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Distinct mechanisms for two amplification systems of insulin release

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The mechanisms whereby activation of the cyclic AMP-dependent protein kinase A or the Ca\(^{2+}\)-phospholipid-dependent protein kinase C amplifies insulin release were studied with mouse islets. Forskolin and the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) were used to stimulate adenylate cyclase and protein kinase C respectively. The sulphonylurea tolbutamide was used to initiate insulin release in the presence of 3 mm-glucose. Tolbutamide alone inhibited \(^{86}\)Rb\(^{+}\) efflux, depolarized \(\beta\)-cell membrane, triggered electrical activity, accelerated \(^{45}\)Ca\(^{2+}\) influx and efflux and stimulated insulin release. Forskolin alone only slightly inhibited \(^{86}\)Rb\(^{+}\) efflux, but markedly increased the effects of tolbutamide on electrical activity, \(^{45}\)Ca\(^{2+}\) influx and efflux, and insulin release. In the absence of Ca\(^{2+}\), only the inhibition of \(^{86}\)Rb\(^{+}\) efflux persisted. TPA (100 nM) alone slightly accelerated \(^{45}\)Ca\(^{2+}\) influx and insulin release without affecting \(^{45}\)Ca\(^{2+}\) influx or \(\beta\)-cell membrane potential. It increased the effects of tolbutamide on \(^{45}\)Ca\(^{2+}\) efflux and insulin release without changing \(^{86}\)Rb\(^{+}\) efflux, \(^{45}\)Ca\(^{2+}\) influx or electrical activity. Omission of extracellular Ca\(^{2+}\) suppressed all effects due to the combination of TPA and tolbutamide, but not those of TPA alone. Though ineffective alone, 10 nM-TPA amplified the releasing action of tolbutamide without affecting its ionic and electrical effects. In conclusion, the two amplification systems of insulin release involve at least partially distinct mechanisms. The cyclic AMP but not the protein kinase C system increases the initiating signal (Ca\(^{2+}\) influx) triggered by the primary secretagogue.

INTRODUCTION

In vivo, the pancreatic \(\beta\)-cell function is mainly regulated by changes in the concentrations of glucose and other nutrients, but is also controlled by complex neuro-hormonal influences. The amount of insulin that is released in response to a given stimulus does not simply depend on the magnitude of the initiating signal triggered by that stimulus. It may be modulated by systems of amplification or of attenuation.

Two systems of amplification have been identified in \(\beta\)-cells, both of which involve protein kinases: the cyclic AMP-dependent protein kinase A and the Ca\(^{2+}\)-phospholipid-dependent protein kinase C (for reviews see [1–6]). Conclusive evidence has long been obtained that cyclic AMP does not initiate insulin release, but strongly amplifies the response to various secretagogues. However, elucidation of the cellular mechanisms whereby cyclic AMP affects \(\beta\)-cell function is relatively new and still incomplete [3–6]. Evidence that activation of protein kinase C also amplifies insulin release is more recent, and the few attempts to elucidate how this amplification occurs remain contradictory [7–10]. The demonstration that distinct islet proteins can be phosphorylated by these two kinases [11–13] raises the possibility that different cellular events underlie the changes in insulin release resulting from an activation of these two pathways.

The aim of the present study was to compare how these two amplification systems affect tolbutamide-induced insulin release by isolated mouse islets. Particular attention was paid to the modifications of ionic and electrical events brought about by the sulphonylurea. The adenylate cyclase activator forskolin [14] was used to increase cyclic AMP in \(\beta\)-cells, and the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) was used to activate protein kinase C [15]. Tolbutamide was chosen as the primary stimulus, because its mode of action on \(\beta\)-cells is relatively simple: by blocking ATP-dependent K\(^{+}\) channels, tolbutamide decreases K\(^{+}\) permeability of the \(\beta\)-cell membrane; this causes a depolarization that activates voltage-dependent Ca\(^{2+}\) channels and permits Ca\(^{2+}\) influx [16–19]. Moreover, the combination of a pharmacological secretagogue with forskolin and TPA should permit instructive comparisons with the recently reported effects of these latter on nutrient-stimulated \(\beta\)-cells [10,20]. Such comparisons are necessary to establish whether the cellular events underlying the amplification of insulin release depend on the nature of the primary stimulus.

