system uses two interconnected neurons to control effector functions and is divided into two systems, the sympathetic and the parasympathetic nervous systems, according to the location of the preganglionic cell bodies. However, there are indications suggesting that these two systems are not always independent of each other, but display anatomical interactions (22) or share similar neurotransmitters (23–25). The endocrine pancreas also receives other types of nerves, the anatomical origin and the function (motor efferent or sensory afferent) of which are not clearly known. These nerves are of peptidergic and nonpeptidergic nature (2, 3).

B. The parasympathetic innervation

The preganglionic fibers of the parasympathetic limb originate from perikarya located in the dorsal motor nucleus of the vagus (26–33) and possibly also in the nucleus ambiguus (26, 34–37), which are both under the control of the hypothalamus. They are organized in well separated branches traveling within the vagus nerves (cranial nerve X), and through the hepatic, gastric (31, 38), and possibly celiac branches of the vagus (39), they reach intrapancreatic ganglia that are dispersed in the exocrine tissue. These ganglia send unmyelinated postganglionic fibers toward the islets (9, 10, 38, 40). Preganglionic vagal fibers release ACh that binds to nicotinic receptors on intraganglionic neurons. Postganglionic vagal fibers release several neurotransmitters: ACh, VIP, gastrin-releasing peptide (GRP), nitric oxide (NO), and pituitary adenylate cyclase-activating polypeptide (PACAP) (3, 27, 41–51). Cholinergic terminals are found in the neighborhood of all islet cell types at the periphery and within the islet (50, 52–56). The importance of the cholinergic innervation of the endocrine pancreas is attested by the presence of a 10-fold higher activity of choline acetyltransferase and acetylcholinesterase (the enzymes involved, respectively, in the synthesis and the degradation of ACh) in the islets than in the surrounding exocrine tissue (57). Cholinergic synapses with endocrine cells have been observed in some species (58, 59).

Understanding the organization of the pancreatic innervation permits correct interpretation of some experiments using different cholinergic antagonists. The stimulation of insulin release occurring upon electrical stimulation of vagal nerves in the dog is abolished by both nicotinic and muscarinic antagonists (60). In the perfused rat pancreas, nicotine produces an increase of insulin secretion that is blocked by atropine (10). These observations can be explained by the presence of nicotinic receptors on pancreatic ganglia and nerves (61–64) and muscarinic receptors on β-cells (see *Section X*).

The overall effect of a parasympathetic stimulation is an increase of insulin secretion (see *Section III*). Because postganglionic fibers contain various neurotransmitters in addition to the classic neurotransmitter ACh, it is important to keep in mind that parasympathetic neurotransmission is the sum of various biological effects. VIP and PACAP stimulate insulin secretion by increasing cAMP levels (3). GRP and its amphibian homolog, bombesin (3), are also insulinotropic (3, 42, 65–68). They act on the same family of receptors (69) and exert their action by two mechanisms, directly by stimulating β-cells through the PLC-PKC pathway (3), and indirectly by activating intrapancreatic postganglionic nerves that stimulate insulin secretion (68). NO synthase has been

detected in nerves in several organs wherein NO is considered a neurotransmitter (70, 71), and in pancreatic nitrergic nerves (45, 48, 49, 67). Various effects of NO on β-cells have been reported (72–75), but it is unclear whether NO is implicated in the parasympathetic modulation of insulin secretion.

The parasympathetic system also controls the secretion of the other islet hormones. Vagal nerve stimulation increases glucagon (31, 41, 60, 76–78) and PP secretion (41). The effect of vagal stimulation on δ-cells is less clear, as it was reported to stimulate (79) or inhibit somatostatin secretion (78, 80). *In vitro* and *in vivo* experiments using various cholinergic agents have shown that ACh stimulates glucagon and PP secretion through atropine-sensitive mechanisms (81–84). The effects of cholinergic agonists on *in vitro* somatostatin secretion are again controversial (2, 80, 82, 85), although this might reflect species differences.

C. The sympathetic innervation

The sympathetic innervation of the pancreas originates from preganglionic perikarya located in the thoracic and upper lumbar segments of the spinal cord (86). The myelinated axons of these cells traverse the ventral roots to form the white communicating rami of the thoracic and lumbar nerves that reach the paravertebral sympathetic chain (87). Preganglionic fibers either communicate with a nest of ganglion cells within the paravertebral sympathetic chain or pass through the sympathetic chain, travel through the splanchnic nerves, and reach the celiac (2, 3, 35, 86, 88) and mesenteric ganglia (86). Ganglia within the paravertebral sympathetic chain, and the celiac and mesenteric ganglia, give off postganglionic fibers that eventually reach the pancreas. The existence of intrapancreatic sympathetic ganglia has also been reported (25, 26, 37). The preganglionic fibers release ACh that acts on nicotinic receptors on intraganglionic neurons, whereas the postganglionic fibers release several neurotransmitters: norepinephrine, galanin, and NPY (3, 51, 89–91). A rich supply of adrenergic nerves in close proximity of the islet cells has been observed in several mammalian species (53–55, 92).

The net physiological effect of splanchnic nerve stimulation is a lowering of plasma insulin concentration (93–96). This effect is attributed to release of norepinephrine from nerve fibers close to β-cells and to elevation of catecholamine (epinephrine and norepinephrine) plasma levels because of the stimulation of the adrenal medulla. Catecholamines have long been known to inhibit insulin secretion *in vivo* (1–3, 97) and *in vitro* (1–3, 98–101). Their action is mediated by α_2-adrenoceptors (102), probably of the α_{2a}- and α_{2c}-subtypes (103), which have been identified in β-cells by both pharmacological (104) and molecular approaches (103, 105). Activation of α_2-adrenoceptors interferes with the secretory process through several mechanisms that are all prevented by pertussis toxin treatment and are, thus, likely mediated by $G_{\alpha i}$ or $G_{\alpha o}$ (106): an inhibition of adenylate cyclase leading to a lowering of β-cell cAMP, an opening of K^+ channels of small conductance leading to partial membrane repolarization and decrease in Ca^{2+} influx, and a major inhibition of a late step of exocytosis (106). Similar pathways are implicated in the inhibitory action of galanin, which may cooperate with catecholamines to inhibit insulin secretion in response to splanchnic nerve stimulation (3, 106). In contrast, an increase in plasma insulin can be evoked by selective β-adrenergic agonists, particularly of the β_2-subtype (2, 95, 107, 108), that activate adenylate cyclase and increase cAMP. However, these usually have little effect on insulin secretion by isolated islets (109). Moreover, the presence of β_2-adrenoceptors in β-cells remains controversial (105, 110). It is also important to emphasize that a number of pharmacological studies have been misinterpreted because antagonists of adrenoceptors can influence insulin secretion by acting on other targets, *e.g.*, on ATP-sensitive K^+ (K^+-ATP) channels (111). The mechanisms by which NPY inhibits insulin release are not clearly known and might involve a decrease in cAMP levels (3).

The sympathetic nervous system exerts profound effects on the secretion of the other islet hormones. Splanchnic nerve stimulation increases glucagon secretion (93–96, 112, 113), and epinephrine stimulates glucagon secretion *in vivo* and *in vitro* (84, 100, 114, 115). This effect results from the activation of β-adrenoceptors (101), probably of the β_2-subtype (110), although one report implicates α-adrenoceptors (96). It has been shown that pancreatic α-cells express α_1-, α_2-, and α_3-subtypes (103). Splanchnic nerve stimulation decreases somatostatin secretion (80, 96, 116), and norepinephrine inhibits somatostatin release by isolated rat islets (100). The results are less clear for PP secretion. Thus, splanchnic nerve stimulation has been reported to increase (3, 113, 117) or inhibit PP secretion (2, 116). Catecholamines stimulate PP release by isolated islets (118).

Overall, the sympathetic nervous system serves to maintain or increase glycemia in various conditions of stress such as neuroglycopenia, hypovolemia, or physical exercise (3). Its pancreatic action not only involves inhibition of insulin secretion, but also stimulation of glucagon secretion (3, 119).

D. Sensory fibers

Calcitonin gene-related peptide (CGRP) and substance P (SP) are thought to report sensory information in many systems (120). CGRP (51, 121, 122)- and SP-immunoreactive (51, 123, 124) nerve fibers have been observed in both the exocrine and endocrine pancreas. Vanilloid receptors, activated by heat, low pH, and various vanilloid agents (such as capsaicin), are localized in sensory fibers and generally report pain information (120). Neonatal treatment of mice with capsaicin destroys the majority of capsaicin-sensitive neurons and has often been used to identify sensory fibers (120). This treatment was followed by a marked reduction of CGRP-immunoreactive fibers in both the endocrine and exocrine pancreas (122) and by a partial reduction in SP-immunoreactive fibers (36, 125).

It is thought that sensory afferents leave the pancreas along the sympathetic fibers within the splanchnic nerves and that the perikarya of the sensory fibers are present in dorsal root ganglia, mainly at the level of the lower thoracic segments of the spinal cord, transmitting noxious information to the central nervous system by synapsing on second-order neurons of the dorsal horn of the spinal cord (2, 86, 126, 127). The existence of such an anatomical route is supported

by experiments of retrograde labeling (36, 86, 126, 128). It has been suggested that the pancreas is also innervated by sensory afferents that run within the vagus nerve, the perikarya of which are in the nodose ganglion and transmit information to the nucleus tractus solitarius (30, 35, 36, 129, 130).

There is no doubt that sensory nerve fibers report pain information associated with diseases of the exocrine tissue, such as pancreatic cancer and pancreatitis (127, 131), but there are no reports of sensations of pain associated with a destruction of the endocrine pancreas. However, it is possible that sensory fibers play a role in the control of insulin secretion. Thus, neonatal treatment of mice with capsaicin (to destroy these fibers) results in more glucose-stimulated insulin secretion than in nontreated mice, suggesting that sensory fibers exert a direct, tonic inhibition of insulin secretion (132). CGRP may inhibit insulin secretion through a direct action on the islets (121, 133), whereas both inhibitory (134) and stimulatory (124, 135) effects of SP have been reported. Indirect effects of capsaicin-sensitive fibers are also possible. Indeed, it has been reported that removal of endogenous sensory neuropeptides by deafferentation of capsaicin-sensitive sensory nerves improves glucose tolerance by increasing *in vivo* insulin sensitivity (136, 137).

E. Other types of nerves

Immunocytochemistry has revealed the presence of neurotransmitters other than those described above in pancreatic nerves: cholecystokinin (138), 5-hydroxytryptamine (5-HT or serotonin) (139, 140), and methionine-enkephalin (3, 51). These might also influence insulin secretion: cholecystokinin stimulates insulin release by activating PLC and PLA_2 (138), but the effects of 5-HT are controversial, as both inhibition (141, 142) and stimulation (143) of insulin secretion have been reported. Enkephalin also exerts variable effects depending on the concentration used and the species studied (144, 145).

The pancreatic innervation presents other interesting features. The section of extrinsic pancreatic nerves has revealed that many of the intrinsic pancreatic neurons are independent of the integrity of the extrinsic nerves (146), suggesting that the pancreatic innervation might behave as an independent system. This is supported by the observation that intrapancreatic ganglia are interconnected with one another, as are enteric ganglia (37, 140). It has also been suggested that intrapancreatic ganglia are connected with the duodenal myenteric plexus by nerve fibers (50), suggesting the existence of an entero-pancreatic innervation. On the other hand, ganglia from the myenteric plexus of the stomach and duodenum send nerve fibers toward the pancreas (50, 139). Many of these nerves are immunoreactive for 5-HT. Whether these innervations play a physiological role in the regulation of hormone secretion by the endocrine pancreas remains to be investigated.

III. Physiological Role of the Parasympathetic Control of β-Cells

In 1927, Zunz and LaBarre (147), using a cross-perfused canine model, showed that stimulation of the vagus nerve in one dog induced hypoglycemia in the other animal. In 1967, three *in vivo* studies performed in the dog and the baboon reported that stimulation of the vagus nerve increased plasma insulin, and that this effect involved muscarinic receptors because it was inhibited by atropine (148–150). At the same time, an *in vitro* study showed that cholinergic agonists stimulated insulin release from pieces of rat pancreas, and this effect was also antagonized by atropine (99).

The most important characteristic of the influence of ACh on insulin secretion is a tight dependence on the ambient glucose concentration. *In vivo*, electrical stimulation of the vagus nerve has little effect on the concentration of plasma insulin during hypoglycemia, but increases it more and more efficiently as the concentration of plasma glucose augments (41, 151–155). Similar observations have been made *in vitro* when the perfused pancreas (81, 156–160) or isolated islets (161–164) were used to study insulin secretion directly (Fig. 1A). This behavior is typical of a potentiating agent. The mechanisms underlying this potentiation will be explained in detail in *Section IX*.

From here, it is important to bear in mind that the majority of *in vitro* experiments were conducted with rodent islets, and that the concentration dependence of glucose-induced insulin secretion is different in rodent and human islets. Indeed, the threshold glucose concentration and the half-maximal effective concentration for insulin secretion are, respectively, around 4 and 9 mM for human islets as compared with 7 and 15 mM for mouse islets (165).

A. Difficulties and pitfalls of in vivo studies

Species differences must be considered when interpreting the effects of ACh on insulin secretion *in vivo*. As already mentioned in *Section II*, vagal stimulation can release at least five neurotransmitters (ACh, VIP, PACAP, GRP, and NO), the relative contribution of which differs between species. In the dog (49, 60, 76, 149, 166), rat (78), albino mouse (167), and calf (41), vagal stimulation of insulin secretion is mediated mainly or exclusively by muscarinic receptors because it is largely or fully prevented by atropine. This is not the case in the pig (113, 168) and in the cat (10, 169), in which neurotransmitters other than ACh are probably implicated.

A second difficulty is linked to the number of physiological events that are under parasympathetic control. Cholinergic agonists or antagonists, stimulation of the vagus nerve, and vagotomy induce multiple effects that can indirectly interfere with insulin secretion. Cholinergic agents influence the secretion of the other islet hormones (see *Section II*), but it is difficult to establish to what extent the observed changes in insulin secretion are influenced by paracrine or endocrine interactions. Such interactions depend on the organization of the microvascularization and the direction of blood flow, which are still a matter of debate (170–172). In addition, clear evidence for intraislet paracrine influences has not yet been reported (172). Importantly, the effects of ACh on insulin secretion are observed at glucose concentrations that are substimulating or stimulating for insulin release but inhibitory for glucagon release.

Several intestinal hormones, particularly glucose-dependent insulin-releasing peptide (GIP) and glucagon-like peptide-1, potently increase insulin secretion (173–175). GIP- and

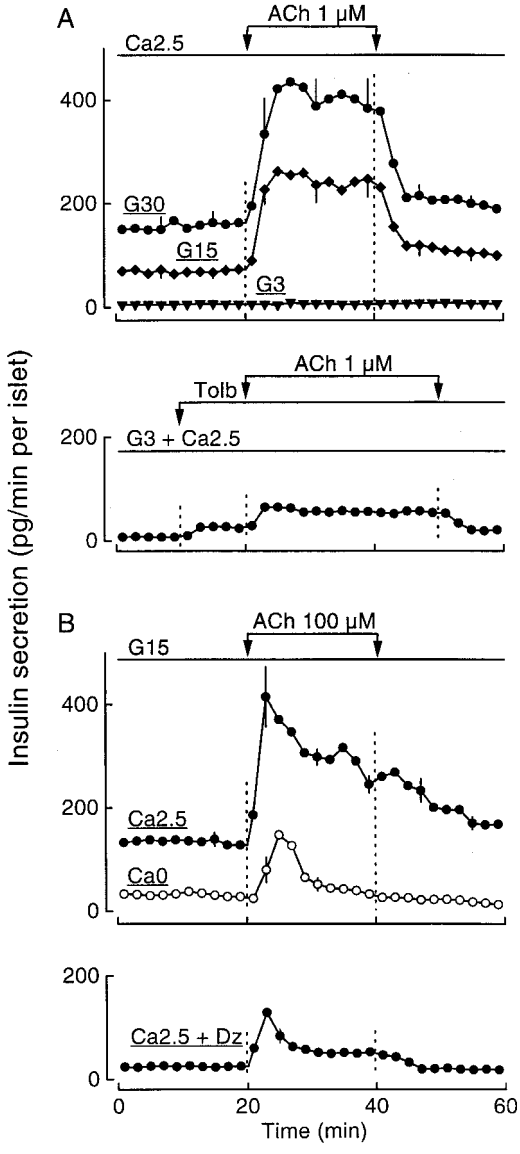


FIG. 1. General characteristics of ACh effects on insulin secretion *in vitro*. Mouse islets were perifused with a medium containing 2.5 mM $CaCl_2$ (Ca2.5) or no $CaCl_2$ (Ca0) and 3, 15, or 30 mM glucose (G3, G15, and G30, respectively). A, Experiments with freshly isolated islets. In the experiments shown in the *lower panel*, 100 μM tolbutamide (Tolb) was added to the medium to close K^+-ATP channels and depolarize the β-cell membrane despite the low glucose concentration. [The *lower panel* was redrawn from M. P. Hermans *et al.*: *Endocrinology* 120: 1765–1773, 1987 (279).] B, Experiments with cultured islets. In the experiments shown in the *lower panel*, the medium was supplemented with 100 μM diazoxide (Dz) to open K^+-ATP channels and hold the membrane hyperpolarized despite the high glucose concentration.

glucagon-like peptide-1-secreting cells possess muscarinic receptors (176, 177), and both the stimulation of the vagus nerve and cholinergic agonists stimulate their release (178).

ACh also stimulates gastric emptying (179), which may affect the rate of glucose absorption, change in glycemia, and hence, insulin secretion (180, 181). It has also been reported that the increase in islet blood flow produced by a rise in blood glucose is mediated by the central nervous system, which senses the changes in glycemia and sends signals to islet vessels through the vagus nerves (182).

B. Physiological situations

1. Role of the vagus nerve on glucose tolerance. In lean animals or humans, basal insulin secretion is not affected or is only slightly decreased by vagotomy or atropinization (9, 149, 183–196), which indicates that there is no significant tonic stimulation of the β-cells by the parasympathetic system in the fasting state. In contrast, it is generally agreed that the vagus nerves participate in the control of insulinemia during the periods of feeding. The difficulty is to assess their contribution to the overall insulin-secretory response. Thus, depending on the study, the tolerance to a glucose load is unaffected or impaired by atropine or vagotomy, whereas the associated insulin response is larger, similar, or smaller. The results become more consistent when the insulinogenic index (Δ insulinemia/Δ glycemia) is calculated, and the mode of administration of glucose (oral or iv) is taken into account (197–199). Thus, when glucose is administered iv, the insulinogenic index is not affected or is hardly modified by atropine or vagotomy (185, 187, 191, 193, 195, 197–201). In contrast, when glucose is given orally, the insulinogenic index is significantly decreased by atropine or vagotomy (184, 197, 199, 200, 202). In addition, the rise in plasma insulin is delayed, which also contributes to the glucose intolerance of vagotomized or atropine-treated rats (1, 199, 203). These results suggest that ACh potentiates the insulin response to glucose after a glucose load, a conclusion that is supported by experiments using animal models without parasympathetic innervation of the β-cells. After destruction of their β-cells by alloxan or streptozotocin, rats were transplanted with isolated islets. The vagus nerves of the receivers were intact, but the transplanted β-cells were presumably denervated at the time of test (203–205). Meal ingestion induced a glucose increase that was larger in transplanted than in normal control rats, and that was associated with a delayed insulin response (40, 206, 207). This confirms that a direct parasympathetic innervation of β-cells improves glucose tolerance.

2. The vagus nerves transmit signals of several origins. When evaluating the physiological role of the muscarinic control of β-cells, it is important to bear in mind that the vagus nerves are the parasympathetic effectors of signals that are all integrated in the brain but come from at least four sources: cephalic sensory organs including those of the oral cavity and the visual and olfactory systems, the gut, the liver, and the brain itself (208). The sequential activation of all these inputs will affect insulin secretion in a time-dependent manner upon meal ingestion.

a. Cephalic sensory organs and the gastrointestinal tract. The preabsorptive insulin phase corresponds to the earliest plasma insulin rise during the first minutes of food ingestion. It does not depend on nutrient assimilation, as it occurs before the glycemia has increased (1, 200, 206, 207, 209–216) and is sometimes associated with a transient hypoglycemia (28, 203, 217). The amplitude of the preabsorptive insulin phase is highly variable from one study to another, but it is consistently much smaller than the postabsorptive insulin phase occurring when glycemia starts increasing. It corresponds to a rise of approximately 20% (28, 216, 217) or more

(1, 206) above basal insulinemia, which may be an underestimation of the reality, because insulin is measured in peripheral or heart blood and not directly in the portal circulation. Insulin is indeed very rapidly degraded by the liver (50% during the first passage of blood), and the amplitude of the changes in insulinemia is much smaller in peripheral than in portal blood (192, 218). The preabsorptive insulin response involves both cholinergic and noncholinergic mechanisms (3, 216). It can be subdivided into the cephalic phase and the enteric phase.

The cephalic phase does not even require ingestion of nutrients, as it can occur in response to oral saccharin or water intake in animals (1, 204), but not in humans (219). Its mechanisms involve stimulation of oropharyngeal receptors (210, 220, 221) and probably also conditioned visual and olfactory reflexes, because an early peak of insulin secretion can be observed in animals that simply see or smell food (28). A cephalic phase exists in humans (Refs. 28 and 222–225, but see Ref. 226), but is less easily conditioned than in animals (227, 228). Because no cephalic phase occurs after vagotomy, nor is this phase observed in diabetic animals transplanted with denervated islets, it is ascribed to a direct stimulation of β-cells by both cholinergic and noncholinergic fibers of the vagus nerves (3, 203–206). The sympathetic nervous system might also contribute to the cephalic phase in the dog by activating β_2-adrenoceptors (229).

The enteric phase has been much less extensively studied because of the difficulty in separating it from the preceding cephalic phase and the following postabsorptive phase, which rapidly causes an increase in glycemia (28, 203, 217, 230, 231). This phase has been observed after direct infusion of a meal into the stomach or the duodenum (176, 203, 232) and is sometimes reflected by a single preabsorptive insulinemia peak (203). Abolition of this phase by vagotomy and atropine implicates the vagus nerve (176, 217, 232), but it remains unclear whether the response is mediated indirectly by a vagally induced release of incretins (203), or more directly by a reflex involving gut glucoreceptors augmenting efferent activity of the pancreatic branch of the vagus nerve (233, 234). Glucoresponsive neurons equipped with K^+-ATP channels similar to those of β-cells have recently been identified in the myenteric plexus of the guinea pig ileum (235).

The role of the preabsorptive phases of insulin secretion was initially addressed by comparing the insulin and glucose responses to oral *vs.* iv glucose administrations (203, 236). However, it was later found that this type of comparison might be misleading because a lesser glucose tolerance after iv administration of the sugar could result from the lack of incretin effect rather than the lack of preabsorptive insulin secretion. The importance of the cephalic phase for glucose homeostasis was established by experiments showing that plasma glucose and insulin concentrations increased more after direct administration into the stomach than after oral intake of the same amount of nutrients (237, 238). These results were confirmed in a more recent study that compared the insulin and glucose responses to gastric glucose administration in humans allowed or not allowed to taste food (239). Prevention of cephalic phase during food intake diminished the glucose tolerance without changing insulin secretion during the 3-h period after the beginning of food intake. This glucose intolerance was attributed to differences in the kinetics of the changes in insulinemia, and possibly also to a larger glucagon secretion over the same period of time (239).

That the cephalic phase exerts a beneficial, long-lasting effect has also been elegantly demonstrated after oral glucose administration to insulin-deficient rats transplanted with denervated islets. The missing cephalic phase in these rats was mimicked by a small premeal iv injection of insulin. This restored early insulin peak did not affect subsequent plasma insulin levels during the period of glucose intake, but attenuated (without normalizing) the rise in plasma glucose levels (40).

With the exception of two reports (196, 240), all the above-described studies suggest that the timing of insulin secretion is important for optimal glucose homeostasis. By promoting anticipatory use of glucose by the liver, muscles, and adipose tissue, and by inhibiting glucose production by the liver, the preabsorptive insulin phases restrain the changes in glycemia and insulinemia within a narrow range. This may serve as a protection against overworking of the β-cells.

b. Liver. Like β-cells, the liver receives efferent vagal stimuli in response to an oral stimulus (220, 241). Neurophysiological studies have also revealed that portal glucose injection increases efferent vagus activity innervating the pancreas, and it has been suggested that hepatic glucosensitive mechanisms may affect pancreatic function by involving hepatic vagus afferents and pancreatic vagus efferents (208). These results are supported by some physiological experiments demonstrating that a rise of the glucose concentration in the portal circulation to the liver induces an increase of insulin secretion that is prevented by vagotomy of the hepatic branch (242) and is mimicked by hepatic vagal stimulation (243). However, other studies have reported that a rise in insulinemia is mimicked by a section of the hepatic branches of the vagus nerve, whereas a drop in insulinemia is induced by electrical stimulation of this branch (241, 244). Therefore, it has been suggested that afferent fibers exert a tonic inhibition in brainstem centers of an efferent vagal branch innervating the pancreas (241, 244). This implies that the afferent hepatic nerve activity is inversely related to the portal glucose level, which is confirmed by neurophysiological data (245–247). It is difficult to establish how glucose homeostasis is influenced by these vagally mediated messages from the liver to the endocrine pancreas.

c. Brain. Several studies suggest that an increase in the glucose concentration in the brain can increase vagal tone. Indeed, injection of glucose in the carotid artery of rats, in an amount insufficient to modify systemic plasma glucose concentration, induced a rapid increase in insulin secretion that was abolished by vagotomy (182). This effect likely involves glucoresponsive neurons in the hypothalamus and the nucleus tractus solitarius (220, 248).

3. Is there a long-lasting vagal stimulus during the absorptive phase? Whereas it is clear that the parasympathetic system contributes to the preabsorptive insulin response, it is less obvious whether a parasympathetic stimulus of the endocrine pancreas persists during the meal. Because ACh is

quickly degraded by cholinesterases in plasma, it is impossible to reliably measure the pancreatic ACh spillover as an index of parasympathetic neural activity of the pancreas (229). Measurements of plasma PP provide an alternative approach to evaluate the parasympathetic activity. Indeed, PP secretion is predominantly under vagal control because its secretion *in vivo* is nearly completely prevented by atropine or vagotomy (249–251). Meal ingestion induces a biphasic PP secretion characterized by a rapid first phase followed by a second sustained response. Both phases are prevented by atropine (229). The first phase has sometimes been correlated to the preabsorptive insulin response (250, 252). The presence of the second sustained phase supports the existence of a long-lasting cholinergic stimulus. Because cholinergic nerve fibers innervating PP and β-cells likely have a common origin, it is highly plausible that β-cells are also under the influence of a long-lasting vagal stimulus during meals. This suggestion is corroborated by the observation that atropine markedly suppressed insulin response to a meal (251). However, recent data obtained with mice lacking the M_3 muscarinic receptor (the main muscarinic receptor on β-cells, see *Section X*) do not clarify the issue. These mice do not show any signs of impaired glucose intolerance after oral or ip glucose administration (253), but it is unclear to which extent other factors that were observed in these mice, such as increased insulin sensitivity, hypoleptinemia, and hypophagia, contributed to glucose tolerance.

C. Pathophysiological situations: hyperinsulinemia, obesity, and insulin resistance

The net effect of the central nervous system on insulin secretion is the result of a balance between the influence of the inhibitory sympathetic system and the stimulatory parasympathetic system. The metabolic consequences of a dysregulation of this subtle balance have been reviewed recently (254). Only the troubles associated with an anomaly of the parasympathetic system will be briefly mentioned here.

Two areas in the brain play a major role in the control of the efferent autonomic pathways, the ventromedial hypothalamic nuclei (VMH, also called the satiety center) and the lateral hypothalamic area (LHA, also called the feeding center) (241, 254). The VMH increases the activity of the sympathetic nervous system and decreases that of the parasympathetic nervous system, whereas opposite effects are produced by the LHA. The VMH and the LHA reciprocally inhibit each other.

Several animal models of hyperinsulinemia are characterized by a dysregulation of the sympathetic and parasympathetic pathways. A lesion of the VMH in the rat causes an exaggerated insulin response to an iv or intragastric glucose load. This hyperinsulinemia occurs rapidly, 10 min after the lesion (255), and is abolished by atropine or vagotomy (256–259). In animal models of hyperinsulinemia associated with a defect in leptin signaling, such as the *ob/ob* mice (abnormal leptin) or *fa/fa* (Zucker) rats (abnormal leptin receptors), the earliest detectable alteration of insulin secretion is a hyperresponsiveness to glucose that occurs before the animals become hyperphagic (193, 260–265). It is mediated by the vagus nerve, as it is abolished by atropine (194, 255, 260, 266) or vagotomy (193, 266). NPY is a potent physiological stimulator of feeding that is present at abnormally high levels in the hypothalamus of *fa/fa* rats and *ob/ob* mice. Rats that undergo a chronic intracerebroventricular infusion of NPY display basal and glucose-induced hyperinsulinemia that is prevented by vagotomy (267, 268). A common feature of all these animal models is a hyperinsulinemia that results from an excessive vagal cholinergic tone and an attenuation of the inhibitory sympathetic tone (254). The chronic influence of hyperglycemia on the autonomic system may also aggravate the syndrome (269).

An increased sensitivity of β-cells to ACh might also contribute to hyperinsulinemia. This has been reported in *ob/ob* mice (262) and in genetically normal mice subjected to a high-fat diet (46, 270, 271). The hyperinsulinemia brought about by an enhanced cholinergic over sympathetic tone and/or an exaggerated sensitivity of β-cells to ACh might be a compensatory mechanism for insulin resistance.

This fairly clear picture obtained in experimental animals cannot readily be extrapolated to human subjects. Although indirect evidence suggests that insulin secretion is more sensitive to cholinergic stimulation in insulin-resistant obese subjects than in lean subjects (272), it is widely agreed that atropine does not correct the hyperinsulinemia of obese subjects (195, 196). Moreover, the preabsorptive phase of insulin secretion was found to be enhanced (273), normal (224, 274, 275), or absent in obese subjects (276). In type 2 diabetes, the initial rise in insulin levels after a meal is often delayed or deficient (277, 278), but it is unknown whether an impaired preabsorptive vagal stimulus contributes to this defect.

IV. General Characteristics of Acetylcholine (ACh) Effects on Insulin Secretion *in Vitro*

The glucose dependence of the effects of ACh on insulin release has already been emphasized (see *Section III* and Refs. 162 and 163) and is illustrated in Fig. 1A. At the concentration of 1 μM, ACh does not affect basal insulin secretion (3 mM glucose), but causes a rapid, marked, and sustained potentiation of insulin secretion induced by 15 mM glucose (the half-maximally effective concentration in this model). The effect of ACh starts to appear between 5 and 7 mM glucose, *i.e.*, around the threshold concentration of the sugar (not illustrated), and persists in the presence of a maximally effective concentration of glucose (30 mM). ACh also increases insulin secretion in the presence of nutrients other than glucose, *e.g.*, leucine (279). However, nutrients can be replaced by tolbutamide to unmask the insulinotropic effect of ACh. As shown in the *lower panel* of Fig. 1A, the addition of 100 μM tolbutamide to a medium containing 3 mM glucose evokes a small increase in insulin secretion (mediated by K^+-ATP channel closure and membrane depolarization), and the subsequent addition of 1 μM ACh slightly potentiates insulin secretion. The larger efficacy of ACh in the presence of high glucose than in the presence of low glucose plus tolbutamide can largely be ascribed to the amplification of insulin secretion (increase in Ca^{2+} efficiency in exocytosis) that the sugar produces (280). This glucose dependence persists when ACh is used at high concentrations (*e.g.*, 100 μM), which, however,

also induce a small sustained elevation of basal insulin secretion (not shown).

The pattern of ACh-induced insulin secretion critically depends on whether Ca^{2+} influx can occur (281–288). In the presence of a control medium containing extracellular Ca^{2+}, the stimulation of secretion is sustained. When Ca^{2+} is omitted from the medium, only high ACh concentrations (≥10 μM) trigger a rapid, transient peak of secretion that also requires the presence of a high concentration of glucose (or other nutrients) (Refs. 162 and 289–291 and Fig. 1B). Ca^{2+} influx can also be prevented by opening K^+-ATP channels with diazoxide and holding the membrane at the resting potential. Under these conditions, the effect of ACh is similar to that produced in the absence of extracellular Ca^{2+} (289) (Fig. 1B, *lower panel*). Blockade of voltage-operated Ca^{2+} channels similarly affects the action of ACh on secretion (not shown) (287).

The mechanisms underlying these glucose, membrane potential, and Ca^{2+} dependencies of ACh-induced insulin secretion will be explained in the following paragraphs.

V. Effects of ACh on β-Cell Phospholipases

A. Activation of PLC

1. Type of PLC and mechanisms of activation. PLC enzymes hydrolyze the phosphodiester bond on the third (sn-3) position of phosphoglyceride molecules to release diacylglycerol (DAG) and a phosphorylated polar head group (Figs. 2 and 3). There exist three main groups of PLC (PLC-β, PLC-γ, and PLC-δ), each containing several subtypes (292–294). PLC-β are activated by heterotrimeric G proteins, whereas PLC-γ are activated by tyrosine kinases. The mechanisms of activation of PLC-δ are unknown (294). All three types hydrolyze phosphatidylinositol (PI), PI 4-phosphate (PIP), and PI 4,5-bisphosphate (PIP_2) in a Ca^{2+}-dependent manner to produce DAG and inositol 1-phosphate (1 IP), inositol 1,4-bisphosphate, and IP3, respectively. At low $[Ca^{2+}]_c$, PIP_2 is the preferred substrate (293). In some tissues, certain PLC isoforms can hydrolyze plasmenylcholine, phosphatidylethanolamine, or phosphatidylcholine (PC) (295–298).

In pancreatic β-cells, PLC is both cytosolic and membrane associated (299–301) and specifically hydrolyzes phosphoinositides (300, 302). Stimulation of normal β-cells (5, 286, 303–309) and insulin-secreting tumor cells (310–313) with cholinergic agonists has long been shown to cause DAG and IP3 accumulation. By analogy with other tissues (314, 315), it is assumed that this results from activation of a PLC-β isozyme. However, it is not known which of the three PLC-β isoforms (β1, β2, or β3) identified in β-cells (316–320) is coupled to the muscarinic receptor. Activation of PLC by cholinergic agonists involves a G protein (310, 321, 322). One single study, performed with rat islets, reported that the G protein activated by carbachol is pertussis toxin sensitive and suggested that it corresponds to a $G_{\alpha o}$ protein (323). All other studies, performed with RINm5F cells (324, 325), rat islets (321, 326, 327), and β-TC3 cells (328), found the G protein coupled to the muscarinic receptor to be pertussis and cholera toxin insensitive. It is thought to belong to the G_q subfamily (328), like the G protein that couples muscarinic receptors to PLC-β in other tissues. Activation of PLC-β has been reported to be directly mediated by the α-subunit of the G_q protein (329). The G_q subfamily contains several members ($G_{\alpha q}$, $G_{\alpha 11}$, $G_{\alpha 14}$, $G_{\alpha 15}$, and $G_{\alpha 16}$) (330, 331) that seem to specifically interact with the different PLC-β subtypes (292). The nature of the G_α-subunit involved in the coupling of the muscarinic receptors to PLC-β in β-cells is unknown.

