Journal of Autonomic Pharmacology, 17, 293–302

Comparative effects of calcium channel blockers and indomethacin on insulin secretion and blood pressure increase derived from α_1-adrenoceptor stimulation in the rabbit

Correspondence:
J. Moratinos

M. J. García-Barrado, M. Reverte, J. Palomero & J. Moratinos

Departamento de Farmacología, Facultad de Medicina, Universidad de Salamanca, Avenida del Campo Charro s/n, 37007 Salamanca, Spain

1 In conscious, fasted rabbits the intravenous infusion of the α_1-adrenoceptor agonist, amidephrine (3 and 10 μg kg^{-1} min^{-1}) induced a dose related increase in insulin plasma levels. This effect was accompanied by a minor hypo- or hyperglycaemic response, depending on the dose of agonist infused.

2 A dose related increase in mean arterial pressure and reduction in heart rate were also found after amidephrine administration.

3 The insulin secretory response to amidephrine was not prevented in rabbits previously treated with atropine (5.26 μg kg^{-1} min^{-1}). However, in the presence of muscarinic receptor blockade the bradycardic effect of amidephrine was either suppressed or attenuated.

4 Pretreatment with the calcium channel antagonist elgodipine (35 ng kg^{-1} min^{-1}) or with indomethacin (0.66 mg kg^{-1} min^{-1}) clearly blocked the effect of amidephrine on insulin secretion.

5 The haemodynamic changes induced by amidephrine were preserved in the presence of either verapamil (0.17 μg kg^{-1} min^{-1}) or indomethacin, whereas the hypertensive response was antagonized by elgodipine.

6 Our results suggest that the metabolic and haemodynamic changes mediated by amidephrine are two independent effects, insulin secretion requiring the presence of extracellular calcium and the synthesis of arachidonic acid metabolites.

Introduction

Selective stimulation of α_1 or β-adrenoceptors induces a significant increase in the levels of plasma immunoreactive insulin (IRI) in conscious rabbits (Moratinos, Carpene, de Pablos & Reverts, 1988). The role of β-adrenoceptors in insulin secretion is well documented, the response resulting from the preferential stimulation of β_2-adrenoceptors (John, Doxey, Walter & Reid, 1990) with subsequent production of cyclic AMP (Malaisse, Malaisse-Lagae & Mayhew, 1967). The pivotal role played by Ca^{2+} in insulin secretion (Malaisse, 1990) is also well established, as is the calcium dependency of α_1-adrenoceptor-mediated responses, including insulin release from an insulin secreting cell line (Ullrich & Wollheim, 1985). An increase in intracellular calcium could be supported by Ca^{2+} influx (Rooney, Jass & Thomas, 1989), because the calcium channel blocker verapamil suppressed the increase in insulin secretion induced by the α_1-adrenoceptor agonist amidephrine (Garcia-Barrado, Reverte & Moratinos, 1992). Therefore, it would be of interest to test, in conscious rabbits, the effect of a more selective calcium channel antagonist, elgodipine (a recent dehydropyridine derivate (Román, de Migual, Giraldez & Galiano, 1990)) on the amidephrine-induced excitatory response.

Several studies have demonstrated the regulatory influences exerted by arachidonate and arachidonic acid metabolites on insulin release (Turk, Gross & Ramanadham, 1993). PGE_2, by decreasing cAMP levels, would inhibit insulin release, whereas PGI_2, acting in an opposite way, would induce insulin secretion (Heaney & Larkins, 1981; Robertson *et al.*, 1987). Interestingly, 6-keto-$PGF_{1\alpha}$, which is produced by spontaneous degradation of prostacyclin, seems to be a major islet metabolic product (Tadayyon, Bonney & Green, 1990), its synthesis being stimulated in peripheral tissues (Negibil & Malik 1992a) by α_1-adrenoceptor agonists. Therefore, it seemed

Journal of Autonomic Pharmacology, 17, 293–302

pertinent to check the effect of the cyclo-oxygenase inhibitor indomethacin on the amidephrine-induced insulin secretory response.

Finally, considering that haemodynamic changes induced by amidephrine could induce a reflex vagal response with subsequent insulin release (Holst, Gronholt, Schaffalitzky & Fahrenkrug, 1981; Ahrèn & Taborsky, 1986) the metabolic effects of the agonist were challenged in the presence of muscarinic blockade. As the drugs employed in the present work could introduce haemodynamic alterations their effects on blood pressure and heart rate in conscious rabbits were recorded.

A preliminary account of this work has been published previously in abstract form (Reverte, Moratinos & Garcia-Barrado, 1994).

Methods

Animals

The experiments were performed in male New Zealand white rabbits aged from 7 to 12 months (body weight between 2.8 and 3.8 kg) and fasted for 24 h before the experiments.

Measurement of plasma glucose and insulin levels

The experimental design has been fully described in earlier publications (Moratinos *et al*., 1988; García-Barrado *et al*., 1992). Briefly, arterial blood was sampled by use of an indwelling cannula placed in the central artery of one ear. Two control samples, separated by an interval of 30 min were always taken to ensure reliable basal measurements before beginning drug infusion. Drug solutions (unless stated otherwise, see below) were infused at a constant rate (0.15 ml min^{-1}) for 30 min through an indwelling cannula in the marginal vein of the contralateral ear. The arterial cannula was kept functional by a slow constant infusion of physiological saline solution (0.07 ml min^{-1}). Resting intervals of 2 weeks between two consecutive experiments on the same animal were used in order to avoid the side-effects of arterial blood sampling.

Analyses

Plasma glucose was estimated by means of the glucose oxidase procedure using a kit from Boehringer–Mannhein, Germany. Immunoreactive insulin (IRI) was determined by using an INSI-PR (CIS-Radioquímica, Spain) radioimmunoassay kit (with human insulin as standard); the detection limit was 3 μIU ml^{-1}.

Drugs

Fresh stock solutions of amidephrine mesylate (Bristol–Myer, USA) were prepared daily. The appropriate dilutions were made in saline just before infusion. D(+)-glucose was obtained from Sigma (Spain). The calcium channel blockers, indomethacin and atropine were diluted in saline and administered immediately after withdrawal of the second control sample. Verapamil hydrochloride (Knoll, Spain) and elgodipine (a gift from Dr A. Galiano, Instituto de Investigación y Desarrollo Químico Biológico, S.A. Spain) were infused for 30 min just before commencing the administration of agonist or saline. Indomethacin (as the sodium salt; Merck Sharp & Dohme, Spain) was infused for 15 min; an interval of 45 min was then allowed before agonist administration. Atropine sulphate (B. Braun Medical, S.A. Spain) was initially infused alone for 15 min, the infusion being maintained for a subsequent 30 min during the infusion of the α_1-adrenoceptor agonist amidephrine (both marginal ear veins were used in this type of experiment). All drug concentrations are expressed in terms of the free base.

