Glucose Dependence of Imidazoline-Induced Insulin Secretion

Different Characteristics of Two ATP-Sensitive K^+ Channel–Blocking Compounds

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The glucose dependence of the insulinotropic action of K_{ATP} channel–blocking imidazoline compounds was investigated. Administration of 100 μmol/l phentolamine, but not 100 μmol/l efaroxan, markedly increased insulin secretion of freshly isolated mouse islets when the perifusion medium contained 5 mmol/l glucose. When the glucose concentration was raised to 10 mmol/l in the continued presence of either imidazoline, a clear potentiation of secretion occurred as compared with 10 mmol/l glucose alone. In the presence of efaroxan, a brisk first-phase–like increase was followed by a sustained phase, whereas a more gradual increase resulted in the presence of phentolamine. Administration of 100 μmol/l phentolamine was somewhat more effective than 100 μmol/l efaroxan to inhibit K_{ATP} channel activity in intact cultured β-cells (reduction by 96 vs. 83%). Both compounds were similarly effective to depolarize the β-cells. When measured by the perforated patch-technique, the depolarization by efaroxan was often oscillatory, whereas that by phentolamine was sustained. In perifused cultured islets, both compounds increased the cytosolic calcium concentration ($[Ca^{2+}]_c$) in the presence of 5 and 10 mmol/l glucose. Efaroxan induced large amplitude oscillations of $[Ca^{2+}]_c$, whereas phentolamine induced a sustained increase. It appears that a K_{ATP} channel block by imidazolines is not incompatible with a glucose-selective enhancement of insulin secretion. The glucose selectivity of efaroxan may involve an inhibitory effect distal to $[Ca^{2+}]_c$ increase and/or the generation of $[Ca^{2+}]_c$ oscillations. *Diabetes* 53 (Suppl. 3): S135–S139, 2004

Imidazolines are a group of investigational antidiabetic drugs. Originally, an antagonism at α-adrenoceptors was believed to be the mechanism of action of the prototypical imidazoline, phentolamine (1,2). When it became clear that the insulinotropic effect of this compound was not due to an α-antagonism but was related to its imidazoline moiety (3,4), it was hypothesized that this effect was mediated by a β-cell imidazoline receptor, as had been described previously for other pharmacological actions of imidazoline compounds (5).

The demonstration that phentolamine and other imidazolines block K_{ATP} channels in pancreatic β-cells (6,7) offered an explanation for their insulinotropic property. However, it remained unclear how the effect on the K_{ATP} channel was related to the hypothetical β-cell imidazoline receptor (8). The demonstration that the imidazoline RX 871024 increased insulin secretion not only by blocking K_{ATP} channels, but also by acting at a site distal to calcium influx, led to the alternative hypothesis that imidazolines have effects at multiple independent sites of the pancreatic β-cell (9). The concept of a β-cell imidazoline receptor mediating a multitude of effects was rendered less likely by the observation that the K_{ATP} channel block by imidazolines was exerted directly at Kir6.2, the pore-forming subunit of the channel (10,11).

Thus, it could be expected that by structural modification it should be possible to separate the blocking effect of the imidazolines on K_{ATP} channels from their more direct effect on exocytosis. An imidazoline devoid of a K_{ATP} channel–blocking activity was expected to stimulate secretion only in the presence of high but not basal glucose concentrations. In fact, several newly synthesized imidazoline compounds were shown to stimulate insulin secretion at concentrations at which they did not affect K_{ATP} channel activity (12,13). Consequently, these compounds were designated as a new generation of imidazoline compounds (14).

However, the mechanism of action of imidazolines appeared worth investigating because, from the beginning, the insulinotropic and glucose-lowering effect differs from that of sulfonylureas in that it is more antihyperglycemic than hypoglycemic (15). In this context, it should be kept in mind that the term "imidazolines" covers a rather heterogeneous group. We have shown earlier that phentol-

amine does in fact stimulate secretion also at basal glucose (16), whereas efaroxan has been described to exhibit a practically ideal glucose sensitivity in that it fails to increase insulin secretion at subthreshold glucose concentrations (17). The glucose dependence of the insulinotropic effect of RX 871024 decreased in a concentration-dependent manner between 10 and 100 μmol/l (9,14). Because all these compounds are blockers of K_{ATP} channels, the question arose whether an imidazoline-induced block of K_{ATP} channels was necessarily associated with a stimulatory effect on insulin secretion at a basal glucose concentration.