EXPERIMENTAL

All experiments were performed with islets of fed female NMRI mice (25–30 g), which had been killed by decapitation. For electrophysiological experiments, a piece of pancreas was fixed in a small perfusion chamber, and the membrane potential of single \(\beta\)-cells was continuously recorded with micro-electrodes [21]. \(\beta\)-Cells were identified by the typical electrical activity that they display in the presence of 10–15 mm-glucose [22]. For all other experiments, islets were isolated after collagenase digestion of the pancreas. The techniques

Abbreviation used: TPA, 12-O-tetradecanoylphorbol 13-acetate.
and the dynamic system of perfusion used to monitor the efflux of $^{45}\text{Ca}^{2+}$ or $^{86}\text{Rb}^{+}$ (used as tracer for $K^+$) from preloaded islets have been described in detail [23]. During the experiments on $^{86}\text{Rb}^{+}$ efflux, a portion of each effluent fraction was taken for measurement of immunoreactive insulin, with rat insulin as standard (Novo Research Institute, Bagsvaerd, Denmark). $^{45}\text{Ca}^{2+}$ uptake by islet cells was measured as described previously [24], by using $[6,6'-3\text{H}]$sucrose as marker of the extracellular space. Islet cyclic AMP concentration was determined by radioimmunoassay with a commercially available kit (New England Nuclear, Boston, MA, U.S.A.), after acetylation of standards and samples [25]. The medium used was a bicarbonate-buffered solution [20], pH 7.4, that was supplemented with bovine serum albumin (1 mg/ml) except for electrophysiological recordings, and that contained 3 mm-glucose in all experiments. $\text{Ca}^{2+}$-free solutions were prepared by replacing $\text{CaCl}_2$ by $\text{MgCl}_2$.

Albumin has a high binding capacity for sulphonylureas [26] and has been shown to lower tolbutamide binding to islet cells [27]. To permit comparisons of the ionic and secretory effects of tolbutamide (measured in the presence of albumin) with its electrical effects (measured in the absence of albumin), different concentrations of the sulphonylurea were used. Most electrophysiological experiments were carried out with only 25 $\mu\text{M}$-tolbutamide, whereas most other experiments were carried out with 100 $\mu\text{M}$-tolbutamide.

Tolbutamide was provided by Hoechst A. G. (Frankfurt/Main, Germany). Forskolin and TPA were from Calbiochem–Behring (San Diego, CA, U.S.A.) and P-L Biochemicals (Milwaukee, WI, U.S.A.) respectively, and were dissolved in dimethyl sulphoxide. Samples of the stock solutions were added to test media immediately before use, whereas dimethyl sulphoxide alone (0.1–0.2 $\mu\text{l}$/ml) was added to control media. The same batch of TPA was used for all experiments. A portion of the original stock solution was transferred from Brussels to Homburg, where it was used for several months before the rest returned to Brussels, where it proved to have retained full activity on insulin release. Radiochemicals were obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). All other reagents were from Merck A.G. (Darmstadt, Germany).

Electrophysiological experiments are illustrated by recordings that are representative of the indicated number of experiments, performed with different mice, or are presented as means (± S.E.M.). All other data are presented as means (± S.E.M.) for a certain number of experiments (different islet preparations) or of batches of islets. The indicated number of batches of islets was obtained in at least three separate experiments. The statistical significance of the effects of forskolin or TPA was assessed by two-tailed Student's $t$ test for unpaired data.

RESULTS

Effects of forskolin on tolbutamide-induced ionic, secretory and electrical events

