Tricyclic antidepressant imipramine reduces the insulin secretory rate in islet cells of Wistar albino rats through a calcium antagonistic action

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Abstract

Aims/hypothesis. Treatments with antidepressants have been associated with modifications in glucose homeostasis. The aim of this study was to assess the effect of imipramine, a tricyclic antidepressant, on insulin-secreting cells.

Methods. Insulin radioimmunoassay, radioisotopic, fluorimetric and patch-clamp methods were used to characterise the effects of imipramine on ionic and secretory events in pancreatic islet cells from Wistar albino rats.

Results. Imipramine induced a dose-dependent decrease in glucose-stimulated insulin output ($IC_{50}=5.2 \mu\text{mol/l}$). It also provoked a concentration-dependent reduction in ^{45}Ca outflow from islets perfused in the presence of 16.7 mmol/l glucose. Moreover, imipramine inhibited

the increase in ^{45}Ca outflow mediated by K^+ depolarisation. Patch-clamp recordings further revealed that imipramine provoked a marked and reversible decrease of the inward Ca^{2+} current. In single islet cells, imipramine counteracted the rise in $[\text{Ca}^{2+}]_i$ evoked by glucose or high K^+ concentrations.

Conclusions/interpretation. These data indicate that imipramine dose-dependently reduces the insulin secretory rate from rat pancreatic beta cells. Such an effect appears to be mediated by the inhibition of voltage-sensitive Ca^{2+} channels with subsequent reduction in Ca^{2+} entry. Thus, it is possible that some adverse effects of imipramine are related, at least in part, to its capacity to behave as a Ca^{2+} entry blocker.

Keywords Ca^{2+} -channel · Imipramine · Insulin release · Pancreatic beta cells

Introduction

Clinical and epidemiological studies have shown that depression is an underestimated disorder in Type 1 and Type 2 diabetic patients [1, 2]. A higher prevalence has been reported in patients with diabetes mel-

litus than in non-diabetic individuals [1, 2]. Moreover, depression may impair control of glycaemia and treatment compliance, as well as increasing the risk of vascular complications [1, 3].

Different classes of antidepressants commonly prescribed to depressed patients are also used in patients with depression and diabetes mellitus [2, 4, 5, 6]. Successful drugs include tricyclic antidepressants (imipramine and imipramine-like compounds, which block the neuronal uptake of serotonin and norepinephrine) and the selective serotonin re-uptake inhibitors (typified by fluoxetine and sertraline). Other compounds such as tetracyclic antidepressants, norepinephrine re-uptake inhibitors and monoamine oxidase inhibitors are also effective in treating the various forms of depressive disorders [2, 4, 5, 6].

Treatment with antidepressants has been reported to affect glucose homeostasis in diabetic and non-dia-

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Abbreviations: FOR, fractional outflow rate

betic individuals [2, 3, 4, 7, 8]. Tricyclic antidepressants but also selective serotonin re-uptake inhibitors have been shown to increase or decrease glycaemia with or without concomitant changes in plasma insulin [2, 3, 4, 7, 9, 10]. Thus, although it is well recognised that antidepressants may affect blood glucose levels, the physiological mechanism(s) underlying these modifications in glucose homeostasis are not completely clear.

The aim of this study, therefore, was to examine, at the insulin-secreting cell level, the ionic and secretory effects of imipramine, a tricyclic antidepressant that has been used clinically for over three decades.

Materials and methods

Measurement of insulin secretion from incubated pancreatic islets. Experiments were performed with pancreatic islets isolated by the collagenase method from fed female Wistar albino rats (180–220 g, Charles River Laboratories, Brussels, Belgium) [11, 12]. Laboratory animal care was approved by the Ethics Committee of the Université Libre de Bruxelles (Free University of Brussels).