Phospholipid hydrolysis and inositol production is larger in β-cells maintained in a Ca^{2+}-containing medium than in a Ca^{2+}-free medium (5, 162, 290, 303, 332), and are markedly reduced in insulin-secreting cells loaded with the Ca^{2+}-chelator 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (333). Two hypotheses have been put forward to explain this Ca^{2+} dependence. Because PLC is strictly Ca^{2+} dependent (293), the enhanced hydrolysis of phosphoinositides observed in cells bathed in a Ca^{2+}-containing medium has sometimes been attributed to a direct activation of PLC by Ca^{2+} (300, 305, 307, 322, 332–338), independently from calmodulin

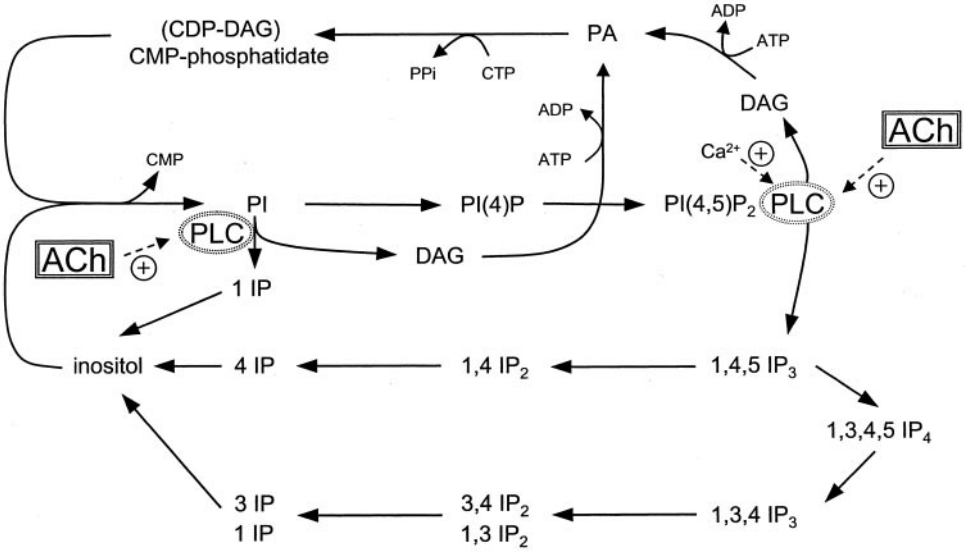


FIG. 2. Influence of ACh on phosphoinositide metabolism in pancreatic β-cells. See text (*Section V.A.2*) for explanations.

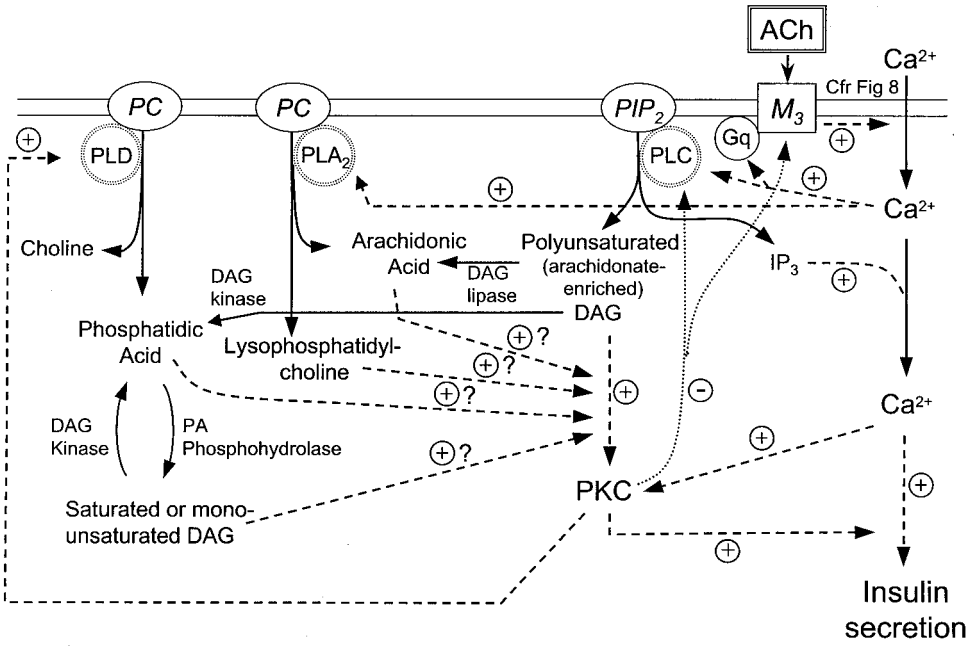


FIG. 3. Influence of ACh on phospholipid metabolism in pancreatic β-cells and its role in the control of insulin secretion. *Arrows with solid lines* represent metabolic or biophysical pathways. *Arrows with dashed and dotted lines* illustrate stimulatory and inhibitory influences. *Question marks* denote PKC-stimulating pathways that are still debated or are not clearly demonstrated in β-cells. See text (*Section V*) for explanations.

(300, 337). This proposal was supported by the observations that high K^+, which induces a large rise in $[Ca^{2+}]_c$, increased IP3 or total IPs levels (332, 339, 340) and accelerated the efflux of radioactivity from rat islets prelabeled with [^{3}H]inositol (335). However, these results must be interpreted with caution. First, the effect of high K^+ on IP3 levels is species dependent and larger in the rat than in the mouse (340), perhaps because of the expression of different PLC isoforms (317, 320, 341, 342). Second, even in the rat, phosphoinositide breakdown is much larger in response to carbachol than to high K^+ (316, 332, 335, 339). Therefore, a second hypothesis suggests that the potentiation by Ca^{2+} of ACh-induced IP3 production results from a synergistic effect between Ca^{2+} and muscarinic activation (309, 316). As G proteins have been reported to enhance the Ca^{2+} sensitivity of PLC (343–346), the Ca^{2+} requirement would be reduced to the resting $[Ca^{2+}]_c$ levels. This would also explain how muscarinic agonists can elicit a significant phosphoinositide hydrolysis in cells bathed in a Ca^{2+}-free medium. Finally, three studies have proposed that PLC activity can also be controlled by the membrane potential, independently from a change in $[Ca^{2+}]_c$. Depolarization in a medium supplemented with methoxyverapamil or in a Ca^{2+}-free medium was reported to increase PLC activity in *ob/ob* islets (347) or in insulin-secreting β-TC3 cells loaded with 1,2-bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (338). The opposite effect, a decrease in PLC activity, was measured in rat islets (339). Thus, the question remains unanswered.

2. Phosphoinositol and phosphoinositide metabolism. Experiments with RINm5F cells or rat islets have shown that IP3 is very rapidly transformed into inositol 1,3,4,5-tetrakisphosphate (307, 332, 348) by a kinase activated by Ca^{2+}-calmodulin (305, 349, 350), and into inositol 1,4-bisphosphate by a phosphatase (305, 348, 351, 352) (Fig. 2). Inositol 1,3,4,5-tetrakisphosphate can then be degraded into inositol 1,3,4-trisphosphate. All inositol phosphate isomers can be further metabolized through complex pathways (350, 353). In several studies in which the different isomers of inositol phosphate were not separated (5, 290, 311, 313), cholinergic agonists caused a monotonic increase of inositol trisphosphate levels to a plateau that was reached within approximately 1 min and thereafter maintained with only a minor decline. When the two major isomers of inositol trisphosphate, *i.e.*, IP3 and inositol 1,3,4-trisphosphate, were separated, very different time courses of accumulation emerged (305, 311, 352). Indeed, IP3 accumulation consisted in a burst, reaching a peak within the first 5 sec of stimulation, followed by a decrease and then a lower sustained phase. By contrast, inositol 1,3,4-trisphosphate levels increased slowly, reached a maximum after approximately 30 sec of stimulation, and plateaued at that level thereafter. The biphasic increase in IP3 probably involves both a negative feedback effect of PKC on PLC activity (see *Section V.A.3*) and a rapid degradation of IP3 (352, 354). Indeed, the two-step conversion of IP3 into inositol 1,3,4-trisphosphate initiated by the Ca^{2+}-calmodulin-sensitive IP3 kinase was markedly attenuated if the rise in $[Ca^{2+}]_c$ that IP3 produces (see *Section VIII.A.1*) was abolished (305). This suggests that the rise in $[Ca^{2+}]_c$ contributes to the rapid degradation of IP3 and, therefore, also contributes to the transient nature of its accumulation (305, 352).

Whereas it is well established that muscarinic stimulation of insulin secretion increases with the glucose concentration, it remains unclear whether glucose, *per se*, potentiates ACh-induced IP3 accumulation. The larger effect that carbachol produces in the presence of high glucose (164, 304, 316, 355) might well result from an additional Ca^{2+}-dependent activation of PLC due to a greater increase in $[Ca^{2+}]_c$ (see *Section VIII.A.3*). This interpretation is supported by the observation that glucose failed to enhance carbachol-induced accumulation of inositol trisphosphate in rat islets incubated in a Ca^{2+}-free medium (339). Glucose itself and various intermediates of its metabolism were without effect on PLC activity in a cytosolic fraction of mouse islet homogenate

(300). However, other reports have suggested that glucose metabolism might interact with phosphoinositol and phosphoinositide metabolism. Thus, glucose metabolites inhibited IP3 degradation (356, 357) and increased IP3 production (358) in rat islet and RINm5F cell homogenates, and directly stimulated Ca^{2+} release from intracellular Ca^{2+} stores of HIT-T15 cell homogenates (359). Moreover, glucose has been reported to stimulate the *de novo* synthesis of DAG, inositol phosphate, phosphoinositides, or polyphosphoinositides (4, 302, 305, 311, 358, 360–366). More experiments must be performed to evaluate the possible direct effects of glucose on ACh-induced IP3 accumulation.

In many tissues, PLC mainly hydrolyzes PIP_2. In pancreatic β-cells, cholinergic agonists also stimulate PI hydrolysis as shown by the rapid accumulation of 1 IP independently from the other phosphoinositol intermediates in rat islets challenged with carbachol (348) (Fig. 2). In agreement with this observation, PI was found to be a better substrate than PIP_2 for PLC from the cytosolic fraction of mouse islet homogenates (300). Surprisingly, carbachol-induced accumulation of 1 IP in rat islets is stimulated by hyperpolarization and is inhibited by depolarization of the plasma membrane (339). The underlying mechanisms are not known. The predominance of PI hydrolysis over that of PIP_2 during prolonged stimulation (348) implies that DAG can be formed independently of IP3 formation. However, the potential importance of this pathway for the stimulation of insulin release has yet to be established.

At the same time cholinergic agonists hydrolyze phospholipids, they also accelerate PI turnover. DAG can be resynthesized back to PI by the following steps (Fig. 2): 1) diacylglycerol kinase converts DAG into phosphatidic acid (PA) at the expense of an ATP; and 2) PA then reacts with CTP to form CMP-phosphatidate (CDP-DAG), which in turn reacts with inositol to form PI (346, 367–369). The enzymes involved in this cycle are present in rat islets (370, 371). Because the cycle requires phosphorylated nucleotides, its turnover was estimated by labeling islets with $^{32}PO_4^{3-}$. It was found that carbachol stimulates the labeling of PA and decreases that of PIP and PIP_2, which suggests that cholinergic agonists accelerate PI turnover after PLC activation in rat islets (5, 312, 360, 372, 373). This effect was strongly inhibited in a Ca^{2+}-free medium, which might result from the Ca^{2+} dependence of PLC (334, 372). Concomitantly, there is an enhanced flux from PI → PIP → PIP_2, which is necessary for resynthesis of PIP_2 (322). This increased flux might result from a direct stimulation of the activity of PI 4-kinase, the enzyme responsible for the synthesis of PIP from PI (322). Because PIP kinase is inhibited by its product, PIP_2, hydrolysis of PIP_2 by PLC could also relieve this inhibition, therefore stimulating the flux for PIP_2 synthesis. This latter mechanism has been demonstrated in other cell types (374), but not in β-cells. Cholinergic agonists not only accelerate PI turnover, they also stimulate *de novo* synthesis of phospholipids, as deduced from the enhanced incorporation of [^{3}H]glycerol into DAG (375), PA, and PI in rat islets (360). Again, this *de novo* synthesis pathway is very much Ca^{2+} dependent (360).

Of all inositol species formed upon ACh stimulation, IP3 is the physiologically more important isomer. Its effects will be described later (see *Section VI.A.1*).

3. Diacylglycerol and PKC. DAG is liposoluble and remains in the plasma membrane. It causes the translocation of its target, PKC, from the cytosol to the membrane. This translocation also requires Ca^{2+} and an acidic phospholipid, such as phosphatidylserine (376, 377). Metabolism of DAG, either by DAG lipase-catalyzed deacylation (which yields arachidonic acid) or by DAG kinase-catalyzed phosphorylation (which yields PA), terminates its action on PKC (299, 378) (Fig. 3).

Cholinergic agonists produce two major species of DAG that are enriched in either arachidonate (a polyunsaturated fatty acid) or palmitate (a saturated fatty acid), and accumulate with different time courses in β-cells (Fig. 3). The concentration of arachidonate-enriched DAG increases quickly during the first seconds of stimulation, before declining and remaining at a lower sustained level. This type of DAG probably originates from PIP_2 that mostly contains arachidonate at the sn-2 position of the phospholipid (302, 379). By contrast, the palmitate-enriched DAG accumulates monotonically during the first minutes of stimulation (302, 375). This species resembles that produced upon glucose stimulation (365, 375), but its source upon ACh stimulation is unknown. It might originate from PLD activation (see *Section V.C*) and hydrolysis of PC, a phospholipid enriched in palmitate in islets, from a synergistic effect with glucose on *de novo* DAG synthesis, or from other undefined pathways (302, 380). A biphasic increase in polyunsaturated DAG and a delayed accumulation of saturated DAG have been documented in many other cell types (381). The differential time course of accumulation of the two DAG species might have an impact on PKC activation. Indeed, polyunsaturated DAG is a much more potent PKC activator than saturated DAG in HIT cells (365, 382) and other cell types (379).

The PKC family comprises a number of phosphatidylserine-binding isoforms that can be classified in four groups. The conventional isoforms, or cPKC, are activated by Ca^{2+} and DAG or phorbol esters; they include α, βI, βII, and γ, of which βI and βII refer to the two gene products resulting from alternative splicing of the same PKCβ gene. The novel isoforms, or nPKC (δ, ε, η, and θ), are unresponsive to Ca^{2+} but are activated by DAG alone or phorbol esters. The atypical isoforms, or aPKC (ζ, and ι/λ; PKCλ is the mouse homolog of human PKCι), are Ca^{2+} independent and do not bind DAG or phorbol esters. The PKCμ isoform is also Ca^{2+} independent and is activated by phorbol esters, but has a structure different from the other isoforms (298, 381, 383–386). The nature of the PKC isoforms present in insulin-secreting cells remains controversial (reviewed in Ref. 376). In pancreatic islets, the isoform α predominates, but one or several of the isoforms β, δ, ε, ζ, and ι may also be present (376, 387–396). This does not necessarily mean that all these isoforms are expressed in β-cells because approximately 20–35% of the islet cells are non-β-cells. Insulin-secreting cell lines express one or several of the isoforms α, β, δ, ε, η, ζ, ι/λ, and μ (382, 387, 392, 394, 397–400).

Several studies demonstrate that cholinergic agonists induce the translocation of PKC to membranes (163, 312, 365, 382, 401), but they do not establish which isoforms are translocated. Because cholinergic agonists induce DAG accumu-

lation (302, 339, 375), it seems reasonable to assume that they stimulate all DAG-sensitive isoforms present in normal and tumoral insulin-secreting cells. However, a recent study suggests that this might not be the case. Carbachol was found to translocate the α, β, and ζ isoforms without affecting the δ, ε, μ, and ι isoforms in RINm5F cells (400). These data must be interpreted with caution because they were obtained with an insulin-secreting cell line whose responses to cholinergic agonists differ from those of normal β-cells (see *Section IX.D*).

PKC phosphorylate their substrates on serine and/or threonine residues. It has been suggested that the targeting of PKC isoforms to particular membranes is mediated by specific anchoring proteins including the receptors for activated C kinases (384, 402), which might explain why a PKC isoform is translocated either to the nucleus or the plasma membrane. It is possible that the multiple phospholipid-derived second messengers produced upon ACh stimulation activate different PKC isoforms that, after being translocated to specific targets, activate different pathways (403). Such a differential activation of PKC isoforms has been reported upon glucose stimulation, with αPKC and εPKC being translocated to the cell periphery and δPKC and ζPKC being translocated to perinuclear sites (396).

Many proteins are phosphorylated by PKC in islets (for review, see Ref. 376), but their nature is largely unknown. One identified target for PKC in β-cells is the myristoylated alanine-rich C kinase substrate (MARCKS) (404), a protein that binds actin and Ca^{2+}-calmodulin and that has been implicated in cell movement and vesicle transport (405, 406). This substrate is phosphorylated in response to carbachol (407, 408). Other PKC substrates might be the G proteins that are associated with the α_2-adrenoceptor and uncouple from the receptor after phosphorylation. This mechanism might explain how phorbol esters and carbachol reduce the ability of adrenoceptors to inhibit glucose-induced insulin secretion from rat islets (409, 410). In view of the importance of the PKC-dependent pathway in the stimulation of insulin secretion by ACh (see *Section IX.B.1*), identification of the targets of PKC in β-cells is an important question.

It has been suggested that PKC activation also exerts a negative feedback control on the signal transduction linked to PLC and activated by ACh. Indeed, stimulation of PKC inhibits the production of inositol phosphates induced by cholinergic agonists (290, 312, 322, 352, 393). This effect occurs within 10 min (perhaps within even less time) of stimulation with phorbol esters (352). It likely contributes to the biphasic time course of accumulation of IP3 and arachidonate-enriched DAG upon stimulation with ACh. This PKC-mediated negative feedback might result from the uncoupling of PLC from the ACh receptor (411), either by a direct phosphorylation of PLC by PKC (412) and/or Ca^{2+}-calmodulin kinase (413), by a modification of the G protein coupling the ACh receptor to PLC (414, 415), or by phosphorylation of the receptor itself (416). A muscarinic receptor kinase has recently been identified that might fulfill this role leading to decreased PLC activity (315, 417). Alternatively, cholinergic stimulation could cause a down-regulation of muscarinic receptors via (312, 418) or independently (419) of PKC activation.

B. Activation of PLA_2

PLA_2 enzymes hydrolyze the sn-2 ester linkages in phosphoglyceride molecules to release a lysophospholipid and a free acid, such as arachidonate (Fig. 3). They can hydrolyze various substrates, such as PC, phosphatidylethanolamine, phosphatidylserine, PI, PA, and plasmalogens (420).

There exist several types of mammalian PLA_2 (421). Types I, II, V, and VII are associated with membranes and, because they are secreted, are referred to as secretory PLA_2 ($sPLA_2$). Types I, II, and V are stimulated by millimolar Ca^{2+} concentrations, whereas type VII is Ca^{2+} independent (421). Types IV, VI, and VIII are cytosolic. Type IV is Ca^{2+} dependent, requiring micromolar Ca^{2+} concentrations to be translocated to the membrane, whereas types VI and VIII are Ca^{2+} independent (421). Types IV and VI PLA_2 display a specificity for phospholipids with arachidonic acid esterified to the second carbon of the glycerol backbone (422, 423), whereas types I and II show little specificity for the hydrolyzed fatty acid chain (293, 423).

The presence of $sPLA_2$ in insulin-secreting cells is well documented, but controversies persist concerning the type of $sPLA_2$ that is expressed (424–426). $sPLA_2$ might be associated with insulin-secretory granules (427). Pancreatic islets and insulin-secreting cell lines also contain type IV PLA_2 and type VI cytosolic, ATP-stimulatable Ca^{2+}-independent PLA_2 (ASCI-PLA_2) (422, 425, 426, 428–432).

Several studies suggest that ACh activates PLA_2 in islets (Fig. 3). Thus, cholinergic agonists stimulate efflux of radioactivity from rat or mouse islets prelabeled with radioactive arachidonic acid (5, 433–435), and arachidonate represents the major metabolite present in the effluent fractions (433). This effect is largely Ca^{2+} dependent, as the stimulated efflux of radioactive arachidonic acid was markedly reduced by verapamil or removal of external Ca^{2+} (433, 434, 436). The persistence of a small stimulation of the efflux in a Ca^{2+}-free medium is compatible with the activation of a Ca^{2+}-independent pathway (433, 434). Similar results were obtained by studying carbachol-induced production of PGE_2, an eicosanoid derived from arachidonic acid (327). Muscarinic activation of PLA_2 is also supported by the demonstration that both lysophosphatidylcholine (434) and arachidonic acid (375, 434, 437) accumulate in rat islets upon stimulation with carbachol. However, because the accumulation of arachidonic acid was larger than that of lysophosphatidylcholine and was approximately 65% inhibited by RG80267, an inhibitor of DAG lipase, it is likely that only a fraction of arachidonic acid accumulation results from PLA_2 activation (434). The other fraction of arachidonic acid could derive from DAG lipase-catalyzed deacylation of DAG formed after PLC activation (299, 434). Because PLC can be activated by ACh in Ca^{2+}-dependent and Ca^{2+}-independent ways, this pathway could explain how some arachidonic acid accumulates even in the absence of Ca^{2+}. Carbachol has also been suggested to activate ASCI-PLA_2, but this proposal was based on the use of haloenol lactone suicide substrate (HELSS), an inhibitor of ASCI-PLA_2 (438) that has since been shown to exert nonspecific effects in β-cells (435).

The transduction mechanisms leading to activation of PLA_2 in β-cells are not known. It is likely that the increase in

$[Ca^{2+}]_c$ produced by cholinergic agonists activates the cytosolic Ca^{2+}-dependent PLA_2 (439). Indeed, high K^+ and the Ca^{2+} ionophore A23187 also increased arachidonic acid accumulation within rat islets and also increased PGE_2 release from rat islets (440). Other mechanisms might also activate PLA_2, including emptying of intracellular Ca^{2+} stores (441), G protein regulation (420), PKC (297, 298), and MAPK (442).

The two primary products formed upon PLA_2 activation are arachidonic acid and lysophosphatidylcholine (Fig. 3). Arachidonic acid has been reported to exert various effects in β-cells (380). These include Ca^{2+} mobilization from the endoplasmic reticulum (Refs. 443–448, but see Refs. 324 and 448), facilitation of voltage-dependent Ca^{2+} entry (380, 449), increase in $[Ca^{2+}]_c$ through voltage-independent Ca^{2+} channels (449), activation of K^+-ATP channels (450), and stimulation of PKC (446, 451). Among all these effects, the last one deserves particular attention because it also exists in other cell types and consists of a direct activation of PKC or a potentiation of the PKC stimulation by DAG (297, 298, 381, 403, 452, 453). Arachidonic acid is also the precursor of cyclooxygenase (PGs, prostacyclins, and thromboxane) and lipoxygenase products (hydroperoxyeicosatetraenoic acids, hydroxyeicosatetraenoic acids, and leukotrienes) (297) that seem to exert various, although not major, modulatory effects on insulin secretion (380, 454, 455). Lysophosphatidylcholine also activates PKC in the presence of DAG in various cell types (381). In β-cells and insulin-secreting cell lines, it stimulates $^{45}Ca^{2+}$ efflux and insulin secretion (456–459).

C. Activation of PLD

PLD catalyzes the hydrolysis of the terminal diester bond of the membrane glycerophospholipids, resulting in the formation of PA and a free polar head group (Fig. 3). Different PLD isoforms hydrolyze various substrates, such as PC, PI, phosphatidylserine, or phosphatidylethanolamine (460, 461). PC is the most abundant phospholipid in pancreatic islets (462–464), and its hydrolysis by PLD yields PA and choline. PLD can be activated by numerous pathways, including tyrosine kinases, PKC, or small G proteins, and seems to require various cofactors, such as fatty acids, or Ca^{2+}, for its activation (461, 465, 466).

The studies of PLD activation in islets have yielded conflicting results. Carbachol has been reported to increase the production of [^{3}H]choline in mouse islets prelabeled with [*methyl*-^{3}H]choline (467), which suggests that PLD was activated. Because this effect was mimicked by sodium fluoride, which activates PLC, and by a phorbol ester, it might result from PKC activation (467, 468). Activation of PLD by PKC has been documented in other cell types (293, 460, 461, 466). However, carbachol did not affect [^{3}H]choline production in rat islets prelabeled with [^{3}H]choline (302). Therefore, the carbachol-induced accumulation of PA in rat islets (437) was not ascribed to PLD activation, but to phosphorylation by DAG kinase of DAG derived from PLC activation (318).

In various cell types, PLD activation is implicated in the regulation of vesicular trafficking, and its main product, PA, is involved in secretion, mitogenesis, and inflammation (461). PA has been reported to directly activate PKC (469). However, early suggestions that PA can act as a second messenger to stimulate insulin release (470, 471) still await confirmation. The action of PA is terminated by its conversion into lyso-PA by a PLA_2 or DAG by PA-phosphohydrolase (472). The resulting DAG accumulation might theoretically activate PKC. However, this mechanism is probably of minor importance because DAGs derived from PLD contain saturated or mono-unsaturated fatty acids at the sn-2 position and are poor activators of PKC (379).

VI. Effects of ACh on the Membrane Potential of β-Cells

To understand how ACh influences the membrane potential of β-cells, it is important to bear in mind the mechanisms by which glucose regulates this membrane potential. Glucose enters the β-cell by a facilitated transport system belonging to the GLUT family (473–475), and its metabolism leads to a rapid increase in the ATP/ADP ratio (476), which closes K^+-ATP channels in the plasma membrane. In the absence of glucose or at a nonstimulating glucose concentration, the ATP/ADP ratio is low. Enough K^+-ATP channels are open to confer a low electrical resistance to the plasma membrane and to keep it at the resting potential, close to the equilibrium potential of K^+. In the presence of a stimulating glucose concentration, the ATP/ADP ratio is high, and K^+-ATP channels are largely closed, which increases the resistance of the membrane. The decrease of the K^+ permeability allows a yet unidentified current to depolarize the plasma membrane. When the threshold potential for activation of voltage-dependent Ca^{2+} channels is reached, an oscillating electrical activity starts (477, 478). Each oscillation of the membrane potential is characterized by a sustained depolarizing phase, commonly called slow wave, on top of which Ca^{2+} spikes occur. The effect of glucose on the membrane potential can be mimicked by other nutrients, *e.g.*, leucine, that are metabolized by the β-cell. It can also be reproduced by a pharmacological agent, such as tolbutamide, that directly closes K^+-ATP channels (479). In contrast, the effect of glucose on the membrane potential can be antagonized by diazoxide, which directly opens K^+-ATP channels even when the ATP/ADP ratio has been increased by glucose.

A. Dependence on the electrical resistance of the plasma membrane

The effects of ACh on the membrane potential depend on the glucose concentration (Fig. 4, A–C). In the presence of a low glucose concentration (<5 mM), when the membrane potential is high (resting potential), cholinergic agonists (1–100 μM) produce only a small and sustained depolarization and do not induce electrical activity (279, 480–483) (Fig. 4A). In contrast, when the membrane has already been partially depolarized by a stimulatory concentration of glucose, the depolarizing effect of ACh is larger and is accompanied by an increase of the electrical activity (Fig. 4B). However, if glucose-induced depolarization is reversed by diazoxide, the effect of ACh on the membrane potential is again similar to that produced in low glucose (Fig. 4C). This difference is not due to the absolute level of the membrane potential before addition of ACh. Thus, the effect of ACh remains small when

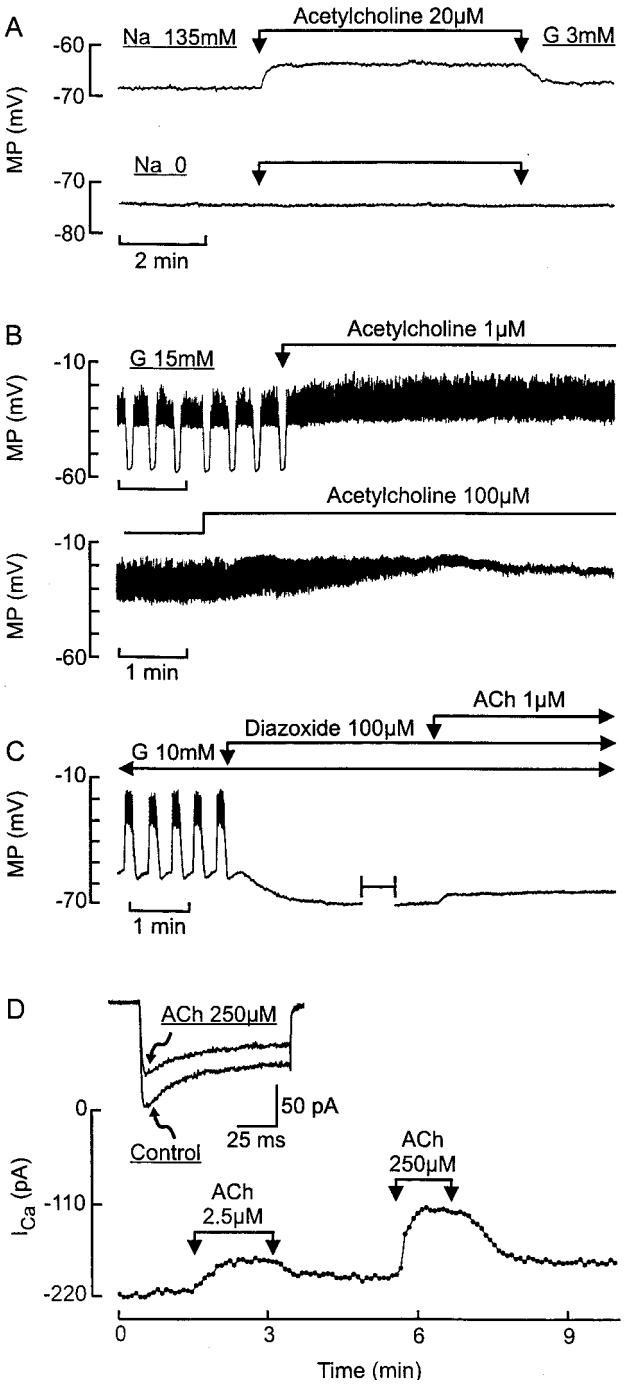


FIG. 4. Effects of ACh on the membrane potential (A–C) and voltage-dependent Ca^{2+} current (D) of mouse pancreatic β-cells. A–C, The membrane potential of a single cell within an islet was recorded with a high resistance microelectrode. A, Sodium dependence of the effect of 20 μM ACh on the membrane potential of β-cells perifused with a medium containing 3 mM glucose (G) and 2.5 mM Ca^{2+}. ACh was added when indicated to a medium containing 135 mM Na^+ (Na 135 mM) or to a medium in which Na^+ has been replaced by *N*-methyl-D-glucamine (Na 0). [Redrawn from J. C. Henquin *et al.*: *Endocrinology* 122:2134–2142, 1988 (480) © The Endocrine Society.] B, Effects of two concentrations of ACh (1 and 100 μM) on the membrane potential of a β-cell perifused with a medium containing 15 mM glucose throughout. The two recordings are shown without interruption. [Redrawn from P. Gilon *et al.*: *Biochem J* 311:259–267, 1995 (545). © the Biochemical Society.] C, Effect of 1 μM ACh on the membrane potential of a β-cell perifused with a medium containing 10 mM glucose throughout. Diazoxide (100 μM) was added when indicated. By reducing the resistance of the plasma membrane, it decreases the depolarizing action of ACh. [Redrawn from M. P. Hermans *et al.*: *Endocrinology* 120:1765–1773, 1987 (279). © The Endocrine Society.] D, Inhibition of voltage-dependent Ca^{2+} current in an isolated β-cell. The current, recorded in the whole-cell mode of the patch-clamp technique, was elicited by a depolarization from −80 to +10 mV every 10 s. The *upper trace* shows control current and current after the application of ACh. The *lower trace* represents the time course of the peak Ca^{2+} current. An *upward deflection* corresponds to a decrease of its amplitude. The inhibitory effect of ACh depends on the concentration used and is reversible. [Redrawn from P. Gilon *et al.*: *J Physiol* 499: 65–76, 1997 (630).]

the membrane is depolarized by high K^+ or arginine in the presence of diazoxide, and is large when the membrane is depolarized by tolbutamide in low glucose (279). These results indicate that the depolarizing action of ACh critically depends on the resistance of the plasma membrane. When K^+-ATP channels are open, either because the glucose concentration is low or because of the presence of diazoxide, the plasma membrane has a low resistance, and ACh produces only a minor depolarization. The depolarizing action of ACh is much larger when the plasma membrane has a high resistance because of the closure of K^+-ATP channels by glucose or tolbutamide. In the presence of a stimulating glucose concentration, cholinergic agonists accelerate the slow waves of membrane potential or produce a sustained depolarization with continuous electrical activity (161, 279, 482, 484–487) (Fig. 4B). This depolarizing effect is already manifest at low concentrations of ACh (~0.1 μM) or cholinergic agonists (161, 279, 482). One report has described a peculiar inhibitory effect of muscarinic agonists on glucose-induced electrical activity in β-cells (488).

B. Mechanisms of the depolarization

Several ionic mechanisms may depolarize the plasma membrane: a decrease of K^+ permeability, an increase of Na^+, Ca^{2+}, or Cl^- permeability, or an inhibition of the electrogenic Na^+ pump.

Before K^+-ATP channels were identified in β-cells and were shown to be the target of glucose metabolism, measurements of $^{86}Rb^+$ efflux (a tracer of K^+ efflux) from mouse islets indicated that ACh depolarizes the β-cell membrane by a mechanism other than a decrease in K^+ conductance (285). Thus, under no experimental condition did ACh decrease $^{86}Rb^+$ efflux as do glucose and tolbutamide. Moreover, the effects of ACh on the electrical activity were very different from those induced by glucose through closure of K^+-ATP channels. Indeed, a rise in the glucose concentration increased the duration of the plateau phase without affecting the frequency of slow waves, whereas low concentrations of ACh increased the frequency of slow waves of the membrane potential without affecting the duration of the plateau phase (279, 484). All available data, except those of one study (486), speak against an effect of ACh on K^+-ATP channels. However, no direct test with the patch-clamp technique has been reported. In view of the recent suggestion that PIP_2 might negatively modulate K^+-ATP channels, and that its hydrolysis by PLC-linked agonists might decrease K^+-ATP channel

activity (489, 490), it would be interesting to evaluate whether ACh indirectly influences K^+-ATP channels in β-cells.

It has been suggested that ACh inhibits Cl^- channels in outside-out patches of β-cell membrane (491). However, ACh was found not to affect $^{36}Cl^-$ efflux from normal mouse islets (492) and $^{36}Cl^-$ retention by *ob/ob* mouse islets (493). Moreover, the depolarization produced by ACh was unaffected in a Cl^--free medium (492). Overall, these observations indicate that Cl^- plays no major role in the effect of ACh on the β-cell membrane potential.

The currently accepted hypothesis is that ACh depolarizes the β-cell membrane by increasing its permeability to Na^+ (480, 493). The cornerstones of this proposal are the abolition of the depolarization by omission of extracellular Na^+ (Fig. 4A) (480) and the activation of a small Na^+-dependent inward current by ACh (494). The hypothesis is also supported by the observations that ACh increases total Na^+ content (495), $^{22}Na^+$ uptake (480, 493), and free cytosolic Na^+ concentration ($[Na^+]_c$) (496) in islet cells. The mechanisms by which ACh activates a Na^+ current are not known. Activation of voltage-dependent Na^+ channels has been ruled out for two reasons: 1) these channels are already completely inactivated at the resting potential in mouse β-cells (497) or at the plateau potential in the rat (498); and 2) tetrodotoxin, a blocker of voltage-dependent Na^+ channels, does not prevent the depolarization, the $^{22}Na^+$ uptake, the $[Na^+]_c$ increase, or the inward current produced by ACh (480, 494, 496). Nicotinic receptors are nonselective cation channels (416, 499). However, these channels are not present in pancreatic β-cells. All effects of ACh on membrane potential, Na^+ current, and $[Na^+]_c$ measurements are completely prevented by atropine, whereas they are unaffected by tubocurarine or hexamethonium, two nicotinic antagonists, and are not mimicked by nicotine (480, 484, 486, 493, 494, 496). In cardiac Purkinje cells, ACh was found to increase $[Na^+]_c$ by blockade of the Na^+ pump (500). This is not the case in β-cells, because ACh- and ouabain-induced $[Na^+]_c$ increases were additive (495, 496).