Tolbutamide (100 $\mu\text{M}$) inhibited $^{86}\text{Rb}^{+}$ efflux, accelerated $^{45}\text{Ca}^{2+}$ efflux and stimulated insulin release from mouse islets perfused with 3 mm-glucose and 2.5 mm-$\text{Ca}^{2+}$ (Fig. 1). Subsequent addition of 1 $\mu\text{M}$-forskolin caused a small but consistent acceleration of $^{86}\text{Rb}^{+}$ efflux, and much larger increases in $^{45}\text{Ca}^{2+}$ efflux and insulin release. These effects were sustained and reversible upon withdrawal of tolbutamide and forskolin. In the absence of extracellular $\text{Ca}^{2+}$, the inhibition of $^{86}\text{Rb}^{+}$ efflux by tolbutamide was faster than in a control medium. On the other hand, its effects on $^{45}\text{Ca}^{2+}$ efflux and insulin release were abolished, as were those of the subsequent addition of forskolin (Fig. 1). In another series of experiments, performed in the presence of $\text{Ca}^{2+}$, tolbutamide was used at the lower concentration of 25 $\mu\text{M}$. Basal insulin release ($9 ± 2$ pg/min per islet) was not modified by the sulphonylurea ($10 ± 2$ pg/min per islet), but subsequent addition of forskolin increased it 3-fold ($29 ± 3$ pg/min per islet; $n = 4$).

Tolbutamide (25 $\mu\text{M}$) caused a rapid depolarization of the $\beta$-cell membrane and evoked continuous spike activity for a few minutes (Fig. 2, upper panel).
Thereafter, the membrane potential started to oscillate in slow waves, with bursts of spikes superimposed on the plateau. These slow waves progressively decreased in duration and frequency, and in two of eight cells ceased after about 20 min. Addition of 1 μM-forskolin 30 min after tolbutamide markedly increased electrical activity in all cells where it was still present (Fig. 2, middle panel), and restored electrical activity in the two cells where it had stopped (results not shown). The average changes in overall electrical activity (percentage of time with spike activity) brought about by tolbutamide and the marked increase by forskolin are shown in the lower panel of Fig. 2. Dibutyryl cyclic AMP (1 mM) increased tolbutamide-induced electrical activity as did forskolin (results not shown).

At the lower concentration of 10 μM, tolbutamide induced slow waves in three of five cells, and forskolin increased the electrical activity (results not shown). In the other two cells, 10 μM-tolbutamide only depolarized the β-cell membrane, and forskolin had no further effect. At the higher concentration of 100 μM, tolbutamide persistently depolarized the β-cell membrane to the plateau potential and evoked continuous spike activity (Fig. 3, upper panel). With time the amplitude of the spikes decreased, but addition of forskolin rapidly increased it again without affecting their frequency (Fig. 3).

**Comparison of the effects of forskolin and TPA**

TPA affects β-cell function more slowly than does forskolin [10,20]. To ensure valid comparison of their effects, the islets were thus treated with either test agent for 30 min before stimulation by tolbutamide.

As shown by Fig. 4, 1 μM-forskolin slightly decreased ⁸⁶Rb⁺ efflux, but did not affect ⁴⁵Ca²⁺ efflux or insulin release in the presence of 3 mM-glucose alone. The presence of forskolin slightly attenuated the inhibition of ⁸⁶Rb⁺ efflux by tolbutamide, increased its stimulation of ⁴⁵Ca²⁺ efflux and considerably amplified the insulin response (Fig. 4).

As shown by Fig. 5, 100 nM-TPA produced a slow and slight inhibition of ⁸⁶Rb⁺ efflux, acceleration of ⁴⁵Ca²⁺...
The medium contained 3 mm-glucose and 2.5 mm-CaCl₂ throughout. Forskolin (1 μM) was added to test solutions (O) from 40 min onwards. Tolbutamide (100 μM) was added to solutions with (O) or without (●) forskolin between 70 and 100 min. Control experiments without forskolin or tolbutamide are shown by the dashed lines. Values are means ± S.E.M. for five experiments.

efflux and stimulation of insulin release, whereas 10 nM-TPA was ineffective. TPA did not alter the decrease in ⁸⁶Rb⁺ efflux brought about by tolbutamide, but attenuated the rebound increase occurring upon removal of the sulphonylurea in a dose-dependent manner. The efflux of ⁴⁵Ca⁺⁺ triggered by tolbutamide was not affected by 10 nM-TPA, but was augmented by 100 nM-TPA, whereas the insulin response was potentiated 5- and 12-fold by the low and the high concentration of TPA respectively (Fig. 5). Though ineffective alone, 25 μM-tolbutamide increased the rate of insulin release from 12 ± 1 to 30 ± 3 pg/min per islet in the presence of 10 nM-TPA. Omission of extracellular Ca⁺⁺ did not suppress the effects of 100 nM-TPA alone, but abolished those of tolbutamide on ⁴⁵Ca⁺⁺ efflux and insulin release, whatever the concentration of TPA (results not shown).