Cardiovascular studies

For studies in conscious animals a catheter was placed in the central artery of the ear; the catheter was connected to a Viggo transducer coupled to a polygraph (model 400b, Letica, Spain) for blood pressure (BP) recording. Heart rate (HR) was calculated from the blood pressure signal. A resting period of at least 30 min was allowed before commencing the experiment. The cardiovascular parameters were recorded during a 5 min period before (10 min), during (30 min) and after (15 min) the i.v. infusion of either saline or amidephrine. When studying the effects of atropine, the calcium channel blockers and indomethacin, whether by themselves or against amidephrine, the protocol described above was adhered to. Blood pressure and heart rate were monitored at 5 min intervals throughout the experiment. Drugs were infused into the marginal vein of the contralateral ear at a rate of 0.075 ml min^{-1}. At the beginning of each experiment rabbits received sodium heparin (500 IU ml^{-1}) as an i.v. bolus.

Statistics and data analysis

Changes in blood glucose, plasma insulin, mean arterial pressure (MAP) and heart rate (HR) from at least five rabbits are expressed as means ± SEM. Statistical analyses employed were the one-way analysis of variance and the Newman–Keuls test. Changes in IRI were referred as a percent of mean control.

Results

Effects of selective stimulation of α_1-adrenoceptors on insulin and plasma glucose levels

The i.v. infusion of 3 and 10 μg kg^{-1} min^{-1} of the very selective α_1-adrenoceptor agonist amidephrine

Journal of Autonomic Pharmacology, 17, 293–302

(Moratinos *et al.*, 1988) induced a dose-related increase in plasma insulin levels (Figs 1b and 2b). Peak effects appeared at 30 min ($\Delta = 52.30 \pm 5.80\%$, $n = 5$, $P < 0.001$ vs. $-2.90 \pm 2.85\%$, $n = 7$ in saline treated animals), and 45 min ($\Delta = 115.80 \pm 36.40\%$, $n = 8$, $P < 0.001$ vs. $1.26 \pm 6.72\%$, $n = 7$, in saline treated rabbits). Preinfusion plasma values were: 11.10 ± 1.25 μIU ml^{-1}, for saline treated animals; 7.15 ± 1.40 μIU ml^{-1}, for animals that received 3 μg kg^{-1} min^{-1} amidephrine; and 6.00 ± 0.57 μIU ml^{-1}, for animals that received 10 μg kg^{-1} min^{-1} amidephrine. At the low infusion rate amidephrine evoked a mild, though significant, hypoglycaemia (Fig. 1a, Δ at 45 min $= -0.47 \pm 0.26$ mM, $n = 8$, $P < 0.001$, vs. 0.36 ± 0.06 mM, $n = 13$ in saline treated animals). At the higher dose amidephrine induced a minor, short-lasting rise in blood glucose (Fig. 2a; Δ values at 30 min were: 0.06 ± 0.13 mM, $n = 12$, in saline treated rabbits; and 0.50 ± 0.30 mM, $n = 6$, in drug treated rabbits; $P < 0.05$). Pre-infusion fasting blood glucose levels for the different groups ranged between 4.95 ± 0.21 mM and 5.70 ± 0.15 mM.

The insulin secretory response exerted by amidephrine was not modified in those animals exposed to a simultaneous administration of atropine (5.26 μg kg^{-1} min^{-1}, for 45 min). Peak effects after the lower and higher amidephrine doses in the presence of atropine were, respectively, $41.20 \pm 11.9\%$ $n = 7$, (Fig. 1b) and $92.80 \pm 35.50\%$, $n = 6$ (Fig. 2b). The muscarinic receptor antagonist when infused alone did not alter basal insulin plasma levels. (Preinfusion plasma levels in these experimental designs ranged between 10.50 ± 1.03 and 11.30 ± 1.40 μIU ml^{-1}).

Atropine by itself moderately raised blood glucose (Δ at 30 min was 0.66 ± 0.26 mM, $n = 5$, $P < 0.001$ vs. 0.06 ± 0.13 mM, $n = 13$ in saline control rabbits; Fig. 1a). In the presence of atropine, changes in blood glucose found with amidephrine did not differ from those induced by the muscarinic antagonist alone. Pre-infusion plasma glucose levels ranged between 5.80 ± 0.40 mM (for atropine) and 6.30 ± 0.30 mM

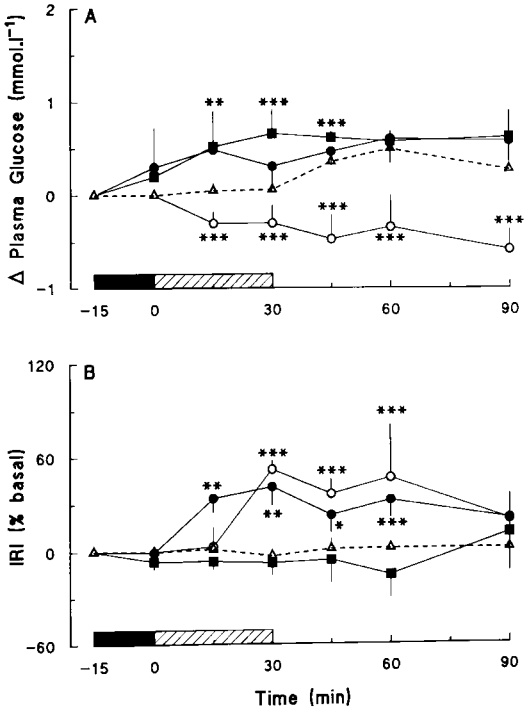

Figure 1 The effects of amidephrine (3 μg kg^{-1} min^{-1}) on plasma glucose (A) and immunoreactive insulin levels (IRI) (B) in the absence (○) and presence (●) of atropine in fasted rabbits. The α_1-adrenoceptor agonist or saline (△) were infused at a constant rate of 0.15 ml min^{-1} over a period of 30 min (cross-hatched bar). Atropine was infused at a rate of 5.26 μg kg^{-1} min^{-1} over 45 min as follows: alone for an initial 15 min period (black bar), then simultaneously with amidephrine for another 30 min (cross-hatched bar). The effects of atropine by itself (a 45 min infusion, ■) on both parameters are also shown. Ordinate scales: Δ mmol l^{-1} plasma glucose refers to the variations from control values. Values of IRI levels are expressed as a % change from the control level (control = %). Mean responses from at least five rabbits are represented. Vertical lines indicate SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, values significantly different from those seen on infusing saline.

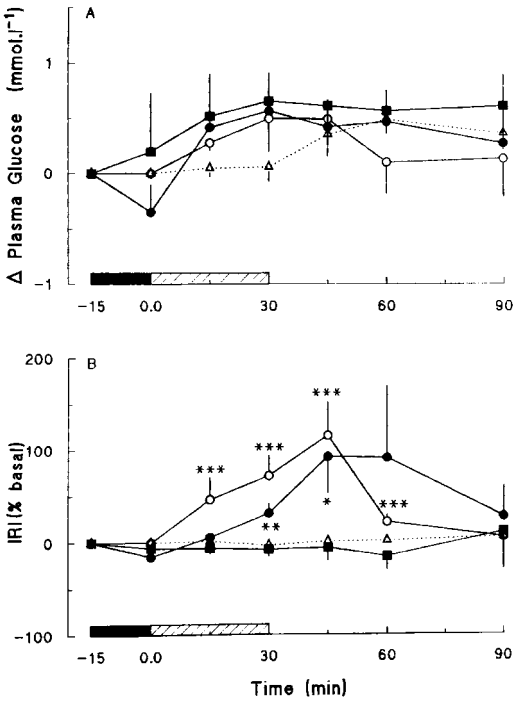

Figure 2 The effects of amidephrine (10 μg kg^{-1} min^{-1}) on plasma glucose (A) and IRI levels (B) in the absence (○) and presence (●) of atropine in fasted rabbits. The α_1-adrenoceptor agonist or saline (△) were infused at a constant rate of 0.15 ml min^{-1} over a period of 30 min (cross-hatched bar). Atropine was infused at a rate of 5.26 μg kg^{-1} min^{-1} over 45 min as follows: alone for an initial 15 min period (black bar), then simultaneously with amidephrine for another 30 min (cross-hatched bar). The effects of atropine by itself (a 45 min infusion, ■) on both parameters are also shown. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, values significantly different from saline.

