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Clonidine inhibits ATP-sensitive K^+ channels in mouse pancreatic β-cells

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1 The effects of clonidine and adrenaline on adenosine 5'-triphosphate (ATP)-sensitive K^+ channels were studied in pancreatic β-cells from normal mice.

2 When perifused with a medium containing 1 mM glucose, many of the ATP-sensitive K^+ channels in the β-cell membrane are open. Under these conditions, clonidine (5–100 μM) reversibly decreased ^{86}Rb efflux from the islets, whereas adrenaline was ineffective at concentrations up to 100 μM.

3 In 6 mM glucose, most of the ATP-sensitive K^+ channels in the β-cell membrane are closed. Opening these channels by diazoxide (100 μM) caused a marked acceleration of ^{86}Rb efflux from the islets, which was attenuated by 100 μM clonidine.

4 ATP-sensitive K^+ currents were measured in single β-cells by the whole cell mode of the patch-clamp technique. At concentrations above 4 μM, clonidine reversibly inhibited the ATP-sensitive K^+ current in a dose-dependent manner.

5 Voltage-sensitive K^+ currents were unaffected by 20 μM but decreased slightly by 100 μM clonidine.

6 Calcium currents, measured by the whole cell or perforated patch technique, were unaffected by clonidine at concentrations up to 100 μM.

7 It is concluded that high concentrations of the α_2-adrenoceptor agonist clonidine, but not of adrenaline, can inhibit ATP-sensitive K^+ channels in pancreatic β-cells. Other ionic channels are only slightly affected or unaffected.

Keywords: α-Adrenoceptors; clonidine; adrenaline; K^+ channels; pancreatic β-cells; insulin release

Introduction

A wide variety of chemically-unrelated substances stimulate insulin secretion by closing adenosine 5'-triphosphate (ATP)-sensitive K^+ channels in pancreatic β-cells whereas others inhibit secretion by opening these channels (Garrino *et al.*, 1989; Cook & Quast, 1990; Henquin, 1990).

We recently observed that the α-adrenoceptor blockers phentolamine (α_1, α_2) and yohimbine (α_2) can inhibit ATP-sensitive K^+ channels in β-cells (Plant & Henquin, 1990). Although this occurs at concentrations of the antagonists higher than those necessary to block the receptors, the effect is intriguing because agonists of the α_2-adrenoceptors appear to hyperpolarize the β-cell membrane by increasing its K^+ permeability (Drews *et al.*, 1990; Rorsman *et al.*, 1991). We therefore felt it of interest to evaluate the effects of two agonists of α_2-adrenoceptors, clonidine and adrenaline, on ATP-sensitive K^+ channels in mouse β-cells.

Methods

^{86}Rb efflux experiments

These experiments were carried out at 37°C with islets obtained by collagenase digestion of the pancreas of fed female NMRI mice. After isolation, the islets were loaded with ^{86}Rb (Rb used as tracer for K) for 90 min in a medium containing 15 mM glucose and supplemented with $^{86}RbCl$ (1.5 to 3 MBq ml^{-1}; sp.act. 7.4 to 18.5 TBq mol^{-1}). The Rb concentration never exceeded 0.4 mM (Garrino & Henquin, 1988). ^{86}Rb efflux was then monitored in a dynamic perifusion system (Henquin, 1978). The radioactivity lost by the islets was measured immediately by the Cerenkov radiation (Henquin, 1978) in the effluent fractions collected at 2 min intervals. From the sum of the radioactivity remaining in the islets at the end of the experiments and the accumulated effluent radioactivity, the fractional efflux rate was calculated for each period (radioactivity lost by tissue during the time interval/radioactivity present in the tissue during that time interval) and expressed as per cent per minute. The solutions used had the following ionic composition (in mM): NaCl 120, KCl 4.8, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 24, and were gassed with O_2/CO_2 (94:6) to maintain a pH of 7.4. They were supplemented with 1 mg ml^{-1} bovine serum albumin fraction V (Boehringer, Mannheim, Germany).