The effects of tolbutamide on ⁴⁵Ca⁺⁺ influx in islet cells were estimated by measuring the 5 min uptake of the tracer after 30 min of incubation with 3 mm-glucose alone, or with forskolin or TPA. Under similar conditions, both forskolin (Fig. 4) and 100 nM-TPA (Fig. 5) accelerated tolbutamide-induced ⁴⁵Ca⁺⁺ influx. ⁴⁵Ca⁺⁺ influx was unaffected by forskolin or TPA alone, but was increased almost 2.5-fold by tolbutamide (Table 1). This effect of tolbutamide was potentiated by forskolin, but not by 10 nM or 100 nM-TPA.

Islet cyclic AMP concentration was increased 5-fold after 30 min of incubation with 1 μM-forskolin, was not affected by 10 nM-TPA and was marginally augmented (15%) by 100 nM-TPA (Table 1).

Stimulation with 25 μM-tolbutamide after 30 min of perfusion with a medium containing 3 mm-glucose depolarized the β-cell membrane and induced electrical activity (Fig. 6). This activity consisted of slow waves of the membrane potential with bursts of spikes originating from the plateau potential. In none of the eight cells studied were these slow waves preceded by a period of
amplification systems of insulin release

Table 1. Effects of forskolin and TPA on islet cyclic AMP concentration and on tolbutamide-induced $^{45}\text{Ca}^{2+}$ uptake by mouse islets

For cyclic AMP measurements, batches of ten islets were incubated for 30 min under the indicated conditions. For $^{45}\text{Ca}^{2+}$-uptake measurements, batches of 10 islets were incubated for 30 min under the indicated conditions, and then for 5 min in the presence of $^{45}\text{CaCl}_2$. When tested, tolbutamide (100 µM) was added at the same time as $^{45}\text{CaCl}_2$. Values are means ± S.E.M. for 10 batches of islets (for cyclic AMP), 12 batches of islets (for $^{45}\text{Ca}^{2+}$-uptake controls) or 16 batches of islets (for $^{45}\text{Ca}^{2+}$ uptake with tolbutamide): *P < 0.05, †P < 0.001 versus glucose (3 mM) alone.

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Cyclic AMP concn. (fmol/islet)</th>
<th>$^{45}\text{Ca}^{2+}$ uptake (pmol/5 min per islet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Tolbutamide</td>
<td></td>
</tr>
<tr>
<td>Glucose (3 mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Forskolin (1 µM)</td>
<td>59.2 ± 3.0†</td>
<td>6.86 ± 0.31</td>
</tr>
<tr>
<td>+ TPA (10 nm)</td>
<td>12.2 ± 0.9</td>
<td>9.62 ± 0.37†</td>
</tr>
<tr>
<td>+ TPA (100 nM)</td>
<td>13.6 ± 0.6*</td>
<td>6.58 ± 0.37†</td>
</tr>
</tbody>
</table>

Fig. 6. Effects of forskolin or TPA on the electrical activity induced by 25 µM-tolbutamide in mouse β-cells

The concentration of glucose was 3 mM throughout. All these experiments lasted at least 70 min: 30 min without, 30 min with, and again 10 min without, tolbutamide. When tested, forskolin (1 µM) or TPA (100 nm) was present during the three periods. Only one test was performed in each preparation, and the three records are thus from different cells. The lower panel shows the percentage of time that β-cells spent at the plateau potential (with spike activity). Values are means for five (forskolin), six (TPA) or eight cells (controls, broken line).

persistent depolarization with continuous spike activity, as when tolbutamide stimulation was applied after a shorter period of perfusion with a low concentration of glucose (cf. Fig. 2). When the whole experiment was performed in the presence of 1 µM-forskolin, tolbutamide depolarized the β-cell membrane to the plateau potential and triggered continuous spike activity for several minutes before slow waves appeared (Fig. 6). In contrast, pretreatment of the islets with 100 nM-TPA before stimulation with tolbutamide had no influence on the changes in membrane potential evoked by tolbutamide. The lower panel of Fig. 6 illustrates the changes in overall electrical activity (percentage of time with spike activity) recorded under the three experimental conditions.