Journal of
Autonomic Pharmacology,
17, 293–302

(for those animals treated with lower amidephrine dose in the presence of the antagonist).

Metabolic effects of amidephrine in the presence of elgodipine

The i.v. infusion of 35 ng kg^{-1} min^{-1} elgodipine evoked a minor and transient reduction in insulin plasma levels (Δ at 15 min = $-10.20 \pm 4.35\%$, $n = 5$, $P < 0.05$ when compared with $1.50 \pm 5.60\%$, $n = 7$ in saline control animals), but a moderate increase in blood glucose (Fig. 3) (Δ at 15 min = 0.75 ± 0.25 mM, $n = 9$, $P < 0.001$ vs. 0.05 ± 0.08 mM, $n = 14$, in saline control animals). Preinfusion plasma values of insulin and glucose for elgodipine treated rabbits were 7.90 ± 1.80 μIU ml^{-1}, and 5.90 ± 0.08 mM, respectively.

The calcium antagonist blocked the insulin secretory response induced by 10 μg kg^{-1} min^{-1} amidephrine (Fig. 3b) (Δ values at 45 min in the absence and presence of elgodipine were, respectively, $115.80 \pm 36.40\%$, $n = 8$, and $28.30 \pm 26.90\%$, $n = 8$; $P < 0.001$). Pre-infusion absolute values for those animals challenged in the presence of the calcium antagonist were 10.93 ± 1.20 μIU ml^{-1}. However, changes in plasma glucose did not differ much from those found in rabbits treated either with amidephrine or elgodipine alone (Fig. 3a). The pre-infusion plasma value in this experimental setting was 6.38 ± 0.40 mM for elgodipine treated rabbits.

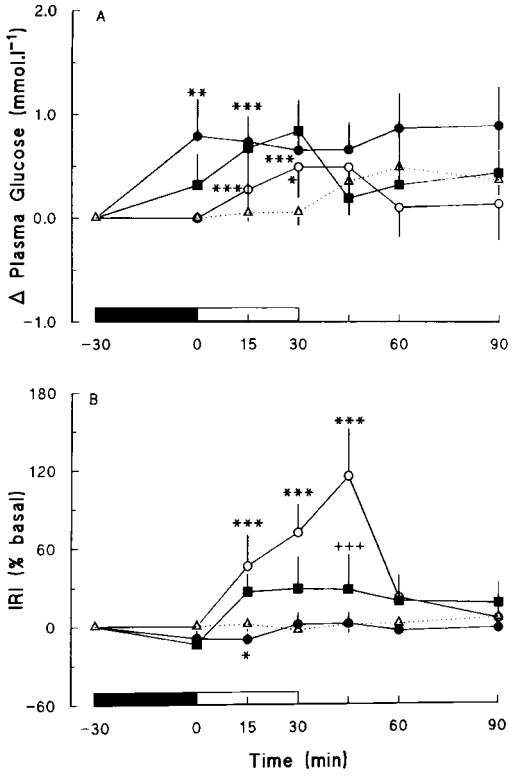

Figure 3 Changes in plasma glucose (A) and IRI (B) levels induced by amidephrine (10 μg kg^{-1} min^{-1}) when infused alone (○) or in the presence of elgodipine (35 ng kg^{-1} min^{-1}) (■) in fasted rabbits. The effects of elgodipine by itself on both parameters are also presented (●). The calcium antagonist was infused for 30 min (black bar), immediately followed by a 30 min infusion of either the agonist or saline (open bar). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, values significantly different from those seen on infusing saline (△), $^{+++}P < 0.001$, when the level of significance was calculated between both experimental groups, calcium channel blocker treated, and non-treated animals.

The effect of calcium channel blockers on glucose induced insulin secretion

Previous work had shown that verapamil (0.17 μg kg^{-1} min^{-1}) also abolished the excitatory secretory response induced by amidephrine (García-Barrado *et al.*, 1992). The selectivity of verapamil for calcium channels at this particular infusion rate was tested in animals challenged with a glucose load.

The iv infusion of a glucose load (10 mg kg^{-1} min^{-1}) evoked the expected increases in plasma glucose (Fig. 4) (Δ at 30 min was 3.87 ± 0.25 mM, $n = 6$, $P < 0.001$ vs. 0.06 ± 0.13 mM, $n = 14$ in saline treated rabbits). The pre-infusion absolute value for glucose treated animals was 5.4 ± 0.20 mM). The

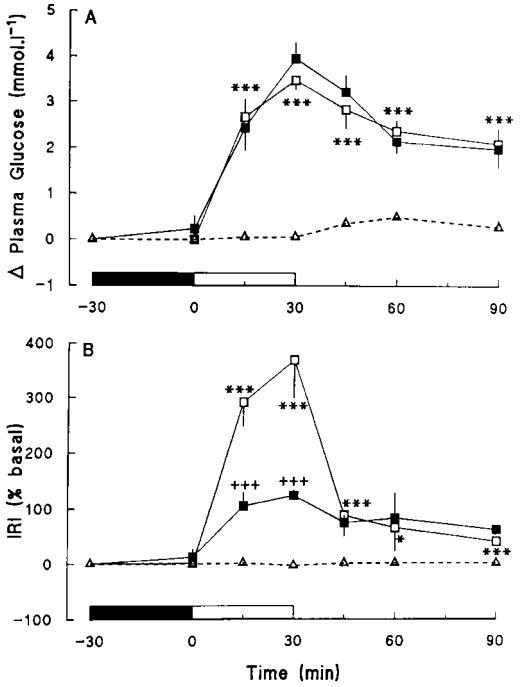

Figure 4 The effects of a glucose load (10 mg kg^{-1} min^{-1}) on plasma glucose (A) and IRI (B) levels in the absence (□) and presence of verapamil (0.17 μg kg^{-1} min^{-1}, ■) in fasted rabbits. Glucose was infused at a constant rate of 0.15 ml min^{-1}. The experimental design was as described in Fig. 3, but with glucose acting as an agonist. $^{*}P < 0.05$, $^{***}P < 0.001$, values significantly different from saline (△). $^{+++}P < 0.001$, when the statistical significance was calculated between both experimental groups, verapamil treated and non-treated animals.