Electrical recordings

After isolation of the islets, islet cells were dispersed and cultured for 1–2 days as previously described (Plant, 1988). Patch-clamp experiments were performed at room temperature (20–24°C) on single β-cells. For measurements of ATP-sensitive and voltage-dependent K^+ currents the bath solution contained (in mM): NaCl 135, KCl 5.6, $CaCl_2$ 2.6, $MgCl_2$ 1.2, HEPES 10, and was titrated to pH 7.4 with NaOH. Pipettes were filled with a solution containing (in mM): KCl 135, $MgCl_2$ 4, $CaCl_2$ 2, EGTA 10, Na_2ATP 0.65, HEPES 20, and titrated to pH 7.15 with KOH. Details of the recording technique and the separation of ATP-sensitive and voltage-dependent currents from other membrane currents have been described previously (Garrino *et al.*, 1989; Plant & Henquin, 1990). In brief, ATP-sensitive K^+ currents were measured by recording the currents at 15 s intervals at the holding potential (−70 mV) and during 100 ms pulses to −60 and −80 mV which were separated by an interval of 100 ms. Under the conditions used, the current which develops with time during dialysis is almost entirely ATP-sensitive K^+ current. To measure voltage-dependent K^+ currents, cells were held at −70 mV and depolarized at 15 s intervals to 0 mV to activate K^+ currents in a bathing solution which was

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supplemented with 100 μM tolbutamide to block ATP-sensitive K^+ currents. A short (50 ms) hyperpolarization to −100 mV, applied 100 ms before the test pulse, was used to estimate the leakage current. Currents were also measured (sampling rate: 2.5 kHz) during voltage ramps of 480 ms duration from −120 to 30 mV, applied at intervals of 15 s.

Calcium currents were recorded by the conventional whole-cell and perforated patch technique (Horn & Marty, 1988). The latter preserves second messenger and metabolic systems of the cell and reduces run-down of the Ca^{2+} current compared to the conventional whole cell technique. Cells were bathed in a solution containing (in mM): NaCl 115, tetraethylammonium chloride (TEACl) 20, $CaCl_2$ or $BaCl_2$ 10, $MgCl_2$ 1.2, tolbutamide 0.1, glucose 15, HEPES 10, which was titrated to pH 7.4 with NaOH. For whole-cell experiments, the pipette solution contained (in mM): CsCl 50, N-methyl-D-glucamine 70, HCl 58, $MgCl_2$ 4, Na_2ATP 3, EGTA 10, $CaCl_2$ 2, HEPES 10, titrated to pH 7.15 with CsOH. For perforated patch experiments, the pipette solution contained (in mM): Cs_2SO_4 70, NaCl 10, KCl 10, $MgCl_2$ 7, HEPES 10, titrated to pH 7.4 with NaOH (Smith *et al.*, 1989). The tip of the pipette was first filled by dipping the pipette briefly in this solution, then back-filled with the same solution supplemented with 100 or 150 μg ml^{-1} nystatin in 0.2% dimethylsulphoxide (DMSO). Perforation of the patch was monitored by the change in the capacity transient during a 10 mV depolarizing pulse from a holding potential of −70 mV, and the value of the series resistance obtained from the capacity transient cancellation on the patch clamp amplifier. Measurements were started when the series resistance was less than 50 MΩ, which was normally within 10 to 20 min after seal formation.

Chemicals

Clonidine hydrochloride was obtained from Sigma (Deisenhofen, Germany) or Boehringer (Ingelheim, Germany), adrenaline D-hydrogentartrate from Serva (Heidelberg, Germany), tolbutamide from Hoechst A.G. (Frankfurt, Germany) and diazoxide was provided by Schering Corp. (Bloomfield, NJ, U.S.A.). $^{86}RbCl$ was purchased from the Radiochemical Centre (Amersham, Bucks, U.K.). When the effects of adrenaline were tested, the solutions were supplemented with 0.5 mM ascorbic acid to prevent oxidation of the catecholamine. The dihydropyridine, CGP 28392, was obtained from Ciba Geigy A.G. (Basle, Switzerland).