The onset of depolarization by tolbutamide was not different in control β-cells (17.3 ± 2.4 s) and in β-cells pre-treated with 1 μM-forskolin (15.3 ± 0.7 s) or with 100 µM-TPA (18.2 ± 1.6 s). On the other hand, the delay in appearance of the first spike was shorter in the presence of forskolin (37 ± 7 s; P < 0.005) than in a control medium (79 ± 6 s) or in the presence of TPA (83 ± 6 s).

Discussion

This study shows that raising cyclic AMP concentration in islet cells (by forskolin) or activating protein kinase C (by TPA) similarly amplifies tolbutamide-induced insulin release, but that at least partially distinct mechanisms underly this amplification.

Effects of forskolin

It has long been known that tolbutamide-induced insulin release can be amplified by stimulating the adenylyl cyclase with glucagon [28] or by inhibiting the cyclic AMP phosphodiesterase by a methylxanthine [29]. The present work extends these findings by showing that an increase in cyclic AMP in β-cells augments the triggering signal induced by tolbutamide.

Though ineffective alone, forskolin modified the effects of tolbutamide on $^{45}\text{Ca}^{2+}$ efflux, $^{45}\text{Ca}^{2+}$ uptake and β-cell membrane potential. First, forskolin augmented the acceleration of $^{45}\text{Ca}^{2+}$ efflux from preloaded islets, provided that extracellular $\text{Ca}^{2+}$ was present. Such an effect is generally interpreted as indirect evidence for a stimulation of $\text{Ca}^{2+}$ influx, with exchange of cellular $^{45}\text{Ca}^{2+}$ for incoming non-radioactive $\text{Ca}^{2+}$. Second, forskolin increased tolbutamide-induced $^{45}\text{Ca}^{2+}$ uptake (5 min). Third, under similar conditions, forskolin also
augmented electrical events (slow waves and spikes), the Ca\(^{2+}\)-dependency of which is well established (for a review, see [30]). These three lines of evidence support the contention that cyclic AMP increases tolbutamide-induced Ca\(^{2+}\) influx in \(\beta\)-cells.

Similar effects of cyclic AMP have been observed previously during stimulation by glucose [20,31,32] or leucine [33], but not during stimulation by alanine or arginine [33]. Since these last two amino acids are practically not metabolized by islet cells [34], it was possible that cyclic AMP could affect Ca\(^{2+}\) permeability of the \(\beta\)-cell membrane only during stimulation by a well-metabolized secretagogue. The present study with tolbutamide rules out that hypothesis. It rather seems that the effect of cyclic AMP requires that the \(\beta\)-cell membrane be depolarized by a decrease in K\(^+\) permeability. This is indeed a common property of glucose, leucine and tolbutamide [18,30,35] that is not shared by alanine and arginine [33,35].

The concentration of cytosolic free Ca\(^{2+}\), estimated by the fluorescent Ca\(^{2+}\)-indicator quin 2, did not increase in RINm5F cells [36] or ob\(\text{ob}\)/ob\(\text{ob}\)-mouse islet cells [37] stimulated by forskolin. This prompted the suggestion that cyclic AMP potentiates insulin release by sensitizing the releasing machinery to Ca\(^{2+}\). A similar conclusion was drawn from experiments using permeabilized rat islet cells [38,39], though the effect of forskolin or exogenous cyclic AMP occurred only at relatively high concentrations of free Ca\(^{2+}\). By no means do our present and previous [20,33] results exclude such a mechanism. On the contrary, the potentiation of insulin release by forskolin was consistently larger than expected from the increases in \(^{45}\text{Ca}^{2+}\) influx and electrical activity, had they been produced by a rise in glucose or tolbutamide concentration. Moreover, forskolin can also increase insulin release without affecting the ionic properties of the \(\beta\)-cell membrane, e.g. in the presence of certain amino acids [33]. On the other hand, the measurements of cytoplasmic Ca\(^{2+}\) are not necessarily incompatible with our experimental evidence for an increase in Ca\(^{2+}\) influx by cyclic AMP. It is possible that the accelerated influx of Ca\(^{2+}\), coupled to an accelerated rate of efflux, raises the concentration of free Ca\(^{2+}\) only beneath the plasma membrane, and that such a localized change escapes detection by the quin 2 technique. Furthermore it should be noted that loading ob\(\text{ob}\)/ob\(\text{ob}\)-mouse \(\beta\)-cells with the indicator decreased the effect of forskolin on insulin release by 75% [37].