Journal of Autonomic Pharmacology, 17, 293–302

glucose load also increased circulating insulin (Δ at 30 min was 370.10 ± 70.70%, $n = 7$, $P < 0.001$ vs. −2.90 ± 2.86%, $n = 7$ in saline control animals). The pre-infusion absolute value for glucose treated animals was 5.60 ± 0.90 μIU ml^{-1}). Pretreatment with verapamil reduced the insulin secretory response just within the 30 min of glucose infusion (Fig. 4) (Δ at 30 min in the presence of verapamil was 125.32 ± 10.00%, $n = 8$, $P < 0.001$ when compared with animals treated with glucose alone) although the resulting hyperglycaemia was very similar in verapamil treated and non-treated animals. Pre-infusion plasma insulin and glucose values were 10.10 ± 1.30 μIU ml^{-1} and 6.10 ± 0.30 mM, respectively.

When the glucose load was explored in elgodipine pre-treated rabbits, the calcium antagonist transiently attenuated the insulin secretory response induced by the nutrient (Fig. 5) (Δ values at 30 min in the absence and presence of elgodipine were, respectively, 362.7 ± 65.0%, $n = 6$, vs. 215.60 ± 40.1%, $n = 6$, $P < 0.05$). Pre-infusion plasma insulin values for saline and glucose treated animals were: 12.6 ± 1.6 μIU ml^{-1}, 7.95 ± 2.8 μIU ml^{-1}, and 10.20 ± 2.55 μIU ml^{-1}. Plasma glucose levels found after the glucose challenge were not altered by previous treatment with elgodipine. Preinfusion plasma glucose values in these experimental designs ranged between 4.66 ± 0.20 mM and 5.85 ± 0.26 mM.

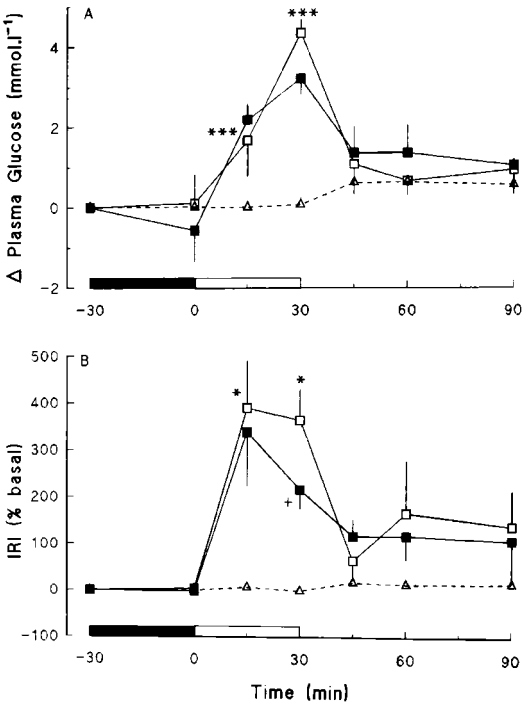

Figure 5 The effects of a glucose load (10 mg kg^{-1} min^{-1}) on plasma glucose (A) and IRI (B) levels in the absence (□) and presence of elgodipine (35 ng kg^{-1} min^{-1}, ■) in fasted rabbits. The experimental design was as described in Fig. 3, but with glucose acting as an agonist. $^{*}P < 0.05$, $^{***}P < 0.001$, values significantly different from saline (△). $^{+}P < 0.05$, when the statistical significance was calculated between both experimental groups, elgodipine treated and non-treated animals.

Effects of indomethacin on amidephrine-induced insulin secretion

A dose of indomethacin (0.66 mg kg^{-1} min^{-1}) that was able to block the increase in plasma levels of 6-ketoPGF$_{1\alpha}$ and TxB_2 in the rabbit (Bottje *et al*., 1991) has been used in these experiments. Indomethacin by itself introduced late minor changes in plasma glucose levels, and transiently reduced plasma insulin (Fig. 6) (Δ at 15 min = −15.87 ± 6.52%, $n = 6$, $P < 0.01$ vs. 1.51 ± 5.60%, $n = 7$ in saline treated animals). Indomethacin blocked the α_1-adrenoceptor mediated insulin secretory rise (Δ at 45 min in the presence of the cyclo-oxygenase inhibitor was −13.80 ± 14.23% $n = 6$, $P < 0.001$). In this experimental design the pre-infusion plasma insulin level was 7.75 ± 1.70 μIU ml^{-1}, whereas the initial value of 6.11 ± 0.85 μIU ml^{-1} was measured in animals receiving indomethacin alone. The levels of

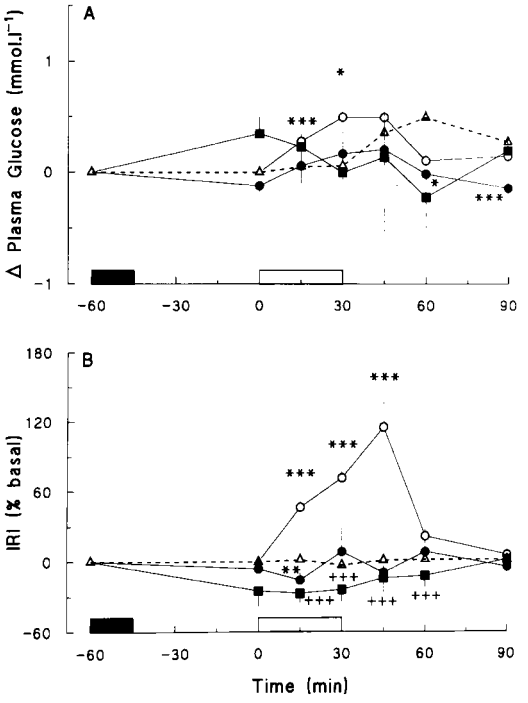

Figure 6 Changes in plasma glucose (A) and IRI (B) levels induced by amidephrine (10 μg kg^{-1} min^{-1}) when infused alone (○) or in the presence of indomethacin (0.66 mg kg^{-1} min^{-1}) (■). The cyclo-oxygenase inhibitor was infused for 15 min (black bar). Forty five min later, agonist infusion began (open bar). The effects of indomethacin by itself on both parameters are also shown (●) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, values significantly different from saline (△). $^{+++}P < 0.001$ when the level of significance was calculated between groups treated with amidephrine either in the absence or the presence of indomethacin.

Journal of Autonomic Pharmacology, 17, 293–302

circulating insulin were slightly inhibited during the 30 min amidephrine infusion. No significant increase in plasma glucose was recorded. (Pre-infusion values in these experiments ranged between 6.20 ± 0.11 mM and 5.10 ± 0.20 mM.)

The cardiovascular effects of amidephrine in the absence and presence of atropine, calcium channel antagonists and indomethacin

Controls. The different groups of animals were highly homogeneous regarding their average blood pressure and heart rate pre-infusion values. Analyses of variance showed significant differences in mean arterial pressure (MAP) only in those animals which later received the low amidephrine dose in the presence of atropine (Table 1, $P < 0.05$), and in heart rate (HR) in rabbits treated either with elgodipine alone or the higher amidephrine dose in atropine pre-treated animals (Table 1, $P < 0.05$). Throughout the experiment blood pressure fluctuated between 72.0 ± 3.0 and 76.2 ± 1.8 mmHg; and heart rate fluctuated between 207 ± 5 and 218 ± 10 beats min^{-1}, in saline treated animals.

