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EFFECT OF CALCIUM ANTAGONISTS ON POTASSIUM CONDUCTANCE IN ISLET CELLS

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Abstract—Verapamil and the ion cobalt, both considered as calcium antagonists, provoked a rapid, dose-related, and rapidly reversible inhibition of ^{86}Rb outflow from prelabelled pancreatic islets. The decrease in ^{86}Rb outflow was not affected by the concentration of extracellular calcium, identical results being obtained at normal (1 mM) and high calcium concentration (5 mM), as well as in the absence of calcium and presence of EGTA. The capacity of glucose in high concentration (11.1–27.2 mM) to inhibit ^{86}Rb outflow was considerably decreased in islets exposed to either verapamil or cobalt. These findings suggest that verapamil and cobalt may not be adequate tools to distinguish between changes in K^+ conductance and calcium inflow, respectively, as determinants of the bioelectrical response of the pancreatic β-cell to glucose.

There is increasing evidence to suggest that changes in membrane potential play a major role in the mechanisms by which glucose stimulates the release of insulin from the pancreatic β-cell [1–4].

A current view is that glucose depolarizes the β-cell membrane and, by doing so, causes the gating of voltage-sensitive Ca^{2+} channels [5]. This leads to a rise in the intracellular concentration of Ca^{2+} which is thought to trigger the release of insulin [6]. The mechanism by which glucose depolarizes the β-cell is not fully elucidated. When the glucose concentration of the extracellular milieu is increased from a non-stimulatory to an insulinotropic value, the membrane first slowly depolarizes from a resting to a threshold potential. When the latter is reached, a rapid and further depolarization is observed which brings the membrane to a plateau potential on which spike activity is superimposed [7]. It was initially proposed that the slow depolarization results mainly from a decrease in K^+ conductance of the β-cell membrane, and that the rapid depolarization reflects the gating of voltage-sensitive Ca^{2+} channels [7, 8]. However, recent observations suggest that both the slow and the rapid depolarization may result from a decrease in K^+ conductance and that the gating of voltage-sensitive Ca^{2+} channels only occurs when the membrane has reached the plateau potential. Thus, when the β-cell membrane resistance is measured at frequent intervals during continuous exposure to glucose, the membrane resistance is highest at the onset of the plateau potential, before spike activity takes place [9]. Moreover, it was observed that in the presence of organic and inorganic Ca^{2+} antagonists (e.g. verapamil or cobalt) glucose is still able to depolarize the β-cell to the plateau potential [7, 10, 11]. The latter finding suggests that the depolarization to the plateau potential does not require the entry of Ca^{2+} into the islet cells, since the latter phenomenon is precisely inhibited by either verapamil [12] or cobalt [13]. However, in interpreting these data, the assumption is implicitly made that neither verapamil or cobalt exert any direct effect upon K^+ conductance.

The aim of the present work is to investigate whether verapamil or cobalt may affect K^+ conductance in islet cells. It is shown that both cobalt and verapamil markedly reduce K^+ conductance in islet cells. In addition, it is shown that these agents, when used at a sufficiently high concentration, considerably decrease the magnitude of the inhibitory effect of glucose on K^+ conductance.

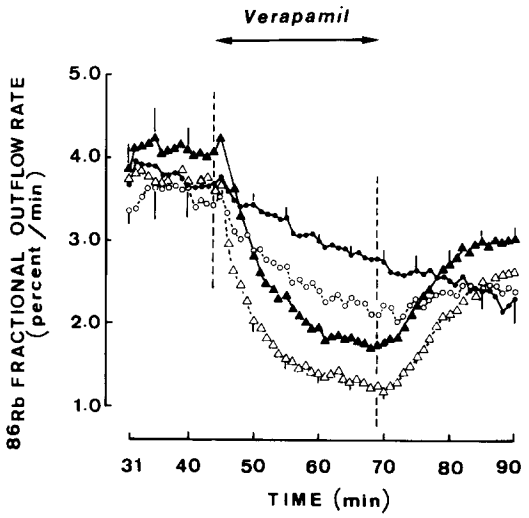

Fig. 1. Effect of increasing concentrations of verapamil (●—●: 1 μM; ○--○: 5 μM; ▲—▲: 10 μM; △--△: 20 μM) upon ^{86}Rb efflux from perfused islets. Mean values (±S.E.M.) for ^{86}Rb efflux are expressed as a fractional outflow rate and refer to 4 individual experiments in each case.