Results

Effects of clonidine and adrenaline on ^{86}Rb efflux

In the presence of a low concentration of glucose (1 mM), many ATP-sensitive K^+ channels in the β-cell membrane are open and the potassium permeability is high (Rorsman & Trube, 1985; Misler *et al.*, 1986; Ashcroft *et al.*, 1988). Under these conditions, the ^{86}Rb efflux rate is high and declines with time (Figure 1a). Addition of clonidine to the bathing medium reversibly inhibited ^{86}Rb efflux. A small but consistent decrease was observed at a clonidine concentration of 5 μM. The difference between test and control efflux rates ranged between 0.15 and 0.21% min^{-1} ($P < 0.05$ by paired *t* test). The inhibition by 100 μM clonidine was clearly larger (0.71% min^{-1}) and similar to that produced by 10 μM tolbutamide (0.66% min^{-1}), a specific blocker of ATP-sensitive K^+ channels (Figure 1b). In contrast, adrenaline (100 μM) was without effect on ^{86}Rb efflux under the same conditions. In 6 mM glucose, most ATP-sensitive K^+ channels are closed (Rorsman & Trube, 1985; Misler *et al.*, 1986; Ashcroft *et al.*, 1988) and ^{86}Rb efflux is low and stable. Diazoxide (100 μM), an opener of ATP-sensitive K^+ channels in β-cells (Trube *et al.*, 1986), caused a marked acceleration of ^{86}Rb efflux. Clonidine

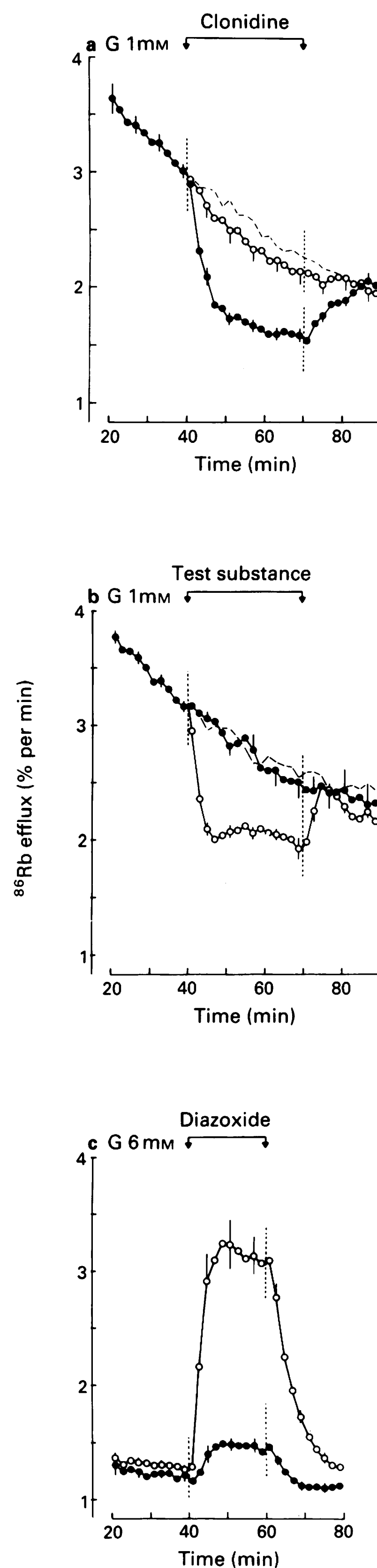

Figure 1 Effects of clonidine and adrenaline on ^{86}Rb efflux from mouse islets. (a) Clonidine was added at a concentration of 5 μM (○) or 100 μM (●) to a medium containing 1 mM glucose (G). (b) Adrenaline, at a concentration of 100 μM, (●), or tolbutamide, at a concentration of 10 μM, (○), was added to a medium containing 1 mM glucose. In (a) and (b), controls without the test substance, are shown by the broken line. (c) Diazoxide (100 μM) was added to a medium containing 6 mM glucose (G) alone (○), or 6 mM glucose plus 100 μM clonidine (●). Values are means for 4–5 experiments with s.e.mean shown by vertical lines.