In various cell types, emptying of intracellular Ca^{2+} pools activates different conductances (Ca^{2+}, Na^+, or K^+) (501–505) carried by a family of channels called store-operated channels (SOCs) (503–505). The tumoral insulin-secreting MIN6 cells express the transient receptor potential 1 gene (506), whose human homolog encodes a nonselective channel permeable to Na^+ and Ca^{2+} and is activated by Ca^{2+} store depletion (507). In platelets and lymphocytes (508, 509), intracellular Ca^{2+} store depletion by thapsigargin and cyclopiazonic acid, two inhibitors of the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump, activates Na^+ influx. It has been hypothesized that ACh, which also depletes intracellular Ca^{2+} stores (see *Section VIII.A.1*), activates Na^+ influx by a similar mechanism (487). However, this pathway accounts for only a small fraction of the influx of Na^+ elicited by ACh in β-cells, because thapsigargin or cyclopiazonic acid, which empty intracellular Ca^{2+} pools much more efficiently than does ACh, did not mimic or abolish the rise in $[Na^+]_c$ produced by ACh (510). Likewise, thapsigargin did not prevent ACh from activating an inward Na^+ current (494).

It is clear that K^+ is the major counterion for the increased Na^+ influx in β-cells. Indeed, ACh induces a sustained stimulation of $^{86}Rb^+$ efflux from mouse islets, which is abolished in a Na^+-free medium (162, 285, 480). This acceleration of K^+ efflux is a very sensitive response to ACh, similar to that of the membrane potential, as it is almost maximally stimulated by 1 μM ACh. Its resistance to omission of extracellular Cl^- and to furosemide rules out the intervention of the $Na^+K^+2Cl^-$ cotransport system (492). It remains unclear whether the channel activated by ACh is highly selective for Na^+ or is nonselective, carrying both K^+ efflux and Na^+ influx.

Activation of a Na^+ conductance by muscarinic receptors is not classical, but it has also been reported in other systems. M_2 receptors induce a tetrodotoxin- and pertussis toxin-resistant Na^+ current in ventricular myocytes (511–513). Muscarinic stimulation activates a nonselective cationic conductance in guinea pig gastric and ileal smooth muscle cells (514–517), rabbit jejunal longitudinal cells (518), canine pyloric circular muscle cells (519), and chromaffin cells (520). Recently, an inward monovalent cation current activated by carbachol has been reported in Chinese hamster ovary (CHO) cells expressing the M_3 receptor (521).

It is important to emphasize here that although the SOC current is not responsible for the influx of Na^+ triggered by ACh, it can depolarize the plasma membrane by stimulating Ca^{2+} influx (capacitative Ca^{2+} entry; see *Section VIII.A.2*). Indeed, thapsigargin has been reported to stimulate Ca^{2+} influx and to depolarize β-cells (522, 523). This mechanism is activated by high concentrations of ACh (100 μM), but contributes much less to the depolarization of the plasma membrane than does the stimulation of Na^+ influx that is already operative at low concentrations of the neurotransmitter (~1 μM).

C. Paradoxical hyperpolarization by ACh

Several (482, 483, 486, 487, 524, 525), but not all (480, 484), studies have reported that high concentrations of cholinergic agonists (≥10 μM) produce an early transient hyperpolarization of β-cells when islets are perifused with a stimulating concentration of glucose. Because this hyperpolarization is blocked by charybdotoxin, it might result from the transient opening of large conductance maxi $K_{(Ca)}$ channels activated during the large $[Ca^{2+}]_c$ increase resulting from Ca^{2+} mobilization (482). Activation of a K^+ current synchronized with Ca^{2+} release from intracellular Ca^{2+} stores is well documented in pancreatic acinar cells (526) and β-cells (527–530).

The rise in $[Na^+]_c$ brought about by ACh activates the sodium pump, which is electrogenic and produces a repolarizing current. However, the impact of this current only becomes evident when the depolarizing current produced by ACh stops. It is responsible for the marked and transient repolarization of the β-cell membrane upon washing of ACh (161, 480, 486, 531). It is also possible that this pump current is involved in the acceleration of the slow waves by ACh (480, 484).

VII. Other Effects of ACh in Islet Cells

Many other effects of ACh in β-cells have been reported, but they have remained controversial. Only those that were

believed to be important for the control of insulin secretion will be mentioned briefly.

A. Effects on glucose metabolism

ACh has been reported to slightly increase glucose utilization (532) and nicotinamide adenine dinucleotide (reduced form) (NADH) content in rat islets (533, 534). This effect might result from the $[Ca^{2+}]_c$ increase produced by ACh. However, other studies found glucose oxidation by mouse islets (493) and reduced nicotinamide-adenine dinucleotide (phosphate) [NAD(P)H] fluorescence (535) and glucose utilization (341) in rat islets to be unaffected by ACh. We have already emphasized that the effects of ACh on ionic fluxes and β-cell membrane potential differ from those induced by an increase in nutrient concentration.

B. Effects on cyclic nucleotides

ACh induced a small, rapid (284), and transient (534) increase in cAMP levels in rat islets incubated in low glucose, probably via the activation of Ca^{2+}-calmodulin-sensitive adenylate cyclase (536, 537). However, in the presence of stimulating glucose concentrations, ACh did not affect islet cAMP levels (161, 284, 286). In contrast to the situation in the exocrine pancreas (538) and various other cell types (539), cholinergic agonists do not increase cyclic GMP (161) and NO production (540) in islets.

C. Effects on cytoplasmic pH

It has been suggested that alkalinization of β-cells increases insulin release under certain conditions (541). ACh slightly increases intracellular pH in mouse β-cells and probably does so through the activation, by PKC, of the Na^+/H^+ exchanger, because the effect was observed in a HEPES-buffered, bicarbonate-free medium (542, 543).

VIII. ACh Controls Free Cytosolic Ca^{2+} Concentration ($[Ca^{2+}]_c$) in β-Cells

The rise of $[Ca^{2+}]_c$ in β-cells serves as a triggering signal for exocytosis of insulin granules. The complex effects of ACh on this triggering signal were first deciphered by $^{45}Ca^{2+}$ efflux measurements. The conclusions of these experiments were later confirmed by more direct approaches using fluorescent probes to measure $[Ca^{2+}]_c$ directly inside the cells (Fig. 5). ACh has only small effects on β-cell $[Ca^{2+}]_c$ in the presence of low, nonstimulatory glucose concentrations (Fig. 5A), but causes a sustained $[Ca^{2+}]_c$ rise in the presence of high glucose (Fig. 5, B and C) (544–546). This sustained response, however, requires the presence of extracellular Ca^{2+} and the possibility for Ca^{2+} to enter β-cells through voltage-operated Ca^{2+} channels (Fig. 5A). At high concentrations, ACh also unexpectedly lowers $[Ca^{2+}]_c$ in β-cells (Fig. 5C) (545). The following paragraphs describe the mechanisms by which ACh produces these changes.

A. Mechanisms by which ACh increases $[Ca^{2+}]_c$

1. Mobilization of Ca^{2+} from intracellular Ca^{2+} stores (Figs. 5A and 6, A and B). Mobilization of Ca^{2+} from intracellular Ca^{2+} stores can be studied by monitoring $^{45}Ca^{2+}$ efflux from or $[Ca^{2+}]_c$ in islets perifused with a Ca^{2+}-free medium, *i.e.*, when no Ca^{2+} influx can occur. Under these conditions, cholinergic agonists increase the $^{45}Ca^{2+}$ efflux rate (162, 279, 285–287, 291, 304, 483, 547, 548) and $[Ca^{2+}]_c$ (545, 546, 549, 550). The mechanisms underlying this $[Ca^{2+}]_c$ rise have been extensively studied with subcellular fractions or permeabilized insulin-secreting cells (324, 351, 354, 357, 443, 547, 551–561). They involve rapid production of IP3, catalyzed by PLC (see *Section V.A.1*), and its binding to specific IP3 receptors located on intracellular Ca^{2+} stores. The concentration of IP3 accumulated in response to maximal concentrations of carbachol has been estimated in experiments performed with RINm5F cells in which phosphoinositides were labeled to isotopic equilibrium with [^{3}H]inositol (311). An increase of IP3 of 1.5 μM was calculated, which is close to the reported half-maximal concentration (0.5–3 μM) that releases Ca^{2+} from the endoplasmic reticulum in permeabilized insulin-secreting cells (351, 552, 562). Accumulation of IP3 was very fast, in keeping with the rapidity of Ca^{2+} mobilization by ACh (311, 333). This Ca^{2+} mobilization is not produced by physiological phosphoinositols other than IP3 (367, 447, 552, 554, 563) and is prevented by injecting β-cells with heparin, an antagonist of IP3 receptors (523, 557, 564, 565).

The response to ACh is different in whole islets and in single cells. In whole islets, ACh induces a concentration-dependent transient peak of $[Ca^{2+}]_c$ followed by a small sustained elevation (545) (Figs. 5A and 6A). A similar biphasic pattern was reported for $^{45}Ca^{2+}$ efflux (162, 279, 285, 287, 547, 548). This contrasts with the two types of responses occurring in single cells: a rapid single $[Ca^{2+}]_c$ transient (510, 546, 550, 566) or a series of $[Ca^{2+}]_c$ oscillations (510, 566, 567) (Fig. 6B). Similar oscillations can be produced by infusing β-cells with guanosine 5′-[γ-thio]triphosphate (527, 529, 568, 569). The reason why islets do not display $[Ca^{2+}]_c$ oscillations in response to ACh in a Ca^{2+}-free medium is attributed to the fact that the recorded Ca^{2+} signal is the average of the $[Ca^{2+}]_c$ responses of all β-cells within the islet. Contrary to glucose-induced $[Ca^{2+}]_c$ oscillations that result from periodic depolarizations of the plasma membrane and are coupled between all β-cells of the islet through gap junctions (289, 570, 571), IP3-induced $[Ca^{2+}]_c$ oscillations are not synchronized between electrically coupled β-cells (572).

The amplitude of the transient peak of $[Ca^{2+}]_c$ or $^{45}Ca^{2+}$ efflux triggered by ACh largely depends on the glucose concentration present before and during ACh stimulation (162, 547, 548, 556, 573–575). It is much smaller at a low glucose concentration than at a high glucose concentration (Fig. 5A). This difference is attributed to the filling of intracellular Ca^{2+} stores by glucose (562, 565, 574). Other mechanisms, such as an enhanced production or a decreased degradation of IP3 in the presence of glucose (see *Section V.A.1.b*), might also be involved.

Three isoforms of the IP3 receptor have been described (I, II, and III) (576) that form both homo- and heterotetramers (577). Rat islets express more type III isoforms than types I

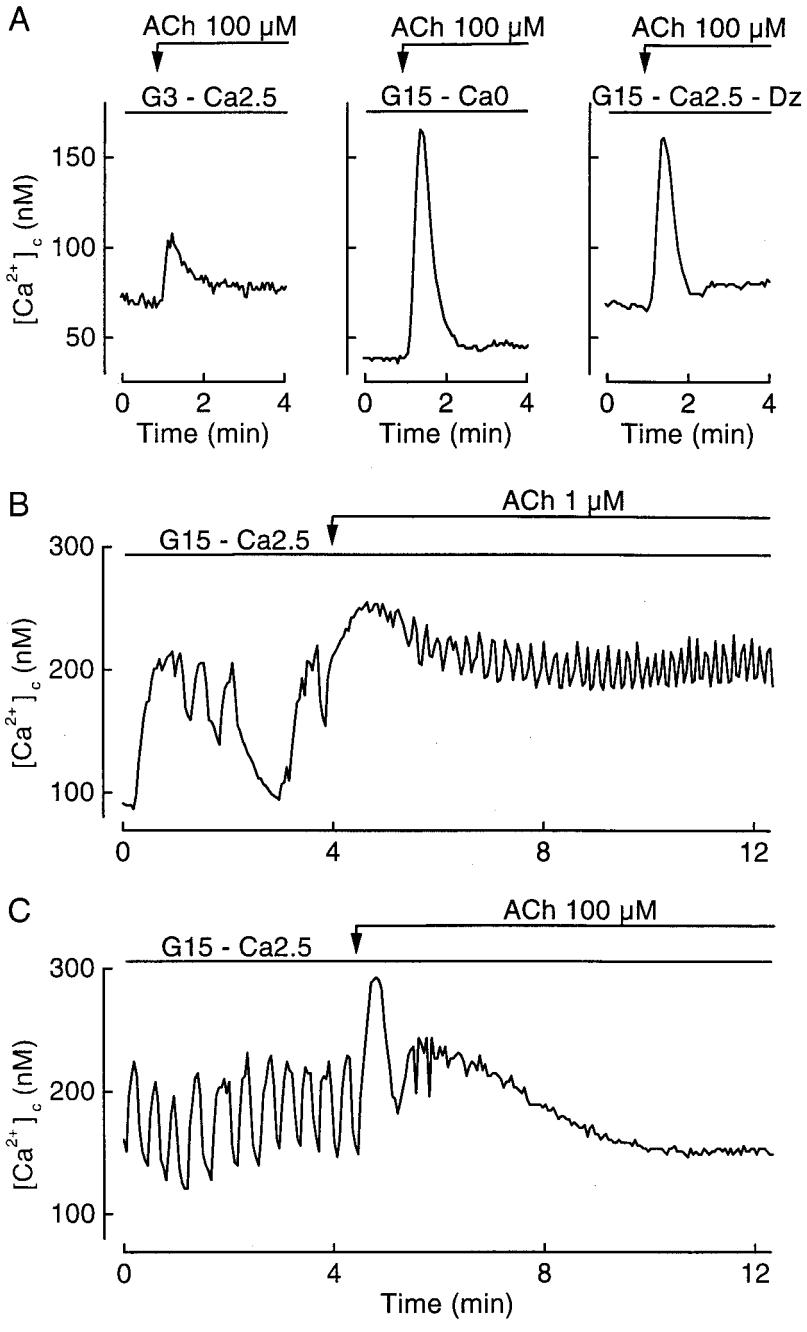
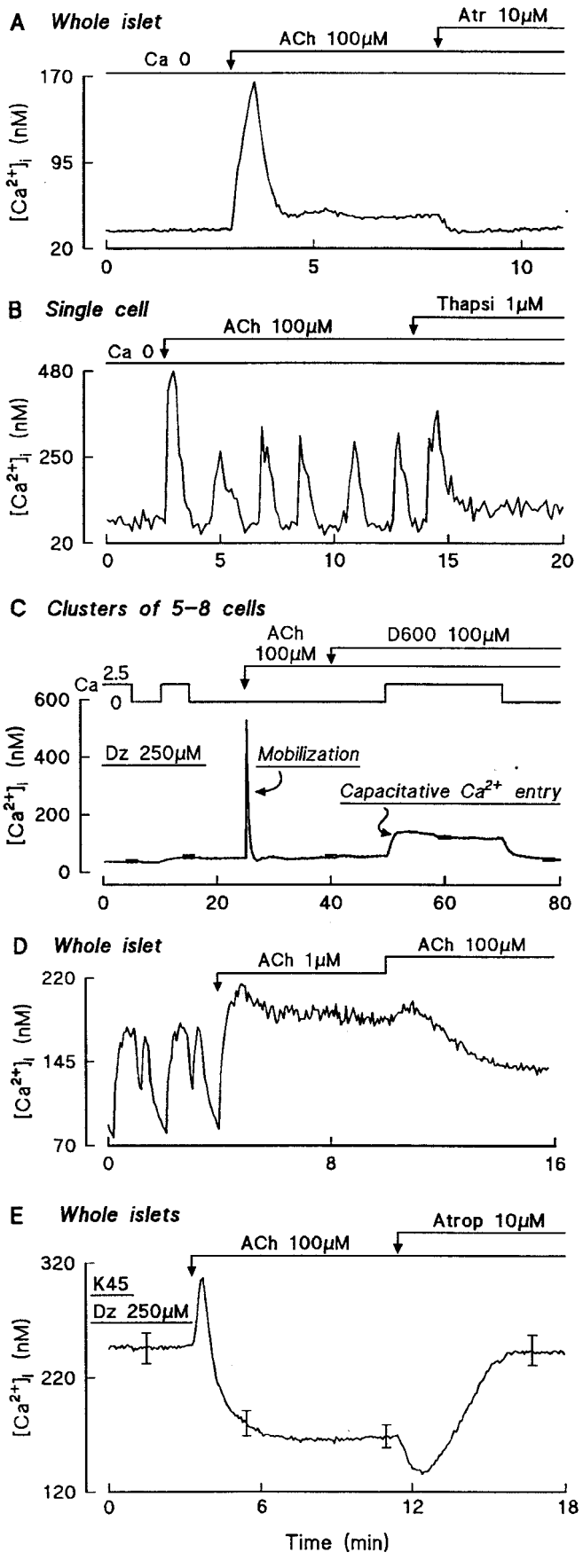


FIG. 5. General characteristics of ACh effects on mouse islet cell $[Ca^{2+}]_c$. Cultured islets were perifused with a medium without Ca^{2+} (Ca0) or with 2.5 mM Ca^{2+} (Ca2.5), and containing 3 or 15 mM glucose (G3 and G15, respectively). A, *Left panel*, The biphasic increase in $[Ca^{2+}]_c$ produced by 100 μM ACh is considerably reduced in a medium containing only 3 mM glucose. *Middle and right panels*, The second sustained $[Ca^{2+}]_c$ phase produced by 100 μM is also strongly reduced when Ca^{2+} influx is prevented by perifusing the islet with a Ca^{2+}-free medium (*middle panel*) or with a medium containing Ca^{2+} and the K^+-ATP channel opener, diazoxide (Dz 250 μM), that keeps the plasma membrane at resting potential. The large initial increase observed under these conditions results from mobilization of Ca^{2+} from intracellular stores as demonstrated by its persistence in a Ca^{2+}-free medium. B and C, In a medium containing Ca^{2+} and 15 mM glucose, ACh induced a biphasic increase in $[Ca^{2+}]_c$, the characteristics of which depended on the concentration of ACh. A low concentration of ACh (1 μM) accelerated the frequency of $[Ca^{2+}]_c$ oscillations induced by 15 mM glucose, whereas a high concentration of ACh (100 μM) transformed $[Ca^{2+}]_c$ oscillations into a sustained phase. Note that 100 μM ACh induced the largest initial increase but the lowest sustained phase.

and II (578–580), and mouse islets express more type I isoforms than types II and III (581, 582). Type II isoform was, however, undetectable in β-cells by immunocytochemistry (582). Because of the use of different techniques, it is unclear whether this difference between the rat and the mouse is real or only apparent. The contribution of non-β-cells in this expression is also unknown.

Studies in various tissues have shown that the three isoforms are differently regulated by cAMP, IP3, ATP, Ca^{2+}, and other factors (583, 584). Type II isoform has a higher affinity for IP3 than types I and III (584–587). Type I isoform contains a regulatory domain for PKA (588, 589), which might explain the observation that cAMP-producing agents enhance the carbachol-induced mobilization of Ca^{2+} in *ob/ob* mouse β-cells (347). All isoforms are regulated by Ca^{2+}, possibly through a Ca^{2+}/calmodulin complex (590). Type I and II isoforms are allosterically modulated by Ca^{2+} so that the Ca^{2+}-mobilizing action of IP3 is markedly amplified when $[Ca^{2+}]_c$ increases from basal (100 nM) to intermediate levels (typically ≤300 nM), whereas it is inhibited when $[Ca^{2+}]_c$ reaches higher concentrations (584, 591–593). These positive and negative feedback mechanisms of Ca^{2+} are considered important for generation of Ca^{2+} oscillations from IP3-sensitive Ca^{2+} stores. In contrast, type III isoform is only positively modulated by Ca^{2+}, and this isoform would not be suitable for $[Ca^{2+}]_c$ oscillations (584, 594).

The subcellular localization of IP3 receptors has not been firmly established in β-cells, although subcellular fraction-



ation experiments show that they are located on Ca^{2+} stores distinct from the mitochondria. An immunocytochemical study using an antibody against IP3 receptors of type III suggested that their preferential localization is on insulin-containing granules (595, 596). However, this conclusion was subsequently shown to be incorrect (597), which is consistent with the observations that IP3 does not release Ca^{2+} from subcellular fractions enriched in secretory granules (552, 598), and that granules do not regulate the ambient free Ca^{2+} concentration (551, 599–602) even though they contain high levels of Ca^{2+} (536, 600, 603). The endoplasmic reticulum appears to be the major source of Ca^{2+} released by IP3. This is consistent with the following two observations: First, ACh- or carbachol-induced mobilization of Ca^{2+} is completely suppressed by thapsigargin and cyclopiazonic acid, two SERCA pump inhibitors (510, 545, 546, 549, 600, 604). Second, a drop in the free Ca^{2+} concentration in the endoplasmic reticulum has recently been visualized upon carbachol stimulation of INS-1 cells expressing the Ca^{2+}-sensitive photoprotein, aequorin, in the endoplasmic reticulum. It is likely that the Golgi apparatus can also release Ca^{2+} upon ACh stimulation (605).

Experiments using INS-1 cells expressing aequorin in the endoplasmic reticulum also revealed that high carbachol concentrations (100 μM) decreased free Ca^{2+} concentration in the endoplasmic reticulum by only 20–25%, in contrast to SERCA pump inhibitors that completely emptied the endoplasmic reticulum (606). This is in agreement with the observation that thapsigargin can still release Ca^{2+} from the

FIG. 6. Mechanisms of the effects of ACh on $[Ca^{2+}]_c$ in mouse pancreatic β-cells. All experiments were performed in the presence of 15 mM glucose. A, Mobilization of intracellular Ca^{2+} in an islet perifused with a Ca^{2+}-free medium. Atropine (Atr) suppressed the ACh-induced small sustained elevation of $[Ca^{2+}]_c$ due to mobilization. B, $[Ca^{2+}]_c$ oscillations due to mobilization of intracellular Ca^{2+} in a single cell perifused with a Ca^{2+}-free medium. Thapsigargin (Thapsi 1 μM), a specific inhibitor of the SERCA pump, abolished the oscillations by preventing uptake of Ca^{2+} into the endoplasmic reticulum and thereby emptying it of Ca^{2+}. [Redrawn from Y. Miura *et al.*: *Biochem Biophys Res Commun* 224:67–73, 1996 (510).] C, Mobilization of intracellular Ca^{2+} followed by capacitative Ca^{2+} entry in clusters of cells whose plasma membrane was hyperpolarized with diazoxide (Dz 250 μM). Ca^{2+} mobilization was observed in a Ca^{2+}-free medium, and capacitative Ca^{2+} entry occurred upon Ca^{2+} readmission to the medium. A blocker of voltage-dependent Ca^{2+} channels, D-600 (100 μM), was added to the medium to ensure that the sustained $[Ca^{2+}]_c$ increase that was observed upon Ca^{2+} readmission resulted exclusively from influx through voltage-independent Ca^{2+} channels. D, Sustained $[Ca^{2+}]_c$ elevation in an islet perifused with a medium containing 2.5 mM Ca^{2+}. This sustained rise resulted essentially from the plasma membrane depolarization that ACh produced. It was lower at a high (100 μM) ACh concentration than at a low (1 μM) ACh concentration because the high concentration of the neurotransmitter activates mechanisms of $[Ca^{2+}]_c$ decrease that oppose to the mechanisms of $[Ca^{2+}]_c$ increase. [Redrawn from P. Gilon *et al.*: *Biochem J* 311:259–267, 1995 (545).] E, Sustained decrease of $[Ca^{2+}]_c$ in islets whose $[Ca^{2+}]_c$ was raised by depolarizing the plasma membrane with 45 mM K^+. Diazoxide (Dz 250 μM) was added to the medium to decrease the plasma membrane resistance and prevent ACh from affecting the membrane potential. The initial $[Ca^{2+}]_c$ peak upon ACh addition reflects Ca^{2+} mobilization from the endoplasmic reticulum, whereas the transient drop induced by atropine (Atrop 10 μM) reflects Ca^{2+} sequestration into the endoplasmic reticulum. [A, D, and E redrawn from P. Gilon *et al.*: *Biochem J* 311:259–267, 1995 (545). © the Biochemical Society.]

endoplasmic reticulum in the presence of ACh (510, 607). It is unclear why ACh is unable to empty the endoplasmic reticulum to the same extent as IP3 itself (50% or more in permeabilized cells) (565, 606, 608). Because desensitization of IP3 receptors does not seem to occur (369, 561, 562, 606, 608), the transient time course of IP3 elevation may be involved.

In agreement with the widespread localization of the endoplasmic reticulum within the cell, mobilization of Ca^{2+} by carbachol produces a rather uniform increase in $[Ca^{2+}]_c$, contrary to agents that stimulate Ca^{2+} influx through voltage-dependent Ca^{2+} channels and raise $[Ca^{2+}]_c$ preferentially in the periphery of the cell (566, 570, 609, 610). This spatial difference has sometimes been taken as an argument to explain the poor insulinotropic effect of ACh in a Ca^{2+}-free medium. Probably because of close contacts between the endoplasmic reticulum and mitochondria (611, 612), high concentrations of carbachol can also increase the mitochondrial free Ca^{2+} concentration in clonal β-cells (613).

It is important to emphasize that the process of Ca^{2+} mobilization by ACh requires relatively high concentrations (≥1 μM) of the neurotransmitter (162, 545). Even in the presence of optimal glucose concentrations, the half-maximal effective concentration of ACh-induced Ca^{2+} mobilization is approximately 10 μM (545). Stimulation of Ca^{2+} influx is much more sensitive to ACh (see *Section VIII.A.3*).

2. Capacitative Ca^{2+} entry (Fig. 6C). In nonexcitable cells, PLC-linked agonists induce a biphasic rise in $[Ca^{2+}]_c$. The first phase corresponds to mobilization of Ca^{2+} from intracellular stores, whereas the second phase corresponds to Ca^{2+} influx through voltage-independent Ca^{2+} channels belonging to the family of SOCs. The process by which emptying of intracellular Ca^{2+} pools activates Ca^{2+} influx has been called capacitative Ca^{2+} entry (614), but the mechanisms linking Ca^{2+} pool depletion to Ca^{2+} influx are still disputed (615, 616).

A capacitative Ca^{2+} entry has been documented in pancreatic β-cells (522, 550, 617). Indeed, cholinergic agonists and thapsigargin activate a Ca^{2+} entry sensitive to La^{3+} but resistant to the blockade of voltage-dependent Ca^{2+} channels by D-600 (methoxyverapamil) (Fig. 6C) or membrane hyperpolarization with diazoxide. However, the rise in $[Ca^{2+}]_c$ that this entry produces is small, approximately 8-fold less than that after the opening of voltage-dependent Ca^{2+} channels by high K^+. Moreover, it decreases when the membrane depolarizes, probably because the driving force for Ca^{2+} diminishes as the membrane potential approaches the equilibrium potential for Ca^{2+} (522). Contrary to other systems (615, 618), the capacitative Ca^{2+} entry in β-cells is not affected by the energy state of the cell, PKC activation, or serine/threonine phosphatase or tyrosine kinase inhibition (550). The situation is different in RINm5F cells in which capacitative Ca^{2+} entry requires activation of PKC (619).

The concentration dependence of the capacitative Ca^{2+} entry elicited by ACh has not been precisely studied, but high concentrations of agonists (100 μM) seem to be necessary (550).

3. Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Figs. 5 and 6D). Under control conditions, when extracellular Ca^{2+} is present, the effects of cholinergic agonists on $[Ca^{2+}]_c$ and $^{45}Ca^{2+}$ efflux largely depend on the glucose concentration or, more exactly, on the β-cell membrane potential set by the glucose concentration. In the presence of a nonstimulatory glucose concentration, when β-cells are hyperpolarized, ACh induces a biphasic change in $[Ca^{2+}]_c$ (Fig. 5A) and $^{45}Ca^{2+}$ efflux (162, 285, 480) characterized by an initial slight peak followed by a small sustained elevation. When β-cells are depolarized by a stimulatory or near-stimulatory glucose concentration, cholinergic agonists also induce a biphasic change in $[Ca^{2+}]_c$ and $^{45}Ca^{2+}$ efflux, but both phases are now much larger than at low glucose (162, 279, 285, 287, 480, 483, 545, 617, 620, 621) (Figs. 5B–C). When Ca^{2+} influx is inhibited by keeping the membrane hyperpolarized with diazoxide or by blocking the voltage-dependent Ca^{2+} channels, the initial peak is only partially reduced, whereas the sustained phase is largely suppressed (Fig. 5A). This indicates that the contribution of Ca^{2+} influx through voltage-dependent Ca^{2+} channels is much more important to the sustained phase than the early phase. When Ca^{2+} influx through voltage-dependent Ca^{2+} channels is prevented, the residual initial peak results from Ca^{2+} mobilization from the endoplasmic reticulum, and the small residual sustained phase is caused by continuous mobilization and capacitative Ca^{2+} entry.

ACh stimulation of Ca^{2+} influx through voltage-dependent Ca^{2+} channels is explained by the effects of the neurotransmitter on the membrane potential (described above). In low glucose or in high glucose plus diazoxide, the depolarization by ACh is too small to activate voltage-dependent Ca^{2+} channels. In contrast, in the presence of high glucose and other depolarizing secretagogues, ACh further activates voltage-dependent Ca^{2+} channels (279, 480, 545, 546, 617, 622). This constitutes the major mechanism by which ACh, already at low concentrations (~0.01 μM) (545, 546), induces a sustained $[Ca^{2+}]_c$ increase (545).

4. Relative importance and physiological relevance of these three mechanisms. Because the rise in $[Ca^{2+}]_c$ resulting from the capacitative Ca^{2+} entry is very small, requires high concentrations of ACh, and decreases when the membrane depolarizes, its contribution to the overall rise in $[Ca^{2+}]_c$ produced by ACh is minimal and will not be discussed further.

Although Ca^{2+} mobilization is by far the most widely studied effect of ACh on $[Ca^{2+}]_c$, its importance in the electrically excitable β-cell must be qualified. In the absence of glucose or in the presence of low concentrations of the sugar (<3 mM), ACh has almost no effect on $[Ca^{2+}]_c$ because little Ca^{2+} can be mobilized from nearly empty intracellular Ca^{2+} pools, and because the membrane depolarization is insufficient to open Ca^{2+} channels. At glucose concentrations (3–6 mM) that allow refilling of intracellular stores with Ca^{2+} (565, 623) but remain below the threshold for generation of electrical activity (7 mM), mobilization of Ca^{2+} is the major mechanism by which ACh increases $[Ca^{2+}]_c$. At near-stimulating glucose concentrations (~6–7 mM), the depolarization that ACh produces also triggers Ca^{2+} influx through voltage-dependent Ca^{2+} channels. At stimulating glucose concentrations, this mechanism contributes even more than the mobilization of Ca^{2+} to the overall increase in $[Ca^{2+}]_c$ brought about by ACh. Moreover, the different concentra-

tion dependencies of Ca^{2+} mobilization and Ca^{2+} influx for ACh at stimulating glucose concentrations reinforce the role of the depolarization in the $[Ca^{2+}]_c$ rise. Indeed, Ca^{2+} influx is already stimulated by low concentrations of the neurotransmitter (~0.1 μM), whereas Ca^{2+} mobilization requires higher ACh concentrations (≥1 μM).

The relative contribution of each mechanism to the action of ACh *in vivo* is difficult to evaluate, but two reasons reinforce the view that the changes in membrane potential play a predominant role. First, without knowing what concentration of ACh can be reached in the vicinity of β-cells upon cholinergic nerve stimulation, it is reasonable to assume that the effect observed with the lower concentrations is likely to be physiological. Second, because of the influence of non-glucose stimuli (which are not present in the experimental buffers), the threshold glucose concentration that triggers depolarization of β-cells is lower *in vivo* than *in vitro* (624).

B. Mechanisms by which ACh decreases $[Ca^{2+}]_c$

When the effects of various concentrations of ACh on $[Ca^{2+}]_c$ were compared in glucose-stimulated islets, it was unexpectedly found that the steady-state $[Ca^{2+}]_c$ was higher in the presence of low concentrations (0.1–1 μM) of ACh than in high (≥10 μM) concentrations (Figs. 5, B and C, and 6D). This suggests that ACh might also decrease $[Ca^{2+}]_c$, an effect that is clearly demonstrated in islets steadily depolarized with high K^+ (545) (Fig. 6E). The $^{45}Ca^{2+}$ efflux measurements indicate that a slight acceleration of Ca^{2+} efflux contributes to this effect. This acceleration may be ascribed to PKC stimulation because phorbol esters also promote Ca^{2+} efflux (407, 625, 626) by activating the plasma membrane Ca^{2+}-ATPase (627) or the Na^+/Ca^{2+} exchanger (628). PA and DAG, which increase in the presence of muscarinic agonists, stimulated Ca^{2+}-ATPase activity in an islet cell plasma membrane-enriched fraction (629).

However, membrane potential measurements also revealed that whereas low ACh concentrations increased the electrical activity elicited by glucose, high concentrations of the neurotransmitter decreased the amplitude of the spikes (Fig. 4B). Because spikes reflect Ca^{2+} influx through voltage-dependent Ca^{2+} channels, this observation suggested that high concentrations of ACh might inhibit these channels (545). This was confirmed by patch-clamp experiments (630) (Fig. 4D). ACh dose dependently inhibited the whole-cell voltage-dependent Ca^{2+} current of the L-type. Maximum inhibition was produced by approximately 100 μM ACh and reached about 35%, whereas the 50% inhibitory concentration was observed at 5 μM ACh. This effect was mediated by a pertussis- and cholera toxin-insensitive G protein. It is unlikely to involve DAG-sensitive PKCs, because phorbol esters increase voltage-dependent Ca^{2+} currents in insulin-secreting cells (393, 631–634). The inhibitory effect of ACh on the Ca^{2+} current is compatible with the inhibition of the L-type current by photorelease of guanosine 5′-[γ-thio] triphosphate in β-cells (635). Inhibition of L-type current by muscarinic receptors has also been observed in smooth muscle (636) and neuronal cells (637–641). In contrast to the situation found in normal β-cells, the muscarinic agonist bethanechol increased the L-type Ca^{2+} current by activating PKC in HIT-T15 cells (634). This discrepancy might be related to the very different responses to muscarinic agents between normal and insulin-secreting cell lines (see *Section IX.D*).

The decrease in $[Ca^{2+}]_c$ occurring in the presence of high concentrations of ACh might constitute a protective mechanism against deleterious Ca^{2+} overload. As will be discussed below, it is not accompanied by an equivalent decrease in insulin secretion.

IX. Mechanisms of the Stimulation of Insulin Secretion by ACh

ACh brings into operation at least two types of Ca^{2+}-dependent mechanisms: the first one involves a rise in $[Ca^{2+}]_c$, and the second one increases the efficacy of Ca^{2+} on exocytosis.