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MATERIALS AND METHODS

All experiments were performed with islets isolated by the collagenase technique [14] and removed from fed albino rats.

Incubation, washing and perfusion media

The media used for incubating, washing or perfusing the islets consisted of a Krebs–Ringer bicarbonate-buffered solution supplemented with 0.5% (w/v) dialyzed albumin (fraction V; Sigma Chemical Company, St. Louis, MO) and equilibrated against a mixture of O_2 (95%) and Co_2 (5%). Some media contained no $CaCl_2$ and were enriched with 0.5 mM ethylene glycol bis-(β-aminoethylether)-*N*,*N*′-tetra-acetic acid (EGTA). The media also contained verapamil (Veride S.A., 1920 Diegem, Belgium), cobalt and glucose, when required.

Experimental procedure

The method used for the measurement of ^{86}Rb efflux from perfused islets has been described elsewhere [15]. In brief, groups of 100 islets each were incubated in the presence of ^{86}Rb (0.3–0.6 mM; 100 μCi/ml) and 16.7 mM glucose. After incubation the islets were washed three times and then perfused. From the 31st to the 90th min, the effluent was continuously collected over successive periods of 1 min each and examined for its radioactive content by scintillation counting. The efflux of ^{86}Rb is expressed as a fractional outflow rate (percentage of instantaneous islet content per min). The validity of ^{86}Rb as a tracer for the study of $^{39}K^+$ handling in the islets has been assessed elsewhere [15].

Calculations and statistics

All results are expressed as the mean (±S.E.M.) together with the number of individual experiments (*n*). The statistical significance of differences between mean experimental and control data was assessed by use of Student's *t*-test.

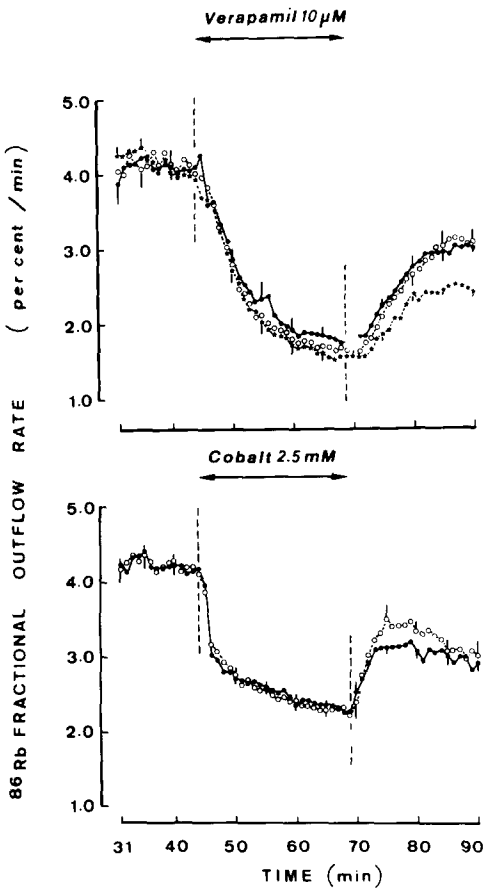

Fig. 2. Upper panel: Effect of 10 μM verapamil upon ^{86}Rb efflux from islets perfused in the absence (○--○) or presence of 1 mM (●—●) or 5 mM (×--×) Ca^{2+}. The Ca^{2+}-deprived media contained EGTA (0.5 mM). Lower panel: Effect of 2.5 mM cobalt upon ^{86}Rb efflux from islets perfused in the absence (○--○) or presence (●—●) of 1 mM Ca^{2+}. The Ca^{2+}-deprived media contained EGTA (0.5 mM). Mean values (±S.E.M.) for ^{86}Rb efflux are expressed as a fractional outflow rate and refer to 4 individual experiments in each case.