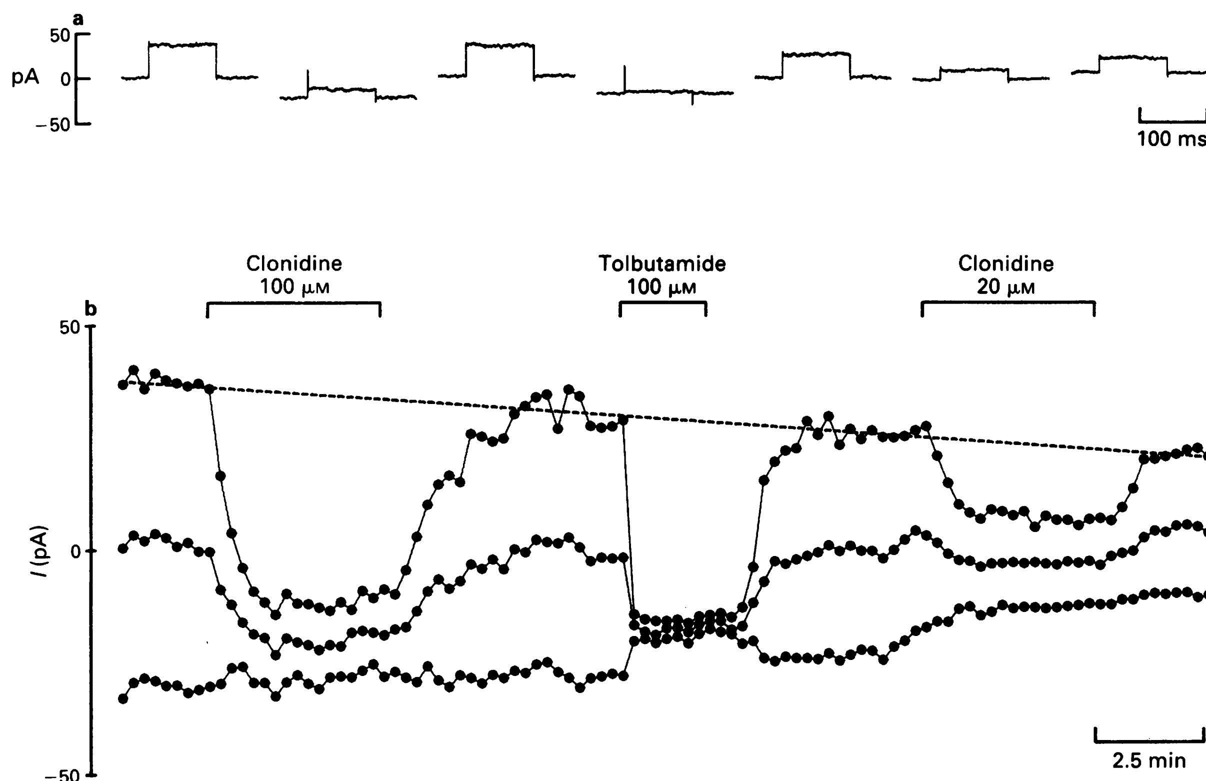

Figure 2 Inhibition of whole cell ATP-sensitive K^+ currents by clonidine. (a) Currents recorded during 100 ms pulses from −70 to −60 mV during the different phases of the experiment shown in (b). Outward currents are denoted positive and shown upwards. (b) Current values recorded at 15 s intervals at −60, −70 and −80 mV (upper, middle and lower traces respectively). The effects of 20 and 100 μM clonidine are compared to those of 100 μM tolbutamide. The time course of run-down of the ATP-sensitive K^+ current at −60 mV is indicated by the broken line. In this experiment, an inward current was observed following inhibition of the ATP-sensitive K^+ current. This probably resulted from leak currents between the pipette surface and the cell membrane.

(100 μM) markedly decreased the effect of diazoxide (Figure 1c).