A. The rise in $[Ca^{2+}]_c$ by ACh triggers exocytosis

When the stimulation by ACh is applied in the presence of diazoxide or a voltage-dependent Ca^{2+} channel blocker, or in a Ca^{2+}-free medium, there exists a tight temporal parallelism between the rise in $[Ca^{2+}]_c$ and insulin secretion. Indeed, insulin release is stimulated only during the transient elevation of $[Ca^{2+}]_c$ (compare trace with *open circles* of Fig. 1B with *middle panel* of Fig. 5A). This indicates that ACh triggers exocytosis by increasing $[Ca^{2+}]_c$. Two effects of Ca^{2+} may be involved: a direct action of Ca^{2+} on the exocytotic machinery close to the zone of fusion of secretory granules with the plasma membrane (642–645), and a Ca^{2+}-mediated acceleration of granule movements to sites of release (646–648). This second effect, which may serve to amplify exocytosis upon subsequent stimulation, could be independent from PKC activation but might involve a Ca^{2+}-calmodulin-dependent protein kinase (376), either myosin light chain kinase (647) or Ca^{2+}/calmodulin-dependent kinase II (648).

The amplitude of the transient secretory peak in a Ca^{2+}-free medium depends on the glucose concentration (162). Two reasons may explain this glucose-dependence: mobilization of Ca^{2+} is greater in the presence of glucose (see *Section VIII.A.1*), and Ca^{2+}-induced insulin secretion is increased by glucose through its amplifying pathway (for a given $[Ca^{2+}]_c$, more insulin is secreted at high glucose than at low glucose) (280, 649).

B. ACh increases the efficacy of Ca^{2+} on exocytosis

In a Ca^{2+}-containing medium, the effects of ACh on insulin secretion result from a balance between multiple mechanisms that increase or decrease $[Ca^{2+}]_c$ and amplify the efficacy of Ca^{2+} on exocytosis. Indeed, there is no good temporal or quantitative relationship between the sustained changes in $[Ca^{2+}]_c$ and insulin secretion induced by ACh in the presence of 15 mM glucose. During the first minutes of stimulation, both $[Ca^{2+}]_c$ and insulin responses (initial peaks) increase with the concentration of the neurotransmitter (Fig. 7). However, during steady-state stimulation, concentrations of ACh that barely increase $[Ca^{2+}]_c$ strongly potentiate glucose-induced insulin secretion. This indicates that one or several mechanisms other than the rise in $[Ca^{2+}]_c$

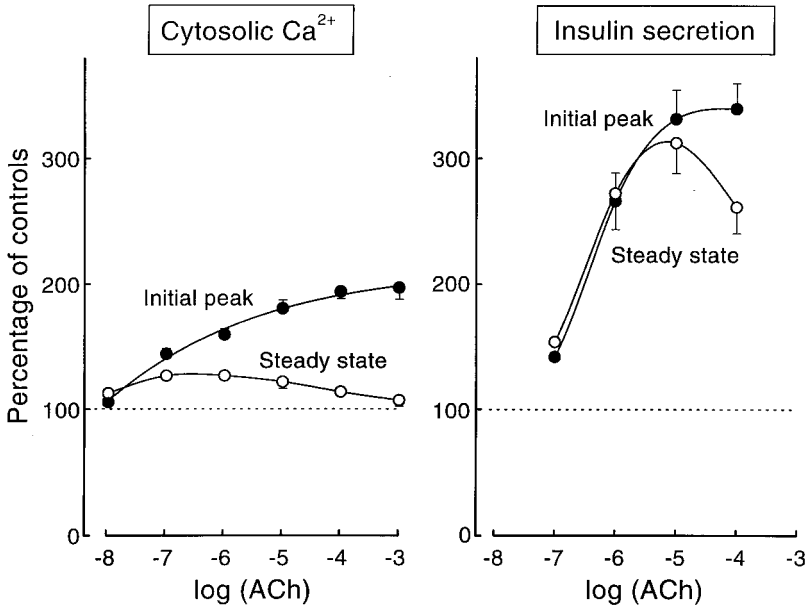


FIG. 7. Comparison of the effects of various concentrations of ACh on $[Ca^{2+}]_c$ and insulin secretion measured during the first minutes of stimulation (integrated over 2 min for $[Ca^{2+}]_c$ and 4 min for insulin secretion) and the steady-state stimulation (integrated over 3 min for $[Ca^{2+}]_c$ and 6 min for insulin secretion) with ACh. The results are presented as percentages of control values, which were computed by integrating $[Ca^{2+}]_c$ and insulin secretion during the last 3 and 6 min, respectively, before addition of ACh. The glucose concentration of the medium was 15 mM throughout. All data were obtained with cultured mouse islets. (Derived from Ref. 545 for $[Ca^{2+}]_c$ experiments.)

become operative and increase the efficacy of Ca^{2+} on the secretory machinery. In patch-clamp experiments using membrane capacitance measurements in which the intracellular Ca^{2+} concentration is artificially clamped (650), it has been clearly demonstrated that ACh sensitizes the secretory machinery to Ca^{2+}. This sensitization is also evident in islets depolarized with high K^+ and diazoxide. Under these conditions, ACh exerts no or minor effects on the membrane potential of β-cells (279, 545), lowers $[Ca^{2+}]_c$ (545), but potentiates insulin secretion (651).

1. The PKC pathway plays a major role. Whereas accumulation of phosphoinositols *per se* is devoid of any stimulatory effect on insulin secretion (311, 319, 341), activation of PKC sensitizes the secretory machinery to Ca^{2+} (326, 625, 632, 652–655).

Involvement of PKC in the stimulation of insulin secretion by ACh is suggested by experiments using various PKC inhibitors, including bisindolylmaleimide, H-7, and staurosporine (400, 408, 656–658). However, these experiments are not conclusive because the inhibitors are nonselective kinase inhibitors or exert nonspecific effects (376, 659). Synthetic pseudosubstrate peptide inhibitors permit more specific inhibition of certain PKC isoforms. The insulin response of rat islets to carbachol was completely prevented by an inhibitory peptide corresponding to the consensus sequence of the pseudosubstrate regions of the PKC isoforms α and β (660). Down-regulation of PKC by prolonged exposure (>20 h) of β-cells to phorbol esters strongly inhibited the insulin response to a subsequent cholinergic stimulation (163, 290, 399, 573, 661, 662). Because the treatment with the phorbol ester down-regulated DAG-sensitive PKC isoforms, with the surprising exception of PKCβII isoform in MIN6 cells (399), it was suggested that one or several of the three PKC isoforms, α, δ, and/or ε play a major role in the stimulatory effect of ACh on insulin release. Because PKCα isoform is the only isoform that has been implicated in experiments with both PKC pseudosubstrates and PKC down-regulation, and it is the major isoform expressed in normal β-cells, it is likely that most of the PKC-dependent effects of ACh on insulin secretion are mediated by this isoform.

A small residual stimulation of insulin secretion by muscarinic agonists was observed in islets with down-regulated PKC (163, 290, 662, 663). It probably results from the increase in $[Ca^{2+}]_c$ that cholinergic agents still produce in such islets (290) and from the activation of PKC-independent pathways. It is important to stress here that translocation of PKC to the plasma membrane by carbachol does not stimulate insulin secretion when $[Ca^{2+}]_c$ is low (163, 657). The PKC-dependent stimulation of insulin secretion only occurs when $[Ca^{2+}]_c$ is elevated (Figs. 1, A and B). It is therefore teleologically understandable that ACh brings into operation separate mechanisms that simultaneously increase $[Ca^{2+}]_c$ (depolarization) and stimulate PKC.

As described above, PKC activation exerts a negative feedback control on the signal transduction linked to PLC, which might explain the biphasic time course of accumulation of arachidonate-enriched DAG upon stimulation by cholinergic agents. However, this feedback control does not determine the time course of insulin secretion; cholinergic agonists can induce a sustained insulin secretion for relatively long periods (30–60 min) without any sign of desensitization (161, 162, 263, 286, 288, 664, 665). This suggests that the decrease in PLC-derived DAG levels is probably not accompanied by a parallel decrease of PKC activation. Low levels of PLC-derived DAG levels might be sufficient to maintain a sustained PKC activation. Other phospholipid-derived products formed during stimulation by ACh (arachidonic acid, lysophosphatidate, phosphatidate, various DAGs, and probably several other metabolites) may, alone or in synergy, stimulate PKC (297, 453) and support the sustained secretion of insulin. Such a time-dependent, multifactorial activation of PKC has been reported in various systems (381).

2. The role of the PLA_2 pathway is uncertain. Whereas the role of the PLC-PKC pathway in the insulinotropic effect of ACh is

firmly established, it remains unclear whether the PLA_2 pathway is also involved, and if so, whether its effects are also mediated by PKC. The reported effects of arachidonic acid on insulin secretion are extremely controversial (666, 667). Exogenous arachidonic acid inhibited, had no effect, or stimulated insulin secretion by mouse or rat islets depending on the glucose concentration used (435, 440, 446, 451, 668, 669). It also induced insulin release from permeabilized islets (670, 671). Arachidonic acid-stimulated insulin secretion has been reported to involve PKC (446), but it has also been reported not to involve PKC (668, 671, 672). At the concentrations that induce insulin secretion, arachidonic acid may also exert toxic effects in islets and inactivate PKC (672). Its insulinotropic effect is not blocked by norepinephrine (670), which, in contrast, prevents ACh-induced insulin secretion (673). Because of all these controversies, the contribution of the PLA_2 pathway to the insulinotropic effect of cholinergic agonists is still unsettled.

C. Delayed effects of ACh on insulin secretion

It has been suggested that cholinergic agonists also exert long-lasting effects on insulin secretion. The phenomenon, referred to as time-dependent potentiation or priming, consists in the enhancement of the β-cell secretion response to various stimuli, including glucose, GIP, cholecystokinin, and tolbutamide, by prior transient stimulation with cholinergic agonists (164, 342, 366, 674, 675). This effect has been observed in the rat and the mouse (658) and might play a role during the preabsorptive phase (see *Section III.B.2.a*). Because it was mimicked by phorbol 12-myristate 13-acetate (PMA) (658, 676, 677), it has been ascribed to a persistent activation of PKC, which can then be readily activated by the rise in $[Ca^{2+}]_c$ that glucose produces. However, in the perfused rat pancreas, the phenomenon could be induced by PMA (678), but not by carbachol (679). Comparison of the effects of both agents is not easy because PMA, unlike carbachol, exerts irreversible activation of PKC even after short application.

Whereas brief stimulation with ACh amplifies insulin secretion, prolonged stimulation might exert adverse affects. Exposure of rat islets to 10 μM carbachol for 3.5 h has been reported to desensitize β-cells to subsequent stimulation by glucose and cholinergic agonists (263, 665, 680). This desensitization might result from an impaired phosphoinositide pathway (342). Ubiquitination is a process whereby ubiquitin, a 76-residue protein, is associated with certain proteins to make them recognizable by the proteasome pathway that degrades them (681). Prolonged exposure (6 h) to carbachol has recently been shown to down-regulate IP3 receptors in mouse islet by the ubiquitin/proteasome pathway (582).

D. Muscarinic responses are often abnormal in insulin-secreting cell lines

Insulin-secreting cell lines have been used extensively to study stimulus-secretion coupling. They can be useful when responses occurring in non-β-cells of the islets complicate interpretation of the results, when large amounts of cells are needed for biochemical determinations, and for transfection experiments. Their use has yielded interesting data that can sometimes be extrapolated to normal β-cells. However, it is important to bear in mind that they are, in many respects, different from normal β-cells. A major difference between normal β-cells and some cell lines that were established long ago is a markedly different glucose dependence (682). Described below are some important differences regarding cholinergic effects.

RINm5F cells (a clonal rat β-cell line) are not depolarized by cholinergic agonists (311, 607), but are depolarized by phorbol esters that activate PKC (607, 654, 683, 684). This is exactly opposite to the situation in normal β-cells, in which ACh depolarizes the plasma membrane, whereas PMA lacks this effect (407, 625). In RINm5F cells, carbachol induces a transient increase in $[Ca^{2+}]_c$ by mobilizing intracellular Ca^{2+}, but causes a sustained secretion of insulin that is independent from a rise in $[Ca^{2+}]_c$ and persists after depleting the Ca^{2+} content of the endoplasmic reticulum with thapsigargin (311, 607). The effect of ACh on insulin secretion is poorly glucose dependent (685), and PKC down-regulation or inhibition does not affect (400, 607) or paradoxically enhances (397) insulin secretion in response to carbachol in RINm5F cells. Some of these peculiar effects might be explained by the fact that carbachol also translocates the phorbol ester-insensitive ζ-isoform of PKC (400).

In MIN6 (a mouse β-cell line) and HIT cells (a clonal hamster β-cell line), ACh and carbachol induce a transient rise in $[Ca^{2+}]_c$, which mainly results from Ca^{2+} influx. Surprisingly, they stimulate insulin secretion even when $[Ca^{2+}]_c$ has returned to basal levels (573, 686). The latter effect is markedly reduced by PKC down-regulation (163, 399, 573). In MIN6 (686) and HIT cells (685), the insulinotropic effect of ACh is poorly dependent on the glucose concentration.

X. Nature of the Muscarinic Receptor Activated by ACh

With the exception of two reports from the same group (687, 688), there is general agreement that all direct effects of ACh on insulin-secreting cells are exclusively mediated by muscarinic receptors (288, 480, 486, 621, 689, 690).

Muscarinic receptors belong to the family of receptors with seven transmembrane domains connected by three cytoplasmic loops and three extracellular loops (691–694). Five muscarinic receptor subtypes, which elicit classical responses, have been cloned so far: the M_1, M_3, and M_5 subtypes are linked to G proteins of the G_q class and activate PLC, and the M_2 and M_4 are linked to pertussis toxin-sensitive G proteins of the G_i or G_o class and initiate several processes such as inhibition of adenylate cyclase or of voltage-dependent Ca^{2+} channels and activation of the atrial cardiac K^+ channel by M_2 (314, 416, 513, 637, 638, 640, 693–696). However, classification of muscarinic receptors on the basis of the signal transduction is unreliable because of the overlap between the transduction pathways activated by the different subtypes (692, 697, 698).

Three strategies have been used to identify the muscarinic receptor subtypes present in β-cells: pharmacological blockade of physiological responses by selective antagonists, binding studies of selective ligands, and molecular biology studies.

A. Pharmacological studies

More than a decade ago, only three muscarinic receptor subtypes were identified and classified as neuronal M_1 (high affinity for pirenzepine), cardiac M_2 ($M_{2\alpha}$, low affinity for pirenzepine/high affinity for AF-DX 116), and glandular M_2 ($M_{2\beta}$, low affinity for both pirenzepine and AF-DX 116). A first study comparing the effects of atropine and pirenzepine on insulin secretion from the perfused rat pancreas suggested that the receptor present in β-cells was different from the M_1 receptor (664). Subsequent experiments testing atropine, pirenzepine, and AF-DX 116 on insulin release, $^{86}Rb^+$ efflux, and Ca^{2+} efflux ruled out the presence of M_1 and cardiac M_2 receptors in mouse islets and suggested that the receptor present in β-cells was a glandular M_2 subtype (699). This was confirmed by studying the effect of other agonists or antagonists on the electrical activity (486) and insulin release (700). Later, when gene receptor analysis revealed the existence of 5 muscarinic subtypes (691, 692, 701), it clearly appeared that the glandular M_2 receptor corresponded to a new subtype, the M_3 receptor (702, 703). The observations that the insulin response to cholinergic agonists is mediated by a M_3 subtype (699) were confirmed *in vitro* in RINm5F cells (704) and rat islets (288) and *in vivo* in the mouse (705) with more specific antagonists.

B. Binding studies

The presence of muscarinic receptors in the endocrine pancreas was clearly demonstrated by measuring the specific binding of the muscarinic antagonists [^{3}H]-methylscopolamine or [^{3}H]quinucliolinyl benzilate (QNB) to rat (321, 700, 706–709), mouse (46, 485), and guinea pig islets (710), or to insulin-secreting tumoral RINr cells (312) and INS-1 cells (711). Scatchard plot analysis revealed a single population of high affinity binding sites without any obvious low affinity binding sites (312, 485, 706, 709). Displacement of the binding of [^{3}H]-methylscopolamine by various antagonists indicated the presence of M_3 receptors in rat islets (700).

C. Molecular identification of the receptor subtypes

Using RT-PCR or ribonuclease protection assays, RNA encoding M_3 and M_1 receptor subtypes was detected in rat islets (288, 711). These two receptor subtypes were much more expressed than the M_5 receptor subtype (711). Although similar results were obtained in INS-1 cells (711), this type of determination does not prove that the three types of receptors are expressed in β-cells because isolated islets contain at least four endocrine cell types (β-cells, α-cells, δ-cells, and PP-cells) as well as vascular muscle and endothelial cells. Immunocytochemical experiments using a specific antibody against the M_3 receptor subtype indeed indicated that both central (mainly β-cells) and peripheral cells (mainly non-β-cells) express the M_3 receptor (711). On the other hand, M_3 (704, 711) and M_4 receptor subtypes (704, 711), but not M_1 (711), were detected in RINm5F cells. Interestingly, although several studies reported the presence of M_3 and non-M_3 receptors, two of these (288, 704) suggested that only M_3 receptors are involved in the secretion of insulin in response to cholinergic agonists.

D. One or several receptor subtypes for several transduction pathways?

On the basis of pharmacological, binding, and RT-PCR studies, it is clear that the M_3 receptor plays a central role in β-cells. The idea that this sole subtype activates several transduction pathways is supported by the observation that three different parameters of the β-cell function (insulin secretion, $^{86}Rb^+$ efflux, and Ca^{2+} efflux) displayed a similar antagonistic profile (699).

Activation of multiple transduction pathways by a single class of muscarinic receptors is not a unique feature of the pancreatic β-cell. Another example of complexity is found in ventricular myocytes in which cholinergic agonists, likely acting solely on the M_2 subtype, inhibit L-type Ca^{2+} current through an inhibition of adenylate cyclase activity and activate a Na^+ current (512, 513). Activation of two different transduction pathways by two different parts of the M_3 receptor has also been documented in A9 fibroblast cells (712). The diversity of the effects mediated by ACh not only depends on the nature of the muscarinic receptor subtype involved, but also on posttranslational modifications (glycosylation, phosphorylation, etc.), which might be different from one cell type to another (314) or on the nature of the effector system present in the cells (315, 512, 695, 697). Indeed, when heart M_2 muscarinic receptors, which classically inhibit adenylate cyclase, are expressed in CHO cells, their activation also produces nonclassical effects such as phosphoinositide breakdown (713). Similar results were found for the M_1 receptor (697). Activation of all five muscarinic receptor subtypes expressed in NIH 3T3 cells has recently been shown to inhibit L-type current of this cell type (714). This suggests that each receptor subtype elicits preferential rather than specific effects, depending on the cell type in which it is expressed.

In the same line of ideas, the different concentration dependencies of the multiple effects of ACh in β-cells do not necessarily imply that several muscarinic receptors are involved. They might result from different sensitivities of the effector systems to G protein activation or from other unidentified mechanisms. Activation of transduction pathways with different concentration dependencies has recently been reported for the M_3 receptor expressed in CHO cells. Moderate concentrations of carbachol (1–10 μM) elicited maximal capacitative Ca^{2+} influx, whereas higher concentrations were necessary to activate an inward monovalent cation current that depolarizes the plasma membrane (521).

XI. Summary and Conclusions

A. The physiological role of ACh

ACh is released by intrapancreatic nerve endings under the control of the vagus nerves during the preabsorptive, cephalic, and enteric phases of feeding and, very likely, also during the absorptive phase. Vagal stimulation occurs after activation of cephalic sensory organs including those of the

oral cavity and the visual and olfactory systems, and after activation of glucoreceptors in the gut, brain, and liver. ACh stimulates insulin secretion in a glucose-dependent manner, becoming more and more effective as the plasma glucose concentration increases. This stimulation appears to be important to ensure optimal glucose tolerance during the periods of feeding.

Several animal models of type 2 diabetes are characterized by an alteration of the autonomic nervous system with an increased ratio of the parasympathetic over sympathetic activities leading to hyperinsulinemia. Hyperinsulinemia is a characteristic of obesity, and the kinetics of insulin secretion is often altered in type 2 diabetes, but it is unclear to which extent these abnormalities result from an impaired activity of the autonomic nervous system. Because their effects on insulin secretion are glucose dependent, cholinergic agonists might theoretically be helpful to improve insulin secretion and glucose homeostasis in certain type 2 diabetic patients (46, 715). Although supported by some animal studies (46), this idea has not been largely tested because of insufficient selectivity of the available muscarinic agents for β-cells.

B. The mechanisms of action of ACh in β-cells

At the β-cell level, ACh binds to M_3 receptors and activates several transduction pathways (Fig. 3); one of the major pathways is PLC, which mainly generates IP3 and diacylglycerol, a potent PKC activator. ACh also stimulates PLA_2, probably secondary to the $[Ca^{2+}]_c$ rise. This leads to accumulation of arachidonic acid and lysophosphatidylcholine. ACh might also activate PLD by a mechanism that possibly depends on PKC activation. Many of the phospholipid-derived messengers are also, alone or in synergy with other lipid messengers such as diacylglycerol, activators of PKC (Fig. 3). Besides these complex effects on lipid metabolism, ACh also depolarizes the plasma membrane of β-cells by a Na^+- or nonspecific cationic-dependent mechanism (Fig. 8A, pathway 3), and possibly also by a mechanism involving SOCs activated by intracellular Ca^{2+} pool emptying (Fig. 8A, pathway 4). This depolarization is small and reaches the threshold for the activation of voltage-dependent Ca^{2+} channels only if the plasma membrane is already depolarized by secretagogues such as glucose. The glucose dependence of this depolarization largely contributes to the glucose-dependence of ACh effects on insulin secretion.

All these transduction pathways modulate $[Ca^{2+}]_c$ in β-cells (Fig. 8). ACh transiently increases $[Ca^{2+}]_c$ by mobilizing Ca^{2+} from IP3-sensitive stores mainly in the endoplasmic reticulum (Fig. 8A, pathway 1). ACh induces a sustained increase in $[Ca^{2+}]_c$ by stimulating Ca^{2+} influx by two pathways: through voltage-independent Ca^{2+} channels that open upon intracellular Ca^{2+} pool emptying (capacitative Ca^{2+} entry; Fig. 8A, pathway 2) and through voltage-dependent Ca^{2+} channels that are activated by depolarization (Fig. 8A, pathways 3 and 4). ACh decreases $[Ca^{2+}]_c$ under certain circumstances (Fig. 8B). This effect, which is detectable only after the initial phase of intracellular Ca^{2+} mobilization and only when $[Ca^{2+}]_c$ is sustained, requires higher ACh concentrations than those depolarizing the plasma membrane. It results from a stimulation of Ca^{2+} efflux that likely involves PKC (Fig. 8B, pathway 6) and a G protein-mediated inhibition of Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Fig. 8B, pathway 5). It might protect β-cells against deleterious Ca^{2+} overload.

The insulinotropic effect of ACh largely depends on the glucose concentration and Ca^{2+} influx. When no Ca^{2+} influx can occur, ACh induces a transient, small, monophasic stimulation of insulin secretion, provided a high concentration of glucose is present. The tight temporal parallelism between the rises in $[Ca^{2+}]_c$ and insulin secretion that occur under these conditions indicates that ACh triggers exocytosis by increasing $[Ca^{2+}]_c$. When Ca^{2+} influx can occur, ACh induces a biphasic stimulation of insulin secretion, the amplitude of which, again, largely depends on the glucose concentration. However, there is no good temporal and quantitative relationship between changes in $[Ca^{2+}]_c$ and insulin secretion because, in the steady state, a large stimulation of insulin secretion occurs with only a moderate increase in $[Ca^{2+}]_c$ (Fig. 7). This suggests that an additional mechanism becomes operative and increases the efficacy of Ca^{2+} on the secretory machinery. The most important amplifying mechanism involves PKC. This PKC-dependent mechanism increases insulin secretion only when $[Ca^{2+}]_c$ is sufficiently elevated above basal levels. Thus, the insulinotropic effect of ACh results from two Ca^{2+}-dependent mechanisms, one that involves a rise in $[Ca^{2+}]_c$ and another that increases the efficacy of Ca^{2+} on exocytosis (Fig. 3).

Although the mechanisms of action of ACh have been extensively studied, many remain incompletely understood. How PKC increases insulin secretion is unclear. Because of the transient accumulation of the PLC-derived arachidonate and the multiple interactions between PKC and various phospholipid-derived products, it is not known which routes require PKC or lead to PKC activation. Interactions between PLA_2-, PLC-, and PLD-derived products and PKC are not well defined. Moreover, it is unclear which PKC isoform is activated by ACh, whether the neurotransmitter translocates specific isoforms to different targets, and which proteins are phosphorylated by PKC. Phorbol esters not only stimulate insulin secretion, they also activate early genes and stimulate cell proliferation (403). It is unknown whether ACh could exert such effects physiologically. Many other questions await clear answers: What are the precise roles of PLA_2 and PLD in the action of ACh? What is the identity of the channel involved in the depolarization produced by ACh? Is the ACh-induced inhibition of the Ca^{2+} current mediated by a cytosolic diffusible messenger or by a direct interaction with Ca^{2+} channels? How can a single subtype of receptor activate so many different transduction pathways with different concentration dependencies for ACh? Answers to this last question might be provided by the use of the recently developed model of muscarinic receptor-knockout mice (253, 641, 716) and of systems expressing truncated muscarinic receptors subtypes.

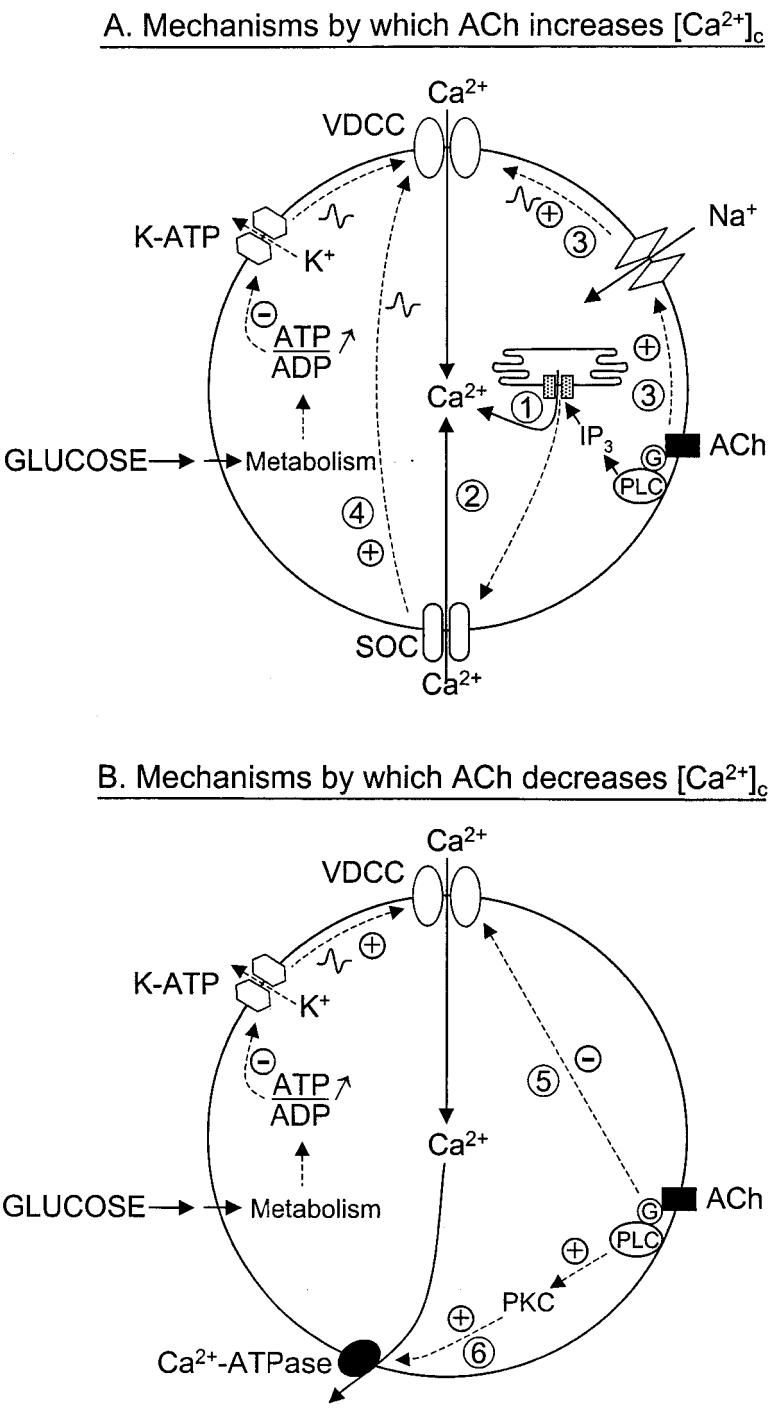


FIG. 8. Mechanisms by which ACh increases (A) or decreases (B) $[Ca^{2+}]_c$ in β-cells. A, ACh induces a transient increase in $[Ca^{2+}]_c$ by mobilizing Ca^{2+} from IP3-sensitive stores (mainly the endoplasmic reticulum; pathway 1). The resulting Ca^{2+} depletion of these stores activates a small sustained Ca^{2+} influx through voltage-independent Ca^{2+} channels (capacitative Ca^{2+} entry; pathway 2). ACh also depolarizes the plasma membrane of β-cells by increasing a specific Na^+- or a nonspecific cationic conductance (pathway 3), and probably also by activating SOCs carrying Ca^{2+} (capacitative Ca^{2+} entry) or other ions (pathway 4). This depolarization is small and reaches the threshold for the activation of voltage-dependent Ca^{2+} channels (VDCC) only if the plasma membrane is already depolarized by secretagogues, such as glucose, that close K^+-ATP channels. Stimulation of Ca^{2+} influx through voltage-dependent Ca^{2+} channels is the main mechanism by which ACh induces a sustained increase in $[Ca^{2+}]_c$ at stimulatory glucose concentrations. B, When $[Ca^{2+}]_c$ is already elevated, ACh also decreases $[Ca^{2+}]_c$ by inhibition of voltage-dependent Ca^{2+} channels (pathway 5) and stimulation of Ca^{2+} efflux, probably via PKC activation (pathway 6).

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References

1. **Strubbe JH, Steffens AB** 1993 Neural control of insulin secretion. Horm Metab Res 25:507–512
2. **Brunicardi FC, Shavelle DM, Andersen DK** 1995 Neural regulation of the endocrine pancreas. Int J Pancreatol 18:177–195
3. **Ahrén B** 2000 Autonomic regulation of islet hormone secretion–implications for health and disease. Diabetologia 43:393–410
4. **Best L, Dunlop M, Malaisse WJ** 1984 Phospholipid metabolism in pancreatic islets. Experientia 40:1085–1091
5. **Malaisse WJ** 1986 Stimulus-secretion coupling in the pancreatic B-cell: the cholinergic pathway for insulin release. Diabetes Metab

Rev 2:243–259
6. **Biden TJ, Wollheim CB** 1989 Generation, metabolism and function of inositol phosphates during nutrient-induced and neurotransmitter-induced insulin secretion. In: Michell RH, Drummond AH, Downes CP, eds. Inositol lipids in cell signalling. London: Academic Press Ltd.; 405–428
7. **Satin LS, Kinard TA** 1998 Neurotransmitters and their receptors in the islets of Langerhans of the pancreas – what messages do acetylcholine, glutamate, and GABA transmit? Endocrine 8:213–223
8. **Kobayashi S, Fujita T** 1969 Fine structure of mammalian and avian pancreatic islets with special reference to D cells and nervous elements. Z Zellforsch Mikrosk Anat 100:340–363
9. **Woods SC, Porte Jr D** 1974 Neural control of the endocrine pancreas. Physiol Rev 54:596–619
10. **Miller RE** 1981 Pancreatic neuroendocrinology peripheral neural mechanisms in the regulation of the islets of Langerhans. Endocr Rev 2:471–494
11. **Legg PG** 1967 The fine structure and innervation of the β and δ cells in the islet of Langerhans of the cat. Z Zellforsch Mikrosk Anat 80:307–321
12. **Watari N** 1968 Fine structure of nervous elements in the pancreas of some vertebrates. Z Zellforsch Mikrosk Anat 85:291–314
13. **Shorr SS, Bloom FE** 1970 Fine structure of islet-cell innervation in the pancreas of normal and alloxan-treated rats. Z Zellforsch Mikrosk Anat 103:12–25
14. **Fujita T, Kobayashi S** 1979 Proposal of a neurosecretory system in the pancreas: an electron microscope study in the dog. Arch Histol Jpn 42:277–295
15. **Bock P** 1986 Fine structure of the neuro-insular complex type II in the cat. Arch Histol Jpn 49:189–197
16. **Radke R, Stach W** 1986 Are the islets of Langerhans neuro-paraneuronal control centers of the exocrine pancreas? Arch Histol Jpn 49:411–420
17. **Radke R, Stach W** 1986 Innervation of the canine pancreas after vagotomy. Acta Anat (Basel) 127:88–92
18. **Orci L, Perrelet A, Ravazzola M, Malaisse-Lagae F, Renold AE** 1973 A specialized membrane junction between nerve endings and B-cells in islets of Langerhans. Eur J Clin Invest 3:443–445
19. **Watanabe T, Yasuda M** 1977 Electron microscopic study on the innervation of the pancreas of the domestic fowl. Cell Tissue Res 180:453–465
20. **Smith PH, Madson KL** 1981 Interactions between autonomic nerves and endocrine cells of the gastroenteropancreatic system. Diabetologia 20(Suppl):314–324
21. **Gardemann A, Jungermann K, Grosse V, Cossel L, Wohlrab F, Hahn HJ, Blech W, Hildebrandt W** 1994 Intraportal transplantation of pancreatic islets into livers of diabetic rats: reinnervation of islets and regulation of insulin secretion by the hepatic sympathetic nerves. Diabetes 43:1345–1352
22. **Berthoud HR, Powley TL** 1993 Characterization of vagal innervation to the rat celiac, suprarenal and mesenteric ganglia. J Auton Nerv Syst 42:153–169
23. **Sheikh SP, Holst JJ, Skak-Nielsen T, Knigge U, Warberg J, Theodorsson-Norheim E, Hokfelt T, Lundberg JM, Schwartz TW** 1988 Release of NPY in pig pancreas: dual parasympathetic and sympathetic regulation. Am J Physiol 255:G46–G54
24. **Verchere CB, Kowalyk S, Koerker DJ, Baskin DG, Taborsky Jr GJ** 1996 Evidence that galanin is a parasympathetic, rather than a sympathetic, neurotransmitter in the baboon pancreas. Regul Pept 67:93–101
25. **Liu HP, Tay SS, Leong S, Schemann M** 1998 Colocalization of ChAT, DβH and NADPH-d in the pancreatic neurons of the newborn guinea pig. Cell Tissue Res 294:227–231
26. **Luiten PG, ter Horst GJ, Koopmans SJ, Rietberg M, Steffens AB** 1984 Preganglionic innervation of the pancreas islet cells in the rat. J Auton Nerv Syst 10:27–42
27. **Ahrén B, Taborsky Jr GJ, Porte Jr D** 1986 Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. Diabetologia 29:827–836
28. **Louis-Sylvestre J** 1987 The cephalic phase of insulin secretion. Diabetes Metab 13:63–73
29. **Ionescu E, Rohner-Jeanrenaud F, Berthoud HR, Jeanrenaud B** 1983 Increase in plasma insulin levels in response to electrical stimulation of the dorsal motor nucleus of the vagus nerve. Endocrinology 112:904–910
30. **Rinaman L, Miselis RR** 1987 The organization of vagal innervation of rat pancreas using cholera toxin-horseradish peroxidase conjugate. J Auton Nerv Syst 21:109–125
31. **Berthoud HR, Fox EA, Powley TL** 1990 Localization of vagal preganglionics that stimulate insulin and glucagon secretion. Am J Physiol 258:R160–R168
32. **Berthoud HR, Powley TL** 1991 Morphology and distribution of efferent vagal innervation of rat pancreas as revealed with anterograde transport of DiI. Brain Res 553:336–341
33. **Chen XH, Itoh M, Sun W, Miki T, Takeuchi Y** 1996 Localization of sympathetic and parasympathetic neurons innervating pancreas and spleen in the cat. J Auton Nerv Syst 59:12–16
34. **Weaver FC** 1980 Localization of parasympathetic preganglionic cell bodies innervating the pancreas within the vagal nucleus and nucleus ambiguus of the rat brain stem: evidence of dual innervation based on the retrograde axonal transport of horseradish peroxidase. J Auton Nerv Syst 2:61–69
35. **Sharkey KA, Williams RG** 1983 Extrinsic innervation of the rat pancreas: demonstration of vagal sensory neurones in the rat by retrograde tracing. Neurosci Lett 42:131–135
36. **Sharkey KA, Williams RG, Dockray GJ** 1984 Sensory substance P innervation of the stomach and pancreas: demonstration of capsaicin-sensitive sensory neurons in the rat by combined immunohistochemistry and retrograde tracing. Gastroenterology 87: 914–921
37. **Luiten PG, ter Horst GJ, Buijs RM, Steffens AB** 1986 Autonomic innervation of the pancreas in diabetic and non-diabetic rats: a new view on intramural sympathetic structural organization. J Auton Nerv Syst 15:33–44
38. **Berthoud HR, Powley TL** 1990 Identification of vagal preganglionics that mediate cephalic phase insulin response. Am J Physiol 258:R523–R530
39. **Kinami S, Miwa K, Sato T, Miyazaki I** 1997 Section of the vagal celiac branch in man reduces glucagon-stimulated insulin release. J Auton Nerv Syst 64:44–48
40. **Berthoud HR, Bereiter DA, Trimble ER, Siegel EG, Jeanrenaud B** 1981 Cephalic phase, reflex insulin secretion. Neuroanatomical and physiological characterization. Diabetologia 20(Suppl): 393–401
41. **Bloom SR, Edwards AV** 1981 Pancreatic endocrine responses to stimulation of the peripheral ends of the vagus nerves in conscious calves. J Physiol 315:31–41
42. **Bloom SR, Edwards AV, Ghatei MA** 1983 Endocrine responses to exogenous bombesin and gastrin releasing peptide in conscious calves. J Physiol 344:37–48
43. **Knuhtsen S, Holst JJ, Jensen SL, Knigge U, Nielsen OV** 1985 Gastrin-releasing peptide: effect on exocrine secretion and release from isolated perfused porcine pancreas. Am J Physiol 248:G281–G286
44. **Knuhtsen S, Holst JJ, Baldissera FG, Skak-Nielsen T, Poulsen SS, Jensen SL, Nielsen OV** 1987 Gastrin-releasing peptide in the porcine pancreas. Gastroenterology 92:1153–1158
45. **Sha L, Miller SM, Szurszewski JH** 1995 Nitric oxide is a neuromodulator in cat pancreatic ganglia: histochemical and electrophysiological study. Neurosci Lett 192:77–80
46. **Ahrén B, Sauerberg P, Thomsen C** 1999 Increased insulin secretion and normalization of glucose tolerance by cholinergic agonism in high fat-fed mice. Am J Physiol 277:E93–E102
47. **Havel PJ, Dunning BE, Verchere CB, Baskin DG, O'Dorisio T, Taborsky Jr GJ** 1997 Evidence that vasoactive intestinal polypeptide is a parasympathetic neurotransmitter in the endocrine pancreas in dogs. Regul Pept 71:163–170
48. **Ekblad E, Alm P, Sundler F** 1994 Distribution, origin and projections of nitric oxide synthase-containing neurons in gut and pancreas. Neuroscience 63:233–248
49. **Wang J, Zheng H, Berthoud HR** 1999 Functional vagal input to chemically identified neurons in pancreatic ganglia as revealed by Fos expression. Am J Physiol 277:E958–E964
50. **Love JA, Szebeni K** 1999 Morphology and histochemistry of the rabbit pancreatic innervation. Pancreas 18:53–64
51. **Myojin T, Kitamura N, Hondo E, Baltazar ET, Pearson GT,**

