Verapamil, a phenylalkylamine Ca^{2+} channel blocker, inhibits ATP-sensitive K^+ channels in insulin-secreting cells from rats

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Summary Radioisotopic and electrophysiological techniques were used to assess the effects of verapamil, a phenylalkylamine Ca^{2+} channel blocker, on K^+ permeability of insulin-secreting cells. Verapamil provoked a concentration-dependent inhibition of ^{86}Rb (^{42}K substitute) outflow from prelabelled and perfused rat pancreatic islets. This property appears to be inherent to the phenylalkylamine Ca^{2+} channel blockers since gallopamil, a methoxyderivative of verapamil, but not nifedipine, a 1,4-dihydropyridine Ca^{2+} channel blocker, inhibited ^{86}Rb outflow. The experimental data further revealed that verapamil interacted with a Ca^{2+} -independent, glucose- and glibenclamide-sensitive modality of ^{86}Rb extrusion. Moreover, verapamil prevented the increase in ^{86}Rb outflow brought about by BPDZ 44; a potent

activator of the ATP-sensitive K^+ channel. Single-channel current recordings by the patch clamp technique confirmed that verapamil elicited a dose-dependent inhibition of the ATP-dependent K^+ channel. Lastly, under experimental conditions in which verapamil clearly inhibited the ATP-sensitive K^+ channels, the drug did not affect ^{45}Ca outflow, the cytosolic free Ca^{2+} concentration or insulin release. It is concluded that the Ca^{2+} entry blocker verapamil inhibits ATP-sensitive K^+ channels in pancreatic beta cells. This effect was not associated with stimulation of insulin release [Diabetologia (1997) 40: 1403–1410].

Keywords Verapamil, ATP-sensitive K^+ channel, rat pancreatic beta cells, insulin release.

Calcium channel blockers may be defined as drugs which inhibit the flow of calcium ions through Ca^{2+} -conducting channels. The wide variety of chemically unrelated substances described as calcium channel blockers includes the organic compound verapamil [1,2]. Verapamil is the prototype phenylalkylamine Ca^{2+} entry blocker (Fig. 1). The drug is known to interact preferentially with a binding site located within the L-type voltage-sensitive Ca^{2+} channel. Moreover, the blocking potency of verapamil, which is

ionized at physiological pH, has been shown to exhibit voltage and use-dependence [1,3,4]. Although the therapeutic indications for the numerous Ca^{2+} entry blockers are not limited to cardiovascular disorders, verapamil is reported to provide effective therapy for patients with angina pectoris, supraventricular tachyarrhythmias, arterial hypertension and hypertrophic cardiomyopathy [1,2].

Several studies, conducted on different cell types, suggested additional actions of Ca^{2+} entry blockers. Thus, some of them might affect the Na^+ and K^+ currents, the Na^+ - Ca^{2+} exchange or ATP-dependent Ca^{2+} transport processes [1–3]. More than a decade ago, long before a detailed characterization of the different types of ionic channels equipping the pancreatic beta-cell membrane was provided, it was reported that verapamil inhibited ^{86}Rb outflow from prelabelled rat pancreatic islets [5,6]. Such findings were taken as indirect evidence that the drug reduced

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Abbreviations: FOR, Fractional outflow rate; K^+_{ATP} channels, ATP-sensitive K^+ channels.

Fig. 1. Chemical structure of different Ca²⁺ entry blockers and K⁺ channel modifiers

the K⁺ permeability of pancreatic beta cells. The identity of K⁺ channels affected by the phenylalkylamine was, however, not determined.

Because K⁺ channels play a crucial role in the control of the insulin releasing process, we have reassessed in the present study the effect of verapamil on beta-cell K⁺ permeability.

Materials and methods

Animals. All experiments were performed with pancreatic islets isolated by the collagenase method from fed female albino rats (± 200 g) (Proefdierencentrum, Heverlee, Belgium). Principles of laboratory animal care as well as Belgian regulations were strictly followed.