RESULTS

Effect of verapamil and cobalt upon ^{86}Rb efflux

Figure 1 shows that verapamil provoked a rapid and dose-related decrease in ^{86}Rb efflux from islets perfused in the absence of glucose. The effect of verapamil was rapidly reversible and statistically significant at all concentrations of the drug tested. Thus, the mean slope of the regression line characterizing the changes in ^{86}Rb fractional outflow rate averaged -0.015 ± 0.004 during basal period (min 33–44) and

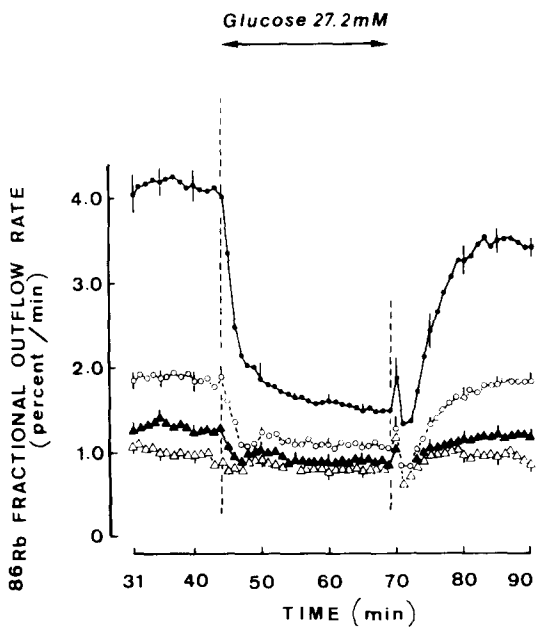

Fig. 3. Effect of 27.2 mM glucose upon ^{86}Rb efflux from islets perfused in the absence (●—●) or presence of 10 μM (○--○), 20 μM (▲—▲), 40 μM (△--△) verapamil. Mean values (±S.E.M.) for ^{86}Rb efflux are expressed as a fractional outflow rate and refer to 4 individual experiments in each case.