Yamada J 2000 Immunohistochemical localization of neuropeptides in bovine pancreas. Anat Histol Embryol 29:167–172
52. **Coupland RE** 1958 The innervation of pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. J Anat 92: 143–149
53. **Esterhuizen AC, Spriggs TL, Lever JD** 1968 Nature of islet-cell innervation in the cat pancreas. Diabetes 17:33–36
54. **Stach W, Radke R** 1982 Innervation of islands of Langerhans: light and electron microscopic studies of the pancreas in laboratory animals. Endokrinologie 79:210–220
55. **Radke R, Stach W** 1986 Electron microscopy and ultrahistochemical studies on the innervation of the vagotomized dog pancreas. J Hirnforsch 27:369–379
56. **Van der Zee EA, Buwalda B, Strubbe JH, Strosberg AD, Luiten PG** 1992 Immunocytochemical localization of muscarinic acetylcholine receptors in the rat endocrine pancreas. Cell Tissue Res 269:99–106
57. **Godfrey DA, Matschinsky FM** 1975 Enzymes of the cholinergic system in islets of Langerhans. J Histochem Cytochem 23:645–651
58. **Voss KM, Herberg L, Kern HF** 1978 Fine structural studies of the islets of langerhans in the Djungarian hamster (*Phodopus sungorus*). Cell Tissue Res 191:333–342
59. **Golding DW, Pow DV** 1990 "Neurosecretion" by synaptic terminals and glandular discharge in the endocrine pancreas: application of tannic acid to the teleost *Xiphophorus helleri*. Neuroendocrinology 51:369–375
60. **Ahrén B, Taborsky Jr GJ** 1986 The mechanism of vagal nerve stimulation of glucagon and insulin secretion in the dog. Endocrinology 118:1551–1557
61. **Stagner JI, Samols E** 1986 Modulation of insulin secretion by pancreatic ganglionic nicotinic receptors. Diabetes 35:849–854
62. **Sha L, Love JA, Ma RC, Szurszewski JH** 1997 Cholinergic transmission in pancreatic ganglia of the cat. Pancreas 14:83–93
63. **Karlsson S, Ahrén B** 1998 Insulin and glucagon secretion by ganglionic nicotinic activation in adrenalectomized mice. Eur J Pharmacol 342:291–295
64. **Kirchgessner AL, Liu MT** 1998 Immunohistochemical localization of nicotinic acetylcholine receptors in the guinea pig bowel and pancreas. J Comp Neurol 390:497–514
65. **Martindale R, Levin S, Alfin Slater R** 1982 Effects of caerulein and bombesin on insulin and glucagon secretion from the isolated, perfused rat pancreas. Regul Pept 3:313–324
66. **Swope SL, Schonbrunn A** 1988 The biphasic stimulation of insulin secretion by bombesin involves both cytosolic free calcium and protein kinase C. Biochem J 253:193–202
67. **Liu HP, Tay SS, Leong SK** 1996 Nitrergic neurons in the pancreas of newborn guinea pig: their distribution and colocalization with various neuropeptides and dopamine-β-hydroxylase. J Auton Nerv Syst 61:248–256
68. **Karlsson S, Sundler F, Ahrén B** 1998 Insulin secretion by gastrin-releasing peptide in mice: ganglionic versus direct islet effect. Am J Physiol 274:E124–E129
69. **Jian X, Sainz E, Clark WA, Jensen RT, Battey JF, Northup JK** 1999 The bombesin receptor subtypes have distinct G protein specificities. J Biol Chem 274:11573–11581
70. **Lundberg JM** 1996 Pharmacology of cotransmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. Pharmacol Rev 48:113–178
71. **Blottner D** 1999 Nitric oxide and target-organ control in the autonomic nervous system: anatomical distribution, spatiotemporal signaling, and neuroeffector maintenance. J Neurosci Res 58: 139–151
72. **Ding Y, Rana RS** 1998 Nitric oxide does not initiate but potentiates glucose-induced insulin secretion in pancreatic β-cells. Biochem Biophys Res Commun 251:699–703
73. **Cunningham JM, Mabley JG, Delaney CA, Green IC** 1994 The effect of nitric oxide donors on insulin secretion, cyclic GMP and cyclic AMP in rat islets of Langerhans and the insulin-secreting cell lines HIT-T15 and RINm5F. Mol Cell Endocrinol 102:23–29
74. **Tsuura Y, Ishida H, Hayashi S, Sakamoto K, Horie M, Seino Y** 1994 Nitric oxide opens ATP-sensitive K^+ channels through suppression of phosphofructokinase activity and inhibits glucose-induced insulin release in pancreatic β cells. J Gen Physiol 104: 1079–1098
75. **Akesson B, Henningsson R, Salehi A, Lundquist I** 1999 Islet constitutive nitric oxide synthase and glucose regulation of insulin release in mice. J Endocrinol 163:39–48
76. **Kaneto A, Miki E, Kosaka K** 1974 Effects of vagal stimulation on glucagon and insulin secretion. Endocrinology 95:1005–1010
77. **Holst JJ, Gronholt R, Schaffalitzky de Muckadell OB, Fahrenkrug J** 1981 Nervous control of pancreatic endocrine secretion in pigs. I. Insulin and glucagon responses to electrical stimulation of the vagus nerves. Acta Physiol Scand 111:1–8
78. **Nishi S, Seino Y, Ishida H, Seno M, Taminato T, Sakurai H, Imura H** 1987 Vagal regulation of insulin, glucagon, and somatostatin secretion *in vitro* in the rat. J Clin Invest 79:1191–1196
79. **Ahrén B, Paquette TL, Taborsky Jr GJ** 1986 Effect and mechanism of vagal nerve stimulation on somatostatin secretion in dogs. Am J Physiol 250:E212–E217
80. **Holst JJ, Jensen SL, Knuhtsen S, Nielsen OV** 1983 Autonomic nervous control of pancreatic somatostatin secretion. Am J Physiol 245:E542–E548
81. **Holst JJ, Schaffalitzky de Muckadell OB, Fahrenkrug J, Lindkaer S, Nielsen OV, Schwartz TW** 1981 Nervous control of pancreatic endocrine secretion in pigs. III. The effect of acetylcholine on the pancreatic secretion of insulin and glucagon. Acta Physiol Scand 111:15–22
82. **Kimura H, Katagiri K, Ohno T, Harada N, Imanishi H, Iwasaki M, Ito M, Takeuchi T** 1982 Effect of acetylcholine and new cholinergic derivative on amylase output, insulin, glucagon, and somatostatin secretions from perfused isolated rat pancreas. Horm Metab Res 14:356–360
83. **Havel PJ, Parry SJ, Curry DL, Stern JS, Akpan JO, Gingerich RL** 1992 Autonomic nervous system mediation of the pancreatic polypeptide response to insulin-induced hypoglycemia in conscious rats. Endocrinology 130:2225–2229
84. **Havel PJ, Parry SJ, Stern JS, Akpan JO, Gingerich RL, Taborsky Jr GJ, Curry DL** 1994 Redundant parasympathetic and sympathoadrenal mediation of increased glucagon secretion during insulin-induced hypoglycemia in conscious rats. Metabolism 43: 860–866
85. **Hermansen K** 1980 Secretion of somatostatin from the normal and diabetic pancreas: studies *in vitro*. Diabetologia 19:492–504
86. **Furuzawa Y, Ohmori Y, Watanabe T** 1996 Anatomical localization of sympathetic postganglionic and sensory neurons innervating the pancreas of the cat. J Vet Med Sci 58:243–248
87. **Chusid JG** 1979 Correlative neuroanatomy and functional neurology. 17th ed. Los Altos: Lange Medical Publications; 1–464
88. **Fox EA, Powley TL** 1986 Tracer diffusion has exaggerated CNS maps of direct preganglionic innervation of pancreas. J Auton Nerv Syst 15:55–69
89. **Dunning BE, Taborsky Jr GJ** 1988 Galanin–sympathetic neurotransmitter in endocrine pancreas? Diabetes 37:1157–1162
90. **Ahrén B, Bottcher G, Kowalyk S, Dunning BE, Sundler F, Taborsky Jr GJ** 1990 Galanin is co-localized with noradrenaline and neuropeptide Y in dog pancreas and celiac ganglion. Cell Tissue Res 261:49–58
91. **Taborsky Jr GJ, Dunning BE, Havel PJ, Ahren B, Kowalyk S, Boyle MR, Verchere CB, Baskin DG, Mundinger TO** 1999 The canine sympathetic neuropeptide galanin: a neurotransmitter in pancreas, a neuromodulator in liver. Horm Metab Res 31:351–354
92. **Ahrén B, Ericson LE, Lundquist I, Loren I, Sundler F** 1981 Adrenergic innervation of pancreatic islets and modulation of insulin secretion by the sympatho-adrenal system. Cell Tissue Res 216: 15–30
93. **Andersson PO, Holst JJ, Jarhult J** 1982 Effects of adrenergic blockade on the release of insulin, glucagon and somatostatin from the pancreas in response to splanchnic nerve stimulation in cats. Acta Physiol Scand 116:403–410
94. **Ahrén B, Veith RC, Taborsky Jr GJ** 1987 Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: 1). Effects on basal release of insulin and glucagon. Endocrinology 121:323–331
95. **Brunicardi FC, Sun YS, Druck P, Goulet RJ, Elahi D, Andersen DK** 1987 Splanchnic neural regulation of insulin and glucagon

secretion in the isolated perfused human pancreas. Am J Surg 153:34–40
96. **Kurose T, Seino Y, Nishi S, Tsuji K, Taminato T, Tsuda K, Imura H** 1990 Mechanism of sympathetic neural regulation of insulin, somatostatin, and glucagon secretion. Am J Physiol 258:E220–E227
97. **Porte Jr D** 1967 A receptor mechanism for the inhibition of insulin release by epinephrine in man. J Clin Invest 46:86–94
98. **Coore HG, Randle PJ** 1964 Regulation of insulin secretion studied with pieces of rabbit pancreas incubated *in vitro*. Biochem J 93: 66–78
99. **Malaisse WJ, Malaisse-Lagae F, Wright PH, Ashmore J** 1967 Effects of adrenergic and cholinergic agents upon insulin secretion *in vitro*. Endocrinology 80:975–978
100. **Sorenson RL, Elde RP, Seybold V** 1979 Effect of norepinephrine on insulin, glucagon and somatostatin secretion in isolated perifused rat islets. Diabetes 28:899–904
101. **Schuit FC, Pipeleers DG** 1986 Differences in adrenergic recognition by pancreatic A and B cells. Science 232:875–877
102. **Nakaki T, Nakadate T, Kato R** 1980 α_2-Adrenoceptors modulating insulin release from isolated pancreatic islets. Naunyn Schmiedebergs Arch Pharmacol 313:151–154
103. **Chan SLF, Perrett CW, Morgan NG** 1997 Differential expression of α_2-adrenoceptor subtypes in purified rat pancreatic islet A- and B-cells. Cell Signal 9:71–78
104. **Angel I, Bidet S, Langer SZ** 1988 Pharmacological characterization of the hyperglycemia induced by α_2 adrenoceptor agonists. J Pharmacol Exp Ther 246:1098–1103
105. **Lacey RJ, Chan SLF, Cable HC, James RFL, Perrett CW, Scarpello JHB, Morgan NG** 1996 Expression of α_2- and β-adrenoceptor subtypes in human islets of Langerhans. J Endocrinol 148:531–543
106. **Sharp GWG** 1996 Mechanisms of inhibition of insulin release. Am J Physiol 271:C1781–C1799
107. **Porte Jr D** 1967 β Adrenergic stimulation of insulin release in man. Diabetes 16:150–155
108. **Ahrén B, Lundquist I** 1981 Effects of selective and non-selective β-adrenergic agents on insulin secretion *in vivo*. Eur J Pharmacol 71:93–104
109. **Garrino MG, Henquin JC** 1990 B cell adrenoceptors and sulphonylurea-induced insulin release in mouse islets. Diabetologia 33: 145–147
110. **Lacey RJ, Berrow NS, Scarpello JHB, Morgan NG** 1991 Selective stimulation of glucagon secretion by β_2-adrenoceptors in isolated islets of Langerhans of the rat. Br J Pharmacol 103:1824–1828
111. **Plant TD, Henquin JC** 1990 Phentolamine and yohimbine inhibit ATP-sensitive K^+ channels in mouse pancreatic β-cells. Br J Pharmacol 101:115–120
112. **Holst JJ, Gronholt R, Schaffalitzky de Muckadell OB, Fahrenkrug J** 1981 Nervous control of pancreatic endocrine secretion in pigs. V. Influence of the sympathetic nervous system on the pancreatic secretion of insulin and glucagon, and on the insulin and glucagon response to vagal stimulation. Acta Physiol Scand 113: 279–283
113. **Holst JJ, Schwartz TW, Knuhtsen S, Jensen SL, Nielsen OV** 1986 Autonomic nervous control of the endocrine secretion from the isolated, perfused pig pancreas. J Auton Nerv Syst 17:71–84
114. **Itoh M, Gerich JE** 1982 Adrenergic modulation of pancreatic somatostatin, insulin, and glucagon secretion: evidence for differential sensitivity of islet A, B, and D cells. Metabolism 31:715–720
115. **Gromada J, Bokvist K, Ding WG, Barg S, Buschard K, Renström E, Rorsman P** 1997 Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the Ca^{2+} current and the number of granules close to the L-type Ca $^{2+}$ channels. J Gen Physiol 110:217–228
116. **Ahrén B, Veith RC, Paquette TL, Taborsky Jr GJ** 1987 Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: 2). Effects on basal release of somatostatin and pancreatic polypeptide. Endocrinology 121:332–339
117. **Brunicardi FC, Druck P, Seymour NE, Sun YS, Gingerich RL, Elahi D, Andersen DK** 1989 Splanchnic neural regulation of pancreatic polypeptide release in the isolated perfused human pancreas. Am J Surg 157:50–57
118. **Meglasson MD, Hazelwood RL** 1983 Adrenergic regulation of avian pancreatic polypeptide secretion *in vitro*. Am J Physiol 244: E408–E413
119. **Taborsky GJ, Ahrén B, Havel PJ** 1998 Autonomic mediation of glucagon secretion during hypoglycemia – implications for impaired α-cell responses in type 1 diabetes. Diabetes 47:995–1005
120. **Szallasi A, Blumberg PM** 1999 Vanilloid (Capsaicin) receptors and mechanisms. Pharmacol Rev 51:159–212
121. **Pettersson M, Ahrén B, Bottcher G, Sundler F** 1986 Calcitonin gene-related peptide: occurrence in pancreatic islets in the mouse and the rat and inhibition of insulin secretion in the mouse. Endocrinology 119:865–869
122. **Sternini C, Brecha N** 1986 Immunocytochemical identification of islet cells and nerve fibers containing calcitonin gene-related peptide-like immunoreactivity in the rat pancreas. Gastroenterology 90:1155–1163
123. **Adeghate E** 1999 Distribution of calcitonin-gene-related peptide, neuropeptide-Y, vasoactive intestinal polypeptide, cholecystokinin-8, substance P and islet peptides in the pancreas of normal and diabetic rats. Neuropeptides 33:227–235
124. **Schmidt PT, Tornoe K, Poulsen SS, Rasmussen TN, Holst JJ** 2000 Tachykinins in the porcine pancreas: potent exocrine and endocrine effects via NK-1 receptors. Pancreas 20:241–247
125. **Karlsson S, Sundler F, Ahrén B** 1992 Neonatal capsaicin-treatment in mice: effects on pancreatic peptidergic nerves and 2-deoxy-D-glucose-induced insulin and glucagon secretion. J Auton Nerv Syst 39:51–60
126. **Su HC, Bishop AE, Power RF, Hamada Y, Polak JM** 1987 Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas. J Neurosci 7:2674–2687
127. **Rossi M, Zaninotto G, Finco C, Codello L, Ancona E** 1995 Thoracoscopic bilateral splanchnicotomy for pain control in unresectable pancreatic cancer. Chir Ital 47:55–57
128. **Won MH, Park HS, Jeong YG, Park HJ** 1998 Afferent innervation of the rat pancreas: retrograde tracing and immunohistochemistry in the dorsal root ganglia. Pancreas 16:80–87
129. **Carobi C** 1987 Capsaicin-sensitive vagal afferent neurons innervating the rat pancreas. Neurosci Lett 77:5–9
130. **Neuhuber WL** 1989 Vagal afferent fibers almost exclusively innervate islets in the rat pancreas as demonstrated by anterograde tracing. J Auton Nerv Syst 29:13–18
131. **Di Sebastiano P, Friess H, Di Mola FF, Innocenti P, Buchler MW** 2000 Mechanisms of pain in chronic pancreatitis. Ann Ital Chir 71:11–16
132. **Karlsson S, Scheurink AJW, Steffens AB, Ahrén B** 1994 Involvement of capsaicin-sensitive nerves in regulation of insulin secretion and glucose tolerance in conscious mice. Am J Physiol 267:R1071–R1077
133. **Ahrén B, Pettersson M** 1990 Calcitonin gene-related peptide (CGRP) and amylin and the endocrine pancreas. Int J Pancreatol 6:1–16
134. **Lundquist I, Sundler F, Ahrén B, Alumets J, Hakanson R** 1979 Somatostatin, pancreatic polypeptide, substance P and neurotensin cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology 104:832–838
135. **Hermansen K** 1980 Effects of substance-P and other peptides on the release of somatostatin, insulin, and glucagon *in vitro*. Endocrinology 107:256–261
136. **Guillot E, Coste A, Angel I** 1996 Involvement of capsaicin-sensitive nerves in the regulation of glucose tolerance in diabetic rats. Life Sci 59:969–977
137. **Koopmans SJ, Leighton B, DeFronzo RA** 1998 Neonatal de-afferentation of capsaicin-sensitive sensory nerves increases *in vivo* insulin sensitivity in conscious adult rats. Diabetologia 41:813–820
138. **Karlsson S, Ahrén B** 1992 Cholecystokinin and the regulation of insulin secretion. Scand J Gastroenterol 27:161–165
139. **Kirchgessner AL, Gershon MD** 1990 Innervation of the pancreas by neurons in the gut. J Neurosci 10:1626–1642
140. **Kirchgessner AL, Pintar JE** 1991 Guinea pig pancreatic ganglia: projections, transmitter content, and the type-specific localization of monoamine oxidase. J Comp Neurol 305:613–631
141. **Quickel KE, Feldman JM, Lebovitz HE** 1971 Inhibition of insulin secretion by serotonin and dopamine: species variation. Endocrinology 89:1295–1302

142. **Lindstrom P, Sehlin J** 1983 Opposite effects of 5-hydroxytryptophan and 5-hydroxytryptamine on the function of microdissected *ob/ob*-mouse pancreatic islets. Diabetologia 24:52–57
143. **Peschke E, Peschke D, Hammer T, Csernus V** 1997 Influence of melatonin and serotonin on glucose-stimulated insulin release from perifused rat pancreatic islets *in vitro*. J Pineal Res 23:156–163
144. **Green IC, Perrin D, Pedley KC, Leslie RDG, Pyke DA** 1980 Effect of enkephalins and morphine on insulin secretion from isolated rat islets. Diabetologia 19:158–161
145. **Hermansen K** 1983 Enkephalins and the secretion of pancreatic somatostatin and insulin in the dog: studies *in vitro*. Endocrinology 113:1149–1154
146. **Huchtebrock HJ, Niebel W, Singer MV, Forssmann WG** 1991 Intrinsic pancreatic nerves after mechanical denervation of the extrinsic pancreatic nerves in dogs. Pancreas 6:1–8
147. **Zunz E, LaBarre J** 1927 Contribution à l'étude des variations physiologiques de le sécrétion interne du pancréas. Arch Int Physiol Biochim 29:265–280
148. **Daniel PM, Henderson JR** 1967 Insulin in bile and other body fluids. Lancet 1:1256–1257
149. **Frohman LA, Ezdinli EZ, Javid R** 1967 Effect of vagotomy and vagal stimulation on insulin secretion. Diabetes 16:443–448
150. **Kaneto A, Kosaka K, Nakao K** 1967 Effects of stimulation of the vagus nerve on insulin secretion. Endocrinology 80:530–536
151. **Bergman RN, Miller RE** 1973 Direct enhancement of insulin secretion by vagal stimulation of the isolated pancreas. Am J Physiol 225:481–486
152. **Blackburn AM, Bloom SR, Edwards AV** 1981 Pancreatic endocrine responses to physiological changes in plasma neurotensin concentration. J Physiol 318:407–412
153. **Bloom SR, Edwards AV** 1985 Effects of certain metabolites on pancreatic endocrine responses to stimulation of the vagus nerves in conscious calves. J Physiol 362:303–310
154. **Edwards AV, Bloom SR** 1986 Autonomic regulation of insulin secretion. Trends Autonom Pharmacol 3:129–145
155. **Havel PJ, Dunning BE, Taborsky Jr GJ** 1994 Autonomic control of insulin release. In: Flatt PR, Lenzen S, eds. Insulin secretion and pancreatic B-cell research. London: Smith-Gordon; 343–351
156. **Iversen J** 1973 Effect of acetylcholine on the secretion of glucagon and insulin from the isolated perfused canine pancreas. Diabetes 22:381–387
157. **Loubatières-Mariani MM, Chapal J, Alric R, Loubatières AL** 1973 Studies of the cholinergic receptors involved in the secretion of insulin using isolated perfused rat pancreas. Diabetologia 9: 439–446
158. **Kaneto A, Kosaka K** 1974 Stimulation of glucagon and insulin secretion by acetylcholine infused intrapancreatically. Endocrinology 95:676–681
159. **Honey RN, Weir GC** 1980 Acetylcholine stimulates insulin, glucagon and somatostatin release in the perfused chicken pancreas. Endocrinology 107:1065–1068
160. **Verchere CB, Kwok YN, Brown JC** 1991 Modulation of acetylcholine-stimulated insulin release by glucose and gastric inhibitory polypeptide. Pharmacology 42:273–282
161. **Gagerman E, Idahl L-Å, Meissner HP, Täljedal IB** 1978 Insulin release, cGMP, cAMP, and membrane potential in acetylcholine-stimulated islets. Am J Physiol 235:E493–E500
162. **Garcia MC, Hermans MP, Henquin JC** 1988 Glucose-, calcium- and concentration-dependence of acetylcholine stimulation of insulin release and ionic fluxes in mouse islets. Biochem J 254:211–218
163. **Persaud SJ, Jones PM, Sugden D, Howell SL** 1989 The role of protein kinase C in cholinergic stimulation of insulin secretion from rat islets of Langerhans. Biochem J 264:753–758
164. **Zawalich WS, Zawalich KC, Rasmussen H** 1989 Cholinergic agonists prime the β-cell to glucose stimulation. Endocrinology 125: 2400–2406
165. **Henquin JC** 1994 Cell biology of insulin secretion. In: Weir GC, Kahn CR, eds. The Joslin's diabetes mellitus. Philadelphia: Lea and Febiger; 56–80
166. **Kaneto A, Kawazu S, Sato H, Kaneko T, Yanaihara C, Yanaihara N, Kosaka K** 1981 Effect of the vagal and splanchnic nerve stimulation on the release of somatostatin, pancreatic polypeptide, glucagon and insulin. Biomed Res 2:166–176
167. **Ionescu E, Jeanrenaud B** 1988 Effect of electrical stimulation of the vagus nerve on insulinemia and glycemia in *Acomys cahirinus* mice. Endocrinology 123:885–890
168. **Holst JJ, Gronholt R, Schaffalitzky de Muckadell OB, Fahrenkrug J** 1981 Nervous control of pancreatic endocrine secretion in pigs. II. The effect of pharmacological blocking agents on the response to vagal stimulation. Acta Physiol Scand 111:9–14
169. **Uvnäs-Wallensten K, Nilsson G** 1978 A quantitative study of the insulin release induced by vagal stimulation in anesthetized cats. Acta Physiol Scand 102:137–142
170. **Samols E, Stagner JI, Ewart RB, Marks V** 1988 The order of islet microvascular cellular perfusion is B-A-D in the perfused rat pancreas. J Clin Invest 82:350–353
171. **Samols E, Stagner JI** 1996 Intra-islet cell-cell interactions and insulin secretion. Diabetes Rev 4:207–223
172. **Brunicardi FC, Stagner J, Bonner-Weir S, Wayland H, Kleinman R, Livingston E, Guth P, Menger M, McCuskey R, Intaglietta M, Charles A, Ashley S, Cheung A, Ipp E, Gilman S, Howard T, Passaro Jr E** 1996 Microcirculation of the islets of Langerhans. Long Beach Veterans Administration Regional Medical Education Center Symposium. Diabetes 45:385–392
173. **Creutzfeldt W, Nauck M** 1992 Gut hormones and diabetes mellitus. Diabetes Metab Rev 8:149–177
174. **Tseng CC, Kieffer TJ, Jarboe LA, Usdin TB, Wolfe MM** 1996 Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide (GIP): effect of a specific glucose-dependent insulinotropic polypeptide receptor antagonist in the rat. J Clin Invest 98:2440–2445
175. **Nauck MA** 1999 Is glucagon-like peptide 1 an incretin hormone? Diabetologia 42:373–379
176. **Schusdziarra V, Bender H, Torres A, Pfeiffer E** 1983 Cholinergic mechanisms in intestinal phase insulin secretion in rats. Regul Pept 6:81–88
177. **Abello J, Ye F, Bosshard A, Bernard C, Cuber JC, Chayvialle JA** 1994 Stimulation of glucagon-like peptide-1 secretion by muscarinic agonist in a murine intestinal endocrine cell line. Endocrinology 134:2011–2017
178. **Rocca AS, Brubaker PL** 1999 Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. Endocrinology 140:1687–1694
179. **Rogers RC, McTigue DM, Hermann GE** 1996 Vagal control of digestion: modulation by central neural and peripheral endocrine factors. Neurosci Biobehav Rev 20:57–66
180. **Helman A, Marre M, Bobbioni E, Poussier P, Reach G, Assan R** 1982 The brain-islet axis: the nervous control of the endocrine pancreas. Diabete Metab 8:53–64
181. **Teff KL, Alavi A, Chen J, Pourdehnad M, Townsend RR** 1999 Muscarinic blockade inhibits gastric emptying of mixed-nutrient meal: effects of weight and gender. Am J Physiol 276:R707–R714
182. **Atef N, Ktorza A, Pénicaud L** 1995 CNS involvement in the glucose-induced increase of islet blood flow in obese Zucker rats. Int J Obes Relat Metab Disord 19:103–107
183. **Miller RE** 1970 Effects of vagotomy or splanchnicotomy on blood insulin and sugar concentrations in the conscious monkey. Endocrinology 86:642–651
184. **Hakanson R, Liedberg G, Lundquist I** 1971 Effect of vagal denervation on insulin release after oral and intravenous glucose. Experientia 27:460–461
185. **Aagaard P, Deckert T, Fenger HJ** 1973 Serum insulin after intravenous administration of glucose before and after total vagotomy. Scand J Gastroenterol 8:699–701
186. **Russell RC, Thomson JP, Bloom SR** 1974 The effect of truncal and selective vagotomy on the release of pancreatic glucagon, insulin and enteroglucagon. Br J Surg 61:821–824
187. **Daniel PM, Henderson JR** 1975 The effect of atropine on insulin release caused by intravenous glucose in the rhesus monkey. Acta Endocrinol (Copenh) 78:736–745
188. **Lund B, Aagaard P, Deckert T** 1975 Effect of vagotomy on insulin release after oral and intravenous glucose administration. Scand J Gastroenterol 10:777–780
189. **Kuzin MI, Morenkova SA, Karelin AA** 1980 Effect of truncal vagotomy on insulin secretion and on the peripheral blood prostaglandin E_2 level in rats. Bull Exp Biol Med 89:412–413

