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Inhibition of Glucose-induced Insulin Secretion by Calcium Channel Blocking Drugs In-vitro but not In-vivo in the Rat

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Abstract—The organic calcium channel blocking drugs darodipine, nicardipine, diltiazem and verapamil inhibited glucose-induced (8·3 mmol L^{-1}) insulin secretion by rat isolated islets in a concentration-dependent manner. The IC50 values (μmol L^{-1}) for nicardipine, diltiazem and verapamil were 0·0025, 1·94 and 3·6, respectively. There were no significant differences between the potencies of any one drug when compared at two different glucose concentrations (27·8 and 8·3 mmol L^{-1}). Isolated islets were also responsive to the calcium channel activating drugs BAY K 8644 and CGP 28392, which enhanced glucose-induced insulin secretion and prevented the inhibitory effect of verapamil. BAY K 8644 was more potent than CGP 28392. In the anaesthetized rat, neither verapamil (1 mg kg^{-1} i.v. 10 min before glucose) nor nicardipine (0·2 mg kg^{-1} i.v.) modified the glucose or insulin response to intravenous glucose whilst producing marked cardiovascular effects. The plasma concentrations of nicardipine ($3·9 \times 10^{-8}$ M at 10 min post injection) were similar to those producing effects in-vitro whereas the plasma concentrations of verapamil (5×10^{-7} M) were lower. It thus appears that the islet B cell calcium channel is less sensitive to calcium channel blocking drugs in-vivo than in-vitro. Moreover, in-vivo, the cardiovascular system is more sensitive to these drugs than are the islet B cells, although the potencies of the calcium channel blocking drug in isolated islets are similar to those reported for cardiac muscle and vascular smooth muscle in-vitro.

The initiation of insulin secretion is thought to be due to the accumulation of Ca^{2+} in the B cell (Malaisse 1973; Herchuelz et al 1980). Ca^{2+} enters the B cell, at least in part, via voltage-operated Ca^{2+} channels (Lebrun et al 1982), which are opened following depolarization of the B cell membrane secondary to a reduction in potassium permeability (Henquin 1978; Atwater et al 1979). Drugs which block voltage-sensitive Ca^{2+} channels potently inhibit glucose-induced insulin secretion in-vitro (Malaisse et al 1976; Lebrun et al 1982) and are used widely in the treatment of angina and hypertension. Although some studies suggest that these drugs may reduce the insulin response to a glucose load and impair glucose tolerance (Giugliano et al 1980; Charles et al 1981; De Marinis & Barbarino 1980) the balance of published work suggests that oral administration of calcium antagonists, in haemodynamically active doses, does not interfere with insulin release or impair glucose tolerance (Donnelly & Harrower 1980; Semple et al 1983; Segrestaa et al 1984; Kanatsuna et al 1985; Marre et al 1985; Shamoon et al 1985). Lebrun et al (1982) suggested that at *low* stimulatory glucose concentrations Ca^{2+} may enter the B cell through non-voltage sensitive mechanisms, since insulin secretion and Ca^{2+} efflux induced by 8·3 mmol L^{-1} glucose were relatively insensitive to inhibition by verapamil. As 8·3 mmol L^{-1} is a physiological (albeit high) glucose concentration, this could explain discrepancies between clinical findings and experimental observations made in-vitro. Most in-vitro studies have used higher glucose concentrations (16·7–27 mmol L^{-1}) to stimulate insulin secretion.

Thus, the present work was undertaken primarily to test the hypothesis that insulin secretion induced by low concentrations of glucose is relatively insensitive to calcium channel blocking drugs in-vitro. Four different agents were examined which represented two of the three different classes of Ca^{2+} channel blocking drugs proposed by Spedding (1985): darodipine and nicardipine (class I) and verapamil and diltiazem (class II). The sensitivity of glucose induced insulin secretion to the dihydropyridine Ca^{2+} channel *activating* drugs BAY K 8644 and CGP 28392 was also examined. In-vivo studies were performed to demonstrate whether calcium antagonists, when given to rats in sufficient doses to exert a hypotensive effect, had any effect on glucose induced insulin secretion. A preliminary account of some of these results was presented at the 1986 spring meeting of the Medical and Scientific Section of the British Diabetic Association (Semple & Furman 1986).

