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INSULIN SECRETION: COMBINED EFFECTS OF PHORBOL ESTER AND A23187

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The effect of the ionophore, A23187, and/or the phorbol ester, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), on insulin secretion were compared with those of glucose. Glucose induces a biphasic pattern of insulin secretion; A23187 a comparable initial spike but no second phase; and TPA a slowly progressive increase. Combined A23187 and TPA evoke a pattern similar to that induced by glucose. Forskolin enhances both phases of glucose-induced and of TPA-A23187-induced insulin secretion. These results are interpreted in terms of a model of cell activation in which two branches of the calcium messenger system, the calmodulin branch and the C-kinase branch, control, respectively, the initial and sustained phases of insulin secretion.

Work by Takai, Kaibuchi and coworkers on platelet activation has led to the discovery that there are two branches by which information flows from the cell surface to the cell interior in the calcium messenger system (1-6). One is mediated by a rise in the cytosolic calcium concentration (7) leading to the modulation of calmodulin-dependent reactions. The other is mediated by a rise in the diacylglycerol content of the plasma membrane leading to the activation of the calcium-activated, phospholipid-dependent protein kinase, C-kinase. These branches can be activated separately: the calmodulin by the divalent ionophore, A23187 (7); and the C-kinase branch by diolein or the phorbol ester, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (5,6). Activation of either branch alone causes a less than maximal release of serotonin from platelets. Activation

Abbreviations: BSA--bovine serum albumin; TPA--12-O-tetradecanoyl-phorbol-13-acetate; EGTA--ethylene glycol bis (β-amino-ethyl ester)-N,N,N;N'-tetra acetic acid; DG--diacylglycerol; EDTA--ethylenediaminetetraacetate.

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of the two branches by the simultaneous addition of A23187 and TPA induces a maximal rate of serotonin secretion comparable to that seen after thrombin-induced platelet activation. These authors conclude that these two pathways have a synergistic effect on platelet activation (6).

In previous work (8), we examined the roles of these branches in the control of aldosterone secretion from the adrenal glomerulosa cell. Ionophore alone caused only a transitory increase, TPA alone a very slowly progressive rise, and their combination a monotonic and sustained increase in aldosterone secretion comparable to that seen after angiotensin II addition (8).

There is now a substantial body of evidence showing that calcium is the major intracellular messenger when glucose regulates insulin secretion, and that cyclic AMP serves as a supplementary or hierarchical messenger in this system (9-14). Of particular relevance to the present study is the fact that glucose-induced insulin secretion is biphasic (10,13): an initial brief increase lasting a few minutes followed by a fall to approximately half the initial maximal rate, and then a slower rise to a plateau which is sustained as long as the plasma glucose is elevated (10). We examined the effects of A23187 and TPA alone or in combination on insulin secretion from perifused islets and compared these responses with the action of glucose. It is already known that islets possess C-kinase (15,16), and that addition of either A23187 (17) or of TPA will induce insulin secretion when studied in a static incubation system (18).

Methods

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Boston, MA) weighing 250-350 grams were used in all studies. The animals were fed standard lab chow ad libitum. Water was always available.

Islet Cell Secretion Studies

The detailed methodology for the islet isolation and perifusion, has been reported elsewhere (19). Briefly, islets were iso-

lated by collagenase treatment (20) of the pancreas obtained from anesthetized (Nembutal, 50 mg/kg) rats. Groups of 20-25 islets were then perifused at a flow rate of 1 ml/min for thirty minutes to stabilize hormone secretion rates. Generally, exogenous fuels were not added to the perifusion medium during this time. In those experiments where islet responsiveness to glucose was assessed, substimulatory glucose (2.75 mM) was included during this stabilization period. In experiments involving phenylephrine or of forskolin, these compounds were usually added to the medium during the last 10 minutes of this stabilization period. During the final five minutes of this period, an aliquot of perifusate was analyzed for insulin content (21). After addition of appropriate agonist, samples were collected at 1 minute intervals for the first ten minutes to assess the first phase of hormone secretion, and then at five minute intervals thereafter for for 60 minutes or longer.

Reagents

The salts used to make the Hanks solution used for islet isolation and the Krebs-Ringer bicarbonate for the perifusion medium were obtained from Sigma Chemical (St. Louis). The bovine serum albumin, used at a final concentration of 0.17 gram/100 ml of perifusion medium, was obtained from Reheis Chemical Co. (Phoenix, Arizona). The phorbol ester, 12-O-tetradecanoylphorbol-13-acetate and the phenylephrine were obtained from Sigma and with the ionophore, A23187 and forskolin, from Calbiochem (La Jolla, CA). New England Nuclear (Boston, MA) was the supplier of the I^{125}-insulin used in the radioimmunoassay.