190. **Sakaguchi T, Yamaguchi K** 1980 Effects of vagal stimulation, vagotomy and adrenalectomy on release of insulin in the rat. J Endocrinol 85:131–136
191. **Lundquist I** 1982 Cholinergic muscarinic effects on insulin release in mice. Pharmacology 25:338
192. **Chap Z, Ishida T, Chou J, Lewis R, Hartley C, Entman M, Field JB** 1985 Effects of atropine and gastric inhibitory polypeptide on hepatic glucose uptake and insulin extraction in conscious dogs. J Clin Invest 76:1174–1181
193. **Lee HC, Curry DL, Stern JS** 1989 Direct effect of CNS on insulin hypersecretion in obese Zucker rats: involvement of vagus nerve. Am J Physiol 256:E439–E444
194. **Fukudo S, Virnelli S, Kuhn CM, Cochrane C, Feinglos MN, Surwit RS** 1989 Muscarinic stimulation and antagonism and glucoregulation in nondiabetic and obese hyperglycemic mice. Diabetes 38:1433–1438
195. **Schneeberger D, Tappy L, Temler E, Jequier E** 1991 Effects of muscarinic blockade on insulin secretion and on glucose-induced thermogenesis in lean and obese human subjects. Eur J Clin Invest 21:608–615
196. **Teff KL, Townsend RR** 1999 Early phase insulin infusion and muscarinic blockade in obese and lean subjects. Am J Physiol 277: R198–R208
197. **Henderson JR, Jefferys DB, Jones RH, Stanley D** 1976 The effect of atropine on the insulin release caused by oral and intravenous glucose in human subjects. Acta Endocrinol (Copenh) 83:772–780
198. **Mattheeuws D, Rottiers R, Kaneko JJ, Vermeulen A** 1980 Glucose assimilation and insulin secretion in I.V. GTT in normal dogs: influence of atropine and pentobarbital. Horm Metab Res 12: 553–554
199. **Trimble ER, Berthoud HR, Siegel EG, Jeanrenaud B, Renold AE** 1981 Importance of cholinergic innervation of the pancreas for glucose tolerance in the rat. Am J Physiol 241:E337–E341
200. **Fischer U, Nowak W, Freyse EJ, Hommel H, Sill U, Lippert H** 1982 The influence of selective pancreatic denervation on plasma insulin and glucose tolerance in dogs. Diabete Metab 8:115–120
201. **Magnan C, Collins S, Berthault MF, Kassis N, Vincent M, Gilbert M, Pénicaud L, Ktorza A, Assimacopoulos-Jeannet F** 1999 Lipid infusion lowers sympathetic nervous activity and leads to increased β-cell responsiveness to glucose. J Clin Invest 103:413–419
202. **Humphrey CS, Dykes JRW, Johnston D** 1975 Effects of truncal, selective, and highly selective vagotomy on glucose tolerance and insulin secretion in patients with duodenal ulcer. Part I. Effect of vagotomy on response to oral glucose. Br Med J 2:112–114
203. **Louis-Sylvestre J** 1978 Relationship between two stages of prandial insulin release in rats. Am J Physiol 235:E103–E111
204. **Berthoud HR, Trimble ER, Siegel EG, Bereiter DA, Jeanrenaud B** 1980 Cephalic-phase insulin secretion in normal and pancreatic islet-transplanted rats. Am J Physiol 238:E336–E340
205. **Strubbe JH** 1982 Effects of pancreas transplantation on insulin secretion in the rat during ingestion of varying glucose loads. Diabetologia 22:354–357
206. **Strubbe JH, Van Wachem P** 1981 Insulin secretion by the transplanted neonatal pancreas during food intake in fasted and fed rats. Diabetologia 20:228–236
207. **Strubbe JH** 1989 Central nervous system and insulin secretion. Neth J Med 34:154–167
208. **Niijima A** 1986 Neural control of blood glucose level. Jpn J Physiol 36:827–841
209. **Fischer U, Hommel H, Ziegler M, Michael R** 1972 The mechanism of insulin secretion after oral glucose administration. I. Multiphasic course of insulin mobilization after oral administration of glucose in conscious dogs: differences to the behaviour after intravenous administration. Diabetologia 8:104–110
210. **Fischer U, Hommel H, Ziegler M, Jutzi E** 1972 The mechanism of insulin secretion after oral glucose administration. III. Investigations on the mechanism of a reflectoric insulin mobilization after oral stimulation. Diabetologia 8:385–390
211. **Strubbe JH, Steffens AB** 1975 Rapid insulin release after ingestion of a meal in the unanesthetized rat. Am J Physiol 229:1019–1022
212. **Flynn FW, Berridge KC, Grill HJ** 1986 Pre- and postabsorptive insulin secretion in chronic decerebrate rats. Am J Physiol 250: R539–R548
213. **Fischer U, Hommel H, Salzsieder E** 1976 Pancreatic blood flow in conscious dogs after oral administration of glucose. Diabetologia 12:133–136
214. **Fischer U, Hommel H, Freyse EJ, Fiedler H** 1975 The mechanism of insulin secretion following oral glucose administration. 4. Inhibition of the early reflectoric of plasma insulin increase by atropine. Endokrinologie 65:91–102
215. **Teff KL, Mattes RD, Engelman K** 1991 Cephalic phase insulin release in normal weight males: verification and reliability. Am J Physiol 261:E430–E436
216. **Ahren B, Holst JJ** 2001 The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. Diabetes 50:1030–1038
217. **Louis-Sylvestre J** 1976 Preabsorptive insulin release and hypoglycemia in rats. Am J Physiol 230:56–60
218. **Hommel HH, Fischer U** 1977 The mechanism of insulin secretion after oral glucose administration. V. Portal venous IRI concentration in dogs after ingestion of glucose. Diabetologia 13:269
219. **Abdallah L, Chabert M, Louis-Sylvestre J** 1997 Cephalic phase responses to sweet taste. Am J Clin Nutr 65:737–743
220. **Niijima A** 1989 Neural mechanisms in the control of blood glucose concentration. J Nutr 119:833–840
221. **Niijima A, Togiyama T, Adachi A** 1990 Cephalic-phase insulin release induced by taste stimulus of monosodium glutamate (Umami taste). Physiol Behav 48:905–908
222. **Parra-Covarrubias A, Rivera-Rodriguez I, Almaraz-Ugalde A** 1971 Cephalic phase of insulin secretion in obese adolescents. Diabetes 20:800–802
223. **Bellisle F, Louis-Sylvestre J, Demozay F, Blazy D, Le Magnen J** 1985 Cephalic phase of insulin secretion and food stimulation in humans: a new perspective. Am J Physiol 249:E639–E645
224. **Simon C, Schlienger JL, Sapin R, Imler M** 1986 Cephalic phase insulin secretion in relation to food presentation in normal and overweight subjects. Physiol Behav 36:465–469
225. **Teff KL, Levin BE, Engelman K** 1993 Oral sensory stimulation in men: effect on insulin, C-peptide and catecholamines. Am J Physiol 265:R1223–R1230
226. **Taylor IL, Feldman M** 1982 Effect of cephalic-vagal stimulation on insulin, gastric inhibitory polypeptide, and pancreatic polypeptide release in humans. J Clin Endocrinol Metab 55:1114–1117
227. **Bruce DG, Storlien LH, Furler SM, Chisholm DJ** 1987 Cephalic phase metabolic responses in normal weight adults. Metabolism 36:721–725
228. **Teff KL, Devine J, Engelman K** 1995 Sweet taste: effect on cephalic phase insulin release in men. Physiol Behav 57:1089–1095
229. **Benthem L, Mundinger TO, Taborsky Jr GJ** 2000 Meal-induced insulin secretion in dogs is mediated by both branches of the autonomic nervous system. Am J Physiol 278:E603–E610
230. **Steffens AB** 1969 Rapid absorption of glucose in the intestinal tract of the rat after ingestion of a meal. Physiol Behav 4:829–832
231. **Hommel H, Fischer U, Retzlaff K, Knofler H** 1972 The mechanism of insulin secretion after oral glucose administration. II. Reflex insulin secretion in conscious dogs beating fistulas of the digestive tract by shamfeeding of glucose or tap water. Diabetologia 8: 111–116
232. **Carlsson PO, Iwase M, Jansson L** 1999 Stimulation of intestinal glucoreceptors in rats increases pancreatic islet blood flow through vagal mechanisms. Am J Physiol 276:R233–R236
233. **Mei N, Arlhac A, Boyer A** 1981 Nervous regulation of insulin release by the intestinal vagal glucoreceptors. J Auton Nerv Syst 4:351–363
234. **Niijima A, Mei N** 1987 Glucose sensors in viscera and control of blood glucose level. News Physiol Sci 2:164–167
235. **Liu M, Seino S, Kirchgessner AL** 1999 Identification and characterization of glucoresponsive neurons in the enteric nervous system. J Neurosci 19:10305–10317
236. **Strubbe JH, Bouman PR** 1978 Plasma insulin patterns in the unanesthetized rat during intracardial infusion and spontaneous ingestion of graded loads of glucose. Metabolism 27:341–351
237. **Steffens AB** 1976 Influence of the oral cavity on insulin release in the rat. Am J Physiol 230:1411–1415
238. **Proietto J, Rohner-Jeanrenaud F, Ionescu E, Jeanrenaud B** 1987

Role of the oropharynx in regulation of glycemia. Diabetes 36: 791–795
239. **Teff KL, Engelman K** 1996 Oral sensory stimulation improves glucose tolerance in humans: Effects on insulin, C-peptide, and glucagon. Am J Physiol 270:R1371–R1379
240. **Andersen HB, Christiansen E, Volund A, Madsbad S, Rasmussen K, Burcharth F, Christensen NJ** 1995 Sham feeding increases glucose tolerance by a mechanism independent of insulin secretion in normal subjects. Digestion 56:253–258
241. **Shimazu T** 1987 Neuronal regulation of hepatic glucose metabolism in mammals. Diabetes Metab Rev 3:185–206
242. **Yamazaki M, Sakaguchi T** 1989 Pancreatic vagal functional distribution in the secretion of insulin evoked by portal infusion of D-glucose. Brain Res 484:357–360
243. **Sakaguchi T, Yamaguchi K** 1979 Effects of electrical stimulation of the hepatic vagus nerve on the plasma insulin concentration in the rat. Brain Res 164:314–316
244. **Lee KC, Miller RE** 1985 The hepatic vagus nerve and the neural regulation of insulin secretion. Endocrinology 117:307–314
245. **Niijima A** 1981 Visceral afferents and metabolic function. Diabetologia 20(Suppl):325–330
246. **Niijima A** 1982 Glucose-sensitive afferent nerve fibres in the hepatic branch of the vagus nerve in the guinea-pig. J Physiol 332: 315–324
247. **Yamatani K, Ohnuma H, Niijima A, Igarashi M, Sugiyama K, Daimon M, Manaka H, Tominaga M, Sasaki H** 1998 Impaired vagus nerve-mediated control of insulin secretion in Wistar fatty rats. Metabolism 47:1167–1173
248. **Yang XJ, Kow LM, Funabashi T, Mobbs CV** 1999 Hypothalamic glucose sensor: similarities to and differences from pancreatic β-cell mechanisms. Diabetes 48:1763–1772
249. **Havel PJ, Taborsky Jr GJ** 1989 The contribution of the autonomic nervous system to changes of glucagon and insulin secretion during hypoglycemic stress. Endocr Rev 10:332–350
250. **Schwartz TW** 1983 Pancreatic polypeptide: a hormone under vagal control. Gastroenterology 85:1411–1425
251. **D'Alessio DA, Kieffer TJ, Taborsky Jr GJ, Havel PJ** 2001 Activation of the parasympathetic nervous system is necessary for normal meal-induced insulin secretion in rhesus macaques. J Clin Endocrinol Metab 86:1253–1259
252. **Teff K** 2000 Nutritional implications of the cephalic-phase reflexes: endocrine responses. Appetite 34:206–213
253. **Yamada M, Miyakawa T, Duttaroy A, Yamanaka A, Moriguchi T, Makita R, Ogawa M, Chou CJ, Xia B, Crawley JN, Felder CC, Deng CX, Wess J** 2001 Mice lacking the M_3 muscarinic acetylcholine receptor are hypophagic and lean. Nature 410:207–212
254. **Rohner-Jeanrenaud F** 1995 A neuroendocrine reappraisal of the dual-centre hypothesis: its implications for obesity and insulin resistance. Int J Obes Relat Metab Disord 19:517–534
255. **Jeanrenaud B** 1985 An hypothesis on the aetiology of obesity: dysfunction of the central nervous system as a primary cause. Diabetologia 28:502–513
256. **Berthoud HR, Jeanrenaud B** 1979 Acute hyperinsulinemia and its reversal by vagotomy after lesions on the ventromedial hypothalamus in anesthetized rats. Endocrinology 105:146–151
257. **Bereiter DA, Rohner Jeanrenaud F, Berthoud HR, Jeanrenaud B** 1981 CNS modulation of pancreatic endocrine function: multiple modes of expression. Diabetologia 20(Suppl):417–425
258. **Tokunaga K, Fukushima M, Kemnitz JW, Bray GA** 1986 Effect of vagotomy on serum insulin in rats with paraventricular or ventromedial hypothalamic lesions. Endocrinology 119:1708–1711
259. **Penicaud L, Kinebanyan MF, Ferre P, Morin J, Kande J, Smadja C, Marfaing-Jallat P, Picon L** 1989 Development of VMH obesity: *in vivo* insulin secretion and tissue insulin sensitivity. Am J Physiol 257:E255–E260
260. **Rohner-Jeanrenaud F, Jeanrenaud B** 1985 Involvement of the cholinergic system in insulin and glucagon oversecretion of genetic preobesity. Endocrinology 116:830–834
261. **Atef N, Brule C, Bihoreau MT, Ktorza A, Picon L, Penicaud L** 1991 Enhanced insulin secretory response to acetylcholine by perifused pancreas of 5-day-old preobese Zucker rats. Endocrinology 129: 2219–2224
262. **Chen NG, Romsos DR** 1995 Enhanced sensitivity of pancreatic islets from preobese 2-week-old *ob/ob* mice to neurohormonal stimulation of insulin secretion. Endocrinology 136:505–511
263. **Zawalich WS, Kelley GG** 1995 The pathogenesis of NIDDM: the role of the pancreatic β cell. Diabetologia 38:986–991
264. **Zawalich WS, Zawalich KC, Kelley GG, Shulman GI** 1995 Islet phosphoinositide hydrolysis and insulin secretory responses from prediabetic *fa/fa* ZDF rats. Biochem Biophys Res Commun 209: 974–980
265. **Chen NG, Swick AG, Romsos DR** 1997 Leptin constrains acetylcholine-induced insulin secretion from pancreatic islets of *ob/ob* mice. J Clin Invest 100:1174–1179
266. **Rohner-Jeanrenaud F, Hochstrasser AC, Jeanrenaud B** 1983 Hyperinsulinemia of preobese and obese *fa/fa* rats is partly vagus nerve mediated. Am J Physiol 244:E317–E322
267. **Jeanrenaud B** 1994 Central nervous system and peripheral abnormalities: clues to the understanding of obesity and NIDDM. Diabetologia 37(Suppl 2):S169–S178
268. **Sainsbury A, Rohner-Jeanrenaud F, Cusin I, Zakrzewska KE, Halban PA, Gaillard RC, Jeanrenaud B** 1997 Chronic central neuropeptide Y infusion in normal rats: status of the hypothalamopituitary-adrenal axis, and vagal mediation of hyperinsulinaemia. Diabetologia 40:1269–1277
269. **N'Guyen JM, Magnan C, Laury MC, Thibault C, Leveteau J, Gilbert M, Pénicaud L, Ktorza A** 1994 Involvement of the autonomic nervous system in the *in vivo* memory to glucose of pancreatic β cell in rats. J Clin Invest 94:1456–1462
270. **Ahrén B, Simonsson E, Scheurink AJW, Mulder H, Myrsén U, Sundler F** 1997 Dissociated insulinotropic sensitivity to glucose and carbachol in high-fat diet-induced insulin resistance in C57BL/6J mice. Metabolism 46:97–106
271. **Simonsson E, Ahrén B** 1998 Potentiated β-cell response to non-glucose stimuli in insulin-resistant C57BL/6Jmice. Eur J Pharmacol 350:243–250
272. **Del Rio G, Procopio M, Bondi M, Marrama P, Menozzi R, Oleandri SE, Grottoli S, Maccario M, Velardo A, Ghigo E** 1997 Cholinergic enhancement by pyridostigmine increases the insulin response to glucose load in obese patients but not in normal subjects. Int J Obes Relat Metab Disord 21:1111–1114
273. **Sjostrom L, Garellick G, Krotkiewski M, Luyckx A** 1980 Peripheral insulin in response to the sight and smell of food. Metabolism 29:901–909
274. **Johnson WG, Wildman HE** 1983 Influence of external and covert food stimuli on insulin secretion in obese and normal persons. Behav Neurosci 97:1025–1028
275. **Teff KL, Mattes RD, Engelman K, Mattern J** 1993 Cephalic-phase insulin in obese and normal-weight men: relation to postprandial insulin. Metabolism 42:1600–1608
276. **Osuna JI, Pages I, Motino MA, Rodriguez E, Osorio C** 1986 Cephalic phase of insulin secretion in obese women. Horm Metab Res 18:473–475
277. **DeFronzo RA, Ferrannini E** 1982 The pathogenesis of non-insulin-dependent diabetes: an update. Medicine (Baltimore) 61:125–140
278. **Bruce DG, Chisholm DJ, Storlien LH, Kraegen EW** 1988 Physiological importance of deficiency in early prandial insulin secretion in non-insulin-dependent diabetes. Diabetes 37:736–744
279. **Hermans MP, Schmeer W, Henquin JC** 1987 Modulation of the effect of acetylcholine on insulin release by the membrane potential of B cells. Endocrinology 120:1765–1773
280. **Henquin JC** 2000 Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49:1751–1760
281. **Griffey MA, Conaway HH, Whitney JE** 1974 Extracellular calcium and acetylcholine-stimulated insulin secretion. Diabetes 23: 494–498
282. **Burr IM, Slonim AE, Burke V, Fletcher T** 1976 Extracellular calcium and adrenergic and cholinergic effects on islet β-cell function. Am J Physiol 231:1246–1249
283. **Hermansen K, Schwartz TW** 1979 The influence of calcium on the basal and acetylcholine-stimulated secretion of pancreatic polypeptide. Endocrinology 105:1469–1474
284. **Wollheim CB, Siegel EG, Sharp GWG** 1980 Dependency of acetylcholine-induced insulin release on Ca^{2+} uptake by rat pancreatic islets. Endocrinology 107:924–929
285. **Nenquin M, Awouters P, Mathot F, Henquin JC** 1984 Distinct

effects of acetylcholine and glucose on 45-calcium and 86-rubidium efflux from mouse pancreatic islets. FEBS Lett 176:457–461
286. **Mathias PCF, Carpinelli AR, Billaudel B, Garcia Morales P, Valverde I, Malaisse WJ** 1985 Cholinergic stimulation of ion fluxes in pancreatic islets. Biochem Pharmacol 34:3451–3457
287. **Hermans MP, Henquin JC** 1989 Relative importance of extracellular and intracellular Ca^{2+} for acetylcholine stimulation of insulin release in mouse islets. Diabetes 38:198–204
288. **Boschero AC, Szpak-Glasman M, Carneiro EM, Bordin S, Paul I, Rojas E, Atwater I** 1995 Oxotremorine-m potentiation of glucose-induced insulin release from rat islets involves M_3 muscarinic receptors. Am J Physiol 268:E336–E342
289. **Gilon P, Shepherd RM, Henquin JC** 1993 Oscillations of secretion driven by oscillations of cytoplasmic Ca^{2+} as evidenced in single pancreatic islets. J Biol Chem 268:22265–22268
290. **Gao ZY, Gilon P, Henquin JC** 1994 The role of protein kinase-C in signal transduction through vasopressin and acetylcholine receptors in pancreatic B-cells from normal mouse. Endocrinology 135: 191–199
291. **Morgan NG, Hurst RD** 1988 Dissociation between intracellular calcium mobilization and insulin secretion in isolated rat islets of Langerhans. FEBS Lett 227:153–156
292. **Rhee SG, Choi KD** 1992 Regulation of inositol phospholipid-specific phospholipase C isozymes. J Biol Chem 267:12393–12396
293. **Rhee SG, Dennis EA** 1996 Function of phospholipases in signal transduction. In: Heldin CH, Purton M, eds. Signal transduction. London: Chapman and Hall; 173–188
294. **Williams RL** 1999 Mammalian phosphoinositide-specific phospholipase C. Biochim Biophys Acta 1441:255–267
295. **Wolf RA, Gross RW** 1985 Identification of neutral active phospholipase C which hydrolyzes choline glycerophospholipids and plasmalogen selective phospholipase A_2 in canine myocardium. J Biol Chem 260:7295–7303
296. **Brown JH, Trilivas I, Trejo J, Martinson E** 1990 Multiple pathways for signal transduction through the muscarinic cholinergic receptor. Prog Brain Res 84:21–29
297. **Naor Z** 1991 Is arachidonic acid a second messenger in signal transduction? Mol Cell Endocrinol 80:C181–C186
298. **Nishizuka Y** 1992 Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607–614
299. **Schrey MP, Montague W** 1983 Phosphatidylinositol hydrolysis in isolated guinea-pig islets of Langerhans. Biochem J 216:433–441
300. **Hedeskov CJ, Thams P, Gembal M, Malik T, Capito K** 1991 Characteristics of phosphoinositide-specific phospholipase C activity from mouse pancreatic islets. Mol Cell Endocrinol 78:187–195
301. **Zawalich WS, Bonnet-Eymard M, Zawalich K** 1998 Glucose-induced desensitization of the pancreatic β-cell is species dependent. Am J Physiol 275:E917–E924
302. **Wolf BA, Easom RA, Hughes JH, McDaniel ML, Turk J** 1989 Secretagogue-induced diacylglycerol accumulation in isolated pancreatic islets: mass spectrometric characterization of the fatty acyl content indicates multiple mechanisms of generation. Biochemistry 28:4291–4301
303. **Best L, Malaisse WJ** 1983 Stimulation of phosphoinositide breakdown in rat pancreatic islets by glucose and carbamylcholine. Biochem Biophys Res Commun 116:9–16
304. **Morgan NG, Rumford GM, Montague W** 1985 Studies on the role of inositol trisphosphate in the regulation of insulin secretion from isolated rat islets of Langerhans. Biochem J 228:713–718
305. **Biden TJ, Wollheim CB** 1986 Ca^{2+} regulates the inositol tris/tetrakisphosphate pathway in intact and broken preparations of insulin-secreting RINm5F cells. J Biol Chem 261:11931–11934
306. **Dunlop ME, Malaisse WJ** 1986 Phosphoinositide phosphorylation and hydrolysis in pancreatic islet cell membrane. Arch Biochem Biophys 244:421–429
307. **Best L, Tomlinson S, Hawkins PT, Downes CP** 1987 Production of inositol trisphosphates and inositol tetrakisphosphate in stimulated pancreatic islets. Biochim Biophys Acta 927:112–116
308. **Petit P, Manteghetti M, Loubatières-Mariani MM** 1988 Differential effects of purinergic and cholinergic activation on the hydrolysis of membrane polyphosphoinositides in rat pancreatic islets. Biochem Pharmacol 37:1213–1217
309. **Wolf BA, Florholmen J, Turk J, McDaniel ML** 1988 Studies of the Ca^{2+} requirements for glucose- and carbachol-induced augmentation of inositol trisphosphate and inositol tetrakisphosphate accumulation in digitonin-permeabilized islets: evidence for a glucose recognition site in insulin secretion. J Biol Chem 263:3565–3575
310. **Blachier F, Malaisse WJ** 1987 Possible role of a GTP-binding protein in the activation of phospholipase C by carbamylcholine in tumoral insulin-producing cells. Res Commun Chem Pathol Pharmacol 58:237–255
311. **Wollheim CB, Biden TJ** 1986 Second messenger function of inositol 1,4,5-trisphosphate: early changes in inositol phosphates, cytosolic Ca^{2+}, and insulin release in carbamylcholine-stimulated RINm5F cells. J Biol Chem 261:8314–8319
312. **Yamatani T, Chiba T, Kadowaki S, Hishikawa R, Yamaguchi A, Inui T, Fujita T, Kawazu S** 1988 Dual action of protein kinase C activation in the regulation of insulin release by muscarinic agonist from rat insulinoma cell line (RINr). Endocrinology 122:2826–2832
313. **Waheed A, Koopmann I, Ammon HPT** 1995 Calmodulin antagonist W7 increases inositol phosphates in insulin secreting RINm5F cells. Exp Clin Endocrinol Diabetes 103:280–284
314. **Caulfield MP** 1993 Muscarinic receptors–characterization, coupling and function. Pharmacol Ther 58:319–379
315. **Nahorski SR, Tobin AB, Willars GB** 1997 Muscarinic M_3 receptor coupling and regulation. Life Sci 60:1039–1045
316. **Kelley GG, Zawalich KC, Zawalich WS** 1995 Synergistic interaction of glucose and neurohumoral agonists to stimulate islet phosphoinositide hydrolysis. Am J Physiol 269:E575–E582
317. **Zawalich WS, Zawalich KC, Kelley GG** 1995 Regulation of insulin release by phospholipase C activation in mouse islets: differential effects of glucose and neurohumoral stimulation. Endocrinology 136:4903–4909
318. **Metz SA** 1994 Roles of phospholipids and phospholipase activation in B-cell function. In: Bittar EE, Howell SL, eds. Advances in molecular and cellular biology. Stamford, CT: JAI Press, Inc.; vol 29: 277–301
319. **Gasa R, Trinh KY, Yu K, Wilkie TM, Newgard CB** 1999 Overexpression of $G_{11\alpha}$ and isoforms of phospholipase C in islet β-cells reveals a lack of correlation between inositol phosphate accumulation and insulin secretion. Diabetes 48:1035–1044
320. **Zawalich WS, Bonnet-Eymard M, Zawalich KC** 2000 Insulin secretion, inositol phosphate levels, and phospholipase C isozymes in rodent pancreatic islets. Metabolism 49:1156–1163
321. **Dunlop ME, Larkins RG** 1986 Muscarinic-agonist and guanine nucleotide activation of polyphosphoinositide phosphodiesterase in isolated islet-cell membranes. Biochem J 240:731–737
322. **Kardasz AM, Thams P, Capito K, Hedeskov CJ** 1997 Carbamoylcholine regulation of polyphosphoinositide synthesis and hydrolysis in cultured, dispersed, digitonin-permeabilized mouse pancreatic islet cells. Eur J Endocrinol 136:539–545
323. **Verspohl EJ, Herrmann K** 1996 Involvement of G proteins in the effect of carbachol and cholecystokinin in rat pancreatic islets. Am J Physiol 271:E65–E72
324. **Wollheim CB, Biden TJ** 1986 Signal transduction in insulin secretion: comparison between fuel stimuli and receptor agonists. Ann NY Acad Sci 488:317–333
325. **Biden TJ, Browne CL** 1993 Cross-talk between muscarinic- and adenosine-receptor signalling in the regulation of cytosolic free Ca^{2+} and insulin secretion. Biochem J 293:721–728
326. **Vallar L, Biden TJ, Wollheim CB** 1987 Guanine nucleotides induce Ca^{2+}-independent insulin secretion from permeabilized RINm5F cells. J Biol Chem 262:5049–5056
327. **Turk J, Mueller M, Bohrer A, Ramanadham S** 1992 Arachidonic acid metabolism in isolated pancreatic islets. VI. Carbohydrate insulin secretagogues must be metabolized to induce eicosanoid release. Biochim Biophys Acta 1125:280–291
328. **Baffy G, Yang L, Wolf BA, Williamson JR** 1993 G-protein specificity in signaling pathways that mobilize calcium in insulin-secreting β-TC3 cells. Diabetes 42:1878–1882
329. **Taylor SJ, Chae HZ, Rhee SG, Exton JH** 1991 Activation of the β 1 isozyme of phospholipase C by α subunits of the Gq class of G proteins. Nature 350:516–518
330. **Hepler JR, Gilman AG** 1992 G proteins. Trends Biochem Sci 17: 383–387

331. **Neer EJ** 1995 Heterotrimeric G proteins: organizers of transmembrane signals. Cell 80:249–257
332. **Biden TJ, Peter-Riesch B, Schlegel W, Wollheim CB** 1987 Ca^{2+}-mediated generation of inositol 1,4,5-triphosphate and inositol 1,3,4,5-tetrakisphosphate in pancreatic islets: studies with K^+, glucose, and carbamylcholine. J Biol Chem 262:3567–3571
333. **Gromada J, Dissing S** 1996 Membrane potential and cytosolic free calcium levels modulate acetylcholine-induced inositol phosphate production in insulin-secreting BTC3 cells. Biochim Biophys Acta 1310:145–148
334. **Laychock SG** 1983 Identification and metabolism of polyphosphoinositides in isolated islets of Langerhans. Biochem J 216: 101–106
335. **Mathias PCF, Best L, Malaisse WJ** 1985 Stimulation by glucose and carbamylcholine of phospholipase C in pancreatic islets. Cell Biochem Funct 3:173–177
336. **Best L** 1986 A role for calcium in the breakdown of inositol phospholipids in intact and digitonin-permeabilized pancreatic islets. Biochem J 238:773–779
337. **Malaisse WJ, Blachier F, Pochet R, Manuel K, Sener A** 1990 Calmodulin and calbindin in pancreatic islet cells. Adv Exp Med Biol 269:127–133
338. **Gromada J, Frøkjaer-Jensen J, Dissing S** 1996 Glucose stimulates voltage- and calcium-dependent inositol trisphosphate production and intracellular calcium mobilization in insulin-secreting βTC3 cells. Biochem J 314:339–345
339. **Biden TJ, Davison AGM, Prugue ML** 1993 Regulation by membrane potential of phosphatidylinositol hydrolysis in pancreatic islets. J Biol Chem 268:11065–11072
340. **Zawalich WS, Zawalich KC** 1997 Regulation of insulin secretion via ATP-sensitive K^+ channel independent mechanisms: role of phospholipase C. Am J Physiol 272:E671–E677
341. **Zawalich WS** 1996 Regulation of insulin secretion by phosphoinositide-specific phospholipase C and protein kinase C activation. Diabetes Rev 4:160–176
342. **Zawalich WS, Zawalich KC** 1996 Regulation of insulin secretion by phospholipase C. Am J Physiol 271:E409–E416
343. **Taylor CW, Merritt JE, Putney Jr JW, Rubin RP** 1986 Effects of Ca^{2+} on phosphoinositide breakdown in exocrine pancreas. Biochem J 238:765–772
344. **Uhing RJ, Prpic V, Jiang H, Exton JH** 1986 Hormone-stimulated polyphosphoinositide breakdown in rat liver plasma membranes: roles of guanine nucleotides and calcium. J Biol Chem 261:2140–2146
345. **Regazzi R, Li G, Ullrich S, Jaggi C, Wollheim CB** 1989 Different requirements for protein kinase C activation and Ca^{2+}-independent insulin secretion in response to guanine nucleotides. Endogenously generated diacylglycerol requires elevated Ca^{2+} for kinase C insertion into membranes. J Biol Chem 264:9939–9944
346. **Rana RS, Hokin LE** 1990 Role of phosphoinositides in transmembrane signaling. Physiol Rev 70:115–164
347. **Liu YJ, Grapengiesser E, Gylfe E, Hellman B** 1996 Crosstalk between the cAMP and inositol trisphosphate-signalling pathways in pancreatic β-cells. Arch Biochem Biophys 334:295–302
348. **Biden TJ, Prugue ML, Davison AGM** 1992 Evidence for phosphatidylinositol hydrolysis in pancreatic islets stimulated with carbamoylcholine: kinetic analysis of inositol polyphosphate metabolism. Biochem J 285:541–549
349. **Biden TJ, Comte M, Cox JA, Wollheim CB** 1987 Calcium-calmodulin stimulates inositol 1,4,5-trisphosphate kinase activity from insulin-secreting RINm5F cells. J Biol Chem 262:9437–9440
350. **Irvine RF, Schell MJ** 2001 Back in the water: the return of the inositol phosphates. Nat Rev Mol Cell Biol 2:327–338
351. **Biden TJ, Prentki M, Irvine RF, Berridge MJ, Wollheim CB** 1984 Inositol 1,4,5-trisphosphate mobilizes intracellular Ca^{2+} from permeabilized insulin-secreting cells. Biochem J 223:467–473
352. **Biden TJ, Vallar L, Wollheim CB** 1988 Regulation of inositol 1,4,5-trisphosphate metabolism in insulin-secreting RINm5F cells. Biochem J 251:435–440
353. **Shears SB** 1998 The versatility of inositol phosphates as cellular signals. Biochim Biophys Acta 1436:49–67
354. **Prentki M, Corkey BE, Matschinsky FM** 1985 Inositol 1,4,5-trisphosphate and the endoplasmic reticulum Ca^{2+} cycle of a rat insulinoma cell line. J Biol Chem 260:9185–9190
355. **Blachier F, Segura MC, Malaisse WJ** 1987 Unresponsiveness of phospholipase C to the regulatory proteins Ns and Ni in pancreatic islets. Res Commun Chem Pathol Pharmacol 55:335–355
356. **Rana RS, Sekar MC, Hokin LE, MacDonald MJ** 1986 A possible role for glucose metabolites in the regulation of inositol-1,4,5-trisphosphate 5-phosphomonoesterase activity in pancreatic islets. J Biol Chem 261:5237–5240
357. **Rana RS, Sekar MC, Mertz RJ, Hokins LE, MacDonald MJ** 1987 Potentiation by glucose metabolites of inositol trisphosphate-induced calcium mobilization in permeabilized rat pancreatic islets. J Biol Chem 262:13567–13570
358. **Blachier F, Malaisse WJ** 1990 Stimulation by D-glucose of the synthesis of polyphosphoinositides in pancreatic islets. Biochimie 72:849–854
359. **Lubell A, Chandarana H, Rana RS** 1999 Glycolytic metabolites and intracellular signaling in the pancreatic β cell. Arch Biochem Biophys 364:178–184
360. **Best L, Malaisse WJ** 1984 Enhanced *de novo* synthesis of phosphatidic acid and phosphatidylinositol in rat pancreatic islets exposed to nutrient or neurotransmitter stimuli. Arch Biochem Biophys 234:253–257
361. **Vara E, Tamarit-Rodriguez J** 1986 Glucose stimulation of insulin secretion in islets of fed and starved rats and its dependence on lipid metabolism. Metabolism 35:266–271
362. **Farese RV, DiMarco PE, Barnes DE, Sabir MA, Larson RE, Davis JS, Morrison AD** 1986 Rapid glucose-dependent increases in phosphatidic acid and phosphoinositides in rat pancreatic islets. Endocrinology 118:1498–1503
363. **Rana RS, Kowluru A, MacDonald MJ** 1986 Secretagogue-responsive and -unresponsive pools of phosphatidylinositol in pancreatic islets. Arch Biochem Biophys 245:411–416
364. **Wolf BA, Easom RA, McDaniel ML, Turk J** 1990 Diacylglycerol synthesis *de novo* from glucose by pancreatic islets isolated from rats and humans. J Clin Invest 85:482–490
365. **Wollheim CB, Regazzi R** 1990 Protein kinase C in insulin releasing cells. Putative role in stimulus secretion coupling. FEBS Lett 268: 376–380
366. **Zawalich WS, Rasmussen H** 1990 Control of insulin secretion: a model involving Ca^{2+}, cAMP and diacylglycerol. Mol Cell Endocrinol 70:119–137
367. **Berridge MJ, Irvine RF** 1984 Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 312:315
368. **Majerus PW, Connolly TM, Deckmyn H, Ross TS, Bross TE, Ishii H, Bansal VS, Wilson DB** 1986 The metabolism of phosphoinositide derived messenger molecules. Science 234:1519–1525
369. **Berridge MJ, Irvine RF** 1989 Inositol phosphates and cell signalling. Nature 341:197–205
370. **Rana RS, Kowluru A, MacDonald MJ** 1986 Enzymes of phospholipid metabolism in rat pancreatic islets: subcellular distribution and the effect of glucose and calcium. J Cell Biochem 32:143–150
371. **Rana RS, MacDonald MJ** 1986 Phosphatidylinositol kinase in rat pancreatic islets: subcellular distribution and sensitivity to calcium. Horm Metab Res 18:659–662
372. **Best L, Malaisse WJ** 1983 Phosphatidylinositol and phosphatidic acid metabolism in rat pancreatic islets in response to neurotransmitter and hormonal stimuli. Biochim Biophys Acta 750:157–163
373. **Best L, Malaisse WJ** 1984 Nutrient and hormone-neurotransmitter stimuli induce hydrolysis of polyphosphoinositides in rat pancreatic islets. Endocrinology 115:1814–1820
374. **Van Rooijen LA, Rossowska M, Bazan NG** 1985 Inhibition of phosphatidylinositol-4-phosphate kinase by its product phosphatidylinositol-4,5-bisphosphate. Biochem Biophys Res Commun 126: 150–155
375. **Peter-Riesch B, Fathi M, Schlegel W, Wollheim CB** 1988 Glucose and carbachol generate 1,2-diacylglycerols by different mechanisms in pancreatic islets. J Clin Invest 81:1154–1161
376. **Jones PM, Persaud SJ** 1998 Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic β-cells. Endocr Rev 19:429–461
377. **Oancea E, Meyer T** 1998 Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. Cell 95:307–318