Measurements of insulin release from incubated pancreatic islets. Groups of 10 islets, each derived from the same batch of islets, were preincubated for 30 min at 37°C in 1 ml of a bicarbonate-buffered solution (in mmol/l: 115 NaCl, 5 KCl, 2.56 CaCl₂, 1 MgCl₂, 24 NaHCO₃) supplemented with 2.8 mmol/l glucose, 0.5% (w/v) dialysed albumin (fraction V) and equilibrated with a mixture of O₂ (95%) and CO₂ (5%). The islets were then incubated at 37°C for a further 90 min in 1 ml of the same bicarbonate buffered medium containing 0, 5.6 or 11.1 mmol/l glucose and, in addition, increasing concentrations of verapamil. Individual experiments were carried out as triplicate measurements with the values corresponding to the number of samples pooled. Due to the islet-related variations, insulin release was expressed as percentage of the value recorded in control experiments (100%), i.e. in the absence of drug and presence of 0, 5.6 or 11.1 mmol/l glucose. The release of insulin was measured radioimmunologically using rat insulin as a standard [7].

Measurements of ⁸⁶Rb, ⁴⁵Ca outflow and insulin release from perfused pancreatic islets. The methods used to measure ⁸⁶Rb

(⁴²K substitute) outflow, ⁴⁵Ca outflow and insulin release from perfused islets have been described previously [8,9]. The outflow of ⁸⁶Rb or ⁴⁵Ca (cpm/min) was expressed as a fractional outflow rate (% of instantaneous islet content/min; FOR). The validity of ⁸⁶Rb as a tracer for the study of K⁺ handling in the islets has been previously assessed [10].

Patch clamp measurements. Electrophysiological studies were carried out with the patch clamp technique [11] on primary cultured rat islet cells, as previously described [12]. Insulin secreting cells were selected on the basis of their larger size [13]. Single-channel currents were recorded using the cell-free, inside-out patch-clamp configuration. Unitary current events from K⁺_{ATP} channels were recorded at a membrane potential of 0 mV. Under these experimental conditions, with the intracellular Ca²⁺ buffered to below 10 nmol/l, openings of Ca²⁺- and voltage-gated K⁺ channels were not observed [14,15]. Modulation of K⁺_{ATP} channels was described in terms of a change in the channel open-state probability [15]. In the record illustrated, upward deflections from the baseline represent outward single-channel events.

Measurements of fura 2 fluorescence from single islet cells. Pancreatic islets were disrupted in a Ca²⁺-deprived medium and then centrifuged through an albumin solution to remove debris and dead cells. Cells were seeded onto glass coverslips and maintained in tissue culture for 72 h. Fura-2 fluorescence of single loaded cells (selected on the basis of their larger size) was measured by use of dual-excitation microfluorimetry with a Spex photometric system (Optilas, Alphen aan den Rijn, Holland [15]). The excitation and emission wavelengths were set at 340/380 and 510 nm, respectively. [Ca²⁺]_i was calculated as previously described [16]. All experiments were repeated at least 3 times, on different days.

Drugs. In some experiments, extracellular Ca²⁺ was eliminated by the omission of CaCl₂ from the physiological medium and the addition of 0.5 mmol/l EGTA (ethylene glycol-bis [β -aminoethyl ether] N,N'-tetraacetic acid) (Sigma Chemical Co., St. Louis, MO, USA). The media also contained (as required) glucose (Merck, Darmstadt, Germany), verapamil hydrochloride (Knoll A.G., Ludwigshafen, Germany), glibenclamide (D600, Knoll A.G., Ludwigshafen), nifedipine (Bayer, Wuppertal, Germany), BPDZ 44 (synthesized at the Department of Medicinal Chemistry, University of

Table 1. Effects of verapamil on insulin secretion from incubated rat pancreatic islets

	No glucose	5.6 mmol/l glucose	11.1 mmol/l glucose
Verapamil 10 μ mol/l	105 $\pm$ 7 (12)	106 $\pm$ 8 (14)	41 $\pm$ 3 (23)
Verapamil 100 μ mol/l	105 $\pm$ 5 (22)	116 $\pm$ 8 (11)	28 $\pm$ 2 (14)

Rat pancreatic islets incubated for 90 min in the absence or presence of extracellular glucose. Insulin release (mean values $\pm$ SEM) expressed as a percentage of the value recorded in control experiments (100%; no verapamil and presence of either 0, 5.6 or 11.1 mmol/l glucose). Figures in parentheses refer to number of samples