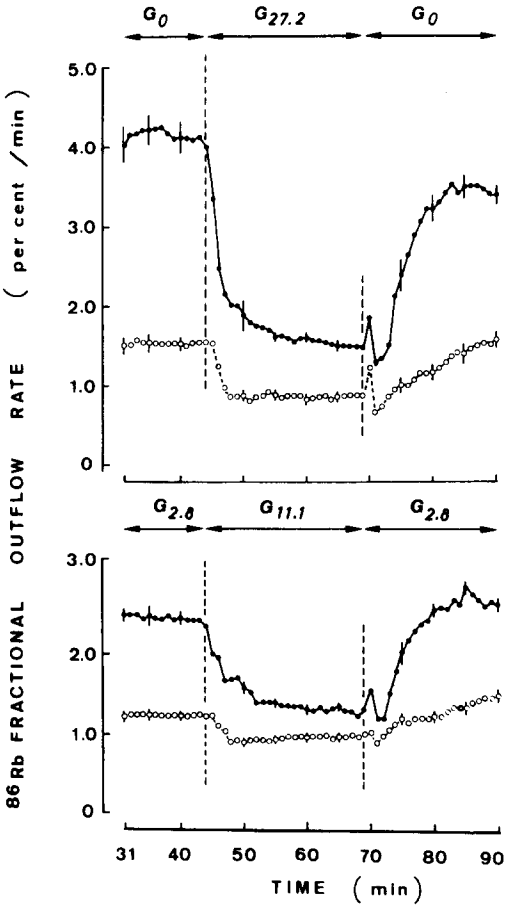

Fig. 4. Upper panel: Effect of 27.2 mM glucose upon ^{86}Rb efflux from islets perfused in the absence (●—●) or presence (○--○) of 2.5 mM cobalt. Lower panel: Effect of a rise in the glucose concentration from 2.8 to 11.1 mM on ^{86}Rb efflux from islets perfused in the absence (●—●) or presence (○--○) of 5 mM cobalt. Mean values (±S.E.M.) for ^{86}Rb efflux are expressed as a fractional outflow rate and refer to 4 individual experiments in each case.

fell to -0.045 ± 0.006 ($P < 0.005$), -0.075 ± 0.03 ($P < 0.001$), -0.189 ± 0.038 ($P < 0.001$) and -0.208 ± 0.019 ($P < 0.001$) in the presence of 1, 5, 10 and 20 μM verapamil (min 44–55), respectively. Likewise, 2.5 mM cobalt provoked a rapid and rapidly reversible decrease in ^{86}Rb efflux (Fig. 2, lower panel). The magnitude of the latter decrease was comparable to that induced by 5 μM verapamil (Fig. 1). The inhibition of ^{86}Rb efflux by verapamil or cobalt was unaffected by external Ca^{2+} (Fig. 2).

Effect of verapamil and cobalt upon the glucose-induced decrease in ^{86}Rb efflux

In the absence of verapamil or cobalt, 27.2 mM glucose provoked a rapid and sustained decrease in ^{86}Rb efflux (Fig. 3, Fig. 4 upper panel). Increasing concentrations of verapamil, when present from the onset of the perfusion, produced a dose-related decrease in the basal ^{86}Rb fractional outflow rate observed in the absence of glucose and progressively decreased the magnitude of the inhibitory effect of glucose on ^{86}Rb efflux (Fig. 3). In agreement with the data presented in Fig. 1, 10 μM verapamil displayed the same ability to reduce ^{86}Rb efflux than 27.2 mM glucose. At a concentration of 40 μM, verapamil abolished the inhibitory effect of 27.2 mM glucose on ^{86}Rb fractional outflow rate, the sugar now producing a small, delayed and transient increase in ^{86}Rb fractional outflow rate. Cobalt (2.5 mM), like verapamil dramatically decreased basal ^{86}Rb fractional outflow rate and markedly reduced the fall in ^{86}Rb efflux evoked by 27.2 mM glucose (Fig. 4, upper panel). In order to mimic the experimental conditions used in electrophysiological studies, the effect of a rise in the glucose concentration from 2.8 to 11.1 mM upon ^{86}Rb efflux, in the absence or presence of 5 mM cobalt was investigated [7]. In the absence of cobalt and presence of 2.8 mM glucose, the efflux of ^{86}Rb slowly decreased with time. A rise in the glucose concentration from 2.8 to 11.1 mM provoked a rapid, sustained and rapidly reversible decrease in ^{86}Rb fractional outflow rate (Fig. 4, lower panel). Cobalt (5 mM), when present from the onset of the perfusion, almost completely suppressed the latter decrease in ^{86}Rb fractional outflow rate.

DISCUSSION

The present data clearly show that cobalt and verapamil, at concentrations commonly used to interfere with Ca^{2+} entry in various biological systems, profoundly inhibit K^+ conductance in islet cells. This confirms and extends previous observations showing inhibition by verapamil of ^{42}K efflux from canine cardiac Purkinje fibres [16] and ^{86}Rb efflux from rat pancreatic islets [17]. Cobalt was also previously shown to inhibit K^+ conductance in squid axon [18]. That cobalt reduces K^+ conductance in islet cells is compatible with the observation that exposure of islet cells simultaneously to cobalt and to a high concentration of extracellular Ca^{2+} (10 mM) slightly reduces ^{86}Rb efflux from perfused islets [19], while exposure of the islets to a high concentration of Ca^{2+} in the absence of cobalt increases ^{86}Rb outflow rate [17, 19].