Groups of ten islets, each derived from the same batch of islets, were pre-incubated for 30 min at 37 °C in 1 ml of a physiological salt medium (in mmol/l: NaCl 115, KCl 5, CaCl₂ 2.56, MgCl₂ 1, NaHCO₃ 24) supplemented with 2.8 mmol/l glucose, 0.5% (w/v) dialysed albumin (Fraction V; Sigma-Aldrich, Bornem, Belgium) and equilibrated against a mixture of O₂ (95%) and CO₂ (5%). The islets were then incubated for 90 min at 37 °C in 1 ml of the same medium containing 16.7 mmol/l glucose and, in addition, increasing concentrations of imipramine. Experiments were repeated on different islets populations. Insulin release was expressed as a percentage of the value recorded in control experiments (100%), i.e. in the absence of drug and presence of 16.7 mmol/l glucose. The release of insulin was measured radioimmunologically using rat insulin as a standard [13].

Measurements of ⁴⁵Ca outflow and insulin release from perfused pancreatic islets. The medium used for incubating, washing and perfusing the islets consisted of a bicarbonate-buffered solution having the following composition (in mmol/l): NaCl 115, KCl 5, CaCl₂ 2.56, MgCl₂ 1, NaHCO₃ 24. The medium was supplemented with 0.5% (w/v) dialysed albumin (Fraction V) and equilibrated against a mixture of O₂ (95%) and CO₂ (5%).

The methods used to measure ⁴⁵Ca outflow and insulin release from perfused islets have been described previously [14, 15]. Briefly, groups of 100 islets were incubated for 60 min in a medium containing 16.7 mmol/l glucose and ⁴⁵Ca ion (0.02–0.04 mmol/l; 3.70×10⁶ Bq/ml). After incubation, the islets were washed four times with a non-radioactive medium and then placed in a perfusion chamber. The perfusate was delivered at a constant rate (1.0 ml/min). From the 31st to the 90th minute of perfusion, the effluent was continuously collected over successive periods of 1 min each. An aliquot of the effluent (0.6 ml) was used for scintillation counting while the remainder was stored at –20 °C for insulin radioimmunoassay [13]. At the end of perfusion, the radioactive content of the islets was also determined. The outflow of ⁴⁵Ca (cpm) was expressed as a fractional outflow rate (FOR; percent of instantaneous islet content per minute).

Electrophysiological measurements. Electrophysiological studies were carried out on isolated rat pancreatic islet cells. Pancreatic islets were disrupted in a Ca²⁺-deprived medium and then centrifuged through an albumin solution to remove debris and dead cells [11, 15]. Insulin-secreting cells were selected on the basis of their larger size [16].

Whole-cell Ca²⁺ currents were recorded using the perforated patch whole-cell configuration [17] of the patch-clamp technique [18]. The standard extracellular solution was composed of (in mmol/l): NaCl 118, tetraethylammonium chloride 20, KCl 5.6, CaCl₂ 2.6, MgCl₂ 1.2, glucose 5 and HEPES 5 (pH adjusted to 7.40 with NaOH). The effect of imipramine was investigated on single beta cells by switching from the standard extracellular solution to the same solution supplemented with 100 µmol/l imipramine. In some experiments, 10 µmol/l tetrodotoxin was added to the extracellular solution to block a potential remaining Na⁺ current. The pipette was filled with (in mmol/l): Cs₂SO₄ 76, KCl 10, NaCl 10, MgCl₂ 1 and HEPES 5 (pH adjusted to 7.35 with CsOH). Amphotericin B (0.24 µg/ml) was included in the pipette solution in order to establish electrical contact with the cell interior. Ionic currents were recorded using an EPC-8 amplifier (HEKA, Lambrecht/Pfalz, Germany) and stored on a computer. The pulse software (HEKA) and an ITC-16 AD/DA converter (Instrutech, New York, USA) were used to acquire the data. The current signals were digitised at 2 kHz. Prior to digitising, the signals were filtered at 500 Hz using an 8-pole low-pass Bessel filter (Frequency Devices, Haverhill, Mass., USA). All experiments were carried out at room temperature. The voltage clamp protocol consisted of 450-ms depolarisation pulses starting from a holding potential of –70 mV and ranging from –40 mV to +30 mV.