RESEARCH DESIGN AND METHODS

Chemicals. Efaroxan was kindly donated by RBI (Natick, MA) and phentolamine by Ciba-Geigy (Lörrach, Germany). Tolbutamide was obtained from Serva (Heidelberg, Germany), collagenase P from Boehringer Mannheim (Mannheim, Germany), and Fura-2/AM from Molecular Probes (Leiden, the Netherlands). Cell culture medium RPMI-1640 was purchased from Gibco BRL (Gaithersburg, MD), and fetal calf serum was obtained from Biochrom (Berlin, Germany). All other reagents of analytical grade were from E. Merck (Darmstadt, Germany). Tolbutamide stock solutions were prepared in 0.1N NaOH.
Tissue culture. Islets were isolated from the pancreas of NMRI mice by a conventional collagenase digestion technique and hand-picked under a stereomicroscope. Single cells were obtained by incubation of the islets for 10 min in a Ca^{2+}-free medium and subsequent vortex mixing for 1 min. Islets and single islet cells were cultured for up to 4 days in RPMI-1640 culture medium with 10% fetal calf serum (5 mmol/l glucose) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.
Electrophysiological recordings. K_{ATP} channel activity was measured by a standard patch-clamp technique using the cell-attached configuration (18). Pipettes were pulled from borosilicate glass (2 mm od, 1.4 mm id; Hilgenberg, Malsfeld, Germany) by a two-stage vertical puller (List Electronic, Darmstadt, Germany) and had resistances between 3 and 6 MOhm when filled with solution. Currents were recorded by an EPC7 patch-clamp amplifier (List Electronic) and were low pass filtered by a 4-pole Bessel filter at 2 kHz and stored on videotape. The pipette holding potential was 0 mV in cell-attached recordings. The membrane potential of β-cells was determined using the whole-cell and perforated patch modes under current clamp condition (19). Test compounds were applied and washed out by changing the bath medium with a slow bath perfusion system. The compositions of the bath and pipette media were as given by Zünkler et al. (20). All experiments were performed at room temperature (20–23°C). Data were analyzed off line using pClamp 6.03 software (Axon Instruments, Foster City, CA).
Microfluorimetric measurements of the cytosolic Ca^{2+} concentration. Islets and islet cells were cultured on glass coverslips in Petri dishes and were used from day 2 to 4 after isolation. Fura-2/AM was loaded at a concentration of 2 μmol/l for 45 min at 37°C. The coverslip with the attached cells or islets was inserted in a purpose-made perifusion chamber on the stage of an epifluorescence microscope fitted with a Zeiss Fluar (40×) objective. The fluorescence (excitation at 340 or 380 nm, emission >470 nm) was recorded by a slow-scan charged-coupled device (CCD) camera (TILL Photonics). All perifusions were performed at 37°C using a HEPES-buffered Krebs-Ringer bicarbonate medium. Image pairs were taken at intervals as indicated in the figures. Illumination time for each image was 800 ms.
Measurement of insulin secretion. Batches of 50 freshly isolated NMRI mouse islets were perifused at 37°C with a HEPES-buffered Krebs-Ringer medium containing various glucose concentrations and the indicated drug. The insulin content in the fractionated effluent was determined by enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden).
Data handling and statistics. Statistical calculations were performed by Prism and Instat software (GraphPad, San Diego CA). If not specified otherwise, differences were considered significant if $P < 0.05$.

RESULTS

Whether K_{ATP} channel–blocking imidazolines necessarily stimulate insulin secretion at a basal glucose concentration was evaluated by comparing the effects of efaroxan and phentolamine on freshly isolated perifused islets. Both compounds were used at a concentration that was expected to strongly depolarize β-cells by inhibition of K_{ATP}

