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Evidence for modulation of cell-to-cell electrical coupling by cAMP in mouse islets of Langerhans

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The effects of forskolin on electrical coupling among pancreatic β-cells were studied. Two microelectrodes were used to measure membrane potentials simultaneously in pairs of islet β-cells. Intracellular injection of a current pulse (ΔI) elicited a membrane response ΔV_1 in the injected cell and also a response ΔV_2 in a nearby β-cell confirming the existence of cell-to-cell electrical coupling among islet β-cells. In the presence of glucose (7 mM), application of forskolin evoked a transient depolarization of the membrane and electrical activity suggesting that the drug induced a partial inhibition of the β-cell membrane K^+ conductance. Concomitant with this depolarization of the membrane there was a marked decrease in β-cell input resistance ($\Delta V_2/\Delta I$) suggesting that exposure to forskolin enhanced intercellular coupling. Direct measurements of the coupling ratio $\Delta V_2/\Delta V_1$ provided further support to the idea that forskolin enhances electrical coupling among islet cells. Indeed, application of forskolin reversibly increased the coupling ratio. These results suggest that cAMP might be involved in the modulation of electrical coupling among islet β-cells.

Forskolin; Electrical coupling; cyclic AMP; (Pancreatic β-cell)

1. INTRODUCTION

Cell-to-cell electrical coupling in mouse pancreatic islets has been shown to improve in the presence of glucose [1]. Glucose also increases the number of dye-coupled cells [2,3] and the density of gap junctions [4]. Furthermore, both glucose and glucagon-receptor stimulation of adenylate cyclase induce an increase in the cAMP content of pancreatic islets [5,6], which in turn stimulates the adhesion between cells and augments the number of gap junctions [7,8]. Experimental conditions known to increase the density of intercellular communications also increase glucose-evoked insulin release [9-11], suggesting that intercellular signalling plays a key role in the regulation of the secretory response of the islet. Intercellular communication may occur through structural coupling between cells in the islet as first suggested by the identification of gap junctions in the islet of Langerhans [12].

Since in other cell systems cAMP-dependent protein kinase raises junctional permeability [13], intercellular communication can be physiologically up-regulated by the cAMP signal system [13-19]. To examine the possibility that cAMP might be involved in the control of intercellular junctions, we studied the effects of forskolin, the most specific stimulator of adenylate cyclase, on electrical coupling. Previous studies of the effects of forskolin on mouse pancreatic β-cell showed a stimulation of electrical activity accompanied by an increase in insulin release [20-22]. However in these studies no use of the intracellular current injection technique was made and, therefore the information provided was restricted to the control of

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the β-cell membrane potential. In the present work we show that forskolin induced a measurable decrease in intercellular resistance which might be caused by cAMP mediated improvement of the electrical coupling among β-cells.

2. MATERIALS AND METHODS

Two modes of β-cell membrane potential recording were used in this work. In the differential mode, the β-cell membrane potential was measured between a pair of Ag/AgCl electrodes, one inside the microelectrode (180–300 MΩ) and the other inside the other microelectrode with the tip immersed in the chamber solution. In the nondifferential recording mode, the β-cell membrane potential was measured between the Ag/AgCl electrode inside the microelectrode and another earthed Ag/AgCl electrode in the chamber solution [1].

Microdissected mouse islets of Langerhans were superfused with modified Krebs solution (mM): 120 NaCl, 5 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$ and 1.1 $MgCl_2$. The solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 to keep the pH at 7.4. Experiments were performed at 37°C.

Input resistance was calculated from the membrane potential responses ΔV_1 to the intracellular application of current pulses ΔI. To study electrical coupling two nearby cells were impaled. Records in the nondifferential mode will be labelled as cell-1 and the membrane responses to