Effects of clonidine and adrenaline on ATP-sensitive K^+ currents

At physiological ATP concentrations, the whole cell ATP-sensitive K^+ current is small because most of the channels are inhibited (Rorsman & Trube, 1985). When the cell is dialysed with a pipette solution containing a lower than physiological ATP concentration, ATP-sensitive K^+ currents activate with time following the establishment of the whole cell configuration and then decline at a rate dependent on the ATP concentration (Trube *et al.*, 1986; Garrino *et al.*, 1989). Drugs were applied during the approximately linear phase of run-down after the current had attained a maximum. Clonidine decreased the ATP-sensitive K^+ current in a dose-dependent manner (Figure 2). Effects of clonidine were visible starting at a concentration of 4 or 5 μM. The changes in current during the potential steps from −70 to −60 mV were used to quantify the effects of drugs. At a concentration of 100 μM, clonidine inhibited the current by $74.5 \pm 2.2\%$ (mean ± s.e.mean, $n = 9$) compared to the current before the addition of the drug. In comparison, tolbutamide (100 μM) applied after clonidine in five of these cells inhibited the current by $93.2 \pm 0.8\%$. In the cell shown in Figure 2, 20 μM clonidine caused an inhibition of 56%. Adrenaline at concentrations up to 100 μM had no effect on the ATP-sensitive K^+ current in six cells (Figure 3).

The effects of clonidine (100 μM) on the responses to voltage ramps from −120 to +30 mV are shown in Figure 4 and compared to the effects of 100 μM tolbutamide. Under the conditions used, the response to voltage ramps has a number of components. Between −120 and −30 mV, the current is mainly ATP-sensitive K^+ current with a small contribution of leakage current. At more positive potentials, voltage-dependent K^+ currents are also activated. Tolbutamide a specific blocker of ATP-sensitive K^+ channels decreased the component between −120 and −30 mV without affecting the voltage-dependent current. Clonidine had similar, though less marked, effects to those of tolbutamide, but in addition appeared to decrease the voltage-dependent component slightly (see below).

Effects of clonidine on voltage-dependent K^+ currents

To determine whether the effects of clonidine are specific for ATP-sensitive K^+ currents, we directly tested the influence of the drug (20 and 100 μM) on the voltage-dependent K^+ current activated during pulses to 0 mV. At a concentration of 20 μM, clonidine was without effect (data not shown), whereas at 100 μM a slight inhibition of the current was observed in all cells studied (Figure 5). The mean inhibition compared to the current before the addition of the drug was $14.0 \pm 1.5\%$ ($n = 4$). The kinetics of the current were not markedly changed. Clonidine had no effect on the leakage current (data not shown).

Effects of clonidine on Ca^{2+} currents

Clonidine, at concentrations between 1 and 100 μM, did not have any effect on Ba^{2+} currents through voltage-sensitive Ca^{2+} channels, measured by the whole cell mode of the patch-clamp technique (data not shown). Owing to the rapid run-down of Ba^{2+} currents in many cells, small effects of clonidine may have been difficult to detect. We therefore measured Ca^{2+} currents by the perforated patch technique (Horn & Marty, 1988; Korn & Horn, 1989; Falke *et al.*, 1989; Smith *et*