channel activity. The effect of each imidazoline was first measured in the presence of 5 mmol/l glucose, a nonstimulatory concentration, before glucose was increased to 10 mmol/l in the continued presence of the imidazoline. Efaroxan did not increase insulin secretion in the presence of 5 mmol/l glucose, but rather caused a small, significant decrease (Fig. 1*A*). When the glucose concentration was raised to 10 mmol/l in the presence of efaroxan, there was a rapid and strong increase of secretion, which was followed by a sustained phase of stimulated secretion. On the whole, this pattern resembled the biphasic increase elicited by 20 mmol/l glucose (Fig. 1*B*), whereas 10 mmol/l glucose alone, in control perifusions, only produced a 50% increase in insulin secretion over baseline (Fig. 1*A*). Phentolamine, after a lag time of about 10 min, markedly increased secretion in the presence of 5 mmol/l glucose. A further increase in secretion occurred when the glucose concentration was raised to 10 mmol/l, but it was slower than that in the presence of efaroxan (Fig. 1*A*).

The effect of both imidazolines on K_{ATP} channel activity was compared using intact cultured β-cells. Administration of 100 μmol/l efaroxan decreased channel activity from a control value of 100% to 18.6 ± 4.2% ($n = 10$). Administration of 100 μmol/l phentolamine was significantly more effective, reducing channel activity to 3.6 ± 1.6% ($n = 5$), which is close to the effect of 500 μmol/l tolbutamide (0.6 ± 0.4%, $n = 4$). The difference between the effects of efaroxan and phentolamine ($P < 0.01$, Welch's t test) raised the possibility that the lack of insulinotropic effect of efaroxan in the presence of 5 mmol/l glucose was due to a less complete channel block by efaroxan.

However, the more complete channel block by phentolamine did not translate into a significantly stronger depolarization. In the standard whole-cell configuration, 100 μmol/l efaroxan depolarized the β-cell plasma membrane potential from a resting value of −73.3 ± 1.7 mV to −28.6 ± 3.4 mV ($n = 6$), and 100 μmol/l phentolamine depolarized from −67.9 ± 4.7 mV to −28.2 ± 5.8 mV ($n = 4$). The effect of efaroxan was reversible within 3 min, whereas the depolarization by phentolamine was of slow onset and only partially reversible during the washout phase. To ascertain that these observations were also valid for metabolically intact β-cells in the presence of 5 mmol/l glucose, the depolarizing effect was measured using the perforated patch configuration. Within 3 min, efaroxan depolarized the membrane potential from a resting value of −63.7 ± 2.6 mV to a plateau value of −47.8 ± 2.7 mV ($n = 6$), whereas phentolamine required at least 8 min to depolarize from −65.4 ± 1.6 mV to a plateau value of −48.4 ± 2.2 mV ($n = 4$). When these plateau values were reached, action potentials occurred regularly. Long-term recordings (Fig. 2) showed oscillations of the plasma membrane potential in 5 of 10 experiments in the presence of efaroxan, whereas phentolamine led to a continuous depolarization (6 of 6 experiments).