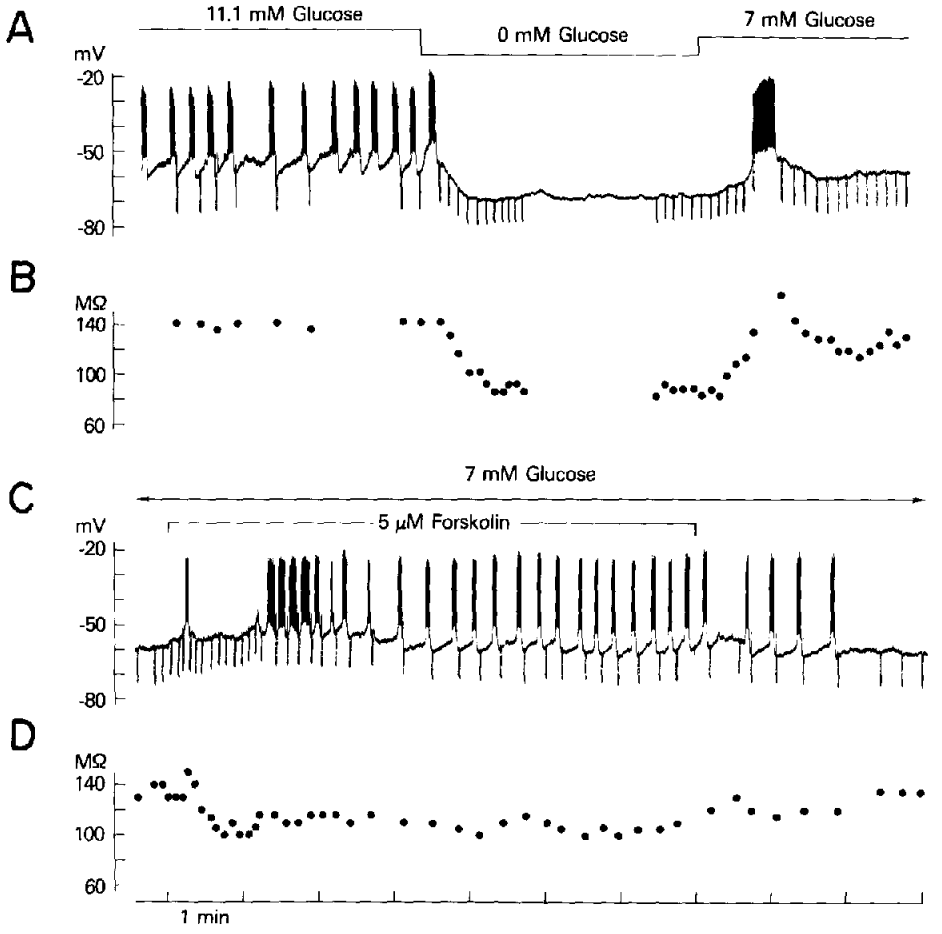

Fig.1. Comparison between the effects of glucose and forskolin on input resistance. (A) Effects of glucose on electrical activity. (B) Effects of glucose on input resistance. (C) Effects of forskolin on electrical activity (continuation of the record in A, 7 min elapsed). (D) Effects of forskolin on input resistance. DMSO 0.05% throughout.

pulses of current will be referred to as ΔV_1. Records in the differential mode will be labelled as cell-2 and the membrane responses to current pulses as ΔV_2.

For the present series of experiments we considered that if an impaled cell responded to 11 mM glucose with the characteristic burst pattern of electrical activity, the impaled cell was a β-cell. That this is a satisfactory criterion has been recently confirmed using the fluorescent dye lucifer yellow to mark an impaled cell responding to 11 mM glucose and a fluorescent antibody against insulin to identify the cell type [23].

3. RESULTS

It is generally accepted that the glucose sensing mechanism of the islet β-cell involves a profound decrease in K^+ conductance of the membrane evoked by an increase in glucose concentration [24]. Recent patch-clamp experiments on pancreatic β-cells in culture have shown that the β-cell membrane potential is under the control of at least two types of K^+ channels. One K^+ channel is activated by an elevation in $[Ca^{2+}]_i$ [25,26] and the other is blocked by an increase in $[ATP]_i$ [27]. The latter K^+ channel is also closed when glucose is present [24]. In this study we compared the physiological response to glucose described above with that produced by forskolin as illustrated in fig.1. In the presence of glucose (11 mM), the typical burst pattern of electrical activity consists of slow oscillations of membrane potential between a silent phase at -60 mV and an active phase at -54 mV (initial part of record in fig.1A). Upon the removal of glucose, the membrane hyperpolarized from -60 to -70 mV (fig.1A) and the β-cell input resistance decreased from 140 to 85 MΩ (fig.1B). Thus, the hyperpolarization of the β-cell membrane might result from the re-activation of K^+ channels closed by glucose. Since cell-to-cell electrical coupling decreases in the absence of glucose [1], the decrease in β-cell input resistance might reflect just the increase in β-cell membrane K^+ conductance. The effects of forskolin on the same electrical parameters are shown below (fig.1C,D). It may be seen that forskolin caused a slight transient depolarization of the membrane (fig.1C) suggesting a reduction in β-cell membrane K^+ conductance. Since this effect of forskolin would tend to increase input resistance and a substantial decrease (from 135 to 110 MΩ; fig.1D) was mesured instead, it seems logical to conclude that the intercellular resistance had been lowered by forskolin. A few minutes after the removal of forskolin, electrical activity ceased and input