Results

A comparison of the insulin secretory responses to various agonists is shown in Figure 1. When the perifusate glucose concentration was raised from 2.75 to 7.0 mM a typical biphasic insulin secretory response was observed (Fig. 1A). When 100 nM TPA was added to the perifusate, there was no immediate increase in insulin secretory rate but a slowly rising rate of insulin secretion (Fig. 1B). This rate eventually reached a value 60-80 percent of that seen after glucose addition (compare Fig. 1B with 1A). When 2.5 μM A23187 was added to the perifusate, there was an immediate sharp increase in insulin secretory rate (Fig. 1C) which was comparable in magnitude to that seen immediately after glucose addition (Fig. 1A). However, the insulin secretory rate then declined to values below the original basal value in spite of the continued presence of the ionophore (Fig. 1C). When both A23187 and TPA were added to the perifusate, there was an initial sharp increase in insulin secretory rate followed by a slight fall and then a slow rise to a

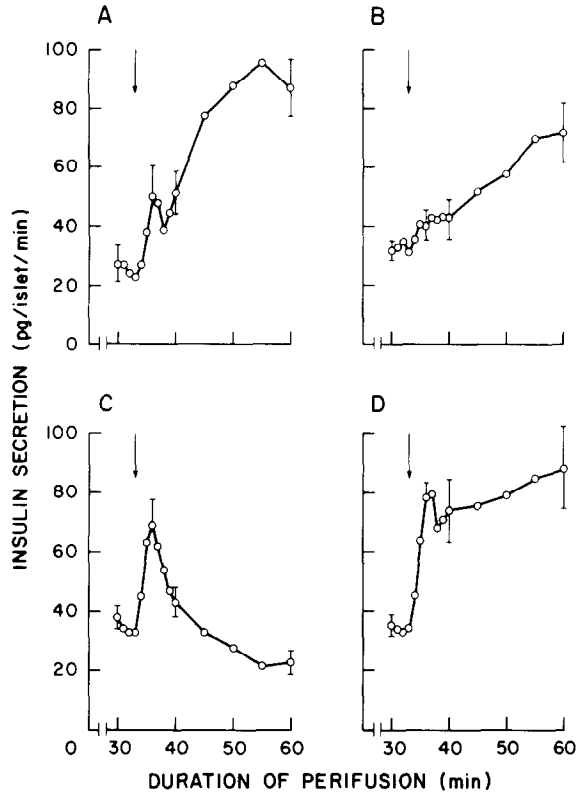

Figure 1. Influence of glucose, TPA, calcium ionophore (A23187), and the combination TPA and A23187 on insulin secretion from perifused islets. A. Islets were perifused with 2.75 mM glucose for thirty minutes and then exposed to 7 mM of the hexose (n = 4). In this and the other parts of the figure mean values of release and, for the sake of clarity, selected SEM's are given. B. The influence of 100 nM TPA on hormone release is demonstrated (n = 5). C. The effect of the calcium ionophore, A23187, used at a concentration of 2.5 μM is depicted (n = 10). D. The effect of combined TPA and ionophore on hormone release is shown (n = 8). Note the similarity between the temporal patterns of secretion depicted in A and D.

sustained plateau (Fig. 1D). Both qualitatively and quantitatively the response to perifusion with combined A23187 and TPA (Fig. 1D) was similar to that seen after perifusion with glucose (Fig. 1A). Both phases of the insulin secretory response to combined TPA and A23187 were dependent on calcium (Fig. 2). The intracellular concentration of cAMP determines the magnitude of the insulin secretory response to a given increase in glucose concentration (14). This is well illustrated by the data shown in Figure 3A. Islets were perifused with normal media containing either no addition, 25 μM forskolin, an agent known to activate adenylate cyclase (22),