The inhibitory effect of cobalt or verapamil on K^+ conductance was comparable in both its magnitude and time course to that exerted by either glucose itself or quinine, a specific inhibitor of K^+ conductance in islet cells [20, 21].

It was reported that a rise in the glucose concentration of the extracellular medium from 2.8 to 11.1 mM simultaneously with the addition of 5 mM cobalt to the latter medium provokes a slow depolarization of the β-cell membrane from the resting to the plateau potential [7]. These data were taken as evidence to indicate that the glucose-induced β-cell depolarization mainly results from a decrease in membrane conductance to K^+ [7]. Although not denying this hypothesis, the present observation that cobalt (or verapamil), when present from the onset of the perfusion almost completely abolished the inhibitory effect of glucose on ^{86}Rb efflux suggests that cobalt or verapamil may not be appropriate tools to demonstrate that glucose depolarizes the β-cell by reducing membrane permeability to K^+.

The fact that in the presence of cobalt or verapamil, glucose depolarizes the membrane to the plateau potential was also taken as evidence that such a depolarization is not attributable to the entry of

Ca^{2+} into the islet cells [11]. In view of the present results, the possibility that cobalt and verapamil may have themselves significantly contributed to the latter depolarization calls for carefulness in the interpretation of experiments in which cobalt or verapamil are used to investigate the ionic basis of glucose-induced membrane depolarization. In any case, we feel that it should not be ruled out that under normal conditions, the rapid depolarization is indeed attributable to Ca^{2+} influx, and that such a process prevents the much slower and progressive depolarization seen in the presence of cobalt from taking place [22].

In view of the existence, in islet cells, of a Ca^{2+}-activated K^+ extrusion, the effect of verapamil or cobalt on K^+ conductance could be viewed as a consequence of inhibited Ca^{2+} influx into the β-cell with a subsequent fall in intracellular Ca^{2+} concentration. Such a view was indeed proposed to explain the depolarizing effect of Ca^{2+} antagonists in the pancreatic β-cell [10]. However, the present finding that the inhibitory effects of verapamil and cobalt on K^+ conductance were not affected in the absence of extracellular Ca^{2+} rather suggests that the Ca^{2+} antagonists reduced K^+ conductance by a direct effect on K^+ channels, rather than by decreasing the intracellular Ca^{2+} concentration. The view that verapamil exerts a primary effect on K^+ transport is compatible with the knowledge that, in an artificial system for the study of ionophoresis verapamil inhibits the translocation of both monovalent and divalent cations mediated by suitable ionophores [23]. Moreover, our experiments indicate that the inhibitory effect of verapamil persisted at a high concentration of Ca^{2+} (4 mM), although high concentrations of extracellular Ca^{2+} competitively antagonize the inhibitory effect of verapamil upon Ca^{2+} inflow into the islets [24].

Although our data clearly demonstrate that cobalt and verapamil do not act solely upon Ca^{2+} movements in the islet cells, the present findings do not detract from the view that the inhibitory effect of these drugs upon insulin release is attributable to their Ca^{2+} antagonistic property [12, 13]. Indeed, if it were to be the sole effect of these drugs, the decrease in ^{86}Rb outflow, which mimics the effect of glucose on K^+ conductance, should cause facilitation rather than inhibition of insulin release.

In conclusion, the present work shows that cobalt and verapamil, often referred to as specific Ca^{2+}-antagonists, markedly reduce K^+ conductance in islet cells. This finding suggests that cobalt and verapamil may not be adequate tools to distinguish between changes in K^+ conductance and Ca^{2+} inflow, respectively, as determinants of the bioelectrical response of the β-cell to glucose.

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