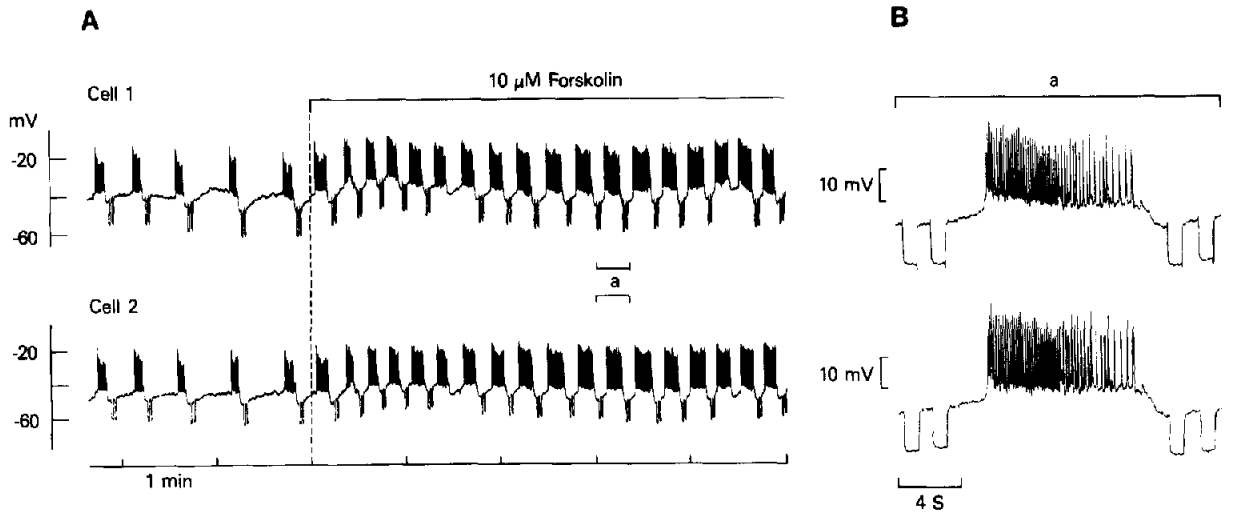

Fig.2. Effects of forskolin on electrical activity, input resistance and coupling between two nearby islet cells. (A) Hyperpolarizing current pulses (0.1 nA, 0.9 s) were applied on cell-1 during the silent phase of the bursts. (B) Bursts from part A (a) on expanded time base. Application of forskolin is indicated by the vertical dashed line (11 mM glucose and DMSO 0.1% throughout). Temperature 37°C.

resistance returned to its control value measured before the application of the drug.

The results presented in fig.1 provide indirect support for the idea that forskolin might stimulate intercellular electrical coupling. Measurements of the effects of forskolin on the values of the coupling ratio $\Delta V_2/\Delta V_1$ can be used to estimate any possible specific effects on the junctional resistance [1]. Fig.2 illustrates the results of one such an experiment. To minimize the contribution from the β-cell membrane resistance to the signal ΔV_1, the experiment was carried out in the presence of 11 mM glucose instead of the 7 mM used in the experiment illustrated in fig.1. At this concentration of glucose the K^+ conductance is greatly reduced. By minimizing the flow of current through non-junctional membrane one should, at least in principle, increase the fraction of the injected current flowing through the junctional membrane and thus, improve the coupling signal ΔV_2 [1]. However, at the concentration of glucose used (11 mM) the enhancement of the coupling ratio evoked by the sugar is close to saturation [1]. In the absence of forskolin the bursts of electrical activity in both cells occurred in perfect synchrony (initial part of records in fig.2). Addition of forskolin caused a clear transient depolarization of the membrane in both cells and a concomitant increase in the duration of the active phase of the bursts. Two segments of the records from cell-1 and cell-2 are shown in fig.2B using an expanded time base. The responses to hyperpolarizing current pulses applied to the β-cell appeared in the records as downward deflections during the silent phases between the bursts (ΔV_1). The time course of the changes in the responses ΔV_1 and ΔV_2 is shown in fig.3 together with the corresponding values for the coupling ratio $\Delta V_2/\Delta V_1$. Exposure of the islet to forskolin evoked a small but statistically significant decrease in ΔV_1 (fig.3A) and thus, a decrease in input resistance. As shown in fig.3C, forskolin induced a small but statistically significant increase in the coupling ratio $\Delta V_2/\Delta V_1$. Fig.4 illustrates the results from another cell pair. Forskolin induced an increase in the coupling ratio which was already apparent one minute after exposure of the islet to the drug. Under steady-state conditions the increase in $\Delta V_2/\Delta V_1$ amounted to 10%. Soon after the removal of forskolin the coupling ratio returned to control values. Two further experiments gave similar results.