Responses to amidephrine. Amideprine increased blood pressure and induced bradycardia. At the lower infusion rate a significant increase in MAP was evident at 20 min ($\Delta = 13 \pm 3$ mmHg, $n = 6$), reaching a peak at the end of drug administration (Table 1). The reduction in heart rate appeared at 15 min ($\Delta = -38 \pm 13$ beats min^{-1}, $n = 6$), with a maximal fall at the end of amidephrine infusion (Table 1). In the presence of atropine, amidephrine did increase blood pressure (see peak effect at 30 min in Table 1), but the reduction in heart rate was completely suppressed (from 15 to 45 min, Table 1).

Amidephrine, at the higher infusion rate, evoked a rise in BP which was evident 10 min within the infusion ($\Delta = 18.45 \pm 3.40$ mmHg) and reached a peak at 30 min (Table 1). The slowing in HR was already apparent after 10 min of drug administration ($\Delta = -76.0 \pm 26.0$ beats min^{-1}), being maximally decreased at the end of the infusion (Table 1). In the presence of atropine amidephrine did not increase blood pressure (see peak effect at 25 min in Table 1). However, a moderate and significant reduction in heart rate was still found during amidephrine infusion, although the bradycardia was certainly less severe (see changes at 25 min in Table 1). In these atropine pretreated animals the reduction in HR persisted 45 min after amidephrine administration ($\Delta = -45.0 \pm 11.0$ beats min^{-1}).

Elgodipine, which by itself evoked small fluctuations in both parameters, blunted the α-adrenoceptor mediated hypertensive response. The observed reduction in heart rate did not differ from the effect described for amidephrine alone (Table 1). Whereas verapamil or indomethacin suppressed the insulin secretory rise induced by amidephrine neither drug blocked the effects of the agonist on BP and HR (Table 1). Verapamil, when infused alone, evoked minor changes in BP, with a transient significant

Table 1. Changes in mean arterial blood pressure (MAP) and heart rate (HR) of conscious rabbits induced by amidephrine when infused alone, or in the presence of drug antagonists

Treatment	MAP (mmHg)		HR (beats min^{-1})	
	Baseline	Δ	Baseline	Δ
Saline, 0.075 ml^{-1} min^{-1}	72.4 ± 2.8 (9)	3.70 ± 1.8	207 ± 5.8	11 ± 10
Amidephrine, 3 μg kg^{-1} min^{-1}	70.1 ± 4.4 (6)	17.20 ± 3.5*	233 ± 23	−58 ± 12*
Atropine, 5.26 μg kg^{-1} min^{-1} + Amidephrine, 3 μg kg^{-1} min^{-1}	92.3 ± 4.5 (5)	16.5 ± 8	226 ± 10	−5 ± 20†
Amidephrine, 10 μg kg^{-1} min^{-1}	64.5 ± 5.2 (5)	32.3 ± 8.2*	200 ± 18	−102 ± 21
Atropine, 5.26 μg kg^{-1} min^{-1} + Amidephrine, 10 μg kg^{-1} min^{-1}	64.4 ± 2 (10)	7.3 ± 3.3†	258 ± 20	−50 ± 16†
Verapamil, 0.17 μg kg^{-1} min^{-1}	71.7 ± 3.4 (10)	−5.4 ± 7.8	230 ± 16	5 ± 21
Verapamil, 0.17 μg kg^{-1} min^{-1} + Amidephrine, 10 μg kg^{-1} min^{-1}	68.7 ± 6.6 (5)	20.5 ± 4*	231 ± 35	−109 ± 20*
Elgodipine, 35 ng kg^{-1} min^{-1}	69.7 ± 7 (5)	−3.2 ± 7	268 ± 27	22 ± 19
Elgodipine, 35 ng kg^{-1} min^{-1} + Amidephrine, 10 μg kg^{-1} min^{-1}	72 ± 4 (5)	−1.9 ± 7.5†	228 ± 8	−72 ± 11*
Indomethacin, 0.66 mg kg^{-1} min^{-1}	65.2 ± 3 (7)	−2.5 ± 3.2	218 ± 8	1 ± 18
Indomethacin, 0.66 mg kg^{-1} min^{-1} + Amidephrine, 3 μg kg^{-1} min^{-1}	63 ± 2.2 (5)	38.7 ± 5*	202.5 ± 24	−85 ± 7*

Following control measurements (baseline) the animals were treated as described. Drugs were administered over a 30 min period, although indomethacin and atropine were infused for 15 or 45 min, respectively. The values are means for n experiments (in parentheses). Δ refers to the change in either parameter from control value. The peak effect, which normally appeared at the end of amidephrine infusion or 5 min earlier, is represented. *$P < 0.05$, values significantly different from saline treated animals †$P < 0.05$, when the level of significance is calculated between animals treated with amidephrine in the absence or presence of the antagonist. For more details see text.

Journal of Autonomic Pharmacology, 17, 293–302

reduction 25 min after its administration (61.5 ± 3.5 mmHg vs. 74.0 ± 1.5 mmHg in saline treated rabbits). In those rabbits treated with indomethacin, the BP pre-infusion value was of 65.2 ± 3.0 mmHg. In the presence of drug, minor fluctuations of this parameter were recorded so that 60 min after its administration a value of 64.4 ± 4.5 mmHg was measured (Table 1).

Discussion

In conscious rabbits the i.v. infusion of the selective α_1-adrenoceptor agonist, amidephrine, increased circulating insulin when administered alone, inducing a potentiated insulin secretory response if simultaneously infused with an equiactive dose of the β-adrenoceptor agonist, isoprenaline (Moratinos *et al*., 1988). Interestingly, this potentiated response was suppressed by calcium channel blockers (García-Barrado *et al*., 1992). The present work used lower doses of amidephrine, and confirms its ability as an insulin secretagogue as well as showing that the agonist effect is dose-related. At the lower infusion rate amidephrine induced a mild but maintained hypoglycaemia, whereas a minor transient increase in blood glucose was found after the larger dose. It is also known that phenylephrine administration induces a well sustained elevation in blood glucose in the rabbit (Moratinos, Olmedilla, de Pablos & Viguera, 1986). An increase in the dose of amidephrine was required to unmask the hyperglycaemic effect of this agonist, although its ability to stimulate insulin secretion would certainly buffer its own hyperglycaemic action. When comparing both α_1-adrenoceptor agonists phenylephrine was more active in inducing hyperglycaemia, while amidephrine was more effective in provoking insulin release. These differences could be explained if each agonist stimulates a different population of α_1-adrenoceptor subtypes. Indeed, whereas amidephrine induced insulin secretion, and elevations in lactate and plasma potassium were prazosin sensitive responses (Moratinos *et al*., 1988; Reverte, Garcia-Barrado & Moratinos, 1991a,b) the blood glucose increase and liver glycogenolysis stimulation derived from phenylephrine administration tended to be prazosin resistant effects (Moratinos *et al*., 1986). Since a high density of low-affinity prazosin binding sites has been described recently in rabbit liver (Ohmura & Muramatsu, 1995) it is tempting to speculate that this receptor population is not easily amenable to amidephrine interaction and would make a significant contribution to liver glycogenolysis in these species.