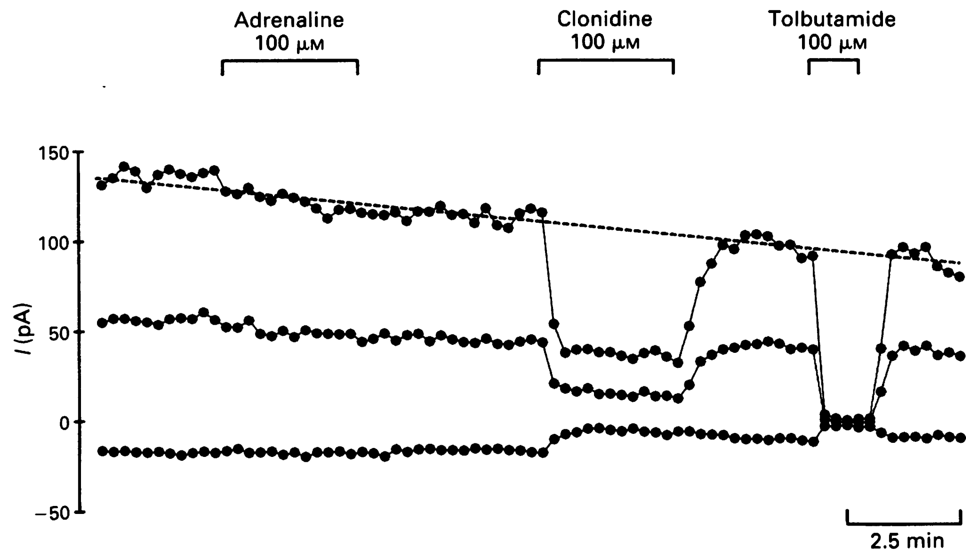

Figure 3 Effects of adrenaline on whole cell ATP-sensitive K^+ currents. Current values measured at −60, −70 and −80 mV (upper, middle and lower traces respectively). The effects of 100 μM adrenaline are compared to those of 100 μM clonidine and 100 μM tolbutamide. The broken line indicates the time course of run-down of the ATP-sensitive current at −60 mV.

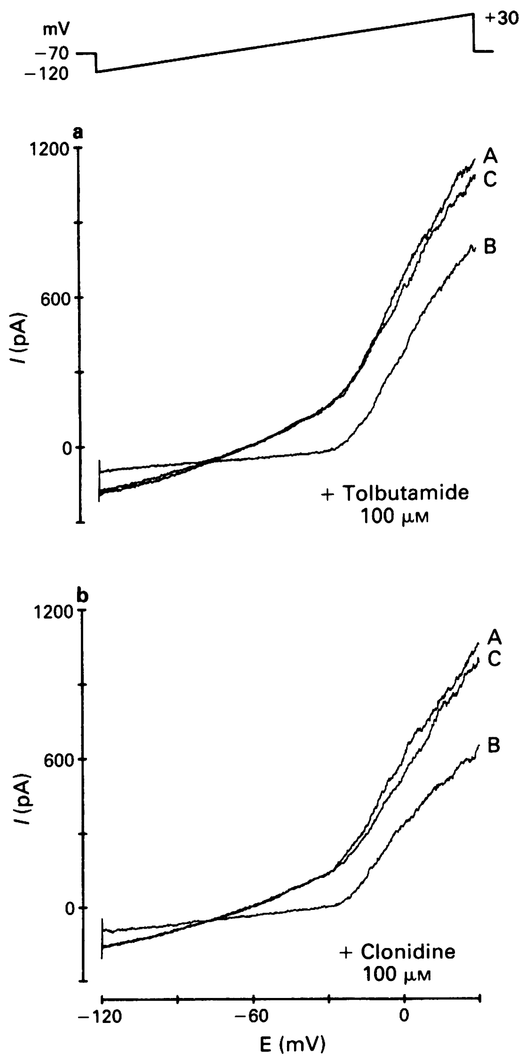

Figure 4 Effects of clonidine and tolbutamide on the current response to voltage ramps. (a) Responses to 480 ms ramps from −120 to +30 mV recorded before addition (A), in the presence (B), and after wash-out (C) of 100 μM tolbutamide. (b) Responses to ramps before addition (A), during the presence (B), and after wash-out (C) of 100 μM clonidine.

al., 1989). With this method calcium currents were much more stable than with the conventional whole cell technique (Plant, 1988), but did show a slow run-down in some cells. They were decreased by 100 μM Cd^{2+} and increased in amplitude by the dihydropyridine, CGP 28392, at a concentration of 5 μM (data not shown). As shown in Figure 6, clonidine (100 μM) had no effect on peak and steady-state Ca^{2+} currents recorded during voltage steps to −10 or 0 mV using this method.