378. **Konrad RJ, Major CD, Wolf BA** 1994 Diacylglycerol hydrolysis to arachidonic acid is necessary for insulin secretion from isolated pancreatic islets: sequential actions of diacylglycerol and monoacylglycerol lipases. Biochemistry 33:13284–13294
379. **Hodgkin MN, Pettitt TR, Martin A, Michell RH, Pemberton AJ, Wakelam MJ** 1998 Diacylglycerols and phosphatidates: which molecular species are intracellular messengers? Trends Biochem Sci 23:200–204
380. **Turk J, Gross RW, Ramanadham S** 1993 Amplification of insulin secretion by lipid messengers. Diabetes 42:367–374
381. **Nishizuka Y** 1995 Protein kinases: protein kinase C and lipid signaling for sustained cellular responses. FASEB J 9:484–496
382. **Regazzi R, Li G, Deshusses J, Wollheim CB** 1990 Stimulus-response coupling in insulin-secreting HIT cells: effects of secretagogues on cytosolic Ca^{2+}, diacylglycerol, and protein kinase C activity. J Biol Chem 265:15003–15009
383. **Dekker LV, Parker PJ** 1994 Protein kinase C–a question of specificity. Trends Biochem Sci 19:73–77
384. **Mochly-Rosen D, Gordon AS** 1998 Anchoring proteins for protein kinase C: a means for isozyme selectivity. FASEB J 12:35–42
385. **Mellor H, Parker PJ** 1998 The extended protein kinase C superfamily. Biochem J 332:281–292
386. **Webb BL, Hirst SJ, Giembycz MA** 2000 Protein kinase C isoenzymes: a review of their structure, regulation and role in regulating airways smooth muscle tone and mitogenesis. Br J Pharmacol 130: 1433–1452
387. **Ito A, Saito N, Taniguchi H, Chiba T, Kikkawa U, Saitoh Y, Tanaka C** 1989 Localization of β II subspecies of protein kinase C in β-cells. Diabetes 38:1005–1011
388. **Onoda K, Hagiwara M, Hachiya T, Usuda N, Nagata T, Hidaka H** 1990 Different expression of protein kinase C isozymes in pancreatic islet cells. Endocrinology 126:1235–1240
389. **Ganesan S, Calle R, Zawalich KC, Smallwood JI, Zawalich WS, Rasmussen H** 1990 Glucose-induced translocation of protein kinase C in rat pancreatic islets. Proc Natl Acad Sci USA 87:9893–9897
390. **Fletcher DJ, Ways DK** 1991 Age-dependent expression of protein kinase C isoforms in rat islets. Diabetes 40:1496–1503
391. **Calle R, Ganesan S, Smallwood JI, Rasmussen H** 1992 Glucose-induced phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS) in isolated rat pancreatic islets. J Biol Chem 267:18723–18727
392. **Selbie LA, Schmitz-Peiffer C, Sheng Y, Biden TJ** 1993 Molecular cloning and characterization of PKCι, an atypical isoform of protein kinase C derived from insulin-secreting cells. J Biol Chem 268: 24296–24302
393. **Arkhammar P, Juntti-Berggren L, Larsson O, Welsh M, Nånberg E, Sjöholm Å, Köhler M, Berggren PO** 1994 Protein kinase C modulates the insulin secretory process by maintaining a proper function of the β-cell voltage-activated Ca^{2+} channels. J Biol Chem 269:2743–2749
394. **Knutson KL, Hoenig M** 1994 Identification and subcellular characterization of protein kinase-C isoforms in insulinoma β-cells and whole islets. Endocrinology 135:881–886
395. **Zaitsev SV, Efendic S, Arkhammar P, Bertorello AM, Berggren PO** 1995 Dissociation between changes in cytoplasmic free Ca^{2+} concentration and insulin secretion as evidenced from measurements in mouse single pancreatic islets. Proc Natl Acad Sci USA 92:9712–9716
396. **Yedovitzky M, Mochly-Rosen D, Johnson JA, Gray MO, Ron D, Abramovitch E, Cerasi E, Nesher R** 1997 Translocation inhibitors define specificity of protein kinase C isoenzymes in pancreatic β-cells. J Biol Chem 272:1417–1420
397. **Li G, Regazzi R, Ullrich S, Pralong WF, Wollheim CB** 1990 Potentiation of stimulus-induced insulin secretion in protein kinase C-deficient RINm5F cells. Biochem J 272:637–645
398. **Knutson KL, Hoenig M** 1996 Regulation of distinct pools of protein kinase C δ in β cells. J Cell Biochem 60:130–138
399. **Tian YM, Urquidi V, Ashcroft SJH** 1996 Protein kinase C in β-cells: expression of multiple isoforms and involvement in cholinergic stimulation of insulin secretion. Mol Cell Endocrinol 119:185–193
400. **Tang SH, Sharp GWG** 1998 Atypical protein kinase C isozyme ζ mediates carbachol-stimulated insulin secretion in RINm5F cells. Diabetes 47:905–912
401. **Persaud SJ, Jones PM, Sugden D, Howell SL** 1989 Translocation of protein kinase C in rat islets of Langerhans: effects of a phorbol ester, carbachol and glucose. FEBS Lett 245:80–84
402. **Mochly-Rosen D** 1995 Localization of protein kinases by anchoring proteins: a theme in signal transduction. Science 268:247–251
403. **Hug H, Sarre TF** 1993 Protein kinase C isoenzymes: divergence in signal transduction. Biochem J 291:329–343
404. **Blackshear PJ** 1993 The MARCKS family of cellular protein kinase C substrates. J Biol Chem 268:1501–1504
405. **Arbuzova A, Murray D, McLaughlin S** 1998 MARCKS, membranes, and calmodulin: kinetics of their interaction. Biochim Biophys Acta 1376:369–379
406. **Chakravarthy B, Morley P, Whitfield J** 1999 Ca^{2+}-calmodulin and protein kinase Cs: a hypothetical synthesis of their conflicting convergences on shared substrate domains. Trends Neurosci 22:12–16
407. **Arkhammar P, Nilsson T, Welsh M, Welsh N, Berggren PO** 1989 Effects of protein kinase C activation on the regulation of the stimulus-secretion coupling in pancreatic β-cells. Biochem J 264: 207–215
408. **Easom RA, Landt M, Colca JR, Hughes JH, Turk J, McDaniel M** 1990 Effects of insulin secretagogues on protein kinase C-catalyzed phosphorylation of an endogenous substrate in isolated pancreatic islets. J Biol Chem 265:14938–14946
409. **Persaud SJ, Jones PM, Howell SL** 1993 Activation of protein kinase C partially alleviates noradrenaline inhibition of insulin secretion. Biochem J 289:497–501
410. **El-Mansoury AM, Morgan NG** 1998 Activation of protein kinase C modulates α_2-adrenergic signalling in rat pancreatic islets. Cell Signal 10:637–643
411. **Vicentini LM, Di Virgilio F, Ambrosini A, Pozzan T, Meldolesi J** 1985 Tumor promoter phorbol 12-myristate 13 acetate inhibits phosphoinositide hydrolysis and cytosolic Ca^{2+} rise induced by the activation of muscarinic receptors in PC12 cells. Biochem Biophys Res Commun 127:310–317
412. **Rhee SG, Suh PG, Ryu SH, Lee SY** 1989 Studies of inositol phospholipid-specific phospholipase C. Science 244:546–550
413. **Hedeskov CJ, Thams P, Gembal M, Malik T, Capito K** 1991 Ca^{2+}- and ATP-dependent reversible inactivation of pancreatic islet phosphoinositide-specific phospholipase C activity. Mol Cell Endocrinol 82:81–88
414. **Orellana S, Solski PA, Brown JH** 1987 Guanosine 5′-*O*-(thiotriphosphate)-dependent inositol trisphosphate formation in membranes is inhibited by phorbol ester and protein kinase C. J Biol Chem 262:1638–1643
415. **Smith CD, Uhing RJ, Snyderman R** 1987 Nucleotide regulatory protein-mediated activation of phospholipase C in human polymorphonuclear leukocytes is disrupted by phorbol esters. J Biol Chem 262:6121–6127
416. **Hosey MM** 1992 Diversity of structure, signaling and regulation within the family of muscarinic cholinergic receptors. FASEB J 6:845–852
417. **Tobin AB, Keys B, Nahorski SR** 1996 Identification of a novel receptor kinase that phosphorylates a phospholipase C-linked muscarinic receptor. J Biol Chem 271:3907–3916
418. **Liles WC, Hunter DD, Meier KE, Nathanson NM** 1986 Activation of protein kinase C induces rapid internalization and subsequent degradation of muscarinic acetylcholine receptors in neuroblastoma cells. J Biol Chem 261:5307–5313
419. **Lai WS, Rogers TB, el Fakahany EE** 1990 Protein kinase C is involved in desensitization of muscarinic receptors induced by phorbol esters but not by receptor agonists. Biochem J 267:23–29
420. **Axelrod J** 1990 Receptor-mediated activation of phospholipase A_2 and arachidonic acid release in signal transduction. Biochem Soc Trans 18:503–507
421. **Dennis EA** 1997 The growing phospholipase A_2 superfamily of signal transduction enzymes. Trends Biochem Sci 22:1–2
422. **Gross RW, Ramanadham S, Kruszka KK, Han X, Turk J** 1993 Rat and human pancreatic islet cells contain a calcium ion independent phospholipase A_2 activity selective for hydrolysis of arachidonate which is stimulated by adenosine triphosphate and is specifically localized to islet β-cells. Biochemistry 32:327–336
423. **Dennis EA** 1994 Diversity of group types, regulation, and function of phospholipase A_2. J Biol Chem 269:13057–13060

424. **Metz S, Holmes D, Robertson RP, Leitner W, Draznin B** 1991 Gene expression of type I phospholipase A_2 in pancreatic β cells: regulation of mRNA levels by starvation or glucose excess. FEBS Lett 295:110–112
425. **Chen M, Yang ZD, Naji A, Wolf BA** 1996 Identification of calcium-dependent phospholipase A_2 isoforms in human and rat pancreatic islets and insulin secreting β-cell lines. Endocrinology 137:2901–2909
426. **Loweth AC, Scarpello JH, Morgan NG** 1995 Phospholipase A_2 expression in human and rodent insulin-secreting cells. Mol Cell Endocrinol 112:177–183
427. **Ramanadham S, Ma ZM, Arita H, Zhang S, Turk J** 1998 Type IB secretory phospholipase A_2 is contained in insulin secretory granules of pancreatic islet β-cells and is co-secreted with insulin from glucose-stimulated islets. Biochim Biophys Acta 1390:301–312
428. **Best L, Sener A, Malaisse WJ** 1984 Does glucose affect phospholipase A_2 activity in pancreatic islets? Biochem Int 8:803–809
429. **Parker KJ, Jones PM, Hunton CH, Persaud SJ, Taylor CG, Howell SL** 1996 Identification and localisation of a type IV cytosolic phospholipase A_2 in rat pancreatic β-cells. J Mol Endocrinol 17:31–43
430. **Ramanadham S, Wolf MJ, Li BB, Bohrer A, Turk J** 1997 Glucose-responsitivity and expression of an ATP-stimulatable, Ca^{2+}-independent phospholipase A_2 enzyme in clonal insulinoma cell lines. Biochim Biophys Acta 1344:153–164
431. **Ma ZM, Ramanadham S, Kempe K, Chi XS, Ladenson J, Turk J** 1997 Pancreatic islets express a Ca^{2+}-independent phospholipase A_2 enzyme that contains a repeated structural motif homologous to the integral membrane protein binding domain of ankyrin. J Biol Chem 272:11118–11127
432. **Ma ZM, Ramanadham S, Hu ZQ, Turk J** 1998 Cloning and expression of a group IV cytosolic Ca^{2+}-dependent phospholipase A_2 from rat pancreatic islets: comparison of the expressed activity with that of an islet group VI cytosolic Ca^{2+}-independent phospholipase A_2. Biochim Biophys Acta 1391:384–400
433. **Mathias PCF, Best L, Malaisse WJ** 1985 Stimulation by glucose and carbamylcholine of phospholipase A_2 in pancreatic islets. Diabetes Res 2:267–270
434. **Konrad RJ, Jolly YC, Major C, Wolf BA** 1992 Carbachol stimulation of phospholipase A_2 and insulin secretion in pancreatic islets. Biochem J 287:283–290
435. **Sato Y, Henquin JC** 1998 The K^+-ATP channel-independent pathway of regulation of insulin secretion by glucose: in search of the underlying mechanism. Diabetes 47:1713–1721
436. **Simonsson E, Karlsson S, Ahrén B** 1998 Ca^{2+}-independent phospholipase A_2 contributes to the insulinotropic action of cholecystokinin-8 in rat islets: dissociation from the mechanism of carbachol. Diabetes 47:1436–1443
437. **Konrad RJ, Jolly YC, Wolf BA** 1991 Glucose and carbachol synergistically stimulate phosphatidic acid accumulation in pancreatic islets. Biochem Biophys Res Commun 180:960–966
438. **Ramanadham S, Gross RW, Han X, Turk J** 1993 Inhibition of arachidonate release by secretagogue-stimulated pancreatic islets suppresses both insulin secretion and the rise in β-cell cytosolic calcium ion concentration. Biochemistry 32:337–346
439. **Jolly YC, Major C, Wolf BA** 1993 Transient activation of calcium-dependent phospholipase A_2 by insulin secretagogues in isolated pancreatic islets. Biochemistry 32:12209–12217
440. **Wolf BA, Pasquale SM, Turk J** 1991 Free fatty acid accumulation in secretagogue-stimulated pancreatic islets and effects of arachidonate on depolarization-induced insulin secretion. Biochemistry 30:6372–6379
441. **Nowatzke W, Ramanadham S, Ma ZM, Hsu FF, Bohrer A, Turk J** 1998 Mass spectrometric evidence that agents that cause loss of Ca^{2+} from intracellular compartments induce hydrolysis of arachidonic acid from pancreatic islet membrane phospholipids by a mechanism that does not require a rise in cytosolic Ca^{2+} concentration. Endocrinology 139:4073–4085
442. **Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A, Davis RJ** 1993 $cPLA_2$ is phosphorylated and activated by MAP kinase. Cell 72:269–278
443. **Wolf BA, Turk J, Sherman WR, McDaniel ML** 1986 Intracellular Ca^{2+} mobilization by arachidonic acid: comparison with myo-inositol 1,4,5-trisphosphate in isolated pancreatic islets. J Biol Chem 261:3501–3511
444. **Wolf BA, Turk J, Comens PG, Sherman WR, McDaniel ML** 1987 Arachidonic acid mobilizes intracellular Ca^{2+} in islets. Ann NY Acad Sci 494:168–170
445. **Metz SA, Draznin B, Sussman KE, Leitner JW** 1987 Unmasking of arachidonate-induced insulin release by removal of extracellular calcium: arachidonic acid mobilizes cellular calcium in rat islets of Langerhans. Biochem Biophys Res Commun 142:251–258
446. **Metz SA** 1988 Exogenous arachidonic acid promotes insulin release from intact or permeabilized rat islets by dual mechanisms: putative activation of Ca^{2+} mobilization and protein kinase C. Diabetes 37:1453–1469
447. **Wolf BA, Colca JR, Turk J, Florholmen J, McDaniel ML** 1988 Regulation of Ca^{2+} homeostasis by islet endoplasmic reticulum and its role in insulin secretion. Am J Physiol 254:E121–E136
448. **Morgan NG, Rumford GM, Montague W** 1987 Mechanisms involved in intracellular calcium mobilization in isolated rat islets of Langerhans. Biochem J 244:669–674
449. **Ramanadham S, Gross R, Turk J** 1992 Arachidonic acid induces an increase in the cytosolic calcium concentration in single pancreatic islet β cells. Biochem Biophys Res Commun 184:647–653
450. **Müller M, Szewczyk A, de Weille JR, Lazdunski M** 1992 ATP-sensitive K^+ channels in insulinoma cells are activated by nonesterified fatty acids. Biochemistry 31:4656–4661
451. **Landt M, Easom RA, Colca JR, Wolf BA, Turk J, Mills LA, McDaniel ML** 1992 Parallel effects of arachidonic acid on insulin secretion, calmodulin-dependent protein kinase activity and protein kinase C activity in pancreatic islets. Cell Calcium 13:163–172
452. **Hansson A, Serhan CN, Haeggstrom J, Ingelman-Sundberg M, Samuelsson B** 1986 Activation of protein kinase C by lipoxin A and other eicosanoids: intracellular action of oxygenation products of arachidonic acid. Biochem Biophys Res Commun 134:1215–1222
453. **Shinomura T, Asaoka Y, Oka M, Yoshida K, Nishizuka Y** 1991 Synergistic action of diacylglycerol and unsaturated fatty acid for protein kinase C activation: its possible implications. Proc Natl Acad Sci USA 88:5149–5153
454. **Robertson RP** 1986 Arachidonic acid metabolite regulation of insulin secretion. Diabetes Metab Rev 2:261–296
455. **Metz SA** 1991 The pancreatic islet as Rubik's cube: is phospholipid hydrolysis a piece of the puzzle? Diabetes 40:1565–1573
456. **Metz SA** 1986 Ether-linked lysophospholipids initiate insulin secretion: lysophospholipids may mediate effects of phospholipase A_2 activation on hormone release. Diabetes 35:808–817
457. **Metz SA** 1986 Putative roles for lysophospholipids as mediators and lipoxygenase-mediated metabolites of arachidonic acid as potentiators of stimulus-secretion coupling: dual mechanisms of *p*-hydroxymercuribenzoic acid-induced insulin release. J Pharmacol Exp Ther 238:819–832
458. **Fujimoto WY, Metz SA** 1987 Phasic effects of glucose, phospholipase A_2, and lysophospholipids on insulin secretion. Endocrinology 120:1750–1757
459. **Fujimoto WY, Teague J** 1989 Phasic effects of glucose, p-hydroxymercuribenzoate, and lysophosphatidylcholine on insulin secretion from HIT cells. Diabetes 38:625–628
460. **Kiss Z** 1996 Regulation of phospholipase D by protein kinase C. Chem Phys Lipids 80:81–102
461. **Gomez-Cambronero J, Keire P** 1998 Phospholipase D: a novel major player in signal transduction. Cell Signal 10:387–397
462. **Hallberg A, Andersson A** 1984 Effects of starvation on phospholipid metabolism of pancreatic islets. Diabetes Res 1:105–110
463. **Turk J, Wolf BA, Lefkowith JB, Stump WT, McDaniel ML** 1986 Glucose-induced phospholipid hydrolysis in isolated pancreatic islets: quantitative effects on the phospholipid content of arachidonate and other fatty acids. Biochim Biophys Acta 879:399–409
464. **Ramanadham S, Bohrer A, Mueller M, Jett P, Gross RW, Turk J** 1993 Mass spectrometric identification and quantitation of arachidonate-containing phospholipids in pancreatic islets: prominence of plasmenylethanolamine molecular species. Biochemistry 32:5339–5351
465. **Cockcroft S** 1997 Phospholipase D: regulation by GTPases and protein kinase C and physiological relevance. Prog Lipid Res 35:345–370

466. **Exton JH** 1999 Regulation of phospholipase D. Biochim Biophys Acta 1439:121–133
467. **Capito K, Hansen SE, Thams P** 1996 Production of [^{3}H]choline-labelled metabolites from endogenously ^{3}H-labelled phosphatidylcholine in mouse pancreatic islets. J Mol Endocrinol 17:101–107
468. **Dunlop M, Metz SA** 1989 A phospholipase D-like mechanism in pancreatic islet cells: stimulation by calcium ionophore, phorbol ester and sodium fluoride. Biochem Biophys Res Commun 163: 922–928
469. **Stasek Jr JE, Natarajan V, Garcia JG** 1993 Phosphatidic acid directly activates endothelial cell protein kinase C. Biochem Biophys Res Commun 191:134–141
470. **Dunlop ME, Larkins RG** 1989 Effects of phosphatidic acid on islet cell phosphoinositide hydrolysis, Ca^{2+}, and adenylate cyclase. Diabetes 38:1187–1192
471. **Metz SA, Dunlop M** 1990 Stimulation of insulin release by phospholipase D: a potential role for endogenous phosphatidic acid in pancreatic islet function. Biochem J 270:427–435
472. **Exton JH** 1997 Phospholipase D: enzymology, mechanisms of regulation, and function. Physiol Rev 77:303–320
473. **Thorens B, Sarkar HK, Kaback HR, Lodish HF** 1988 Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and β-pancreatic islet cells. Cell 55:281–290
474. **Schuit FC** 1997 Is GLUT2 required for glucose sensing? Diabetologia 40:104–111
475. **Sweet IR, Matschinsky FM** 1997 Are there kinetic advantages of GLUT2 in pancreatic glucose sensing? Diabetologia 40:112–119
476. **Detimary P, Jonas JC, Henquin JC** 1995 Possible links between glucose-induced changes in the energy state of pancreatic B cells and insulin release: unmasking by decreasing a stable pool of adenine nucleotides in mouse islets. J Clin Invest 96:1738–1745
477. **Henquin JC, Meissner HP** 1984 Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic B-cells. Experientia 40:1043–1052
478. **Ashcroft FM, Rorsman P** 1989 Electrophysiology of the pancreatic β-cell. Prog Biophys Mol Biol 54:87–143
479. **Henquin JC** 1988 ATP-sensitive K^+ channels may control glucose-induced electrical activity in pancreatic B-cells. Biochem Biophys Res Commun 156:769–775
480. **Henquin JC, Garcia MC, Bozem M, Hermans MP, Nenquin M** 1988 Muscarinic control of pancreatic B cell function involves sodium-dependent depolarization and calcium influx. Endocrinology 122:2134–2142
481. **Shen XM, Tao F, Su QF, Zhang JR** 1994 Analysis of the acetylcholine action on the electrical activities of pancreatic islet B-cells in mice. Acta Physiol Sin 46:105–111
482. **Bordin S, Boschero AC, Carneiro EM, Atwater I** 1995 Ionic mechanisms involved in the regulation of insulin secretion by muscarinic agonists. J Membr Biol 148:177–184
483. **Bordin S, Carneiro EM, Boschero AC** 1997 Modulation of Ca^{2+} and K^+ permeabilities by oxotremorine-m (Oxo-m) in rodent pancreatic B-cells. Exp Physiol 82:967–976
484. **Cook DL, Crill WE, Porte Jr D** 1981 Glucose and acetylcholine have different effects on the plateau pacemaker of pancreatic islet cells. Diabetes 30:558–561
485. **Palafox I, Sanchez-Andres JV, Sala S, Ferrer R, Soria B** 1986 Muscarinic receptors and the control of glucose-induced electrical activity in the pancreatic β-cell. In: Atwater I, Rojas E, Soria B, eds. Biophysics of the pancreatic B-cell. New York: Plenum Publishing Corp.; 351–358
486. **Santos RM, Rojas E** 1989 Muscarinic receptor modulation of glucose-induced electrical activity in mouse pancreatic B-cells. FEBS Lett 249:411–417
487. **Bertram R, Smolen P, Sherman A, Mears D, Atwater I, Martin F, Soria B** 1995 A role for calcium release-activated current (CRAC) in cholinergic modulation of electrical activity in pancreatic β-cells. Biophys J 68:2323–2332
488. **Sanchez-Andres JV, Soria B** 1991 Muscarinic inhibition of pancreatic B-cells. Eur J Pharmacol 205:89–91
489. **Baukrowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ, Ruppersberg JP, Fakler B** 1998 PIP_2 and PIP as determinants for ATP inhibition of K_{ATP} channels. Science 282:1141–1144
490. **Shyng SL, Nichols CG** 1998 Membrane phospholipid control of nucleotide sensitivity of KATP channels. Science 282:1138–1141
491. **Murayama K, Ohara A, Marunaka Y, Kitasato H** 1987 Acetylcholine suppresses activity of large-conductance Cl^--selective channels in mouse pancreatic B-cells. Med Sci Res 15:993–994
492. **Hermans MP, Schmeer W, Gerard M, Henquin JC** 1991 Effects of chloride deficiency on the pancreatic B-cell response to acetylcholine. Biochim Biophys Acta 1092:205–210
493. **Gagerman E, Sehlin J, Täljedal IB** 1980 Effects of acetylcholine on ion fluxes and chlorotetracycline fluorescence in pancreatic islets. J Physiol 300:505–514
494. **Rolland JF, Henquin JC, Gilon P** Activation of an inward Na^+ current by acetylcholine in mouse pancreatic β-cells. Diabetologia 44(Suppl 1):A132 (Abstract)
495. **Saha S, Hellman B** 1991 Carbachol has opposite effects to glucose in raising the sodium content of pancreatic islets. Eur J Pharmacol 204:211–215
496. **Gilon P, Henquin JC** 1993 Activation of muscarinic receptors increases the concentration of free Na^+ in mouse pancreatic B-cells. FEBS Lett 315:353–356
497. **Plant TD** 1988 Na^+ currents in cultured mouse pancreatic B-cells. Pflugers Arch 411:429–435
498. **Hiriart M, Matteson DR** 1988 Na channels and two types of Ca channels in rat pancreatic B cells identified with the reverse hemolytic plaque assay. J Gen Physiol 91:617–639
499. **Hucho F** 1986 The nicotinic acetylcholine receptor and its ion channel. Eur J Biochem 158:211–226
500. **Iacono G, Vassalle M** 1989 Acetylcholine increases intracellular sodium activity in sheep cardiac Purkinje fibers. Am J Physiol 256:H1407–H1416
501. **Parekh AB, Terlau H, Stuhmer W** 1993 Depletion of $InsP_3$ stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger. Nature 364:814–818
502. **Fasolato C, Innocenti B, Pozzan T** 1994 Receptor-activated Ca^{2+} influx: how many mechanisms for how many channels? Trends Pharmacol Sci 15:77–83
503. **Clapham DE** 1996 TRP is cracked but is CRAC TRP? Neuron 16:1069–1072
504. **Friel DD** 1996 TRP: its role in phototransduction and store-operated Ca^{2+} entry. Cell 85:617–619
505. **Hoth M** 1996 Depletion of intracellular calcium stores activates an outward potassium current in mast and RBL-1 cells that is correlated with CRAC channel activation. FEBS Lett 390:285–288
506. **Sakura H, Ashcroft FM** 1997 Identification of four *trp*1 gene variants murine pancreatic β-cells. Diabetologia 40:528–532
507. **Zitt C, Zobel A, Obukhov AG, Harteneck C, Kalkbrenner F, Luckhoff A, Schultz G** 1996 Cloning and functional expression of a human Ca^{2+}-permeable cation channel activated by calcium store depletion. Neuron 16:1189–1196
508. **Tepel M, Kühnapfel S, Theilmeier G, Teupe C, Schlotmann R, Zidek W** 1994 Filling state of intracellular Ca^{2+} pools triggers trans plasma membrane Na^+ and Ca^{2+} influx by a tyrosine kinase-dependent pathway. J Biol Chem 269:26239–26242
509. **Tepel M, Wischniowski H, Zidek W** 1994 Thapsigargin-induced $[Ca^{2+}]_i$ increase activates sodium influx in human platelets. Biochim Biophys Acta 1220:248–252
510. **Miura Y, Gilon P, Henquin JC** 1996 Muscarinic stimulation increases Na^+ entry in pancreatic B-cells by a mechanism other than the emptying of intracellular Ca^{2+} pools. Biochem Biophys Res Commun 224:67–73
511. **Matsumoto K, Pappano AJ** 1989 Sodium-dependent membrane current induced by carbachol in single guinea-pig ventricular myocytes. J Physiol 415:487–502
512. **Matsumoto K, Pappano AJ** 1991 Carbachol activates a novel sodium current in isolated guinea pig ventricular myocytes via M_2 muscarinic receptors. Mol Pharmacol 39:359–363
513. **Shirayama T, Matsumoto K, Pappano AJ** 1993 Carbachol-induced sodium current in guinea pig ventricular myocytes is not regulated by guanine nucleotides. J Pharmacol Exp Ther 265:641–648
514. **Inoue R, Kitamura K, Kuriyama H** 1987 Acetylcholine activates single sodium channels in smooth muscle cells. Pflugers Arch 410: 69–74
515. **Inoue R, Isenberg G** 1990 Acetylcholine activates nonselective

cation channels in guinea pig ileum through a G protein. Am J Physiol 258:C1173–C1178
516. **Zholos AV, Bolton TB** 1997 Muscarinic receptor subtypes controlling the cationic current in guinea-pig ileal smooth muscle. Br J Pharmacol 122:885–893
517. **Rhee JC, Rhee PL, Park MK, So I, Uhm DY, Kim KW, Kang TM** 2000 Muscarinic receptors controlling the carbachol-activated nonselective cationic current in guinea pig gastric smooth muscle cells. Jpn J Pharmacol 82:331–337
518. **Benham CD, Bolton TB, Lang RJ** 1985 Acetylcholine activates an inward current in single mammalian smooth muscle cells. Nature 316:345–347
519. **Vogalis F, Sanders KM** 1990 Cholinergic stimulation activates a non-selective cation current in canine pyloric circular muscle cells. J Physiol 429:223–236
520. **Inoue M, Sakamoto Y, Imanaga I** 1995 Phosphatidylinositol hydrolysis is involved in production of Ca^{2+}-dependent currents, but not non-selective cation currents, by muscarine in chromaffin cells. Eur J Pharmacol 276:123–129
521. **Carroll RC, Peralta EG** 1998 The m3 muscarinic acetylcholine receptor differentially regulates calcium influx and release through modulation of monovalent cation channels. EMBO J 17:3036–3044
522. **Miura Y, Henquin JC, Gilon P** 1997 Emptying of intracellular Ca^{2+} stores stimulates Ca^{2+} entry in mouse pancreatic β-cells by both direct and indirect mechanisms. J Physiol 503:387–398
523. **Gilon P, Arredouani A, Gailly P, Gromada J, Henquin JC** 1999 Uptake and release of Ca^{2+} by the endoplasmic reticulum contribute to the oscillations of the cytosolic Ca^{2+} concentration triggered by Ca^{2+} influx in the electrically excitable pancreatic B-cell. J Biol Chem 274:20197–20205
524. **Sanchez-Andres JV, Ripoll C, Soria B** 1988 Evidence that muscarinic potentiation of insulin release is initiated by an early transient calcium entry. FEBS Lett 231:143–147
525. **Sanchez-Andrés JV, Nadal A, Martin F, Soria B** 1994 Sequential effects of muscarinic agonists on glucose-induced electrical activity and cytosolic $(Ca^{2+})_i$ in the pancreatic B-cell. In: Flatt PR, Lenzen S, eds. Insulin secretion and pancreatic B-cell research. London: Smith-Gordon; 353–358
526. **Petersen OH, Findlay I** 1987 Electrophysiology of the pancreas. Physiol Rev 67:1054–1116
527. **Ämmälä C, Larsson O, Berggren PO, Bokvist K, Juntti-Berggren L, Kindmark H, Rorsman P** 1991 Inositol trisphosphate-dependent periodic activation of a Ca^{2+}-activated K^+ conductance in glucose-stimulated pancreatic β-cells. Nature 353:849–852
528. **Lund P-E, Hellman B** 1993 Activation of G-proteins induces Ca^{2+} oscillations with hyperpolarizing K^+ currents in pancreatic β-cells. Second Messengers Phosphoproteins 14:173–183
529. **Lund P-E, Gylfe E** 1994 Caffeine inhibits cytoplasmic Ca^{2+} oscillations induced by carbachol and guanosine 5′-*O*-(3-thiotriphosphate) in hyperpolarized pancreatic β-cells. Naunyn Schmiedebergs Arch Pharmacol 349:503–509
530. **Kozak JA, Misler S, Logothetis DE** 1998 Characterization of a Ca^{2+}-activated K^+ current in insulin-secreting murine βTC-3 cells. J Physiol 509:355–370
531. **Debuyser A, Drews G, Henquin JC** 1991 The influence of temperature on the effects of acetylcholine and adrenaline on the membrane potential and ^{86}Rb efflux in mouse pancreatic B-cells. Exp Physiol 76:553–559
532. **Meglasson MD, Najafi H, Matschinsky FM** 1986 Acetylcholine stimulates glucose metabolism by pancreatic islets. Life Sci 39: 1745–1750
533. **Trus MD, Hintz CS, Weinstein JB, Williams AD, Pagliara AS, Matschinsky FM** 1978 Effects of glucose and acetylcholine on islet tissue NADH and insulin release. Life Sci 22:809–816
534. **Trus MD, Hintz CS, Weinstein JB, Williams AD, Pagliara AS, Matschinsky FM** 1979 A comparison of the effects of glucose and acetylcholine on insulin release and intermediary metabolism in rat pancreatic islets. J Biol Chem 254:3921–3929
535. **Best L, Elliott AC** 1995 Changes in 2′,7′-bis(carboxyethyl) 5′(6′)-carboxyfluorescein-, fura-2 and autofluorescence in intact rat pancreatic islets in response to nutrients and non-nutrients. Mol Cell Endocrinol 111:191–198
536. **Hellman B, Gylfe E** 1986 Calcium and the control of insulin secretion. In: Calcium and cell function. New York: Academic Press; vol VI: 253–326
537. **Cooper DMF, Mons N, Karpen JW** 1995 Adenylyl cyclases and the interaction between calcium and cAMP signalling. Nature 374: 421–424
538. **Gilon P, Obie JF, Bian X, Bird GS, Putney Jr JW** 1995 Role of cyclic GMP in the control of capacitative Ca^{2+} entry in rat pancreatic acinar cells. Biochem J 311:649–656
539. **Schimerlik MI** 1989 Structure and regulation of muscarinic receptors. Annu Rev Physiol 51:217–227
540. **Jones PM, Persaud SJ, Bjaaland T, Pearson JD, Howell SL** 1992 Nitric oxide is not involved in the initiation of insulin secretion from rat islets of Langerhans. Diabetologia 35:1020–1027
541. **Lindstrom P, Sehlin J** 1986 Effect of intracellular alkalinization on pancreatic islet calcium uptake and insulin secretion. Biochem J 239:199–204
542. **Grapengiesser E, Gylfe E, Hellman B** 1989 Regulation of pH in individual pancreatic β-cells as evaluated by fluorescence ratio microscopy. Biochim Biophys Acta 1014:219–224
543. **Juntti-Berggren L, Arkhammar P, Nilsson T, Rorsman P, Berggren PO** 1991 Glucose-induced increase in cytoplasmic pH in pancreatic β-cells is mediated by Na^+/H^+ exchange, an effect not dependent on protein kinase C. J Biol Chem 266:23537–23541
544. **Wang J, Baimbridge KG, Brown JC** 1992 Glucose- and acetylcholine-induced increase in intracellular free Ca^{2+} in subpopulations of individual rat pancreatic β-cells. Endocrinology 131:146–152
545. **Gilon P, Nenquin M, Henquin JC** 1995 Muscarinic stimulation exerts both stimulatory and inhibitory effects on the concentration of cytoplasmic Ca^{2+} in the electrically excitable pancreatic B-cell. Biochem J 311:259–267
546. **Yada T, Hamakawa N, Yaekura K** 1995 Two distinct modes of Ca^{2+} signalling by ACh in rat pancreatic β-cells: concentration, glucose dependence and Ca^{2+} origin. J Physiol 488:13–24
547. **Hellman B, Gylfe E, Wesslen N** 1986 Inositol 1,4,5-trisphosphate mobilizes glucose-incorporated calcium from pancreatic islets. Biochem Int 13:383–389
548. **Hellman B, Gylfe E** 1986 Mobilization of different intracellular calcium pools after activation of muscarinic receptors in pancreatic β-cells. Pharmacology 32:257–267
549. **Liu YJ, Grapengiesser E, Gylfe E, Hellman B** 1995 Glucose induces oscillations of cytoplasmic Ca $^{2+}$, Sr^{2+} and Ba^{2+} in pancreatic β-cells without participation of the thapsigargin-sensitive store. Cell Calcium 18:165–173
550. **Liu YJ, Gylfe E** 1997 Store-operated Ca^{2+} entry in insulin-releasing pancreatic β-cells. Cell Calcium 22:277–286
551. **Prentki M, Janjic D, Wollheim CB** 1984 Coordinated regulation of free Ca^{2+} by isolated organelles from a rat insulinoma. J Biol Chem 259:14054–14058
552. **Prentki M, Biden TJ, Janjic D, Irvine RF, Berridge MJ, Wollheim CB** 1984 Rapid mobilization of Ca^{2+} from rat insulinoma microsomes by inositol-1,4,5-trisphosphate. Nature 309:562–564
553. **Joseph SK, Williams RJ, Corkey BE, Matschinsky FM, Williamson JR** 1984 The effect of inositol trisphosphate on Ca^{2+} fluxes in insulin-secreting tumor cells. J Biol Chem 259:12952–12955
554. **Wolf BA, Comens PG, Ackermann KE, Sherman WR, McDaniel ML** 1985 The digitonin-permeabilized pancreatic islet model. Effect of myo-inositol 1,4,5-trisphosphate on Ca^{2+} mobilization. Biochem J 227:965–969
555. **Wollheim CB, Biden TJ, Lew PD, Schlegel W** 1986 Calcium mobilization by inositol 1,4,5-trisphosphate during activation of islet, pituitary, and myeloid cells. J Cardiovasc Pharmacol 8(Suppl 8): S65–S70
556. **Nilsson T, Arkhammar P, Hallberg A, Hellman B, Berggren PO** 1987 Characterization of the inositol 1,4,5-trisphosphate-induced Ca^{2+} release in pancreatic β-cells. Biochem J 248:329–336
557. **Nilsson T, Zwiller J, Boynton AL, Berggren PO** 1988 Heparin inhibits IP_3-induced Ca^{2+} release in permeabilized pancreatic β-cells. FEBS Lett 229:211–214
558. **Islam MS, Nilsson T, Rorsman P, Berggren PO** 1991 Interaction with the inositol 1,4,5-trisphosphate receptor promotes Ca^{2+} sequestration in permeabilised insulin-secreting cells. FEBS Lett 288: 27–29
559. **Islam MS, Berggren PO** 1993 Mobilization of Ca^{2+} by thapsigargin

and 2,5-di-(t-butyl)-1,4-benzohydroquinone in permeabilized insulin-secreting RINm5F cells: evidence for separate uptake and release compartments in inositol 1,4,5-trisphosphate-sensitive Ca^{2+} pool. Biochem J 293:423–429