Cardiovascular changes, such as an increase in blood pressure and a subsequent reflex vagal response, would be expected in response to the administration of an α_1-adrenoceptor agonist. Thus, acetylcholine released from parasympathetic nerve terminals would induce insulin secretion (Holst *et al*., 1981). Amidephrine, indeed, even at the lower infusion rate, increased blood pressure and induced a significant reduction in heart rate. However, atropine, at a dose that blocked insulin secretion resulting from vagal stimulation (Ahrèn & Taborky, 1986), antagonized the amidephrine-mediated bradycardia but not the insulin secretory response. In addition, at this particular concentration, amidephrine, when simultaneously infused with isoprenaline, induced a potentiated plasma insulin secretory rise (Reverte *et al*. 1994), even in the presence of a maintained tachycardia, (Palomero & Moratinos, unpublished data). We do not have a clear explanation for the antagonistic effect induced by atropine against the blood pressure response mediated by the higher amidephrine dose, although an inhibition of noradrenaline-induced contraction of rabbit aorta by this muscarinic blocker has been described (Satake *et al*., 1992). Certainly in the absence of any significant increase in blood pressure a reflex mediated bradycardia could not be expected. The persistent reduction in heart rate would then be the result of a direct negative chronotropic response mediated by α_1-adrenoceptor activation, as reported previously (Dukes & Williams, 1984; Saito, Suetsugu, Oku, Kuroda & Tanaka, 1994). However, although the bradycardic response was significantly attenuated, the insulin secretory rise was of the same magnitude in atropine treated and non-treated animals. It is also known that vagal activated responses could elicit the release of vasoactive intestinal polypeptide (VIP) and/or ATP, both of which are positive modulators of insulin secretion. (Wahl, Straub & Ammon, 1993; Hillaire-Buys, Chapel, Bertrand, Petit & Loubatières-Mariani, 1994). Interestingly, in the presence of 1 mg kg^{-1} hexamethonium, amidephrine (10 μg kg^{-1} min^{-1}) was still able to increase IRI plasma levels (Moratinos & Reverte, unpublished observations), confirming the direct role played by α_1-adrenoceptors on insulin secretion. Finally, it is conceivable that an increase in glucagon secretion derived from α_1-adrenoceptor stimulation would subsequently elicit insulin release. However, phenylephrine, while elevating plasma glucagon levels in mice, failed to induce a rise in insulin secretion (Skoglund, Lundquist & Ahrèn, 1987).

Considering the role played by the parasympathetic nervous system in glucose homeostasis (increased glycogen synthesis and reduction in glucose release from the liver; Niijima, 1985) the effect of atropine on plasma glucose could well reflect the predominance of endogenous sympathetic tone in the presence of muscarinic blockade. Thus, a tendency for a modest hyperglycaemia would be released. This enhanced sympathetic activity, with the concomitant increase in glucagon levels (Ahrèn, Veith & Taborsky, 1987), would be preserved during amidephrine infusion restraining the hypoglycaemic effect of the agonist.

Data from an insulin secreting cell line has shown that phenylephrine was able to increase intracellular Ca^{2+} and insulin release (Ullrich & Wollheim, 1985),

Journal of Autonomic Pharmacology, 17, 293–302

confirming the well established relation between α_1-adrenoceptor stimulation and calcium mediated responses (Malaisse, 1990). Amidephrine acting on b cells depolarized by the ambient glucose concentration ($\cong$ 5 mM) could augment Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels. Suppression by calcium channel antagonists of the insulin secretory rise elicited by the higher amidephrine dose is thus consistent with present knowledge. The reported effect of verapamil (Garcia-Barrado *et al*., 1992) could not be ascribed to an interaction with α_1-adrenoceptors because low doses were required to obtain the blockade (Reverte *et al*., 1991b); and at the same concentration verapamil, as expected, clearly attenuated the insulin secretory response evoked by a glucose challenge (Ohta, Nelson, Nelson, Meglasson & Erecinsk, 1993; Skoglund, Gross, Ahrèn & Loubatières-Mariani, 1993). Similar results were found using elgodipine, a new second generation dihydropyridine derivative, which at the infusion rate used in the present study also attenuated glucose induced insulin secretion (at lower concentrations elgodipine could behave as an agonist, enhancing glucose induced insulin secretion (García-Barrado & Moratinos, unpublished observations). Therefore, extracellular calcium seems to be necessary for α_1-adrenoceptor induced elevation of plasma IRI, supporting previous *in vivo* and *in vitro* studies (Fehmann, Stöckmann, Haverich & Crfeldt, 1988; García-Barrado *et al*., 1992; Ohta *et al*., 1993). Despite the marked effects on amidephrine-mediated insulin release, the calcium antagonist did not clearly modify its weak hyperglycaemic response, suggesting the poor ability of amidephrine to promote glucose release from rabbit liver.

At first sight, changes in plasma glucose found in rabbits exposed to a glucose challenge in the presence of Ca^{2+} antagonists are surprising. Conflicting reports have appeared regarding the effects of Ca^{2+} antagonists on glucose metabolism, because these drugs can impair glucose tolerance (Horl, Haag, Riegel & Heidland, 1987), they fail to produce any deleterious effect (Giordano, Matsuda, Sanders, Canessa & de Fronzo, 1995), or increase the rate of glucose transport into muscle fibres (Foot & Leighton, 1994).

Previous data had shown that *in vivo* pressor responses and vascular smooth muscle contraction mediated by α_1-adrenoceptor agonists seemed to be resistant to inhibition by calcium channel blockers (Van Meel *et al*., 1981; Cavero, Shepperson, Lefervre-Borg & Langer, 1983). Our results with verapamil would thus support such interpretation. However, later reports (and the present study) using more selective vascular calcium channel blockers indicate that both the increase in blood pressure and contraction of vascular smooth muscle derived from α_1-adrenoceptor stimulation are dependent upon the entry of Ca^{2+} ions through dihydropyridine-sensitive Ca^{2+} channels (McGrath & O'Brien, 1987; Dunn, Daly, McGrath & Wilson, 1991; Noguera & D'Ocon, 1993). The results found with elgodipine show again that in the absence of an increase in blood pressure amidephrine induced bradycardia. This reduction of heart rate could well be the result of a direct α_1-adrenoceptor mediated response. Interestingly, despite the different effects on blood pressure both types of Ca^{2+} antagonists blocked amidephrine induced insulin secretion.