Discussion

The evidence presented in this study shows that the α_2-adrenoceptor agonist clonidine, like the antagonists phentolamine and yohimbine (Plant & Henquin, 1990), inhibits ATP-sensitive K^+ channels in pancreatic β-cells. This is supported by three lines of evidence: (1) clonidine inhibits ^{86}Rb

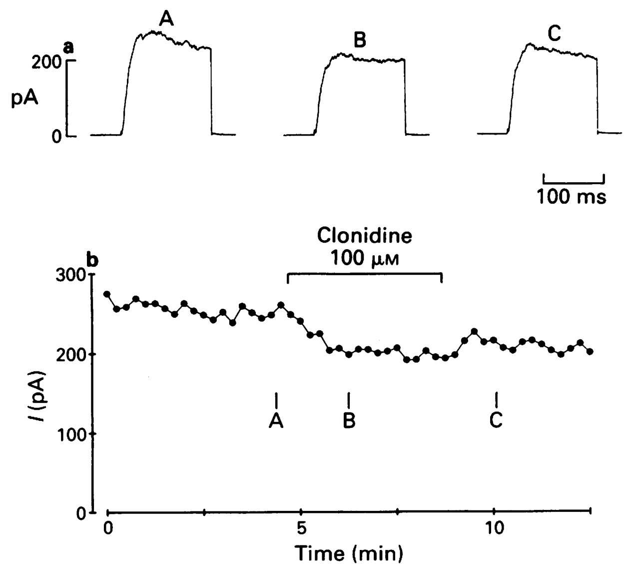

Figure 5 Effects of clonidine on voltage-dependent K^+ currents. (a) Currents recorded during 150 ms voltage steps from −70 to 0 mV at the times shown in (b). (b) Time-course of the effects of clonidine on the voltage-dependent K^+ current. Values of the maximum K^+ current were measured during pulses to 0 mV, applied at 15 s intervals, showing the effect of 100 μM clonidine. The bath solution contained tolbutamide (100 μM) to inhibit ATP-sensitive K^+ currents.

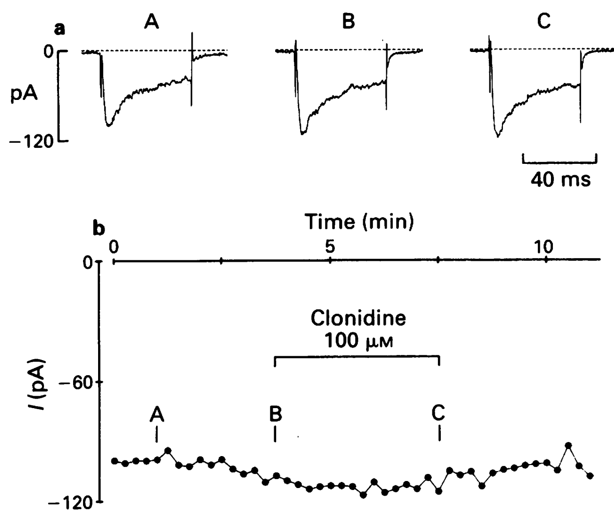

Figure 6 Effects of clonidine on Ca^{2+} currents recorded by the perforated patch technique. (a) Currents recorded in 10 mM Ca^{2+} during steps from −70 to 0 mV at the times shown in (b). (b) Peak Ca^{2+} current recorded at 15 s intervals plotted against time after the start of recording, showing the effect of the addition of 100 μM clonidine. To block K^+ currents the bath solution contained tetraethylammonium ions (20 mM) and tolbutamide (100 μM), and the pipette solution Cs^+ (140 mM).

efflux under conditions where only K^+ channels that are open in the β-cell membrane are the ATP-sensitive ones; (2) clonidine inhibits K^+ currents measured by the whole cell mode of the patch clamp technique, under conditions where almost all current is ATP-sensitive K^+ current, and (3) clonidine markedly decreases the acceleration of ^{86}Rb efflux induced by diazoxide, an opener of ATP-sensitive K^+ channels (Trube *et al.*, 1986). This closure of ATP-sensitive K^+ channels may explain the ability of clonidine to increase insulin release after irreversible blockade of α_2-adrenoceptors by benextramine (Schulz & Hasselblatt, 1989b).