Liège, Belgium) and glibenclamide (Hoechst Roussel, Brussels, Belgium). BPDZ 44, nifedipine and glibenclamide were dissolved in dimethylsulphoxide which was added to both control and test media at final concentrations not exceeding 0.1 % (v/v). At this concentration, dimethylsulphoxide fails to affect islet function [15, 17]. Experiments were conducted with minimal light to prevent photodegradation of the dihydropyridines. When high concentrations of extracellular K⁺ were used, the concentration of extracellular NaCl was lowered to keep the osmolarity constant.

Calculations. The basal value for ⁸⁶Rb or ⁴⁵Ca outflow was computed from min 40–44 of perfusion inclusive. The inhibitory effect of verapamil on ⁸⁶Rb outflow was taken as the difference between the mean value for ⁸⁶Rb outflow recorded in each individual experiment between the min 40–44 and 60–68 of perfusion. The magnitude of the increase in ⁸⁶Rb or ⁴⁵Ca outflow was estimated in each individual experiment from the integrated outflow of ⁸⁶Rb or ⁴⁵Ca observed during stimulation (min 45–68) after correction for basal value (min 40–44).

Statistical analysis. Results are expressed as mean $\pm$ SEM. The statistical significance of differences between mean data was assessed by using Student's *t*-test or by analysis of variance for multiple comparisons followed by a Scheffe test procedure.

Results

Effects of verapamil on insulin release from incubated rat pancreatic islets. The addition of verapamil (10 and 100 μ mol/l) to islets incubated in a glucose-free medium did not modify the insulin output ($p > 0.05$) (Table 1). In islets exposed to 5.6 mmol/l glucose, 10 μ mol/l verapamil failed to affect ($p > 0.05$) while 100 μ mol/l verapamil tended to slightly, although not significantly, increase insulin release ($p > 0.05$) (Table 1). When the incubation medium was enriched with an insulintropic glucose concentration (11.1 mmol/l), verapamil provoked a marked decrease in insulin output ($p < 0.05$) (Table 1). This inhibitory effect of verapamil was concentration-dependent.

Effects of verapamil on ⁸⁶Rb, ⁴⁵Ca outflow and insulin release from pancreatic islets perfused in the absence of extracellular glucose. In the absence of glucose in

Fig. 2. Effect of verapamil (10 μ mol/l) on ⁸⁶Rb (upper panel), ⁴⁵Ca outflow (middle panel) and insulin release (lower panel) from islets perfused throughout in the presence (●) or absence (○) of extracellular Ca²⁺. Basal media contained no glucose. Mean values $\pm$ SEM refer to 4 individual experiments

the extracellular medium, verapamil (10 μ mol/l) provoked a rapid and sustained inhibition of ⁸⁶Rb outflow (Fig. 2, upper panel). On removal of verapamil from the perfusate, an increase in ⁸⁶Rb outflow rate was consistently noticed. The capacity of verapamil (10 μ mol/l) to inhibit ⁸⁶Rb FOR was identical whether the perfusing medium contained or was deprived of extracellular Ca²⁺ (Fig. 2, upper panel). Thus, the paired difference in ⁸⁶Rb FOR before (min 40–44) and during (min 60–68) exposure to verapamil averaged 2.43 ± 0.12 %/min in the presence and 2.57 ± 0.08 %/min in the absence of extracellular Ca²⁺, respectively ($p > 0.05$).

In the absence or presence of extracellular Ca²⁺, verapamil (10 µmol/l) did not affect ⁴⁵Ca outflow from perfused pancreatic islets (Fig. 2, middle panel).

Verapamil (10 µmol/l) also failed to modify the insulin output from islets exposed to a glucose-deprived and Ca²⁺-containing medium (Fig. 2, lower panel).