The results found in the presence of the cyclo-oxygenase inhibitor indomethacin, suggest that the amidephrine effect on insulin secretion is linked to prostaglandin synthesis. Prostaglandin production in response to α_1-adrenoceptor stimulation has been described in several tissues, prostacyclin being the major product of arachidonic acid generated in this interaction (Nebigil & Malik, 1992a,b). It is also known that prostacyclin seems to be the main cyclo-oxygenase metabolite accumulated in the islets (Tadayyon *et al*., 1990) and that prostacyclin itself can stimulate insulin release (Heaney & Larkins, 1981). However, in addition to prostacycline, lipoxygenase products and arachidonic acid itself are able to evoke insulin secretion (Drews, Garrino & Henguin, 1992; Ramanadham, Gross & Turk, 1992; Eddlestone, 1995). It cannot be excluded that indomethacin, at the dose used in the present work, could also inhibit phospholipase A_2 and lipoxygenase activities (Turk, Colca, Kotagal & Daniel, 1984; Drews *et al*., 1992), in this way suppressing the synthesis of most of these excitatory mediators (Schmitt *et al*., 1980). Combined data using calcium channel antagonists and indomethacin would indicate that calcium influx through L-type calcium channels in response to amidephrine, would result in phospholipase A_2 activation, arachidonic acid release, activation of the arachidonate cascade, and subsequent insulin secretion. However, reports about the effects of indomethacin and some other cyclo-oxygenase inhibitors on insulin secretion are not very consistent (Drews *et al*., 1992). Lack of consistency could be the result of species variations and the nature of the secretagogue employed in the experiments (glucose instead of amidephrine).

On the other hand indomethacin failed to alter the effects of amidephrine on blood pressure and heart rate. Similarly, noradrenaline-induced contractile responses resistant to indomethacin have been described in a number of vascular tissues (Lamb, Schwartz, Rohn & Kaiser, 1994). Therefore, a lack of correlation between metabolic and haemodynamic responses is shown again.

In our opinion the findings reported in the present work indicate that in conscious rabbits amidephrine induces a dose related increase in insulin plasma levels. The response seems to require extracellular calcium and the synthesis of an arachidonic acid metabolite. As the ability of amidephrine to increase circulating insulin was atropine resistant, we suggest that stimulation of α_1-adrenoceptors directly mediates insulin release *in vivo*.

Journal of Autonomic Pharmacology, 17, 293–302

Acknowledgements

We are very grateful for financial support from the Spanish Comisión Asesora de Investigación, grant n° PM92-0237.

References

AHRÈN, B. & TABORSKY, G. (1986). The mechanism of vagal nerve stimulation of glucagon and insulin secretion in the dog. *Endocrinology*, **118**, 1551–1557.

AHRÈN, B., VEITH, R. & TABORSKY, G. (1987). Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog. I. Effects on basal release of insulin and glucagon. *Endocrinology*, **121**, 323–331.

BOTTJE, W., GLANH, R., BEERS, K., NEJAD, H., GRAUPNER, W. & HOLMES, K. (1991). Indomethacin attenuation of hepatic perfusion and plasma 6-keto $PGF_{1\alpha}$ elevations following gluthatione depletion in rabbits. *Biochem. Biophys. Acta*, **1073**, 168–176.

CAVERO, I., SHEPPERSON, N., LEFERVRE-BORG, F. & LANGER, S. (1983). Differential inhibition of vascular smooth muscle responses for α_1 and α_2-adrenoceptor agonist by diltiazem and verapamil. *Circ. Res.,* **52**(**suppl. I**), 69–76.

DREWS, G., GARRINO, M.G. & HENQUIN, J.C. (1992). Ionic, electrical, and secretory effects of inhibitors of arachidonic acid metabolism in mouse pancreatic beta-cells. *Diabetes,* **41**, 620–626.

DUKES, Y. & VAUGHAN WILLIAMS, E. (1994). Effects of selective α_1, α_2, β_1 and β_2-adrenoceptor stimulation on potentials and contractions in the rabbit heart. *J. Physiol.*, **355**, 523–546.

DUNN, W., DALY, C., MCGRATH, J. & WILSON, V. (1991). The effects of nifedipine on α_2 adrenoceptor mediated contractions in several isolated blood vessels from the rabbit. *Br. J. Pharmacol.,* **103**, 1493–1499.

EDDLESTONE, G. (1995). ATP-sensitive K channel modulation by products of PLA_2 action in the insulin-secreting HIT cell line. *Am. J. Physiol.*, **268**, C181–C190.

FEHMANN, H., STÖCKMANN, F., HAVERICH, R. & CRFELDT, W. (1988). Diltiazen inhibition of glucose-induced insulin secretion from the isolated perfused rat pancreas. *Eur. J. Pharmacol.*, **156**, 415–417.

FOOT, E. & LEIGHTON, B. (1994). Effects of calcium antagonists on insulin mediated glucose metabolism in skeketal muscle. *Diabetes,* **43**, 73–79.

GARCÍA-BARRADO, M.J., REVERTE, M. & MORATINOS, J (1992). Role of Ca^{2+} channel blockers in insulin secretion resulting from α_1 and β-adrenoceptor stimulation in the rabbit. *Eur. J. Pharmacol.*, **219**, 461–464.

GIORDANO, M., MATSUDA, M., SANDERS, L., CANESSA, M. & DE FRONZO, R. (1995). Effects of angiotensin-converting enzyme inhibitors, Ca^{2+}-channel antagonists, and α-adrenergic blockers on glucose and lipid metabolism in NIDDM patients with hypertension. *Diabetes,* **44**, 665–671.

HEANEY, T.P. & LARKIN, R.G. (1981). The effect of prostacyclin and 6-keto-prostaglandin F1α on insulin secretion and cyclic adenosil 3′5′-monophosphate content in isolated rat islets. *Diabetes*, **30**, 824–828.

HILLAIRE-BUYS, D., CHAPAL, J., BERTRAND, G., PETIT, P. & LOUBATIÈRES-MARIANI, M. (1994). Purinergic receptors on insulin-secreting cells. *Fundam. Clin. Pharmacol.*, **8**, 117–127.

HOLST, J., GRONHOLT, R., SCHAFFALITZKY, O. & FAHRENKRUG, J. (1981). Nervous control of pancreatic endocrine secretion in pigs. I. Insulin and glucagon response to electrical stimulation of the vagus nerves. *Acta Physiol. Scand.*, **111**, 1–7.

HORL, W., HAAG, M., RIEGEL, W. & HEIDLAND, A. (1987). Effect of nifedipine on carbohydrate metabolism in the rat. *Res. Exp. Med.*, **187**, 203–210.

JOHN, G.W., DOXEY, J.C., WALTER, D.S. & REID, J.L. (1990). The role of α and β adrenoceptor subtypes in mediating the effects of catecholamines on fasting glucose and insulin concentrations in the rat. *Br. J. Pharmacol.*, **100**, 699–704.

LAMB, V., SCHWARTZ, A., ROHN, W. & KAISER, L. (1994). Cyclo-oxygenase inhibitors depress norepinephrine constriction of rat abdominal, but not thoracic aorta. *Eur. J. Pharmacol.*, **256**, 221–226.

MALAISSE, W., MALAISSE-LAGAE, F. & MAYHEW, D (1967). A possible role for the adenyl cyclase system in insulin secretion. *J. Clin. Invest.*, **46**, 1724–1734.

MALAISSE, W. (1990). Regulation of insulin release by the intracellular mediators cyclic AMP, Ca^{2+}, inositol 1,4,5-triphosphate, and diacylglycerol. In: *Insulin,* pp. 113–124. Eds. Cuatrecasas, P. & Jacobs, S. Springer-Verlag, Berlin, Heidelberg, New York.