560. **Rutter GA, Theler JM, Li G, Wollheim CB** 1994 Ca^{2+} stores in insulin-secreting cells: lack of effect of cADP ribose. Cell Calcium 16:71–80
561. **Tengholm A, Hellman B, Gylfe E** 2000 Mobilization of Ca^{2+} stores in individual pancreatic β-cells permeabilized or not with digitonin or α-toxin. Cell Calcium 27:43–51
562. **Tengholm A, Hellman B, Gylfe E** 2001 The endoplasmic reticulum is a glucose-modulated high-affinity sink for Ca^{2+} in mouse pancreatic β-cells. J Physiol 530:533–540
563. **Prentki M, Wollheim CB** 1984 Cytosolic free Ca^{2+} in insulin secreting cells and its regulation by isolated organelles. Experientia 40:1052–1060
564. **Willmott NJ, Galione A, Smith PA** 1995 A cADP-ribose antagonist does not inhibit secretagogue-, caffeine- and nitric oxide-induced Ca^{2+} responses in rat pancreatic β-cells. Cell Calcium 18:411–419
565. **Tengholm A, Hellman B, Gylfe E** 1999 Glucose regulation of free Ca^{2+} in the endoplasmic reticulum of mouse pancreatic β cells. J Biol Chem 274:36883–36890
566. **Theler JM, Mollard P, Guérineau N, Vacher P, Pralong WF, Schlegel W, Wollheim CB** 1992 Video imaging of cytosolic Ca^{2+} in pancreatic β-cells stimulated by glucose, carbachol, and ATP. J Biol Chem 267:18110–18117
567. **Prentki M, Glennon MC, Thomas AP, Morris RL, Matschinsky FM, Corkey BE** 1988 Cell-specific patterns of oscillating free Ca^{2+} in carbamylcholine-stimulated insulinoma cells. J Biol Chem 263: 11044–11047
568. **Lund P-E, Grapengiesser E, Gylfe E, Hellman B** 1991 Intracellular ATP mimics GTP-γ-S in generating Ca^{2+} oscillations in pancreatic β-cells. Biochem Biophys Res Commun 177:777–783
569. **Penner R, Neher E** 1988 The role of calcium in stimulus-secretion coupling in excitable and non-excitable cells. J Exp Biol 139:329–345
570. **Gylfe E, Grapengiesser E, Hellman B** 1991 Propagation of cytoplasmic Ca^{2+} oscillations in clusters of pancreatic β-cells exposed to glucose. Cell Calcium 12:229–240
571. **Nadal A, Quesada I, Soria B** 1999 Homologous and heterologous asynchronicity between identified α-, β- and δ-cells within intact islets of Langerhans in the mouse. J Physiol 517:85–93
572. **Jonkers FC, Jonas JC, Gilon P, Henquin JC** 1999 Influence of cell number on the characteristics and synchrony of Ca^{2+} oscillations in clusters of mouse pancreatic islet cells. J Physiol 520:839–849
573. **Hughes SJ, Chalk JG, Ashcroft SJH** 1990 The role of cytosolic free Ca^{2+} and protein kinase C in acetylcholine-induced insulin release in the clonal β-cell line, HIT-T15. Biochem J 267:227–232
574. **Gylfe E** 1991 Carbachol induces sustained glucose-dependent oscillations of cytoplasmic Ca^{2+} in hyperpolarized pancreatic β cells. Pflugers Arch 419:639–643
575. **Hamakawa N, Yada T** 1995 Interplay of glucose-stimulated Ca^{2+} sequestration and acetylcholine-induced Ca^{2+} release at the endoplasmic reticulum in rat pancreatic β-cells. Cell Calcium 17:21–31
576. **Taylor CW** 1998 Inositol trisphosphate receptors: Ca^{2+}-modulated intracellular Ca^{2+} channels. Biochim Biophys Acta 1436:19–33
577. **Nucifora Jr FC, Sharp AH, Milgram SL, Ross CA** 1996 Inositol 1,4,5-trisphosphate receptors in endocrine cells: localization and association in hetero- and homotetramers. Mol Biol Cell 7:949–960
578. **Blondel O, Takeda J, Janssen H, Seino S, Bell GI** 1993 Sequence and functional characterization of a third inositol trisphosphate receptor subtype, IP_3R-3, expressed in pancreatic islets, kidney, gastrointestinal tract, and other tissues. J Biol Chem 268:11356–11363
579. **Lee B, Bradford PG, Laychock SG** 1998 Characterization of inositol 1,4,5-trisphosphate receptor isoform mRNA expression and regulation in rat pancreatic islets, RINm5F cells and βHC9 cells. J Mol Endocrinol 21:31–39
580. **Lee B, Jonas JC, Weir GC, Laychock SG** 1999 Glucose regulates expression of inositol 1,4,5-trisphosphate receptor isoforms in isolated rat pancreatic islets. Endocrinology 140:2173–2182
581. **De Smedt H, Missiaen L, Parys JB, Bootman MD, Mertens L, Van Den Bosch L, Casteels R** 1994 Determination of relative amounts of inositol trisphosphate receptor mRNA isoforms by ratio polymerase chain reaction. J Biol Chem 269:21691–21698
582. **Lee B, Laychock SG** 2001 Inositol 1,4,5-trisphosphate receptor isoform expression in mouse pancreatic islets: effects of carbachol. Biochem Pharmacol 61:327–336
583. **Missiaen L, Parys JB, Sienaert I, Maes K, Kunzelmann K, Takahashi M, Tanzawa K, De Smedt H** 1998 Functional properties of the type-3 $InsP_3$ receptor in 16HBE14o-bronchial mucosal cells. J Biol Chem 273:8983–8986
584. **Miyakawa T, Maeda A, Yamazawa T, Hirose K, Kurosaki T, Iino M** 1999 Encoding of Ca^{2+} signals by differential expression of IP_3 receptor subtypes. EMBO J 18:1303–1308
585. **Newton CL, Mignery GA, Sudhof TC** 1994 Co-expression in vertebrate tissues and cell lines of multiple inositol 1,4,5-trisphosphate ($InsP_3$) receptors with distinct affinities for $InsP_3$. J Biol Chem 269:28613–28619
586. **Parys JB, Missiaen L, De Smedt H, Sienaert I, Casteels R** 1996 Mechanisms responsible for quantal Ca^{2+} release from inositol trisphosphate-sensitive calcium stores. Pflugers Arch 432:359–367
587. **Wojcikiewicz RJ, Luo SG** 1998 Differences among type I, II, and III inositol-1,4,5-trisphosphate receptors in ligand-binding affinity influence the sensitivity of calcium stores to inositol-1,4,5-trisphosphate. Mol Pharmacol 53:656–662
588. **Joseph SK, Ryan SV** 1993 Phosphorylation of the inositol trisphosphate receptor in isolated rat hepatocytes. J Biol Chem 268:23059–23065
589. **Nakade S, Rhee SK, Hamanaka H, Mikoshiba K** 1994 Cyclic AMP-dependent phosphorylation of an immunoaffinity-purified homotetrameric inositol 1,4,5-trisphosphate receptor (type I) increases Ca^{2+} flux in reconstituted lipid vesicles. J Biol Chem 269: 6735–6742
590. **Missiaen L, Parys JB, Weidema AF, Sipma H, Vanlingen S, De Smet P, Callewaert G, De Smedt H** 1999 The bell-shaped Ca^{2+} dependence of the inositol 1,4, 5-trisphosphate-induced Ca^{2+} release is modulated by Ca^{2+}/calmodulin. J Biol Chem 274:13748–13751
591. **Iino M** 1990 Biphasic Ca^{2+} dependence of inositol 1,4,5-trisphosphate-induced Ca release in smooth muscle cells of the guinea pig taenia caeci. J Gen Physiol 95:1103–1122
592. **Bezprozvanny I, Watras J, Ehrlich BE** 1991 Bell-shaped calcium-response curves of $Ins(1,4,5)P_3$- and calcium-gated channels from endoplasmic reticulum of cerebellum. Nature 351:751–754
593. **Finch EA, Turner TJ, Goldin SM** 1991 Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. Science 252: 443–446
594. **Hagar RE, Burgstahler AD, Nathanson MH, Ehrlich BE** 1998 Type III $InsP_3$ receptor channel stays open in the presence of increased calcium. Nature 396:81–84
595. **Blondel O, Bell GI, Moody M, Miller RJ, Gibbons SJ** 1994 Creation of an inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store in secretory granules of insulin-producing cells. J Biol Chem 269:27167–27170
596. **Blondel O, Moody MM, Depaoli AM, Sharp AH, Ross CA, Swift H, Bell GI** 1994 Localization of inositol trisphosphate receptor subtype 3 to insulin and somatostatin secretory granules and regulation of expression in islets and insulinoma cells. Proc Natl Acad Sci USA 91:7777–7781
597. **Ravazzola M, Halban PA, Orci L** 1996 Inositol 1,4,5-trisphosphate receptor subtype 3 in pancreatic islet cell secretory granules revisited. Proc Natl Acad Sci USA 93:2745–2748
598. **Prentki M, Janjic D, Wollheim CB** 1983 The regulation of extramitochondrial steady state free Ca^{2+} concentration by rat insulinoma mitochondria. J Biol Chem 258:7597–7602
599. **Prentki M, Janjic D, Biden TJ, Blondel B, Wollheim CB** 1984 Regulation of Ca^{2+} transport by isolated organelles of a rat insulinoma: studies with endoplasmic reticulum and secretory granules. J Biol Chem 259:10118–10123
600. **Scheenen WJ, Wollheim CB, Pozzan T, Fasolato C** 1998 Ca^{2+} depletion from granules inhibits exocytosis. A study with insulin-secreting cells. J Biol Chem 273:19002–19008
601. **Rutter GA, Fasolato C, Rizzuto R** 1998 Calcium and organelles: a two-sided story. Biochem Biophys Res Commun 253:549–557
602. **Pouli AE, Karagenc N, Wasmeier C, Hutton JC, Bright N, Arden**

S, Schofield JG, Rutter GA 1998 A phogrin-aequorin chimaera to image free Ca^{2+} in the vicinity of secretory granules. Biochem J 330:1399–1404
603. **Hutton JC, Penn EJ, Peshavaria M** 1983 Low-molecular-weight constituents of isolated insulin-secretory granules: bivalent cations, adenine nucleotides and inorganic phosphate. Biochem J 210: 297–305
604. **Zhou YP, Teng DL, Dralyuk F, Ostrega D, Roe MW, Philipson L, Polonsky KS** 1998 Apoptosis in insulin-secreting cells: evidence for the role of intracellular Ca^{2+} stores and arachidonic acid metabolism. J Clin Invest 101:1623–1632
605. **Pinton P, Pozzan T, Rizzuto R** 1998 The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store, with functional properties distinct from those of the endoplasmic reticulum. EMBO J 17:5298–5308
606. **Maechler P, Kennedy ED, Sebo E, Valeva A, Pozzan T, Wollheim CB** 1999 Secretagogues modulate the calcium concentration in the endoplasmic reticulum of insulin-secreting cells. Studies in aequorin-expressing intact and permeabilized ins-1 cells. J Biol Chem 274:12583–12592
607. **Tang SH, Yaney GC, Sharp GWG** 1995 Unusual carbachol responses in RINm5F cells: evidence for a "distal" site of action in stimulus-secretion coupling. Mol Pharmacol 47:863–870
608. **Tengholm A, Hagman C, Gylfe E, Hellman B** 1998 *In situ* characterization of nonmitochondrial Ca^{2+} stores in individual pancreatic β-cells. Diabetes 47:1224–1230
609. **Bokvist K, Eliasson L, Ämmälä C, Renström E, Rorsman P** 1995 Co-localization of L-type Ca^{2+} channels and insulin-containing secretory granules and its significance for the initiation of exocytosis in mouse pancreatic B-cells. EMBO J 14:50–57
610. **Martin F, Ribas J, Soria B** 1997 Cytosolic Ca^{2+} gradients in pancreatic islet-cells stimulated by glucose and carbachol. Biochem Biophys Res Commun 235:465–468
611. **Rizzuto R, Brini M, Murgia M, Pozzan T** 1993 Microdomains with high Ca^{2+} close to IP_3-sensitive channels that are sensed by neighboring mitochondria. Science 262:744–747
612. **Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T** 1998 Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. Science 280:1763–1766
613. **Rutter GA, Theler JM, Murgia M, Wollheim CB, Pozzan T, Rizzuto R** 1993 Stimulated Ca^{2+} influx raises mitochondrial free Ca^{2+} to supramicromolar levels in a pancreatic β-cell line: possible role in glucose and agonist-induced insulin secretion. J Biol Chem 268: 22385–22390
614. **Putney Jr JW** 1986 A model for receptor-regulated calcium entry. Cell Calcium 7:1–12
615. **Berridge MJ** 1995 Capacitative calcium entry. Biochem J 312:1–11
616. **Putney Jr JW, McKay RR** 1999 Capacitative calcium entry channels. Bioessays 21:38–46
617. **Schöfl C, Borger J, Mader T, Waring M, von zur Muhlen A, Brabant G** 2000 Tolbutamide and diazoxide modulate phospholipase C-linked Ca^{2+} signaling and insulin secretion in β-cells. Am J Physiol Endocrinol Metab 278:E639–E647
618. **Parekh AB, Penner R** 1997 Store depletion and calcium influx. Physiol Rev 77:901–930
619. **Bode HP, Göke B** 1994 Protein kinase C activates capacitative calcium entry in the insulin secreting cell line RINm5F. FEBS Lett 339:307–311
620. **Liu YJ, Grapengiesser E, Gylfe E, Hellman B** 1994 Glucose-induced oscillations of Ba^{2+} in pancreatic β-cells occur without involvement of intracellular mobilization. Arch Biochem Biophys 315:387–392
621. **Rojas E, Carroll PB, Ricordi C, Boschero AC, Stojilkovic SS, Atwater I** 1994 Control of cytosolic free calcium in cultured human pancreatic β-cells occurs by external calcium-dependent and independent mechanisms. Endocrinology 134:1771–1781
622. **Schofl C, Borger J, Lange S, von zur Muhlen A, Brabant G** 2000 Energetic requirement of carbachol-induced Ca^{2+} signaling in single mouse β-cells. Endocrinology 141:4065–4071
623. **Nilsson T, Arkhammar P, Berggren PO** 1988 Dual effect of glucose on cytoplasmic free Ca^{2+} concentration and insulin release reflects the β-cell being deprived of fuel. Biochem Biophys Res Commun 153:984–991
624. **Sánchez-Andrés JV, Gomis A, Valdeolmillos M** 1995 The electrical activity of mouse pancreatic β-cells recorded *in vivo* shows glucose-dependent oscillations. J Physiol 486:223–228
625. **Bozem M, Nenquin M, Henquin JC** 1987 The ionic, electrical, and secretory effects of protein kinase C activation in mouse pancreatic B-cells: studies with a phorbol ester. Endocrinology 121:1025–1033
626. **Berggren PO, Arkhammar P, Nilsson T** 1989 Activation of protein kinase C assists insulin producing cells in recovery from raised cytoplasmic Ca^{2+} by stimulating Ca^{2+} efflux. Biochem Biophys Res Commun 165:416–421
627. **Wang KKW, Wright LC, Machan CL, Allen BG, Conigrave AD, Roufogalis BD** 1991 Protein kinase C phosphorylates the carboxyl terminus of the plasma membrane Ca^{2+}-ATPase from human erythrocytes. J Biol Chem 266:9078–9085
628. **Iwamoto T, Pan Y, Nakamura TY, Wakabayashi S, Shigekawa M** 1998 Protein kinase C-dependent regulation of Na^+/Ca^{2+} exchanger isoforms NCX1 and NCX3 does not require their direct phosphorylation. Biochemistry 37:17230–17238
629. **Kotagal N, Colca JR, Buscetto D, McDaniel ML** 1985 Effect of insulin secretagogues and potential modulators of secretion on a plasma membrane (Ca^{2+} + Mg^{2+})-ATPase activity in islets of Langerhans. Arch Biochem Biophys 238:161–169
630. **Gilon P, Yakel J, Gromada J, Zhu Y, Henquin JC, Rorsman P** 1997 G protein-dependent inhibition of L-type Ca^{2+} currents by acetylcholine in mouse pancreatic B-cells. J Physiol 499:65–76
631. **Velasco JM, Petersen OH** 1989 The effect of a cell-permeable diacylglycerol analogue on single Ca^{2+} (Ba^{2+}) channel currents in the insulin-secreting cell line RINm5F. Q J Exp Physiol 74:367–370
632. **Ämmälä C, Eliasson L, Bokvist K, Berggren PO, Honkanen RE, Sjöholm Å, Rorsman P** 1994 Activation of protein kinases and inhibition of protein phosphatases play a central role in the regulation of exocytosis in mouse pancreatic β cells. Proc Natl Acad Sci USA 91:4343–4347
633. **Platano D, Pollo A, Carbone E, Aicardi G** 1996 Up-regulation of L- and non-L, non-N-type Ca^{2+} channels by basal and stimulated protein kinase C activation in insulin-secreting RINm5F cells. FEBS Lett 391:189–194
634. **Love JA, Richards NW, Owyang C, Dawson DC** 1998 Muscarinic modulation of voltage-dependent Ca^{2+} channels in insulin-secreting HIT-T15 cells. Am J Physiol 274:G397–G405
635. **Ämmälä C, Berggren PO, Bokvist K, Rorsman P** 1992 Inhibition of L-type calcium channels by internal GTP-γS in mouse pancreatic β cells. Pflugers Arch 420:72–77
636. **Satoh H, Sperelakis N** 1995 Modulation of L-type Ca^{2+} current by isoprenaline, carbachol and phorbol ester in cultured rat aortic vascular smooth muscle (A7r5) cells. Gen Pharmacol 26:369–379
637. **Mathie A, Bernheim L, Hille B** 1992 Inhibition of N- and L-type calcium channels by muscarinic receptor activation in rat sympathetic neurons. Neuron 8:907–914
638. **Bernheim L, Mathie A, Hille B** 1992 Characterization of muscarinic receptor subtypes inhibiting Ca^{2+} current and M current in rat sympathetic neurons. Proc Natl Acad Sci USA 89:9544–9548
639. **Howe AR, Surmeier DJ** 1995 Muscarinic receptors modulate N-, P-, and L-type Ca^{2+} currents in rat striatal neurons through parallel pathways. J Neurosci 15:458–469
640. **Hille B** 1994 Modulation of ion-channel function by G-protein-coupled receptors. Trends Neurosci 17:531–536
641. **Shapiro MS, Loose MD, Hamilton SE, Nathanson NM, Gomeza J, Wess J, Hille B** 1999 Assignment of muscarinic receptor subtypes mediating G-protein modulation of Ca^{2+} channels by using knockout mice. Proc Natl Acad Sci USA 96:10899–10904
642. **Wollheim CB, Lang J, Regazzi R** 1996 The exocytotic process of insulin secretion and its regulation by Ca^{2+} and G-proteins. Diabetes Rev 4:276–297
643. **Burgoyne RD, Morgan A** 1998 Calcium sensors in regulated exocytosis. Cell Calcium 24:367–376
644. **Regazzi R** 1999 Mechanism of insulin exocytosis. In: Bittar EE, Howell SL, eds. Advances in molecular and cell biology. Stamford, CT: JAI Press, Inc.; 151–172
645. **Lang JC** 1999 Molecular mechanisms and regulation of insulin

exocytosis as a paradigm of endocrine secretion. Eur J Biochem 259:3–17
646. **Hisatomi M, Hidaka H, Niki I** 1996 Ca^{2+}/calmodulin and cyclic 3,5′ adenosine monophosphate control movement of secretory granules through protein phosphorylation/dephosphorylation in the pancreatic β-cell. Endocrinology 137:4644–4649
647. **Niwa T, Matsukawa Y, Senda T, Nimura Y, Hidaka H, Niki I** 1998 Acetylcholine activates intracellular movement of insulin granules in pancreatic β-cells via inositol triphosphate-dependent mobilization of intracellular Ca^{2+}. Diabetes 47:1699–1706
648. **Gromada J, Hoy M, Renström E, Bokvist K, Eliasson L, Göpel S, Rorsman P** 1999 CaM kinase II-dependent mobilization of secretory granules underlies acetylcholine-induced stimulation of exocytosis in mouse pancreatic B-cells. J Physiol 518:745–759
649. **Gembal M, Gilon P, Henquin JC** 1992 Évidence that glucose can control insulin release independently from its action on ATP-sensitive K^+ channels in mouse B cells. J Clin Invest 89:1288–1295
650. **Mariot P, Gilon P, Nenquin M, Henquin JC** 1998 Tolbutamide and diazoxide influence insulin secretion by changing the concentration but not the action of cytoplasmic Ca^{2+} in β-cells. Diabetes 47: 365–373
651. **Ravier MA, Henquin JC** 2001 Oscillations de l'activité des protéine kinases A et C dans les cellules β et oscillations de la sécrétion d'insuline. Diabetes Metab 27(Suppl 1):1S24 (Abstract)
652. **Jones PM, Stutchfield J, Howell SL** 1985 Effects of Ca^{2+} and a phorbol ester on insulin secretion from islets of Langerhans permeabilised by high-voltage discharge. FEBS Lett 191:102–106
653. **Tamagawa T, Niki H, Niki A** 1985 Insulin release independent of a rise in cytosolic free Ca^{2+} by forskolin and phorbol ester. FEBS Lett 183:430–432
654. **Arkhammar P, Nilsson T, Berggren PO** 1986 Stimulation of insulin release by the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate in the clonal cell line RINm5F despite a lowering of the free cytoplasmic Ca^{2+} concentration. Biochim Biophys Acta 887:236–241
655. **Hughes SJ, Christie MR, Ashcroft SJH** 1987 Potentiators of insulin secretion modulate Ca^{2+} sensitivity in rat pancreatic islets. Mol Cell Endocrinol 50:231–236
656. **Malaisse WJ, Sener A** 1985 Inhibition by 1-(5-isoquinolinesulfonyl)2-methylpiperazine (H-7) of protein kinase C activity and insulin release in pancreatic islets. IRCS (Int Res Commun Syst) Med Sci 13:1239–1240
657. **Jones PM, Persaud SJ, Howell SL** 1991 Protein kinase C and the regulation of insulin secretion from pancreatic B cells. J Mol Endocrinol 6:121–127
658. **Zawalich WS, Zawalich KC** 1996 Species differences in the induction of time-dependent potentiation of insulin secretion. Endocrinology 137:1664–1669
659. **Persaud SJ, Jones PM, Howell SL** 1993 Staurosporine inhibits protein kinases activated by Ca^{2+} and cyclic AMP in addition to inhibiting protein kinase C in rat islets of Langerhans. Mol Cell Endocrinol 94:55–60
660. **Harris TE, Persaud SJ, Saermark T, Jones PM** 1996 A myristoylated pseudosubstrate peptide inhibitor of protein kinase C: effects on glucose- and carbachol-induced insulin secretion. Mol Cell Endocrinol 121:133–141
661. **Thams P, Capito K, Hedeskov CJ, Kofod H** 1990 Phorbol-ester-induced down-regulation of protein kinase C in mouse pancreatic islets: potentiation of phase 1 and inhibition of phase 2 of glucose-induced insulin secretion. Biochem J 265:777–787
662. **Persaud SJ, Jones PM, Howell SL** 1991 Activation of protein kinase C is essential for sustained insulin secretion in response to cholinergic stimulation. Biochim Biophys Acta 1091:120–122
663. **Verspohl EJ, Wienecke A** 1998 The role of protein kinase C in the desensitization of rat pancreatic islets to cholinergic stimulation. J Endocrinol 159:287–295
664. **Otsuki M, Nakamura T, Okabayashi Y, Oka T, Fujii M, Baba S** 1985 Comparative inhibitory effects of pirenzepine and atropine on cholinergic stimulation of exocrine and endocrine rat pancreas. Gastroenterology 89:408–414
665. **Zawalich WS, Zawalich KC, Kelley GG** 1996 Time-dependent effects of cholinergic stimulation on β cell responsiveness. Pflugers Arch 432:589–596
666. **Safayhi H, Koopmann I, Ammon HPT** 1993 Insulin secretion without the participation of arachidonic acid. Mol Cell Endocrinol 91: 143–148
667. **Jones PM, Persaud SJ** 1993 Arachidonic acid as a second messenger in glucose-induced insulin secretion from pancreatic β-cells. J Endocrinol 137:7–14
668. **Band AM, Jones PM, Howell SL** 1992 Arachidonic acid-induced insulin secretion from rat islets of Langerhans. J Mol Endocrinol 8:95–101
669. **Loweth AC, Scarpello JHB, Morgan NG** 1996 A specific inhibitor of cytosolic phospholipase A_2 activity, $AACOCF_3$, inhibits glucose-induced insulin secretion from isolated rat islets. Biochem Biophys Res Commun 218:423–427
670. **Band AM, Jones PM, Howell SL** 1993 The mechanism of arachidonic acid-induced insulin secretion from rat islets of Langerhans. Biochim Biophys Acta 1176:64–68
671. **Basudev H, Jones PM, Persaud SJ, Howell SL** 1993 Arachidonic acid-induced insulin secretion from rat islets of Langerhans is not mediated by protein phosphorylation. Mol Cell Endocrinol 91: 193–199
672. **Thams P, Hedeskov CJ, Capito K** 1993 Exogenous arachidonic acid inactivates protein kinase C in mouse pancreatic islets. Acta Physiol Scand 149:227–235
673. **Ito K, Hirose H, Maruyama H, Fukamachi S, Tashiro Y, Saruta T** 1995 Neurotransmitters partially restore glucose sensitivity of insulin and glucagon secretion from perfused streptozotocin-induced diabetic rat pancreas. Diabetologia 38:1276–1284
674. **Zawalich WS, Zawalich KC, Rasmussen H** 1989 Interactions between cholinergic agonists and enteric factors in the regulation of insulin secretion from isolated perifused rat islets. Acta Endocrinol (Copenh) 120:702–707
675. **Fehmann HC, Goke R, Goke B, Arnold R** 1990 Carbachol priming increases glucose- and glucagon-like peptide-1 (7–36)amide-, but not arginine-induced insulin secretion from the isolated perfused rat pancreas. Z Gastroenterol 28:348–352
676. **Niki I, Tamagawa T, Niki H, Niki A, Koide T, Sakamoto N** 1988 Possible involvement of diacylglycerol-activated, Ca^{2+}-dependent protein kinase in glucose memory of the rat pancreatic B-cell. Acta Endocrinol (Copenh) 118:204–208
677. **Zawalich WS, Zawalich KC, Ganesan S, Calle R, Rasmussen H** 1991 Effects of the phorbol ester phorbol 12-myristate 13-acetate (PMA) on islet-cell responsiveness. Biochem J 278:49–56
678. **Sorenson RL** 1986 Islet priming by phorbol ester. Horm Metab Res 18:353–354
679. **Brelje TC, Sorenson RL** 1988 Nutrient and hormonal regulation of the threshold of glucose-stimulated insulin secretion in isolated rat pancreases. Endocrinology 123:1582–1590
680. **Zawalich WS, Zawalich KC** 1996 Glucagon-like peptide-1 stimulates insulin secretion but not phosphoinositide hydrolysis from islets desensitized by prior exposure to high glucose or the muscarinic agonist carbachol. Metabolism 45:273–278
681. **Oberdorf J, Webster JM, Zhu CC, Luo SG, Wojcikiewicz RJ** 1999 Down-regulation of types I, II and III inositol 1,4,5-trisphosphate receptors is mediated by the ubiquitin/proteasome pathway. Biochem J 339:453–461
682. **Persaud SJ** 1999 Pancreatic B-cell lines: their roles in B-cell research and diabetes therapy. In: Bittar EE, Howell SL, eds. Advances in molecular and cell biology. Stamford, CT: JAI Press, Inc.; 21–46
683. **Wollheim CB, Dunne MJ, Peter-Riesch B, Bruzzone R, Pozzan T, Petersen OH** 1988 Activators of protein kinase C depolarize insulin-secreting cells by closing K^+ channels. EMBO J 7:2443–2449
684. **Yada T, Russo LL, Sharp GWG** 1989 Phorbol ester-stimulated insulin secretion by RINm5F insulinoma cells is linked with membrane depolarization and an increase in cytosolic free Ca^{2+} concentration. J Biol Chem 264:2455–2462
685. **Lambert DG, Atkins TW** 1989 Cholinergic stimulation of insulin release from cloned B-cell lines HIT-T15 and RINm5F. Acta Diabetol Lat 26:27–34
686. **Weng L, Davies M, Ashcroft SJH** 1993 Effects of cholinergic agonists on diacylglycerol and intracellular calcium levels in pancreatic β-cells. Cell Signal 5:777–786
687. **Ejiri K, Taniguchi H, Baba S** 1989 Participation of nicotinic receptor in hormone release from isolated rat islets of Langerhans. Diabetes Res Clin Pract 6:53–59

688. **Ejiri K, Taniguchi H, Ishihara K, Hara Y, Baba S** 1990 Possible involvement of cholinergic nicotinic receptor in insulin release from isolated rat islets. Diabetes Res Clin Pract 8:193–199
689. **Sharp R, Culbert S, Cook J, Jennings A, Burr IM** 1974 Cholinergic modification of glucose-induced biphasic insulin release *in vitro*. J Clin Invest 53:710–716
690. **Gylfe E, Hellman B** 1986 Glucose-stimulated sequestration of Ca^{2+} in clonal insulin-releasing cells. Evidence for an opposing effect of muscarinic-receptor activation. Biochem J 233:865–870
691. **Ramachandran J, Peralta EG, Ashkenazi A, Winslow JW, Capon DJ** 1989 The structural and functional interrelationships of muscarinic acetylcholine receptor subtypes. Bioessays 10:54–57
692. **Goyal RK** 1989 Muscarinic receptor subtypes. Physiology and clinical implications. N Engl J Med 321:1022–1029
693. **Hulme EC, Curtis CAM, Page KM, Jones PG** 1993 Agonist activation of muscarinic acetylcholine receptors. Cell Signal 5:687–694
694. **Felder CC** 1995 Muscarinic acetylcholine receptors: signal transduction through multiple effectors. FASEB J 9:619–625
695. **Jones SV** 1993 Muscarinic receptor subtypes: modulation of ion channels. Life Sci 52:457–464
696. **Krapivinsky G, Gordon EA, Wickman K, Velimirovic B, Krapivinsky L, Clapham DE** 1995 The G-protein-gated atrial K^+ channel IKACh is a heteromultimer of two inwardly rectifying K^+-channel proteins. Nature 374:135–141
697. **Stein R, Pinkas Kramarski R, Sokolovsky M** 1988 Cloned M_1 muscarinic receptors mediate both adenylate cyclase inhibition and phosphoinositide turnover. EMBO J 7:3031–3035
698. **Richards MH** 1991 Pharmacology and second messenger interactions of cloned muscarinic receptors. Biochem Pharmacol 42:1645–1653
699. **Henquin JC, Nenquin M** 1988 The muscarinic receptor subtype in mouse pancreatic B-cells. FEBS Lett 236:89–92
700. **Verspohl EJ, Tacke R, Mutschler E, Lambrecht G** 1990 Muscarinic receptor subtypes in rat pancreatic islets: binding and functional studies. Eur J Pharmacol 178:303–311
701. **Peralta EG, Winslow JW, Ashkenazi A, Smith DH, Ramachandran J, Capon DJ** 1988 Structural basis of muscarinic acetylcholine receptor subtype diversity. Trends Pharmacol Sci(Suppl):6–11
702. **Bonner TI, Buckley NJ, Young AC, Brann MR** 1987 Identification of a family of muscarinic acetylcholine receptor genes. Science 237:527–532
703. **Ladinsky H, Schiavi GB, Monferini E, Giraldo E** 1990 Pharmacological muscarinic receptor subtypes. Prog Brain Res 84:193–200
704. **Tang SH, Sharp GW** 1997 Identification of muscarinic receptor subtypes in RINm5F cells by means of polymerase chain reaction, subcloning, and DNA sequencing. Diabetes 46:1419–1423
705. **Karlsson S, Ahrén B** 1993 Muscarinic receptor subtypes in carbachol-stimulated insulin and glucagon secretion in the mouse. J Auton Pharmacol 13:439–446
706. **Grill V, Östenson CG** 1983 Muscarinic receptors in pancreatic islets of the rat. Demonstration and dependence on long-term glucose environment. Biochim Biophys Acta 756:159–162
707. **Grill V, Fak K** 1985 Influence of thiol groups, calcium, and glucose metabolism on cholinergic-induced insulin release and on methylscopolamine binding to muscarinic receptors in pancreatic islets of the rat. Acta Endocrinol (Copenh) 109:355–360
708. **Malaisse WJ, Mahy M, Mathias PCF** 1985 Binding of (^{3}H)-methylscopolamine to rat pancreatic islets. IRCS (Int Res Commun Syst) Med Sci 13:503–504
709. **Östenson CG, Grill V** 1987 Evidence that hyperglycemia increases muscarinic binding in pancreatic islets of the rat. Endocrinology 121:1705–1710
710. **Östenson CG, Grill V** 1985 Glucose exerts opposite effects on muscarinic receptor binding to A and B cells of the endocrine pancreas. Endocrinology 116:1741–1744
711. **Iismaa TP, Kerr EA, Wilson JR, Carpenter L, Sims N, Biden TJ** 2000 Quantitative and functional characterization of muscarinic receptor subtypes in insulin-secreting cell lines and rat pancreatic islets. Diabetes 49:392–398
712. **Felder CC, Poulter MO, Wess J** 1992 Muscarinic receptor-operated Ca^{2+} influx in transfected fibroblast cells is independent of inositol phosphates and release of intracellular Ca^{2+}. Proc Natl Acad Sci USA 89:509–513
713. **Ashkenazi A, Winslow JW, Peralta EG, Peterson GL, Schimerlik MI, Capon DJ, Ramachandran J** 1987 An M_2 muscarinic receptor subtype coupled to both adenylyl cyclase and phosphoinositide turnover. Science 238:672–674
714. **Pemberton KE, Jones SVP** 1997 Inhibition of the L-type calcium channel by the five muscarinic receptors (m1–m5) expressed in NIH 3T3 cells. Pflugers Arch 433:505–514
715. **Henquin JC** 1990 Established, unsuspected and novel pharmacological insulin secretagogues. In: Bailey CJ, Flatt PR, eds. New antidiabetic drugs. London: Smith-Gordon; 93–106
716. **Gainetdinov RR, Caron MG** 1999 Delineating muscarinic receptor functions. Proc Natl Acad Sci USA 96:12222–12223