At a higher concentration (100 µmol/l), verapamil again provoked a rapid, sustained and reversible decrease in ⁸⁶Rb outflow. The magnitude of the inhibitory effect of 100 µmol/l verapamil (3.67 ± 0.12 %/min) was unaffected by the removal of extracellular Ca²⁺ (4.10 ± 0.33 %/min; $p > 0.05$) but was more pronounced than that recorded at a lower concentration (10 µmol/l; $p < 0.05$) (data not shown). Moreover, exposure to verapamil (100 µmol/l) did not increase ⁴⁵Ca outflow or the insulin secretory rate (data not shown).

Incidentally, verapamil (10 µmol/l) inhibited the increase in ⁴⁵Ca outflow brought about by a rise in the extracellular K⁺ concentration from 5 to 50 mmol/l (data not shown). The integrated outflow of ⁴⁵Ca observed during K⁺ stimulation averaged 1.35 ± 0.12 %/min in the absence and 0.87 ± 0.17 %/min in the presence of 10 µmol verapamil ($p < 0.05$).

Effects of gallopamil and nifedipine on ⁸⁶Rb outflow from pancreatic islets perfused in the absence of extracellular glucose. Gallopamil (10 µmol/l), a methoxy derivative of verapamil, induced a marked reduction in ⁸⁶Rb outflow from islets perfused in the absence of extracellular glucose (Fig. 3). The pattern of such an inhibitory effect was not altered in the absence of extracellular Ca²⁺ (data not shown, $p > 0.05$). The slow increase in ⁸⁶Rb outflow recorded on removal of the drug reflects relief from inhibitory effect of gallopamil.

By contrast, the 1,4-dihydropyridine nifedipine did not affect the ⁸⁶Rb FOR which slowly decreased throughout the perfusion period (Fig. 3). Whether the physiological medium contained or was deprived of extracellular Ca²⁺ (data not shown), nifedipine (10 µmol/l) failed to modify the rate of ⁸⁶Rb outflow. The addition of nifedipine (10 µmol/l) also failed to affect the ⁴⁵Ca FOR from islets exposed throughout to a glucose-free medium and perfused in the presence or absence of extracellular Ca²⁺ (data not shown).

Effects of verapamil on ⁸⁶Rb, ⁴⁵Ca outflow and insulin release from islets perfused in the absence and presence of glucose or glibenclamide. As mentioned above, addition of verapamil (10 µmol/l) to a glucose-free medium elicited a rapid, marked and slowly reversible decrease in ⁸⁶Rb outflow (Fig. 4, left panel). The presence of increasing concentrations of glucose in the physiological medium reduced in a dose-dependent manner the basal ⁸⁶Rb outflow ($p < 0.05$, in each case) and attenuated the effect of verapamil (Fig. 4, left

Fig. 3. Effect of gallopamil (●; 10 µmol/l) and nifedipine (○; 10 µmol/l) on ⁸⁶Rb outflow from perfused pancreatic islets. Basal media contained extracellular Ca²⁺ and no glucose. Mean values $\pm$ SEM refer to 8 individual experiments.

panel). Indeed, in the presence of 5.6 and 11.1 mmol/l glucose throughout, the magnitude of the inhibitory effect of verapamil (10 µmol/l) was reduced by 65 % ($p < 0.05$) and 86 % ($p < 0.05$), respectively.

Verapamil (10 µmol/l) did not affect ⁴⁵Ca outflow or insulin output from islets perfused throughout in the presence of 5.6 mmol/l glucose and extracellular Ca²⁺ (data not shown). However, the addition of verapamil (10 and 100 µmol/l) induced a concentration-dependent decrease in both the ⁴⁵Ca outflow and the insulin secretory rate from islets perfused throughout in the presence of 11.1 mmol/l glucose and extracellular Ca²⁺ (data not shown). In islets exposed to a Ca²⁺-free medium containing either 5.6 or 11.1 mmol/l glucose, the addition of verapamil (10 µmol/l) failed to cause any modification in ⁴⁵Ca outflow rate (data not shown).

The continuous presence of the hypoglycaemic sulfonylurea glibenclamide in the perfusing medium modified the cationic response to verapamil in a manner similar to extracellular glucose (Fig. 4, right panel). Firstly, the basal rate of ⁸⁶Rb outflow was lower in the presence than in the absence of glibenclamide (10 µmol/l) throughout ($p < 0.05$). Secondly, with glibenclamide (10 µmol/l) in the basal medium, the capacity of verapamil (10 µmol/l) to inhibit ⁸⁶Rb FOR averaged only 41.6 ± 3.2 % of that recorded in the absence of the hypoglycaemic sulfonylurea ($p < 0.05$).