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Materials and Methods

In-vitro

Pancreatic islets were isolated from freely fed, male Sprague-Dawley rats (250–350 g) (Lacy & Kostianovsky 1967). Groups of five islets were preincubated with shaking (64 oscillations min^{-1}) for 30 min in Krebs-bicarbonate buffer (37 °C, pH 7·4, 95% O_2/5% CO_2, 3 mmol L^{-1} glucose, 3 mg mL^{-1} bovine serum albumin). The preincubation medium was removed and replaced by 1·0 mL of fresh medium containing glucose in various concentrations with various concentrations of drug or appropriate vehicle. After 1 h incubation 25 μL of medium was removed, diluted 1 in 6 with phosphate assay buffer and an aliquot frozen for later assay of immunoreactive insulin.

In-vivo

Male Sprague-Dawley rats (250–300 g) were fasted for 18 h and then anaesthetized with pentobarbitone sodium

(60 mg kg^{-1} i.p.). The trachea was cannulated and the animal ventilated on pure oxygen (53 strokes min^{-1}; 1 mL/100 g). A cannula was placed in the aortic arch via the right common carotid artery for blood pressure recording via a Statham pressure transducer connected to the strain gauge coupler of a Washington Chart Recorder. Cannulae were also placed in the left femoral artery for blood sampling and the left femoral vein for intravenous injection. Temperature was monitored via a rectal thermometer and maintained at 37 ± 0·5 °C, using a lamp over the animal. After 30 min stabilization drug or appropriate vehicle was injected i.v. A blood sample was removed 10 min later and glucose injected (0·25 g kg^{-1} i.v.). Further blood samples (0·1 mL) were removed 5, 10, 20 and 25 min later. Before removing a blood sample, 0·2 mL blood was removed to allow for the cannula dead space and this blood was re-injected to minimize blood loss. The net blood loss across the whole experiment was 0·5 mL. Blood samples were centrifuged immediately (Beckman microfuge) and 25 μL plasma frozen for subsequent insulin (Immunoreactive insulin) assay. The remaining plasma was used for glucose estimation.

Biochemical determinations

Glucose was determined using a Beckman glucose analyzer 2. Immunoreactive insulin was determined (Hales & Randle 1963) with materials obtained in a kit (Amersham International), membrane filters (N25 45 UP) obtained from Oxoid and a rat insulin standard (Novo).

In some experiments plasma concentrations of verapamil or nicardipine were determined at 1 and 10 min after injection. For these experiments plasma was separated and frozen immediately after removal of the blood. The tubes containing frozen plasma were packed in dry ice and sent to Dr D. Holt, Poisons Unit, New Cross Hospital, London for verapamil estimation and to Syntex Research Centre, Research Park, Heriot-Watt University, Riccarton, Edinburgh for determination of nicardipine by HPLC methods.

Calculations and statistical treatment of results

Insulin secretion from isolated islets was expressed as ng insulin per islet secreted in an hour. The molar concentrations of Ca^{2+} channel blocking drugs required to produce 50% maximum inhibition of insulin secretion (IC50), were determined from the pooled data for each drug and compared using linear regression analysis on a Ferranti PC micro-computer. The % maximum inhibition was calculated for each dose of each drug as follows: % maximum inhibition of insulin secretion produced by a given dose of drug = $(I_A - I_I)/(I_A - I_{100}) \times 100$. Where I_A = mean insulin secretion in the absence of drug; I_I = insulin secretion in the presence of a given dose of drug; I_{100} = insulin secretion in the presence of a maximal inhibitory concentration of drug. All insulin secretion values were corrected by subtracting the basal insulin secretion value obtained in 3 mmol L^{-1} glucose.