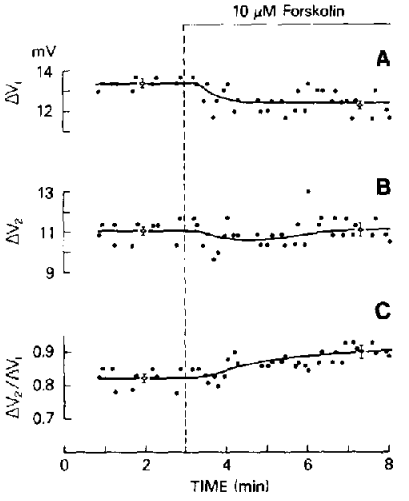

Fig.3. Effects of forskolin on coupling ratio. (A) Time course of the cell-1 responses (ΔV_1) to hyperpolarizing pulses (0.1 nA, 0.9 s). (B) Time course of ΔV_2 (responses from cell-2). (C) Time course of the coupling ratio ($\Delta V_2/\Delta V_1$). (◇) Averages of the control values and of the 10 last points of each graph. Vertical bars represent ± SE.

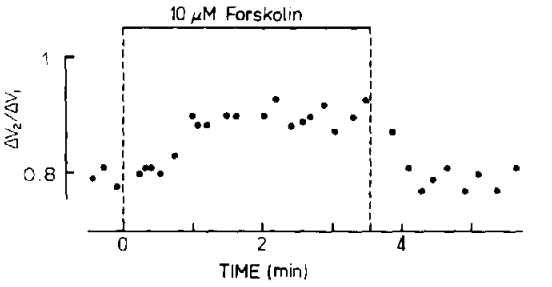

Fig.4. Reversibility of the effect of forskolin on cell-to-cell electrical coupling. Experiment performed in the presence of 11 mM glucose, at 37°C. DMSO 0.1% throughout.

4. DISCUSSION

The data presented here strongly suggest that activation of the catalytic subunit of the β-cell adenylate cyclase enhances cell-to-cell electrical coupling. The simplest interpretation of this result is to assume that exposure of the islet to forskolin stimulates cAMP production and that the enhancement of the electrical coupling is mediated by

cAMP. It has already been shown that forskolin increases cAMP in mouse islets of Langerhans.

In other cell systems, stimulated levels of cAMP have been correlated with an increase in the number of gap junctions as well as intercellular dye spread [16]. In cultured cells from neonatal rat islets, the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) was found to increase the number of dye-coupled cells [3]. While stimulated levels of cAMP enhance coupling, reduced levels of cAMP by somatostatin-evoked inhibition of adenylate cyclase appear to decrease the number of dye-coupled cells [3]. Consistently, cultured rat islets exhibit a low density of gap junctions as well as a decreased level of cytosolic cAMP. When these islets were incubated in the presence of external dbcAMP or theophylline they recovered the levels of cAMP and the number of gap junctions [8]. All these effects described in inslets cells have involved cAMP in a long term regulation of intercellular communications probably by controlling the formation of gap junctions and the number of particles per gap junction. However, our study shows, for the first time, a substantial improvement in cell-to-cell electrical coupling soon after the application of forskolin thus, suggesting that cAMP might also be involved in the short term regulation of intercellular communications which determines the number of open channels. On the bases of these data, we propose that phosphorylation of junctional proteins through a cAMP-dependent protein kinase might be involved. Saez and collaborators [18] have recently shown in hepatocytes that junctional conductance was increased by cAMP-dependent phosphorylation of the junctional polypeptide.

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