Fig. 4. *Left panel:* Effect of verapamil (10 $\mu\text{mol/l}$) on ^{86}Rb outflow from islets perfused in the absence ($\circ$) and presence of 5.6 mmol/l ($\bullet$) or 11.1 mmol/l ($\blacktriangle$) glucose. *Right panel:* Effect of verapamil (10 $\mu\text{mol/l}$) on ^{86}Rb outflow from islets perfused in the absence of glucose and in the absence ($\circ$) or presence of glibenclamide ($\bullet$; 10 $\mu\text{mol/l}$). Basal media contained extracellular Ca^{2+} . Mean values $\pm$ SEM refer to 4–8 individual experiments

Effects of verapamil on BPDZ 44-induced changes in ^{86}Rb outflow. As previously described [18], the $\text{K}^{+}_{\text{ATP}}$ channel opener BPDZ 44 (50 $\mu\text{mol/l}$) increased ^{86}Rb FOR from islets perfused in the presence of 5.6 mmol/l glucose and extracellular Ca^{2+} (Fig. 5). When the same experiment was repeated with verapamil (10 $\mu\text{mol/l}$) present throughout, the basal rate of ^{86}Rb outflow was lower. The basal ^{86}Rb outflow (min 40–44) averaged 2.45 ± 0.20 %/min in the absence and 1.50 ± 0.24 %/min in the presence of verapamil throughout ($p < 0.05$). Moreover, verapamil reduced the cationic response to BPDZ 44. Thus, the integrated outflow of ^{86}Rb measured during exposure to 50 $\mu\text{mol/l}$ BPDZ 44 averaged 2.02 ± 0.27 %/min and 0.55 ± 0.07 %/min in the absence and presence of 10 $\mu\text{mol/l}$ verapamil, respectively ($p < 0.05$).

Fig. 5. Effect of BPDZ 44 (50 $\mu\text{mol/l}$) on ^{86}Rb outflow from islets perfused in the absence ($\circ$) or presence of verapamil ($\bullet$; 10 $\mu\text{mol/l}$) throughout. Basal media contained 5.6 mmol/l glucose and extracellular Ca^{2+} . Mean values $\pm$ SEM refer to 4–8 individual experiments

Effects of verapamil on $\text{K}^{+}_{\text{ATP}}$ channel activity. The effects of verapamil on $\text{K}^{+}_{\text{ATP}}$ channel activity were further studied using the inside-out configuration of the patch-clamp technique. Under these experimental conditions, verapamil consistently inhibited $\text{K}^{+}_{\text{ATP}}$ channels at 10 ($n = 13/14$ occasions in 14 patches) and 100 $\mu\text{mol/l}$ ($n = 17/17$ in 17 patches) concentrations (Fig. 6, upper panel). In 6 patches which were suitable for a detailed quantification of channel activity, 10 $\mu\text{mol/l}$ verapamil decreased the $\text{K}^{+}_{\text{ATP}}$ channels' open state probability to 26.2 ± 15.4 % and 100 $\mu\text{mol/l}$ verapamil to 9.5 ± 1.5 % of the control value recorded immediately prior to the addition of the drug (Fig. 6, lower panel). The effects of verapamil were sustained but not readily reversible at either concentration (Fig. 6, lower panel). Open state probability averaged 57.5 ± 16.0 % and 34.6 ± 16.5 % of the control value 2–3 min after the withdrawal of 10 and 100 $\mu\text{mol/l}$ verapamil, respectively. Following removal of verapamil, the addition of ATP (500 $\mu\text{mol/l}$) on the inside face of the beta-cell membrane was

Fig. 6A, B. Effect of verapamil on K⁺_{ATP} channels. *Upper panel* shows a continuous recording of K⁺_{ATP} channels from a cell-free inside-out patch at 0 mV voltage-clamp. Verapamil inhibits K⁺ channels at 10 and 100 μmol/l concentrations. The data also show inhibition and reactivation of K⁺_{ATP} channels by 500 μmol/l ATP. *Lower panel* summarises data from six separate patch recordings exposed to both 10 and 100 μmol/l verapamil. Average values for changes in K⁺_{ATP} channel open-state probability have been expressed as a fraction of the control value recorded immediately prior to the addition of verapamil (100 %). Recovery values were obtained under steady-state conditions, generally between 2 and 3 min following removal of verapamil

associated with a reduction in K channel openings (Fig. 6, upper panel).