The ability of both imidazolines to inhibit K_{ATP} channel activity and to depolarize β-cells suggested that they should also increase the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) by opening of L-type Ca^{2+} channels. As expected, both compounds at 100 μmol/l increased $[Ca^{2+}]_c$ in *single* β-cells in the presence of 5 mmol/l glucose. The extent of
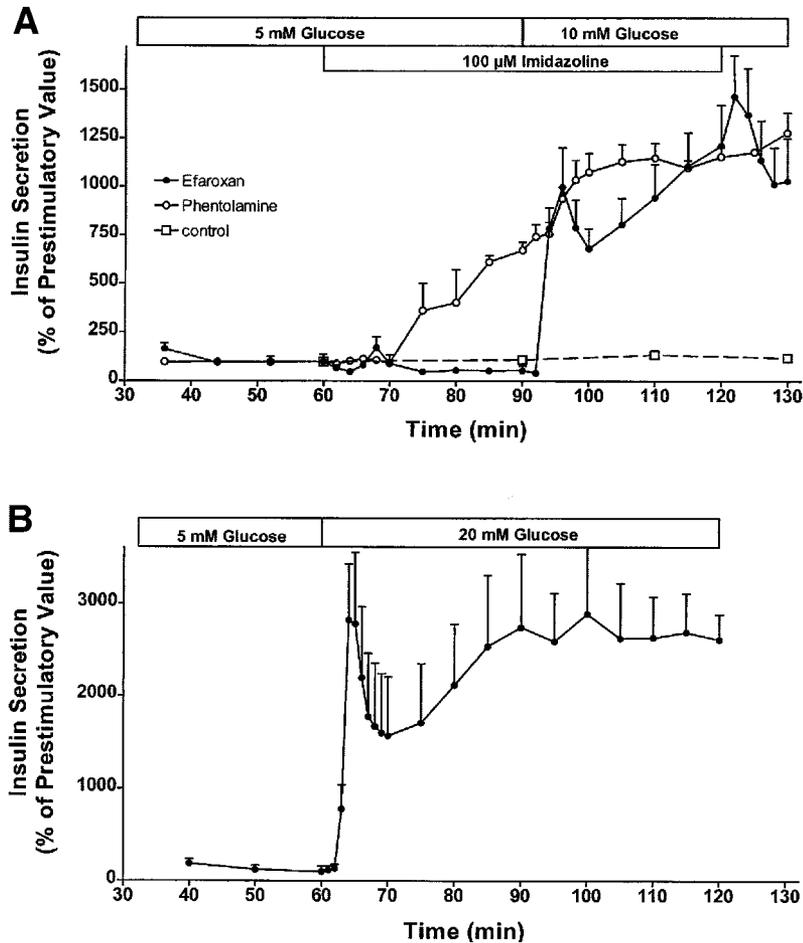
FIG. 1. *A*: Comparison of the glucose-dependent insulinotropic effects of efaroxan and phentolamine. Freshly isolated mouse islets were perifused with a Krebs-Ringer medium containing 5 mmol/l glucose from min 0 to min 90. Then the glucose concentration was raised to 10 mmol/l from min 90 to min 130. Either 100 μmol/l efaroxan (●) or 100 μmol/l phentolamine (○) was present from min 60 to 120. □, the effect of 5 and 10 mmol/l glucose alone. Values are means ± SEM of four experiments. *B*: Effect of raising glucose concentration from 5 to 20 mmol/l. The secretory rate in 20 mmol/l glucose is about twice that evoked by 10 mmol/l glucose in the presence of efaroxan. Note that the kinetics of the first-phase increases are similar. Values are means ± SEM of three experiments.

the increase corresponded roughly to the steady-state increase achieved by a depolarization with 40 mmol/l K^+ under the same conditions (data not shown). $[Ca^{2+}]_c$ was also measured in cultured islets perifused following the same experimental protocol (Fig. 3*A–D*) as that for insulin secretion measurements. An interesting difference between efaroxan and phentolamine became apparent: efaroxan induced an oscillatory pattern of $[Ca^{2+}]_c$ increase, whereas phentolamine led to a sustained increase in $[Ca^{2+}]_c$ with

superimposed low-amplitude oscillations. The large-amplitude oscillations induced by efaroxan (amplitude corresponding to that of a depolarization with 40 mmol/l K^+, frequency between 0.3 and 0.5 × min^{-1}) are obscured by presenting the mean values (Fig. 3*B*) but are obvious in individual experiments (Fig. 3*D*). Raising the glucose concentration in the presence of efaroxan altered the synchronized oscillatory pattern, which resumed after periods of 10–20 min. Raising glucose from 5 to 10 mmol/l affected
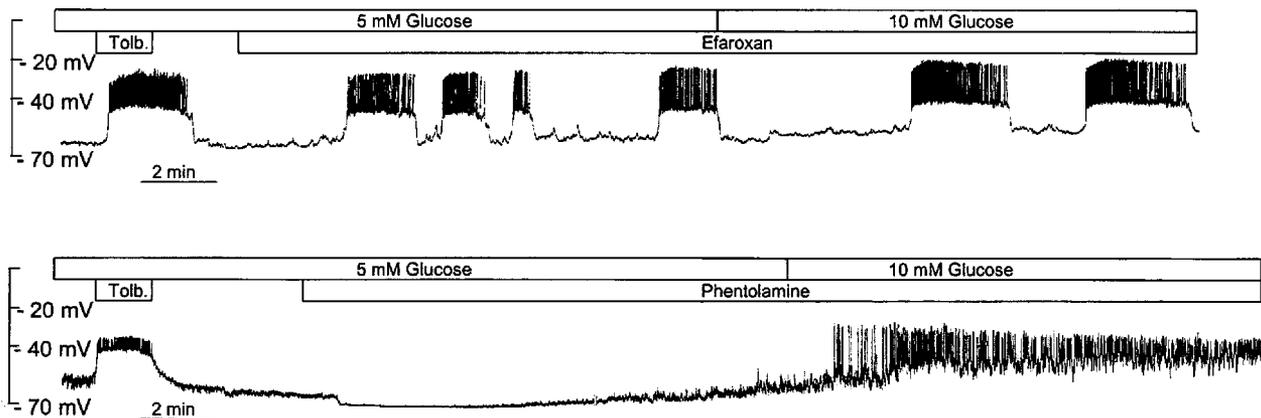