Measurements of fura-2 fluorescence from single rat pancreatic islet cells. The cells were placed on glass coverslips and maintained in tissue culture for 72 h before use. Islet cells were cultured in RPMI 1640 culture medium (Invitrogen, Merelbeke, Belgium) supplemented with 10% (v/v) newborn calf serum and containing glutamine (2.3 mmol/l), penicillin G (100 U/ml) and streptomycin (100 µg/ml). The cells were then incubated with fura-2 AM (final concentration: 2 µmol/l) for 1 h and, after washing, the coverslips with the cells were mounted as the bottom of an open chamber (1 ml) placed on the stage of the microscope. The medium used to perfuse the cells contained (in mmol/l): NaCl 115, KCl 5, CaCl₂ 2.56, MgCl₂ 1, NaHCO₃ 24, glucose 2.8. It was gassed with O₂ (95%) and CO₂ (5%). Fura-2 fluorescence of single loaded cells (selected on the basis of their larger size) was measured by dual-excitation microfluorimetry with a Spex photometric system (Optilas, Alphen aan den Rijn, The Netherlands). The excitation wavelengths (340 nm and 380 nm) were alternated at a frequency of 1 Hz, the length of time for data collection at each wavelength being 0.05 seconds. The emission wavelength was set at 510 nm. We calculated [Ca²⁺]_i as described previously [11, 15]. Individual experiments were repeated at least four times, on different cell populations.

Drugs. In some experiments, extracellular Ca²⁺ was eliminated by omitting CaCl₂ from the physiological medium and adding 0.5 mmol/l EGTA (Sigma-Aldrich). Depending on the experiment, the media were enriched with glucose (Merck, Darmstadt, Germany), imipramine (Sigma-Aldrich) or tetrodotoxin (Acros Organics, Kortrijk, Belgium). When high concentrations of extracellular K⁺ were used, the concentration of NaCl was lowered to keep osmolarity constant.

Calculations. Results are expressed as means ± SEM. The increase in ⁴⁵Ca outflow was estimated in each individual experi-

ment from the integrated outflow of ^{45}Ca observed during stimulation (45th–68th min) after correction for basal value (40th–44th min). Peak ^{45}Ca outflow was estimated from the difference in ^{45}Ca outflow between the highest value recorded during stimulation and the mean basal value (40th–44th min) within the same experiment. The inhibitory effect of imipramine on ^{45}Ca outflow and insulin release from islets perfused in the presence of 16.7 mmol/l glucose was taken as the difference between the mean value for ^{45}Ca outflow or insulin output recorded in each individual experiment between the 40th and 44th and the 60th and 68th min of perfusion. The statistical significance of differences between mean data was assessed by Student's *t* test or by analysis of variance followed by a Scheffe test procedure. A *p* value of less than 0.05 was considered significant.

Results

Effects of imipramine and fluoxetine on insulin release from incubated rat pancreatic islets. In rat pancreatic islets exposed to 5.6 mmol/l glucose, the addition of 100 $\mu\text{mol/l}$ of imipramine did not affect insulin output (data not shown).

By contrast, imipramine inhibited insulin release from pancreatic islets incubated in the presence of an intermediate insulinotropic glucose concentration. Thus, in the simultaneous presence of 8.3 mmol/l glucose and 100 $\mu\text{mol/l}$ imipramine in the incubation medium, the insulin output averaged $55.6 \pm 5.4\%$ of the control experiments ($p < 0.05$).

Moreover, the addition of increasing concentrations of imipramine to pancreatic islets incubated in the presence of 16.7 mmol/l glucose provoked a concentration-dependent decrease in insulin release (Fig. 1). After the addition of 1 $\mu\text{mol/l}$, 10 $\mu\text{mol/l}$ and 100 $\mu\text{mol/l}$ imipramine, the residual insulin release averaged $90.6 \pm 5.8\%$ ($p > 0.05$), $38.3 \pm 2.6\%$ ($p < 0.05$) and $9.9 \pm 0.7\%$ ($p < 0.05$) of the control value. The IC_{50} value (drug concentration evoking a 50% reduction of the secretory response to 16.7 mmol/l glucose) amounted to 5.2 $\mu\text{mol/l}$.