Glucose disappearance constants after i.v. glucose were determined from the regression line relating the logarithm of the glucose excess above fasting and time after glucose (Amatuzio et al 1953). The total plasma insulin response to glucose administration was determined by calculating the area under the plot of plasma insulin versus time and expressed as arbitrary units. (This was done using a program devised by Dr I. J. Zeitlin.)

Data were examined for deviation from normality using Rankit Analysis (Wardlaw 1985). Clear deviation from normality was found for plasma insulin concentrations which were, however, found to approximate to a log normal distribution as noted previously (Furman et al 1981) for serum insulin concentrations in the mouse. Accordingly, plasma insulin concentrations were expressed as geometric means with 95% confidence limits. These data were analysed after conversion to logarithms to the base 10. All other data were expressed as arithmetic means ± s.e.m. Differences among groups were detected using one way analysis of variance and specific group comparisons made using Student's *t*-test for unpaired data. Statistical significance was accepted where $P < 0.05$.

Drugs used

Verapamil was obtained from Sigma Chemical Co., nicardipine, darodipine, diltiazem, BAY K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) and CGP 28392 (4-[2-difluoromethoxyphenyl]-1,4,5,7-tetrahydro-2-methyl-5-oxofuro[3,4-*b*]pyridine-3-carboxylic acid ethyl ester) were gifts, respectively, from Syntex Research, Sandoz (Basle), Dr R. M. Wadsworth (Dept of Physiology and Pharmacology, Strathclyde University), Dr K. Schramm (Bayer) and Ciba-Geigy. Verapamil and diltiazem were obtained as the hydrochlorides and dissolved in saline. The other drugs were water-insoluble and were dissolved in polyethylene glycol 400 (PEG 400) and then diluted with Krebs bicarbonate buffer. BAY K 8644 was dissolved in dimethylsulphoxide (DMSO). The appropriate concentrations of DMSO (10^{-5}–10^{-2} M) or PEG 400 (10^{-5}–10^{-2} M) were used as control solutions. In these concentrations neither solvent modified basal or glucose-induced insulin secretion.

Results

In-vitro

Darodipine, nicardipine, diltiazem and verapamil each inhibited glucose-induced insulin secretion in a concentration-dependent manner (Fig. 1). In the cases of darodipine, nicardipine and verapamil, maximal inhibitory concentrations produced a complete suppression of glucose-induced insulin secretion in either concentration of glucose. Diltiazem, however, produced only an 80% inhibition. The IC50 concentrations of each of the drugs were, in 8·3 mmol L^{-1} glucose 0·025, 1·94 and 3·6 for nicardipine, diltiazem and verapamil, respectively, and in 27·8 mmol L^{-1} glucose 0·01, 2·04 and 3·1, respectively. The unusual shape of the darodipine dose–response curves did not allow calculation of IC50 values but the drug is clearly equipotent with or, probably, more potent than nicardipine. Thus, the rank order of potency in each concentration of glucose (8·3 or 27·8 mmol L^{-1}) was darodipine > nicardipine ≫ diltiazem > verapamil. The IC50 values for each drug when determined in