FIG. 2. Comparison of the depolarizing effect of efaroxan and phentolamine. The membrane potential was measured in the perforated patch configuration under current-clamp condition. Initially, 500 μmol/l tolbutamide was applied in the presence of 5 mmol/l glucose as a positive control. After washout of tolbutamide, 100 μmol/l efaroxan or 100 μmol/l phentolamine was added to the bath medium. After exposure to the drug for at least 10 min, the glucose concentration was raised from 5 to 10 mmol/l. In 5 of 10 experiments, the presence of 100 μmol/l efaroxan resulted in an oscillatory depolarization pattern with superimposed action potentials (upper graph). Administration of 100 μmol/l phentolamine produced a sustained depolarization in six of six experiments (lower graph).
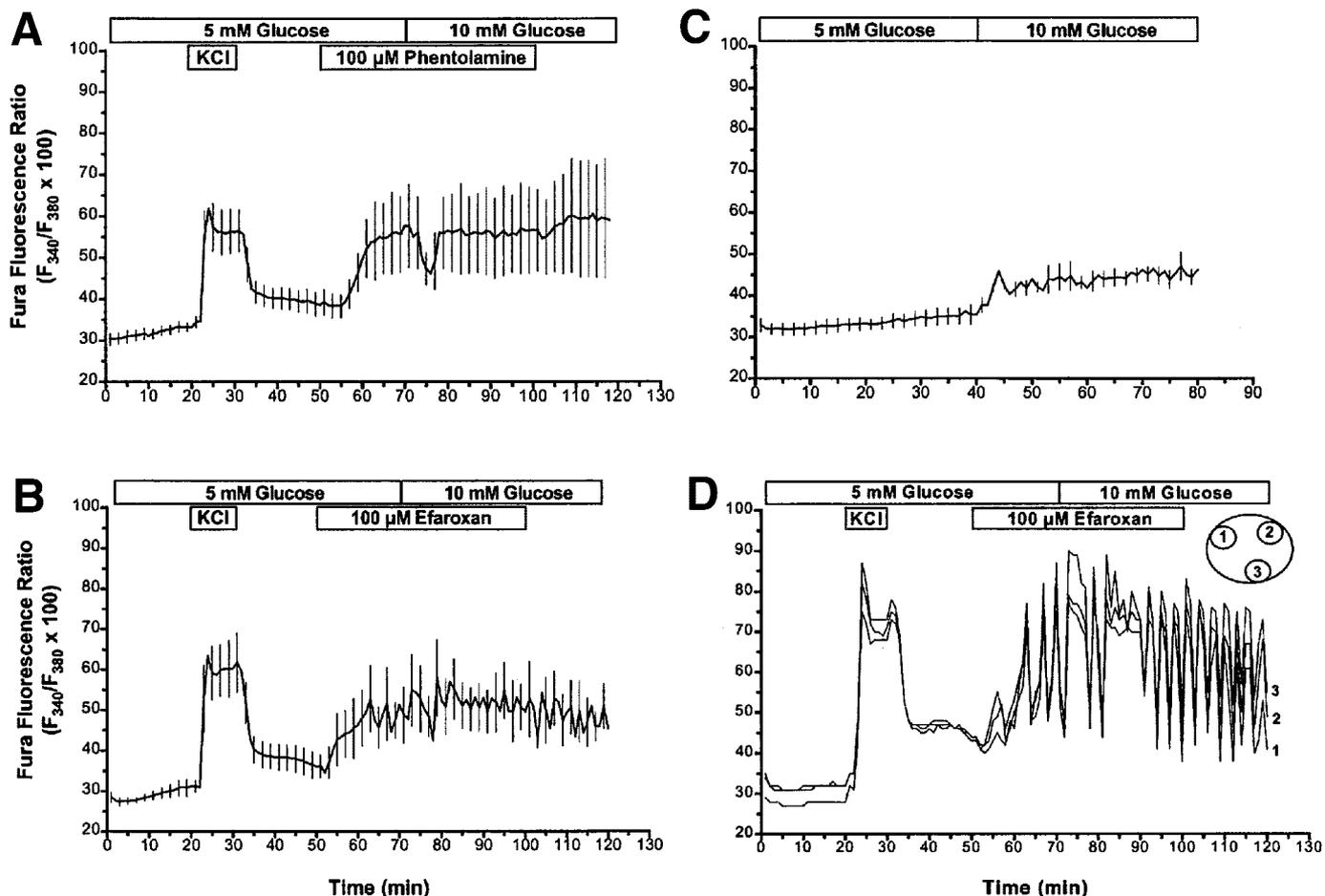
FIG. 3. Comparison of the effect of efaroxan and phentolamine on $[Ca^{2+}]_c$. Fura 2–loaded islets were perifused with Krebs-Ringer medium containing 5 mmol/l glucose. After depolarization with 40 mmol/l K^+ for 10 min, the islets were perifused with 100 μmol/l phentolamine (*A*) or 100 μmol/l efaroxan (*B*). In the continued presence of the imidazolines, the glucose concentration was raised from 5 to 10 mmol/l. Data are means ± SEM of five experiments each. The increase in $[Ca^{2+}]_c$ caused by 10 mmol/l glucose alone is clearly smaller (*C*). Data are means ± SEM of three experiments. Exposure to efaroxan consistently (five of five experiments) caused large amplitude oscillations of $[Ca^{2+}]_c$, which are obscured in the graph showing mean values but clearly visible in a single experiment (*D*). Here, the numbering of the $[Ca^{2+}]_c$ traces refers to the subregions of the islet in which they were determined.