Effect of verapamil on the cytosolic free Ca²⁺ concentration. The addition of 10 or 100 μmol/l verapamil did not affect the fluorescence intensity of fura 2-loaded islet cells perfused in the absence (data not shown) or presence of 2.8 mmol/l extracellular glucose (Fig. 7a, b). By contrast, in single cells exposed to an insulinotropic glucose concentration (11.1 mmol/l), verapamil (10 and 100 μmol/l) provoked a marked and sustained reduction in the cytosolic Ca²⁺ concentration (Fig. 7c, d).

Discussion

The results clearly show that verapamil provokes a concentration-related inhibition of ⁸⁶Rb outflow from perfused rat pancreatic islets. This response was not due to any direct toxicity of the drug since the inhibitory effect on ⁸⁶Rb outflow was reversible. Thus, the present findings suggest that the phenylalkylamine reduces the K⁺ permeability of the pancreatic beta cell. This

property appears to be inherent to the phenylalkylamine Ca²⁺ channel blockers since gallopamil, another phenylalkylamine derivative, but not nifedipine, a 1,4-dihydropyridine Ca²⁺ channel blocker, reproduced the inhibitory effects of verapamil.

Our data also provide information about the type of K⁺ channel affected by verapamil. Firstly, the inhibitory effect of verapamil on ⁸⁶Rb FOR was shown to be identical whether the medium contained or was deprived of extracellular Ca²⁺. Secondly, the hypoglycaemic sulfonylurea glibenclamide reduced the capacity of verapamil to inhibit ⁸⁶Rb outflow, indicating that the effects of verapamil and the hypoglycaemic sulfonylurea were not additive. Thirdly, the magnitude of the inhibitory effect of verapamil was reduced in a concentration-dependent manner by glucose. These findings reveal that verapamil interacts with a Ca²⁺-independent, glucose- and hypoglycaemic sulfonylurea-sensitive ⁸⁶Rb efflux pathway. Such features suggest indirectly that the phenylalkylamine derivative decreases the activity of the K⁺_{ATP} channels in beta cells [19–21].

Several other observations also favour verapamil-induced inhibition of K⁺_{ATP} channels. Indeed, verapamil prevented the stimulatory effect of BPDZ 44 on ⁸⁶Rb outflow from prelabelled and perfused rat pancreatic islets. BPDZ 44 (Fig. 1) is a compound structurally related to both diazoxide and pinacidil and which has previously been shown to be a potent and specific activator of the beta-cell K⁺_{ATP} channel [18]. Direct confirmation that verapamil modulates K⁺_{ATP} channels was obtained by single-channel patch-clamp recordings, which revealed that verapamil elicited a dose-dependent inhibition of the K⁺_{ATP} currents. Taken together, these results support the hypothesis that the beta-cell K⁺_{ATP} channel is a target for the action of verapamil. Our findings, however, cannot exclude the possibility that verapamil

Fig. 7. Effect of verapamil 10 μ mol/l (left panels) and 100 μ mol/l (right panels) on the cytosolic Ca^{2+} concentration of single cells. Basal media contained extracellular Ca^{2+} and either 2.8 (A, B), or 11.1 mmol/l glucose (C, D). Each graph is a representative experiment conducted on a single cell

might also interact with other K⁺ channels equipping the beta-cell plasma membrane. Blockade by verapamil of ATP- and/or glibenclamide-sensitive K⁺ currents has been previously described in heart cells [22,23] and follicle-enclosed *Xenopus* oocytes [24].