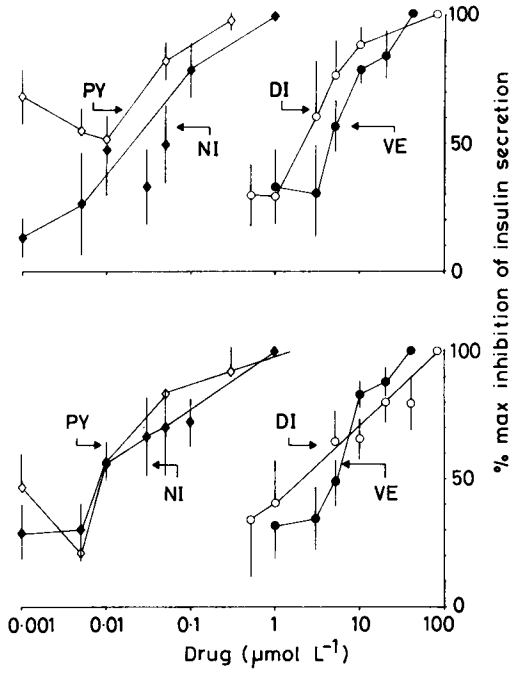

FIG. 1. Effect of various concentration of darodipine (PY), nicardipine (NI), diltiazem (DI) or verapamil (VE) on the secretion of insulin, from rat isolated islets, induced by glucose in a concentration of 8·3 mmol L^{-1} (upper panel) or 27·8 mmol L^{-1} (lower panel). The results are expressed in terms of the % maximum response to each drug. Each value is the mean ± s.e.m. of 8–12 observations.

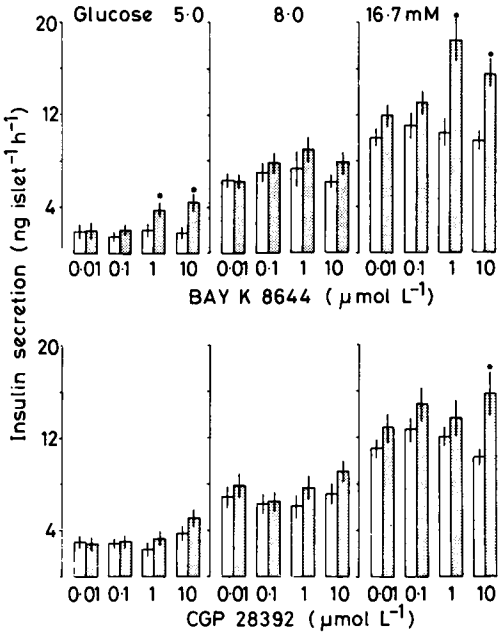

FIG. 2. Effect of BAY K 8644 (upper panel) or CGP 28392 (lower panel) on insulin secretion induced by 5, 8 or 16·7 mmol L^{-1} glucose. Shaded columns refer to islets incubated with drugs and open columns refer to islets incubated in the presence of the appropriate concentration of the vehicle. Each column represents the mean ± s.e.m. of 9–12 observations. * Denotes a statistically significant difference from appropriate control value, $*P < 0.05$.

8·3 mmol L^{-1} glucose were not significantly different from the corresponding IC50 values determined in 27·8 mmol L^{-1} glucose.

BAY K 8644 (10^{-7}–10^{-5} M) augmented glucose-induced insulin secretion but this was only pronounced and statistically significant at glucose concentrations of 5 and 16·7 mmol L^{-1}. The absence of significant effect in 8 mmol L^{-1} glucose was found in several different experiments (Fig. 2). CGP 28392 also augmented glucose-induced insulin release but a significant effect of the drug could be observed only using 10^{-5} M in the presence of 16·7 mmol L^{-1} glucose. In 8 mmol L^{-1} glucose each drug prevented the inhibitory effect of verapamil (2×10^{-5} M) with BAY K 8644 being more potent than CGP 28392. (Insulin secretion, ng $islet^{-1} h^{-1}$, control 7·8 ± 0·9; verapamil 3·4 ± 0·4; verapamil + BAY K8644, 10^{-7} M, 5·2 ± 1·3 ($P < 0.05$); verapamil + BAY K8644, 10^{-6} M, 7·6 ± 1·7 ($P < 0.01$); verapamil + CGP 28392, 10^{-5} M, 5·8 ± 0·6 ($P < 0.05$).)