the phentolamine-induced increase in $[Ca^{2+}]_c$: a marked transient decrease first occurred, followed by a return to the sustained high $[Ca^{2+}]_c$ (Fig. 3*A*). The ability of efaroxan to increase $[Ca^{2+}]_c$ in the presence of 5 mmol/l glucose was verified with freshly isolated islets (data not shown).

DISCUSSION

The present experiments show that efaroxan, a K_{ATP} channel–blocking imidazoline, does not increase insulin secretion in the presence of a nonstimulatory glucose concentration but exerts a marked enhancing effect when the glucose concentration is raised. The interest in imidazolines as potential antidiabetic drugs was always motivated by a higher glucose dependence of their insulinotropic effect as compared with that of sulfonylureas (5,17), but published data on the effects of efaroxan have regularly documented a stimulatory effect at basal glucose concentrations (7,21). The reason for this discrepancy is unclear; it could reflect species differences (rat versus mouse islets). However, the secretory effects of phentolamine are clearly less dependent on stimulatory glucose concentrations than those of efaroxan, confirming our earlier observations of heterogeneity within the group of imidazolines (16).

The requirement of a high glucose concentration for efaroxan to be an effective secretagogue is surprising because of mechanistic considerations. It is generally accepted that compounds with a direct blocking effect on K_{ATP} channels act as both initiators and enhancers of insulin secretion. For this reason sulfonylureas are regarded as stimulators of secretion in their own right, even though there is some debate as to whether a minimum of exogenous fuel is needed for the insulinotropic effect (22,23). One could thus logically expect that only imidazolines devoid of effects on K_{ATP} channels and acting exclusively at distal steps of stimulus-secretion coupling require the presence of stimulatory glucose concentrations to influence insulin secretion (14).

In the present study, 100 μmol/l efaroxan blocked K_{ATP} channels slightly less effectively than did 100 μmol/l phentolamine. However, both compounds showed similar depolarizing efficacy and efaroxan was even slightly more effective to increase $[Ca^{2+}]_c$ in single β-cells. Our conclusion, therefore, is that the greater glucose dependence of efaroxan effects on insulin secretion is not simply a function of a less complete K_{ATP} channel closure. This view is supported by previous observations that phentolamine also exerts an enhancing effect on secretion in the

presence of 5 mmol/l glucose, when used at the lower concentration of 32 μmol/l (16).