Because the primary effect of most insulin secretagogues is regarded as inhibition of the K⁺_{ATP} channel, a phenomenon which in turn causes membrane depolarization with subsequent activation of voltage-sensitive Ca^{2+} channels, it was essential to quantify the effects of verapamil on Ca^{2+} fluxes and insulin release. Under experimental conditions in which verapamil clearly inhibited the K⁺_{ATP} channels (i.e. in the absence of glucose or in the presence of a non-insulinotropic glucose concentration), the drug did not affect Ca^{2+} movements, cytosolic free Ca^{2+} concentration or insulin release.

There are two possible explanations of why verapamil inhibited K⁺_{ATP} channels but neither elevated cytosolic Ca^{2+} or initiated insulin release. Firstly, K⁺_{ATP} channel inhibition might not depolarize the

beta-cell membrane potential sufficiently to open voltage-sensitive Ca^{2+} channels. Alternatively, and perhaps more likely, we believe that the intrinsic Ca^{2+} channel blocking properties of verapamil counteract the potential of the drug to elicit Ca^{2+} entry by membrane depolarization.

The present results do not challenge the concept that, under marked depolarizing conditions, verapamil is a potent inhibitor of Ca^{2+} entry in pancreatic beta cells. In agreement with such a view, the drug was shown to inhibit, in a dose-dependent manner, ⁴⁵Ca outflow and insulin output from islets incubated or perfused throughout in the presence of 11.1 mmol/l glucose and extracellular Ca^{2+} . Under such experimental conditions, a decrease in ⁴⁵Ca FOR is likely to represent a reduction in ⁴⁰Ca²⁺ influx through voltage-dependent Ca^{2+} channels [8,25]. In contrast, verapamil did not affect ⁴⁵Ca outflow and insulin release from islets exposed to a glucose-deprived medium or to a medium containing non-insulinotropic glucose concentrations. The same lack of verapamil effect on ⁴⁵Ca FOR was observed when experiments were conducted with Ca^{2+} -depleted media. The ability of verapamil to inhibit Ca^{2+} influx was further confirmed by its capacity to reduce the cytosolic Ca^{2+} concentration in islet cells incubated in the presence of 11.1 mmol/l glucose. Finally, verapamil was also shown to inhibit the increase in ⁴⁵Ca outflow brought about by a sudden rise in the

extracellular K⁺ concentration. This cationic response to K⁺ depolarization is known to result from the opening of voltage-sensitive Ca²⁺ channels [8]. Collectively, these observations clearly indicate that, whenever a membrane depolarization promotes the activation of voltage-sensitive Ca²⁺ channels, the major effect of verapamil is to block the influx of Ca²⁺ ions.

The precise mechanism(s) by which the phenylalkylamine reduces the K⁺_{ATP} channel activity remain(s), however, unclear. The inhibitory effect of verapamil on K⁺_{ATP} channels appears to be dependent on the phenylalkylamine structure and is not associated with the Ca²⁺ channel blocking activity of the drug. Despite recent findings that verapamil increases the ATP/ADP 22 ratio in rat pancreatic islets [26], it is unlikely that such an increase in the cellular energy state mediates the inhibition of K⁺_{ATP} channels. Indeed, the verapamil-induced changes in nucleotide levels were ascribed to result from the reduction in Ca²⁺ entry. Moreover, verapamil remained an effective K⁺_{ATP} channel blocker in isolated inside-out patches where cell metabolism has been disrupted. The similarity between the effects of the Ca²⁺ entry blocker verapamil and hypoglycaemic sulfonylureas [9,21] on ⁸⁶Rb outflow and K⁺_{ATP} currents might suggest the interaction with a target site exhibiting close and/or similar properties. In support of this idea, both verapamil and hypoglycaemic sulfonylureas have been shown to interact with membrane proteins belonging to the family of the ATP-binding cassette proteins [27].

In conclusion, the present results clearly indicate that verapamil reduces the K⁺ permeability of pancreatic beta cells. This effect is concentration-dependent, Ca²⁺-independent and appears to be inherent to the phenylalkylamine type Ca²⁺ channel blockers. Our radioisotopic and electrophysiologic observations further reveal that the "antagonistic" action of verapamil on K⁺ permeability mainly results from the inhibition of ATP-sensitive K⁺ channels. Such an inhibition was not associated with any increase in the secretory response.

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