In-vivo

Neither nicardipine (0·2 mg kg^{-1} i.v.), nor verapamil (1 mg kg^{-1} i.v.), significantly modified fasting plasma concentrations of glucose and insulin, the peak plasma glucose concentrations after glucose injection or the rate of glucose disappearance (K_g) (Table 1). Moreover, the increase in plasma insulin produced by glucose injection was not modified by either drug. This was true both for the peak plasma insulin concentrations and the area under the curve relating plasma insulin to time after glucose injection.

At these doses each drug produced a marked decrease in mean arterial blood pressure measured at 10 min after injection (blood pressure mm Hg; control 151 ± 5; before nicardipine 150 ± 3; after nicardipine 69·6 ± 9 ($P < 0.001$); before verapamil 137 ± 6; after verapamil 107 ± 6 ($P < 0.01$)). Decreases occurred within seconds of injection and the blood pressure remained reduced for the duration of the experiment (25 min after glucose injection). Neither drug significantly changed the heart rate from the control of 401 ± 15 beats min^{-1}.

Plasma concentrations of verapamil in two separate rats were 4×10^{-6} and $3{\cdot}3 \times 10^{-6}$ M at 1 min post injection and $5{\cdot}5 \times 10^{-7}$ and $5{\cdot}2 \times 10^{-7}$ M at 10 min post injection.

Plasma concentrations of nicardipine in pooled plasma from two rats were $0{\cdot}14 \times 10^{-6}$ and $3{\cdot}9 \times 10^{-8}$ M at 1 and 10 min, respectively, post-injection.

Discussion

The present work shows that glucose-induced insulin secretion from isolated islets of Langerhans is sensitive to inhibition by a range of organic calcium channel blocking drugs. This is in agreement with previous findings using verapamil (Malaisse et al 1977), gallopamil (D-600, Malaisse et al 1976) and a number of dihydropyridine

Table 1. Plasma concentrations of glucose (mmol L^{-1}, mean ± s.e.m.) and insulin (ng mL^{-1}, geometric means, 95% confidence limits) at various times after injection of glucose (0·25 g kg^{-1} i.v.), which was injected 10 min after control solution, nicardipine (0·2 mg kg^{-1} i.v.) or verapamil (1 mg kg^{-1} i.v.). Also shown are the glucose disappearance constants (K_g) and the areas under the curves relating plasma insulin concentration and time after glucose. The values in parentheses are the numbers of observations.

	Plasma glucose (mmol L^{-1})					
	Fasting*	5 min	10 min	20 min	25 min	K_g (% min^{-1})
Control (8)	5·6 ± 0·25	16·1 ± 0·4	13·3 ± 0·28	9·9 ± 0·3	8·6 ± 0·4	6·2 ± 0·73
Nicardipine (8)	5·5 ± 0·26	16·8 ± 0·8	14·4 ± 0·4	11·4 ± 0·7	10·3 ± 0·8	4·4 ± 0·7
Verapamil (7)	5·9 ± 0·5	16·9 ± 1·3	14·2 ± 0·6	11·1 ± 0·4	8·7 ± 1·3	5·3 ± 0·6
	Plasma insulin (ng mL^{-1})					Area under curve (arbitrary units)
Control (8)	1·6 (1·1, 1·9)	11·5 (7·9, 16·5)	7·5 (5·5, 10·4)	4·5 (3·9, 5·3)	3·5 (2·8, 4·4)	164 (130, 207)
Nicardipine (8)	1·3 (0·9, 1·9)	8·3 (5·2, 13·2)	8·9 (5·5, 14·3)	5·4 (3·2, 9·1)	6·0 (3·6, 9·5)	175 (122, 251)
Verapamil (7)	1·4 (1·0, 1·9)	11·0 (7·1, 16·9)	8·3 (4·9, 14·1)	6·2 (4·1, 9·2)	6·0 (3·2, 11·1)	191 (129, 281)

* Measured just before drug injection.