In view of the depolarizing effect of both imidazolines, the increase in $[Ca^{2+}]_c$ of single β-cells in the presence of 5 mmol/l glucose is most likely due to an influx of Ca^{2+} via L-type Ca^{2+} channels. This process normally results in stimulation of insulin secretion, but the latter can be antagonized by inhibition of metabolism (24). An inhibitory effect of efaroxan on energy metabolism does not seem compatible with the large enhancement of secretion in the presence of 10 mmol/l glucose. The lack of increase of secretion by efaroxan in 5 mmol/l glucose in spite of an increase in $[Ca^{2+}]_c$ might be due to an inhibition by the drug (and not by phentolamine) of a step distal to Ca^{2+} influx. The stimulatory effect of both compounds on secretion in the presence of 10 mmol/l glucose can be viewed as an enhancement of the K_{ATP} channel–independent actions of glucose (25).

The results of the $[Ca^{2+}]_c$ measurements in intact islets added a further degree of complexity to this picture. The oscillations of $[Ca^{2+}]_c$ induced by efaroxan in the presence of 5 mmol/l glucose resemble the slow large-amplitude oscillations produced by a stimulatory glucose concentration. In contrast, phentolamine caused a sustained increase of $[Ca^{2+}]_c$. These features parallel those of the changes in membrane potential: efaroxan often caused oscillatory depolarizations, whereas the depolarization evoked by phentolamine was sustained. The less frequent occurrence of oscillations in the electrophysiological than in the microfluorimetric experiments may be due to the lower temperature at which the latter was performed.

The transient decrease in $[Ca^{2+}]_c$ caused by the rise in glucose concentration in the presence of the imidazolines is most likely due to the activation of an ATP-dependent uptake of Ca^{2+} into internal stores (26). A similar transient decrease occurs when the glucose concentration is increased to a stimulatory value in the presence of sulfonylureas (27). Because the $[Ca^{2+}]_c$ oscillations elicited by efaroxan continued when increasing glucose from 5 to 10 mmol/l, it is unlikely that $[Ca^{2+}]_c$ represents the decisive signal for the first phase-like increase of secretion under this condition. A possible explanation is that a glucose-dependent metabolic signal overcomes the hypothetical inhibitory action of efaroxan distal to $[Ca^{2+}]_c$ influx. Complete elucidation of the mechanisms of action of imidazolines will require investigation of their interplay with the metabolic regulation of insulin secretion.