Effects of imipramine on ^{45}Ca outflow and insulin release from perfused rat pancreatic islets. In the presence of 5.6 mmol/l glucose throughout, and regardless of whether the medium contained or was deprived of extracellular Ca^{2+} , the addition of 10 $\mu\text{mol/l}$ imipramine did not affect the rate of ^{45}Ca outflow from prelabelled and perfused rat pancreatic islets (data not shown).

By contrast, when the physiological medium contained 16.7 mmol/l instead of 5.6 mmol/l glucose, exposure to 10 or 100 $\mu\text{mol/l}$ imipramine provoked an immediate and reversible inhibition of ^{45}Ca outflow from islets perfused in the presence of extracellular Ca^{2+} (Fig. 2a, b). The imipramine-induced reduction in the ^{45}Ca FOR was more rapid and more pronounced at the higher concentration. The paired difference in the ^{45}Ca FOR before (40–44 min) and during

Fig. 1. Effect of increasing concentrations of imipramine (Im) on insulin release from pancreatic islets incubated in the presence of 16.7 mmol/l glucose (Glu). Insulin release (means $\pm$ SEM) is expressed as a percent of the value for control experiments (100%, no added drug, presence of 16.7 mmol/l glucose). The upright lines at the top of the bars correspond to the SEM. Number of samples: 0 $\mu\text{mol/l}$ Im: 31; 1 $\mu\text{mol/l}$ Im: 23; 10 $\mu\text{mol/l}$ Im: 23; 100 $\mu\text{mol/l}$ Im: 25

(60–68 min) exposure to 10 $\mu\text{mol/l}$ and 100 $\mu\text{mol/l}$ imipramine averaged $0.72 \pm 0.01\%/min$ and $1.02 \pm 0.07\%/min$ respectively ($p < 0.05$).

On removal of the drug from the perfusing medium, ^{45}Ca outflow increased. This increase was delayed after removal of the higher drug concentration (compare Fig. 2a and b).

The decreases in the ^{45}Ca FOR induced by the addition of 10 $\mu\text{mol/l}$ and 100 $\mu\text{mol/l}$ imipramine were accompanied by concentration-dependent reductions in insulin output (Fig. 2c, d). In the presence of 10 $\mu\text{mol/l}$ and 100 $\mu\text{mol/l}$ imipramine (60–68 min), the release of insulin represented $31.10 \pm 1.08\%$ and $18.62 \pm 1.75\%$ of the secretory rate recorded before administration of the drug (40–44 min) ($p < 0.05$). The withdrawal of 10 $\mu\text{mol/l}$ imipramine was followed by a rapid increase whilst the withdrawal of 100 $\mu\text{mol/l}$ imipramine was followed by a weak increase in insulin output (Fig. 2c, d).

To further investigate the effects of imipramine on radioisotopic fluxes, the experiments were repeated in the absence of extracellular Ca^{2+} (Fig. 2a, b). In islets exposed throughout to 16.7 mmol/l glucose and Ca^{2+} -depleted media, the rate of ^{45}Ca outflow before drug administration (40–44 min) was lower ($p < 0.05$). Under the latter conditions, 10 $\mu\text{mol/l}$ imipramine did not affect ^{45}Ca outflow (Fig. 2a). The addition of 100 $\mu\text{mol/l}$ imipramine, however, provoked a modest but sustained and reversible rise in the ^{45}Ca FOR (Fig. 2b).

Effects of imipramine on KCl-induced changes in ^{45}Ca outflow from perfused rat pancreatic islets. In the presence of 2.8 mmol/l glucose and extracellular Ca^{2+} ,

Fig. 2. Effect of 10 $\mu\text{mol/l}$ (a, c) and 100 $\mu\text{mol/l}$ (b, d) imipramine on ^{45}Ca outflow (a, b) and insulin release (c, d) from pancreatic islets perfused throughout in the presence of 16.7 mmol/l glucose. Basal media contained extracellular Ca^{2+}

(●) or were deprived of Ca^{2+} and enriched with EGTA (○). Mean values ($\pm\text{SEM}$) refer to four or five individual experiments. FOR, fractional outflow rate