Effects of TPA

It has been reported that 200 nM-TPA markedly increased insulin release by rat islets stimulated with gliclazide [7] or tolbutamide [40], that it potentiated certain changes in ionic fluxes brought about by gliclazide in rat islets [7], and that it enhanced the electrical response of mouse \(\beta\)-cells to glibenclamide [8].

The present work partially agrees with these reports. It confirms that a high concentration of TPA (100 nM) amplifies the stimulation of insulin release and \(^{45}\text{Ca}^{2+}\) efflux by a sulphonylurea, but not that it potentiates the inhibition of \(^{45}\text{Rb}^{+}\) efflux [7]. Both studies agree that the effects of the sulphonylurea on insulin release and \(^{45}\text{Ca}^{2+}\) efflux remain dependent on extracellular Ca\(^{2+}\) in the presence of TPA, even though the genuine effects of the phorbol ester persist in the absence of Ca\(^{2+}\). In contrast, the electrical effects of tolbutamide (depolarization, electrical activity), unlike those of glibenclamide [8], were not modified by TPA. The reason for this discrepancy may be that glibenclamide differs from other sulphonylureas by several of its properties [41].

A major observation of the present study is that TPA markedly increased insulin release without affecting the inhibition of \(^{45}\text{Rb}^{+}\) efflux, the stimulation of \(^{45}\text{Ca}^{2+}\) influx and the Ca\(^{2+}\)-dependent electrical activity brought about by tolbutamide. Only the acceleration of \(^{45}\text{Ca}^{2+}\) efflux was increased by the high concentration of TPA. A similar observation was made in the presence of a stimulatory concentration of glucose, although Ca\(^{2+}\) influx was also apparently unaffected [10]. This may reflect the ability of the phorbol ester either to mobilize cellular Ca\(^{2+}\) [7] or to activate Ca\(^{2+}\) extrusion at the plasma-membrane level [9]. It is clear, however, that this change in \(^{45}\text{Ca}^{2+}\) efflux is not a prerequisite for the potentiation of insulin release, since the low concentration of TPA amplified the secretory response 5-fold, without changing Ca\(^{2+}\) fluxes at all.

To explain the synergism between TPA and sulphonylureas, it has been suggested [7,8] that the phorbol ester induces certain physical changes in the plasma membrane, which could facilitate either the access of the sulphonylurea to its target site or the ionic responses to its action. By showing that TPA does not amplify the triggering signals of tolbutamide, the present study rules out that hypothesis.

It has been shown that TPA attenuated the rise in cytosolic Ca\(^{2+}\) occurring upon depolarization of RINm5F cells by a high concentration of K\(^+\) [9,42], but also that high K\(^+\) did not trigger insulin release from these cells, whether TPA was present or not [9]. Extrapolation to possible effects of the phorbol ester on cytosolic Ca\(^{2+}\) of normal \(\beta\)-cells stimulated by tolbutamide would thus be speculative. Experiments using permeabilized islet cells have shown that TPA increases insulin release at a fixed, sub-micromolar, concentration of Ca\(^{2+}\) [38,43] and that this increase is attenuated by polymyxin B, a protein kinase C inhibitor [44]. Taken together, these results and our reports on the interaction of TPA with glucose [10] and tolbutamide (the present study) suggest that the potentiation of insulin release by the phorbol ester results from intracellular events or non-electrogenic membrane events, that sensitize the releasing machinery to the action of Ca\(^{2+}\).

Conclusion

If one accepts that, at the low concentrations purposely used, forskolin [11] and TPA [12] rather specifically activate the adenylate cyclase and the protein kinase C respectively, the following conclusion seems warranted. Pancreatic \(\beta\)-cells are equipped with two systems that serve to amplify the release of insulin in response to a primary stimulus. However, their coexistence does not merely constitute a redundant control system. At least partially distinct mechanisms are involved to reach the ultimately similar effect. This makes it possible for different signals to act in concert or in sequence to modulate positively the \(\beta\)-cell function.

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