ACKNOWLEDGMENTS

REFERENCES

1. Robertson RP, Porte D: Adrenergic modulation of basal insulin secretion in man. *Diabetes* 22:1–8, 1973
2. Efendic S, Cerasi E, Luft R: Effect of phentolamine and preperfusion with glucose on insulin release from the isolated perfused pancreas from fasted and fed rats. *Diabetologia* 11:407–410, 1975
3. Östenson CG, Pigon J, Doxey JC, Efendic S: α₂-Adrenoceptor blockade does not enhance glucose-induced insulin release in normal subjects or patients with non insulin-dependent diabetes. *J Clin Endocrinol Metab* 67:1054–1059, 1988
4. Schulz A, Hasselblatt A: An insulin-releasing property of imidazoline derivatives is not limited to compounds that block α-receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 340:712–714, 1989
5. Eglen RM, Hudson AL, Kendall DA, Nutt DJ, Morgan NG, Wilson VG, Dillon MP: Seeing through a glass darkly: casting light on imidazoline "I" sites. *Trends Pharmacol Sci* 19:381–390, 1998
6. Plant TD, Henquin JC: Phentolamine and yohimbine inhibit ATP-sensitive K⁺-channels in mouse pancreatic β-cells. *Br J Pharmacol* 101:115–120, 1990
7. Chan SLF, Morgan NG: Stimulation of insulin secretion by efaroxan may involve interaction with potassium channels. *Eur J Pharmacol* 176:97–101, 1990
8. Rustenbeck I, Herrmann C, Ratzka P, Hasselblatt A: Imidazoline/guanidinium binding sites and their relation to inhibition of K_ATP channels in pancreatic B-cells. *Naunyn-Schmiedeberg's Arch Pharmacol* 356:410–417, 1997
9. Zaitsev SV, Efanov AM, Efanova IB, Larsson O, Östenson C-G, Gold G, Berggren PO, Efendic S: Imidazoline compounds stimulate insulin release by inhibition of K_ATP channels and interaction with the exocytotic machinery. *Diabetes* 45:1610–1618, 1996
10. Proks P, Ashcroft F: Phentolamine block of K_ATP channels is mediated by Kir6.2. *Proc Natl Acad Sci U S A* 94:11716–11720, 1997
11. Grosse Lackmann T, Zünkler BJ, Rustenbeck I: Specificity of nonadrenergic imidazoline binding sites in insulin-secreting cells and relation to the block of ATP-sensitive K⁺ channels. *Ann N Y Acad Sci* 1009:371–377, 2003
12. Efanov AM, Zaitsev SV, Mest HJ, Raap A, Appelskog IB, Larsson O, Berggren PO, Efendic S: The novel imidazoline compound BL11282 potentiates glucose-induced insulin secretion in pancreatic β-cells in the absence of modulation of K_ATP channel activity. *Diabetes* 50:797–802, 2001
13. Hoy M, Olsen HL, Andersen HS, Bokvist K, Buschard K, Hansen J, Jacobsen P, Petersen JS, Rorsman P, Gromada J: Imidazoline NNC77–0074 stimulates insulin secretion and inhibits glucagon release by control of Ca²⁺-dependent exocytosis in pancreatic α- and β-cells. *Eur J Pharmacol* 446:213–221, 2003
14. Efendic S, Efanov A, Berggren PO, Zaitsev A: Two generations of insulinotropic imidazoline compounds. *Diabetes* 51 (Suppl. 3):S448–S454, 2002
15. Hasselblatt A, Schulz A: Phentolamine, a deceptive tool to investigate sympathetic nervous control of insulin release. *Naunyn-Schmiedeberg's Arch Pharmacol* 337:637–643, 1988
16. Rustenbeck I, Leupolt L, Kowalewski R, Hasselblatt A: Heterogeneous characteristics of imidazolines-induced insulin secretion. *Naunyn-Schmiedeberg's Arch Pharmacol* 359:235–242, 1999
17. Morgan NG, Chan SLF: Imidazoline binding sites in the endocrine pancreas: can they fulfill their potential as targets for the development of new insulin secretagogues? *Curr Pharm Design* 7:1413–1431, 2001
18. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recordings from cells and cell-free membrane patches. *Pflügers Arch* 391:85–100, 1981
19. Smith PA, Ashcroft FM, Rorsman P: Simultaneous recordings of glucose-dependent electrical activity and ATP-regulated K⁺-currents in isolated mouse pancreatic β-cells. *FEBS Lett* 261:187–190, 1990
20. Zünkler BJ, Lins S, Ohno-Shosaku T, Panten U: Cytosolic ADP enhances the sensitivity to tolbutamide of ATP-dependent K⁺-channels from pancreatic B-cells. *FEBS Lett* 239:241–244, 1988
21. Chan SLF, Mourtada M, Morgan NG: Characterization of a K_ATP channel-independent pathway involved in potentiation of insulin secretion by efaroxan. *Diabetes* 50:340–347, 2001
22. Panten U, Zünkler BJ, Scheit S, Kirchhoff K, Lenzen S: Regulation of energy metabolism in pancreatic islets by glucose and tolbutamide. *Diabetologia* 29:648–654, 1986
23. Henquin JC: A minimum of fuel is necessary for tolbutamide to mimic the effects of glucose on electrical activity in pancreatic β-cells. *Endocrinology* 139:993–998, 1998
24. Rustenbeck I, Herrmann C, Grimmsmann T: Energetic requirement of insulin secretion distal to calcium influx. *Diabetes* 46:1305–1311, 1997
25. Henquin JC: Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 49:1751–1760, 2000
26. Liu Y, Tengholm A, Grapengiesser E, Hellman B, Gylfe E: Origin of slow and fast oscillations of Ca²⁺ in mouse pancreatic islets. *J Physiol* 508:471–481, 1998
27. Anello M, Gilon P, Henquin JC: Alterations of insulin secretion from mouse pancreatic islets treated with sulphonylureas: perturbations of Ca²⁺ regulation prevail over changes in insulin content. *Br J Pharmacol* 127:1883–1891, 1999