MCGRATH, J. & O'BRIEN, J. (1987). Blockade by nifedipine of responses to intravenous bolus inyection or infusion of α_1-and α_2-adrenoceptor agonists in the pithed rat. *Br. J. Pharmacol.*, **91**, 355–365.

MORATINOS, J., OLMEDILLA, B., DE PABLOS, I. & VIGUERA, M. (1986). α-adrenoceptor involvement in catecholamine-induced hyperglycaemia in conscious fasted rabbits. *Br. J. Pharmacol.*, **89**, 55–66.

MORATINOS, J., CARPENE, C., DE PABLOS, I. & REVERTE, M. (1988). Role of α_1-and α_2-adrenoceptors in catecholamine induced hyperglycaemia, lipolysis and insulin secretion in conscious fasted rabbits. *Br. J. Pharmacol.*, **94**, 299–310.

NEBIGIL, C. & MALIK, K. (1992a). Prostaglandin synthesis elicited by adrenergic stimuli is mediated by alpha-2C and alpha-1A adrenergic receptors in cultured smooth muscle cells of rabbit aorta. *J. Pharmacol. Exp. Ther.*, **260**, 849–858.

NEBIGIL, C. & MALIK, K. (1992b). Alpha adrenergic receptor subtypes involved in prostaglandin synthesis are coupled to Ca^{2+} channels through a pertussis toxin-sensitive guanine nucleotide-binding protein. *J. Pharmacol. Exp. Ther.*, **266**, 1113–1124.

NIIJIMA, A. (1985). Blood glucose levels modulate efferent activity in the vagal supply to the rat liver. *J. Physiol.*, **364**, 105–112.

NOGUERA, N. & D'OCON, P. (1993). Evidence that deplection of internal calcium stores sensitive to adrenaline elicits a contractile response dependent on extracellular calcium in rat aorta. *Br. J. Pharmacol.*, **110**, 861–867.

OHMURA, T. & MURAMATSU, I. (1995). Two distinct a_1 adrenoceptor subtypes in rabbit liver, a binding study. *Br. J. Pharmacol.*, **116**, 2591–2596.

OHTA, M., NELSON, J., NELSON, D., MEGLASSON, M. & ERECINSKA, M. (1993). Effect of Ca^{2+} channel blockers on energy level and stimulated insulin secretion in isolated rat islets of Langerhans. *J. Pharmacol. Exp. Ther.*, **264**, 35–40.

RAMANADHAM, S., GROSS, R. & TURK, J. (1992). Arachidonic acid induces an increase in the cytosolic calcium concentration in single pancreatic islet β cell. *Biochem. Biophys. Res. Commum.*, **184**, 647–653.

REVERTE, M., GARCÍA-BARRADO, M.J. & MORATINOS, J. (1991a). Changes in plasma lactate evoked by α and β adrenoceptor stimulation in conscious fasted rabbits. *Fundam. Clin. Pharmacol.*, **5**, 663–676.

REVERTE, M., GARCÍA-BARRADO, M.J. & MORATINOS, J. (1991b). Role of α-adrenoceptors in control of plasma potassium in conscious rabbits. *J. Auton. Pharmacol.*, **11**, 305–313.

Journal of Autonomic Pharmacology, 17, 293–302

REVERTE, M., MORATINOS, J. & GARCÍA-BARRADO, M.J. (1994). Role of α_2 adrenoceptors on insulin secretion and blood lactate increase resulting from α_1 and β adrenoceptor stimulation in rabbits. *Br. J. Pharmacol.*, **111**, 109P.

ROBERTSON, R.P., TSAI, P., LITTLE, S., ZHANG, H. & WALSETH, T. (1987). Receptor mediated adenylate cyclase coupled mechanism for PGE_2 inhibiton of insulin secretion in HIT cells. *Diabetes*, **36**, 1047–1053.

ROMAN, C., DE MIGUEL, B., GIRALDEZ, A. & GALIANO, G. (1990). Study of agonism and antagonism of elgodipine on different types receptors. *Eur. J. Pharmacol.*, **183**, 2411–2414.

ROONEY, T., JASS, E. & THOMAS, A. (1989). Characterization of cytosolic calcium oscillations induced by phenylephrine and vasopressin in single fura-2-loaded hepatocytes. *J. Biol. Chem.,* **264**, 17131–17141.

SAITO, K., SUETSUGU, T., OKU, G., KURODA, A. & TANAKA, H. (1994). α_1-adrenoceptors in the conduction system of rat hearts. *Br. J. Pharmacol.*, **111**, 465–468.

SATAKE, N., KIYOTO, S., SHIBATA, S., GANDHI, V., JONES, D. & MORIKAWA, M. (1992). Possible mechanisms of inhibition with atropine against noradrenaline-induced contraction in the rabbit-aorta. *Br. J. Pharmacol.*, **107**, 553–558.

SCHMITT, J., DAVIS, J., LORENZI, M., BENET, L., BURNS, A. & KARAM, J. (1980). Inhibition by indomethacin of the glycemic response to arginine in man. *Proc. Soc. Exp. Biol. Med.*, **163**, 237–239.

SKOGLUND, G., LUNDQUIST, Y. & AHRÈN, B. (1987). α_1 and α_2 adrenoceptor activation increases plasma glucagon levels in the mouse. *Eur. J. Pharmacol.*, **143**, 83–89.

SKOGLUND, G., GROSS, R., AHRÈN, B. & LOUBATIÈRES-MARIANI, M. (1993). Different mechanisms are involved in neuropeptide Y-induced pancreatic vasoconstriction and inhibition of insulin secretion. *Eur. J. Pharmacol.*, **236**, 69–74.

TADAYYON, M., BONNEY, R. & GREEN, I. (1990). Starvation decreases insulin secretion, prostaglandin E production and phospholipase A_2 activity in rat pancreatic islet. *J. Endocrinol.*, **124**, 455–461.

TURK, J., COLCA, J., KOTAGAL, N. & DANIEL, M. (1984). Arachidonic acid metabolism in isolated pancreatic islets. II. The effects of glucose and inhibitors of arachidonate metabolism on insulin secretion and metabolite synthesis. *Biochim. Biophys. Acta*, **794**, 125–136.

TURK, J., GROSS, R. & RAMANADHAM, S. (1993). Amplification of insulin secretion by lipid messangers. *Diabetes*, **42**, 367–374.

ULLRICH, S. & WOLLHEIM, C.B. (1985). Expression of α_1 and α_2 adrenoceptors in an insulin-secreting cell line. Parallel studies of cytosolic free Ca^{2+} and insulin release. *Mol. Pharmacol.*, **28**, 100–106.

VAN MEEL, J. DE JONGE, A., KALKMAN, H., WILFFERT, B., TIMMERMANS, P. & VAN ZWEITEN, P. (1981). Organic and inorganic calcium antagonists reduce vasoconstriction *in vivo* mediated by postsynaptic α_2-adrenoceptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **316**, 288–293.

WAHL, M., STRAUB, S. & AMMON, H. (1993). Vasoactive intestinal polypeptide-augmented insulin release: actions on ionic fluxes and electrical activity of mouse islets. *Diabetologia*, **36**, 920–925.

(Received 20 December 1996
Revised 12 May 1997
Accepted 9 June 1997)