Fig. 3. Effect of a rise in the extracellular K^+ concentration from 5 to 50 mmol/l on ^{45}Ca outflow from pancreatic islets perfused throughout in the absence (○) (a, b) and the presence (●) of 10 $\mu\text{mol/l}$ (a) or 100 $\mu\text{mol/l}$ (b) imipramine. Basal media contained 2.8 mmol/l glucose and extracellular Ca^{2+} . Mean values ($\pm\text{SEM}$) refer to four to six individual experiments. FOR, fractional outflow rate

a sudden rise in the extracellular concentration of K^+ provoked an immediate and pronounced increase in ^{45}Ca outflow from prelabelled and perfused rat pancreatic islets (Fig. 3a, b).

When the same experiment was repeated with either 10 $\mu\text{mol/l}$ (Fig. 3a) or 100 $\mu\text{mol/l}$ (Fig. 3b) imipramine in the medium, the basal rate of ^{45}Ca outflow was unaffected ($p>0.05$). However, the presence of imipramine in the physiological medium markedly reduced the cationic response to K^+ . The increase in ^{45}Ca outflow evoked by 50 mmol/l K^+ averaged $1.18\pm0.14\%/ \text{min}$ in the absence and $0.18\pm0.06\%/ \text{min}$ in the presence of 10 $\mu\text{mol/l}$ imipramine ($p<0.05$). The peak ^{45}Ca outflow observed during exposure to K^+ averaged $1.66\pm0.20\%/ \text{min}$ and $0.92\pm0.08\%/ \text{min}$ in the absence and presence of 10 $\mu\text{mol/l}$ imipramine respectively ($p<0.05$).

Fig. 4. Effect of 100 $\mu\text{mol/l}$ imipramine on the voltage dependence of the inward Ca^{2+} current. **a.** Typical recordings of whole-cell Ca^{2+} currents observed during a 450-ms depolarising pulse from a holding potential of -70 mV to 0 mV on a single islet cell. **b.** Corresponding I-V relationships obtained with voltage clamp protocol consisting of 450-ms depolarisation pulses starting from a holding potential of -70 mV and ranging from -40 mV to $+30$ mV in the absence (control condition, $\circ$) and presence ($\bullet$) of 100 $\mu\text{mol/l}$ imipramine. Mean values ($\pm\text{SEM}$) refer to five individual experiments

In islets exposed throughout to 100 $\mu\text{mol/l}$ imipramine, the stimulatory effect of high extracellular K^+ was completely abolished (Fig. 3b).

Effect of imipramine on the inward Ca^{2+} current in single rat pancreatic islet cells. This effect was investigated by means of the perforated patch configuration of the patch-clamp technique. We investigated the dependence of the Ca^{2+} current on the voltage using 450 ms depolarisation pulses. Each depolarising pulse was separated from the following by 20 seconds to allow return to the basal calcium level [19]. Depolarisations from a holding potential of -70 mV to voltages between -40 mV and $+30$ mV evoked an inward current that reached a maximum amplitude around 0 mV, with gating properties consistent with the slowly deactivating Ca^{2+} current (L-type Ca^{2+} channels) described in rat beta cells [20]. This inward current was insensitive to the application of 10 $\mu\text{mol/l}$ tetrodotoxin (data not shown), thereby confirming that it was not due to the activation of Na^+ channels.

Figure 4a illustrates the effect of 100 $\mu\text{mol/l}$ imipramine on the inward current evoked by depolarisation

Fig. 5. Effect (a) of 20 mmol/l glucose on $[\text{Ca}^{2+}]_i$. **b.** Effect of 10 $\mu\text{mol/l}$ imipramine on glucose-induced (20 mmol/l) increase in $[\text{Ca}^{2+}]_i$. Basal media contained 2.8 mmol/l glucose and extracellular Ca^{2+} . Each graph is a representative experiment conducted on a single cell

tions between -70 and 0 mV in a single islet cell. The decrease of the peak current was marked and reversible.