The inhibitory effect of clonidine on the ATP-sensitive K^+ channels does not seem to be associated with the activation of α_2-adrenoceptors. Adrenaline, at concentrations up to 100 μM, was without effect on ATP-sensitive K^+ channels. Furthermore, clonidine acts on α_2-adrenoceptors to inhibit insulin release at much lower concentrations (1–100 nM) than those needed to affect the ATP-sensitive K^+ channels (Leclercq-Meyer *et al.*, 1980; Nakaki *et al.*, 1981; Langer *et al.*, 1983; Bertrand & Henquin, 1990). A similar dissociation exists between the dose-dependency of the effects of phentolamine or yohimbine on α_2-adrenoceptors and on ATP-sensitive K^+ channels (Plant & Henquin, 1990). It has been suggested that clonidine may exert non-specific membrane effects at high concentrations (Starke *et al.*, 1972). This trivial explanation does not hold here. Our results show that the effects of clonidine are selective for the ATP-sensitive K^+ channels. At high concentrations voltage-dependent K^+ channels are only slightly affected, while voltage-sensitive Ca^{2+} channels are unaffected.

The similarity of the effects of clonidine and phentolamine on ^{86}Rb fluxes and ATP-sensitive K^+ currents might indicate the involvement of an imidazoline binding site (Michel & Insel, 1989) in the regulation of ATP-sensitive K^+ channels in β-cells. This proposal would also be suggested by the observations that, like phentolamine (Henquin *et al.*, 1982), efaroxan antagonizes the inhibition of insulin release by diazoxide (Chan & Morgan, 1990), and that several other imidazoline derivatives increase glucose-induced insulin release (Schulz & Hasselblatt, 1989a). On the other hand, the fact that yohimbine, which is not recognized by the imidazoline receptors (Michel & Insel, 1989), also inhibits ATP-sensitive K^+ channels (Plant & Henquin, 1990) may cast doubt on this hypothesis. However, since these channels can be influenced by a host of chemically-unrelated substances (Cook & Quast, 1990; Henquin, 1990), it is possible that yohimbine and phentolamine fortuitously share the property of blocking both α_2-adrenoceptors and ATP-sensitive K^+ channels.

Clonidine also affects ionic currents in other tissues independently of an action on α_2-adrenoceptors. In bovine chromaffin cells, clonidine blocks the current through acetylcholine receptor channels, at similar concentrations to those at which it blocked the ATP-sensitive K^+ current in this study, whilst not affecting other voltage-dependent currents in these cells (Cull-Candy *et al.*, 1988). In vascular smooth muscle cells, however, clonidine does not share the property of phentolamine and some other imidazolines of preventing the hyperpolarization and relaxation induced by cromakalim, a putative opener of ATP-sensitive K^+ channels in this tissue (McPherson & Angus, 1989; Standen *et al.*, 1989).

Activation of α_2-adrenoceptors in β-cells results in a hyperpolarization of the membrane, associated with a decrease in electrical activity, and an inhibition of insulin release. The hyperpolarization of the membrane is accompanied by a decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels. The decrease in Ca^{2+} entry could be either the primary effect resulting in the hyperpolarization, or occur as a result of the hyperpolarization caused by an increase in K^+ permeability. Most data obtained in normal β-cells support the latter hypothesis (Drews *et al.*, 1990; Rorsman *et al.*, 1991), but there is some evidence for a decrease in the Ca^{2+} current in the insulin-secreting HIT cell line (Keahey *et al.*, 1989). The present results from the whole cell and perforated patch experiments do not support the involvement of a receptor-mediated reduction in calcium current in the hyperpolarization induced by α_2-agonists.

In conclusion, high concentrations of the α_2-adrenoceptor agonist clonidine, but not of adrenaline, inhibit ATP-sensitive K^+ channels in pancreatic β-cells. Other ionic channels are only weakly affected or unaffected. The effect of clonidine resembles that of phentolamine. It is possible that an imidazoline-binding site is involved in the control of ATP-sensitive K^+ channels in β-cells.

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