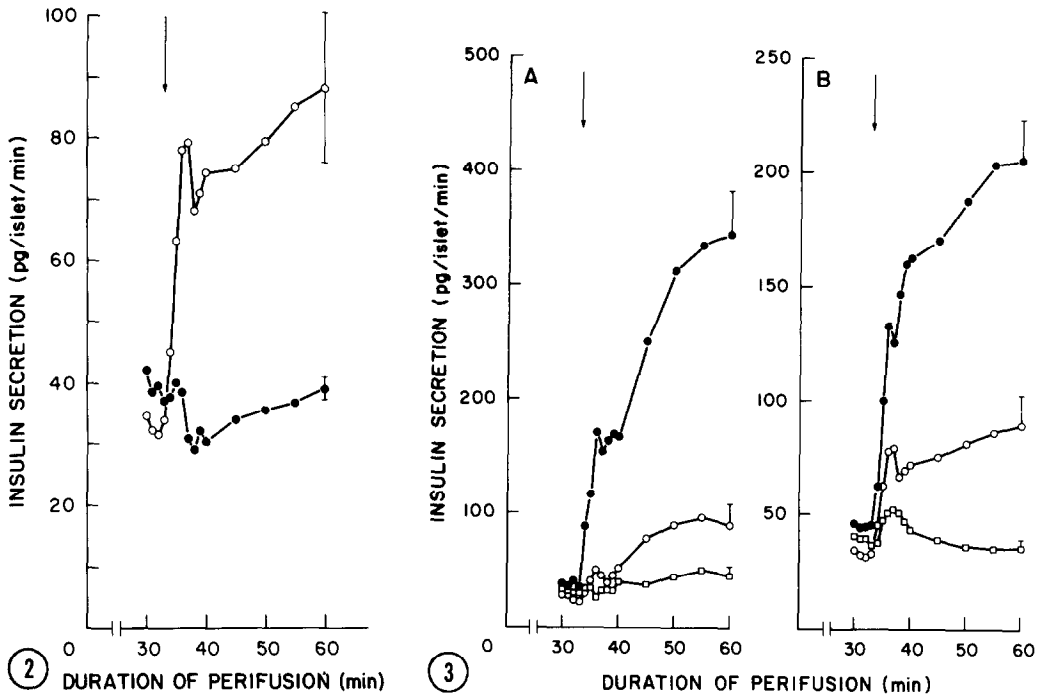

Figure 2. Influence of external calcium on the action of combined TPA and A23187. The effects of the addition of 100 nM TPA plus 2.5 μM A23187 in the presence of 2.2 mM $CaCl_2$ (○—○) or absence (●—●) of external calcium plus 1 mM EDTA on the time course of insulin secretion are shown (n = 3).

Figure 3. Influence of phenylephrine and forskolin on hormone release provoked by either glucose or the combination of TPA and ionophore.

A. Islets were perifused with 2.75 mM glucose for thirty minutes and then perifusate was changed to 7 mM glucose alone (○—○), 7 mM glucose and forskolin (●—●) or 7 mM glucose and phenylephrine (□—□). In those experiments using forskolin, both the forskolin and higher glucose level were presented to the islets at the same time. In the case of the phenylephrine perifusions, this compound was present during the final ten minutes of the 30 min stabilization period as well as during the stimulatory period (n = 4).

B. Islets were perifused for 30 minutes in the absence of any exogenous fuel. During the last 10 minutes of this period, islets were exposed to either 25 μM forskolin (●—●) or 25 μM phenylephrine (□—□). After this, the islets were perifused with a combination of 100 nM TPA and 2.5 μM A23187 either alone (○—○), with forskolin or with phenylephrine (n = 4). Note difference in scale of insulin secretion rates in part A and part B.

or phenylephrine, an agent known to inhibit adenylate cyclase (23). Islets pretreated with forskolin showed a greatly exaggerated response to glucose (Fig. 3A). Conversely, in islets pretreated with phenylphrine, both phases of the response were markedly inhibited (Fig. 3A). Similar experiments were carried out using combined TPA

and A23187 as secretagogues rather than glucose. When islets pretreated with forskolin were exposed to a combination of TPA and A23187, both phases of the insulin secretory response were enhanced 2-2.5-fold (Fig. 3B). Conversely, in islets pretreated with phenylephrine the ability of TPA plus A23187 to induce a rise in the rate of insulin secretion was impaired (Fig. 3B).

Discussion

Our previous studies demonstrated that the regulation of the secretion of a steroid hormone, aldosterone, from the adrenal cortex, appears to involve the flow of information through two branches of the calcium messenger system (8), and that these two branches serve distinct temporal roles: the calmodulin branch being largely responsible for the initiation of cellular response, the C-kinase branch for sustaining it. A similar relationship appears to pertain in the control of insulin secretion by glucose (Fig. 4). The flow of information through one branch, the calmodulin branch, can be initiated by addition of A23187 and is responsible for the initial phase of insulin secretion, and the flow through the second, the C-kinase branch, can be initiated by TPA addition and is responsible for the sustained phase of insulin secretion. The temporal integration of the flow of information via these two branches is responsible for the observed cellular response seen after glucose addition. Furthermore, each phase of the response is dependent on calcium (Fig. 2), and is influenced by the cAMP content of the islet (Fig. 3).

The C-kinase pathway provides a means by which a sustained cellular response can be achieved in the calcium messenger system under conditions of relatively low free intracellular Ca^{2+} (8,24) in which case the danger of mitochondrial (and cellular) calcium overload can be minimized (25,26). The C-kinase provides a type of gain control in this cellular control system. This conclusion leads to several further conclusions. First, factors which in-

fluence the initial component of a sustained cellular response are not necessarily the same as those influencing the sustained component of this response. Second, it is very likely that this role of the C-kinase pathway is widespread in both neural, endocrine, and exocrine secretion, smooth muscle contraction, and in the regulation of transcellular transport processes. Third, the combined use of A23187 and TPA should prove of considerable value in exploring the functions of the two separate branches of the calcium messenger system, and their tempoeral relationships. Finally, it should be emphasized that Ca^{2+} regulates the flow of information through both branches of this system (Fig. 2), but that the concentration of Ca^{2+} needed to activate the C-kinase branch is less than that needed to activate the calmodulin branch (1,26,27) because of the positive sensitivity modulation (25) of the C-kinase enzyme by diacylglycerol (or TPA) and membrane phospholipids (27).

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