The amplitude of the inward currents was used to construct I-V plots. The application of 100 $\mu\text{mol/l}$ imipramine induced (Fig. 4b) a robust decrease of the Ca^{2+} current without altering its voltage-dependence. At 0 mV, the peak current intensity dropped from -48.4 ± 4.1 pA in control conditions to -10.9 ± 2.4 pA in the presence of 100 $\mu\text{mol/l}$ imipramine ($n=5$, $p<0.05$).

Effects of imipramine on the cytosolic free Ca^{2+} concentration of single rat pancreatic islet cells. A rise in the extracellular glucose concentration from 2.8 to 20.0 mmol/l provoked a biphasic increase in $[\text{Ca}^{2+}]_i$, consisting of an initial peak followed by a long-lasting plateau (Fig. 5a). The addition of imipramine (10 $\mu\text{mol/l}$) during stimulation with 20 mmol/l glucose reduced the cytosolic free Ca^{2+} concentration (Fig. 5b). The inhibitory effect of the drug was sustained and slowly reversible.

Figure 6 illustrates the effect of depolarising concentrations of K^+ on the cytosolic Ca^{2+} concentration of single islet cells. An increase in the extracellular concentration of K^+ from 5 to 50 mmol/l provoked in most cells a monophasic and marked increase in $[\text{Ca}^{2+}]_i$ (Fig. 6a). In some cells, the $[\text{Ca}^{2+}]_i$ response to

Fig. 6. Effect (a) of 50 mmol/l KCl on $[Ca^{2+}]_i$. b. Effect of 10 μ mol/l imipramine on KCl-induced (50 mmol/l) increase in $[Ca^{2+}]_i$. Basal media contained 5 mmol/l K^+ , 2.8 mmol/l glucose and extracellular Ca^{2+} . Each graph is a representative experiment conducted on a single cell

50 mmol/l K^+ was biphasic, an initial peak of short duration being followed by a plateau (Fig. 6b). Whatever the pattern of the K^+ response, the subsequent addition of imipramine (10 μ mol/l) markedly reduced the $[Ca^{2+}]_i$ (Fig. 6b). In the latter condition, the inhibitory effect of imipramine was less readily reversible (Fig. 6b).

In another series of experiments, we examined the effects of 100 μ mol/l imipramine on the rises in $[Ca^{2+}]_i$ induced by glucose (20.0 mmol/l) and K^+ (50 mmol/l) (data not shown). In both cases, the addition of 100 μ mol/l imipramine again induced marked reductions in $[Ca^{2+}]_i$. At this concentration, however, the drug acted faster. The removal of 100 μ mol/l imipramine from the physiological medium was followed by a delayed increase in $[Ca^{2+}]_i$.

Discussion

We showed that the tricyclic antidepressant imipramine provoked a concentration-dependent inhibition of insulin release from rat pancreatic islets incubated in the presence of insulinotropic (8.3 and 16.7 mmol/l) glucose concentrations.

Dynamic experiments using perfused rat pancreatic islets confirmed the capacity of imipramine to reduce the insulin secretory rate. The latter experimental con-

dition further revealed that the secretory response to imipramine was reversible, suggesting that the antidepressant did not damage insulin-secreting cells.

The dose-dependent inhibitory effect of imipramine on the glucose-induced insulin release was reproduced by fluoxetine, a selective serotonin re-uptake inhibitor antidepressant (data not shown). Altogether, these findings confirm those of another study [21] and extend previous observations, indicating that the *in vitro* reduction of the insulin secretory rate evoked by antidepressants is not restricted to tricyclic antidepressants.

Additional experimental data support the idea that the secretory response to imipramine might be related to modifications in Ca^{2+} inflow.

Radioisotopic experiments conducted on prelabelled rat pancreatic islets indicated that imipramine elicited a concentration-dependent decrease in ^{45}Ca outflow from islets perfused in the presence of 16.7 mmol/l glucose and extracellular Ca^{2+} . In islets exposed throughout to calcium and insulinotropic glucose concentrations, the ^{45}Ca FOR is known to reflect a sustained stimulation of isotopic exchange between influent ^{40}Ca and effluent ^{45}Ca [22]. Thus, under such experimental conditions, the inhibitory effect of imipramine on ^{45}Ca outflow can be viewed as the result of a reduction of ^{40}Ca entry into the islet cells.