calcium channel blocking drugs (Al-Mahmood et al 1986). Moreover, the absolute potencies, and rank order of potencies, of the individual agents in isolated islets in the present study corresponded closely to their potencies in blocking voltage-sensitive Ca^{2+} channels in certain vascular beds and in cardiac tissue in-vitro (Cauvin et al 1983; Clarke et al 1983). The similarity between isolated islets and cardiac and smooth muscle tissue in-vitro is further indicated by the effect of the two dihydropyridine Ca^{2+} channel activating drugs, which are known to contract smooth muscle and exert positive inotropic effects (Ishii et al 1985; Allen et al 1985). These drugs were found to stimulate insulin secretion, with BAY K 8644 being more potent than CGP 28392. Others have also reported similar stimulatory effects of these drugs on insulin secretion in isolated islets (Henquin et al 1985; Malaisse & Mathias 1985; Panten et al 1985). Each drug was found to prevent the inhibitory effect of verapamil on insulin secretion, a finding noted previously for CGP 28392 (Malaisse & Mathias 1985). This antagonism of verapamil has been attributed in other tissues to allosteric modification of the verapamil binding site (Ishii et al 1985), or to the production of the opposite effect by activation of the dihydropyridine receptor (Allen et al 1985).

Despite the high sensitivity in-vitro, we were unable to demonstrate inhibition of glucose-induced hyperinsulinaemia in-vivo. This agrees with some, but not all, findings in patients (see introduction). Few in-vivo studies have been carried out in experimental animals. One study using nifedipine showed a marked impairment of glucose-induced hyperinsulinaemia in the rat (Kanatsuna et al 1985). Verapamil has been shown to inhibit glucose-induced elevations in plasma insulin in the dog (Dominic et al 1980). However, neither of these studies attempted to relate the doses producing these effects to doses producing cardiovascular changes. The difference between the in-vivo and in-vitro findings cannot be explained readily. The present work, using four different agents, does not support the hypothesis that insulin secretion induced by low (physiological) concentrations of glucose is relatively insensitive to organic Ca^{2+} channel blocking drugs, as there was no significant difference between the potencies of the drugs when different concentrations of glucose were used (8·3 and 16·7 mmol L^{-1}). Thus, it can be speculated that Ca^{2+} is entering the beta cell using the same mechanisms at both glucose concentrations. This contrasts with the work of Lebrun et al (1982), who showed verapamil to be much more potent in inhibiting insulin secretion induced by high potassium or by 27·8 mmol L^{-1} glucose than that induced by 8·3 mmol L^{-1} glucose. The difference between our findings and those of Lebrun et al (1982) may depend on their use of perifused, rather than incubated, islets and the pre-perifusion of their islets in zero glucose, compared with our pre-incubation in 3·0 mmol L^{-1} glucose.

Another possible explanation for the discrepancy between the in-vitro and in-vivo findings is that the two drugs failed to reach the concentrations in-vivo corresponding to the in-vitro concentrations. However, after their i.v. injection in our experiments, verapamil and nicardipine were clearly present in pharmacologically active concentrations, as evidenced by the profound and sustained decrease in blood pressure produced by each drug. In the case of verapamil, this concentration may have been too low to influence insulin secretion as evidenced by the plasma verapamil concentration of about 5×10^{-7} M, at the time of the glucose injection. On the other hand, the plasma nicardipine concentration was actually higher than its in-vitro IC50 concentration. Thus, assuming that plasma concentrations of the drugs reflect the concentration of drugs in contact with the islet B cell, it appears that the rat cardiovascular system in-vivo, may be more sensitive to Ca^{2+} channel blocking drugs than is the pancreatic islet B cell. This difference in sensitivity does not appear to be predictable from in-vitro studies (comparing the present work on isolated islets with that of others using cardiovascular tissues in-vitro). If the rat is a reasonable model for the human in this respect, this could explain the general absence of deleterious metabolic effects in patients receiving those compounds in usual therapeutic doses.

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