The effects of imipramine on the ^{45}Ca FOR responses to high extracellular K^+ concentrations also suggest that imipramine dose-dependently reduces Ca^{2+} inflow. Indeed, in ^{45}Ca -loaded pancreatic islets, the increment in ^{45}Ca FOR evoked by high K^+ again results from the stimulation of a $^{40}Ca/^{45}Ca$ exchange process [12, 22].

A further argument in support of an inhibitory effect of imipramine on Ca^{2+} entry can be found in the drug's lack of inhibitory effect on ^{45}Ca outflow from islets exposed to non-insulinotropic glucose concentrations and/or to Ca^{2+} -depleted media.

The electrophysiological experiments conducted on single islet cells confirmed the ability of imipramine to inhibit a tetrodotoxin-insensitive inward current.

The present findings also provide information about the modality of Ca^{2+} entry affected by imipramine. Indeed, imipramine failed to affect the rate of ^{45}Ca outflow from islets perfused in the presence of 5.6 mmol/l glucose but markedly inhibited the ^{45}Ca FOR response to 16.7 mmol/l glucose. Under the latter condition, the depolarising effect of glucose reaches the threshold potential for activation of voltage-sensitive Ca^{2+} channels.

The inhibitory effects of imipramine on the KCl-induced changes in ^{45}Ca outflow further suggest that the drug interfered with a voltage-sensitive modality of Ca^{2+} entry.

Moreover, patch-clamp experiments revealed that imipramine decreased the amplitude of a voltage-sensitive inward current with gating properties similar to the L-type Ca^{2+} channels equipping rat beta cells [20].

Incidentally, the effects of imipramine on the cationic responses to glucose and KCl depolarisation are reminiscent of those evoked by Cd^{2+} or verapamil, two calcium channel blockers known to interact with the L-type Ca^{2+} channels [12, 22, 23, 24].

Thus, all these observations support the view that imipramine reduced Ca^{2+} entry by inhibiting the L-type voltage-sensitive Ca^{2+} channels. Previous studies on cardiac and neuronal cells also revealed that imipramine has an inhibitory effect on an inward Ca^{2+} current [25, 26].

A decrease in Ca^{2+} entry as mediated by imipramine should be followed by a reduction in the cytosolic Ca^{2+} concentration. In agreement with this, calcium fluorimetry experiments conducted on single islet cells confirmed the ability of imipramine to counteract the increase in $[\text{Ca}^{2+}]_i$ elicited either by insulinotropic concentrations of glucose or by high K^+ concentrations.

This effect on $[\text{Ca}^{2+}]_i$, a key element in the stimulus–secretion coupling process, probably triggers the decrease in insulin output. Such a view is also substantiated in the observation that the secretory responses to imipramine mirrored the ^{45}Ca FOR data.

In addition to inhibitory effects on plasma membrane Ca^{2+} channels, 100 $\mu\text{mol/l}$ imipramine also elicited a small, sustained and slowly reversible increase in ^{45}Ca FOR from islets exposed to 16.7 mmol/l glucose and Ca^{2+} -free media. This suggests that a high concentration of imipramine might also provoke intracellular calcium redistribution with subsequent changes in ^{45}Ca outflow [11, 27, 28].

In conclusion, our data indicate that the tricyclic antidepressant imipramine reduces the glucose-sensitive insulin output from rat pancreatic beta cells. This effect results from the inhibition of voltage-sensitive Ca^{2+} channels with subsequent reduction in Ca^{2+} entry.

Although serum concentrations of imipramine at typical clinical doses are lower (~ 0.5 – 1.0 $\mu\text{mol/l}$), it should be kept in mind that the plasma concentrations of this tricyclic antidepressant can reach higher levels, e.g. through overdosage, deficient elimination and drug interactions [29]. Moreover, numerous data indicate that imipramine, like other antidepressants, is extensively bound to tissue protein and accumulates in the cells [30, 31, 32]. From a clinical viewpoint, therefore, and taking into account the species differences, it is possible that the side-effect profile of imipramine is related, at least in part, to its ability to act as a Ca^{2+} entry blocker.

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