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Effects of verapamil and elgodipine on isoprenaline-induced metabolic responses in rabbits

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Abstract

Verapamil (0.17 μg kg^{-1} min^{-1} intravenous, i.v.) but not elgodipine (35 ng kg^{-1} min^{-1}) modestly enhanced the weak blood glucose increase induced by the i.v. infusion of isoprenaline (0.3 μg kg^{-1} min^{-1}) in conscious rabbits. However, elgodipine but not verapamil suppressed the increase in circulating insulin evoked by the agonist. Both drugs enhanced the rise in plasma lactate mediated by isoprenaline but only elgodipine potentiated the lipolytic effect of the agonist. In isolated islets elgodipine (10^{-6} M) blocked forskolin (10^{-6} M)-induced insulin release. However, in rabbit adipocytes elgodipine potentiated both glycerol release and cAMP accumulation induced by isoprenaline (10^{-8}–10^{-6} M). Excess K^+ (40–60 mM) did not alter basal lipolysis or the response to isoprenaline in either rabbit or mouse adipocytes. Therefore, Ca^{2+} influx through L-type Ca^{2+} channels does not seem to play a significant role in the lipolytic effect of isoprenaline. Metabolic alterations found with Ca^{2+} channel antagonists were of minor intensity and probably devoid of pathological implications.

Keywords: Ca^{2+} channel antagonist; Glucose homeostasis; Insulin secretion; Isoprenaline; Lipolysis; (Rabbit)

1. Introduction

It is well known that Ca^{2+} plays an essential role in hormonal metabolism, especially in carbohydrate homeostasis, and that Ca^{2+} channel-blocking agents might interfere with metabolic control. Reports of the effects of these drugs on glucose and lipid metabolism are rather complex and conflicting. A large body of evidence suggests that these drugs do not alter glucose handling in non-diabetic and diabetic subjects (Teuscher and Weidman, 1997; Gress et al., 2000), but Ca^{2+} channel antagonists (either short-acting drugs or sustained release formulations) are associated with the development of diabetes, impaired glucose tolerance and insulin resistance (Kaplan, 1992; Gurwitz et al., 1993; Foot and Leighton, 1994; Giordano et al., 1995), which limits their therapeutic applications (Califf and Kramen, 1998; Pahor et al., 1998). Thus, patients with diabetes mellitus may represent a special group for whom therapy with a Ca^{2+} channel antagonist increases the risk of cardiovascular complications (see also: Abernathy and Schwartz, 1999; Kuusisto and Laaklo, 1999 for reviews).

Catecholamines, by stimulating α- and β-adrenoceptors, induce responses that either promote glucose production or increase glucose utilisation. Extracellular Ca^{2+} seems to be a main requirement for α_1-adrenoceptor-mediated responses, such as liver glycogenolysis and insulin release (Maroto et al., 1992; García-Barrado et al., 1997). However, adrenaline and noradrenaline inhibit glucose clearance predominantly by β-adrenoceptor-mediated mechanisms: liver and muscle glycogenolysis activation, lipolysis, gluconeogenic stimulation and peripheral glucose uptake inhibition (Marette and Bukowiecki, 1989). The role played by extracellular Ca^{2+} and Ca^{2+} channel blockers in some of these metabolic effects has been only partially studied (Maroto et al., 1992; Pershadsingh et al., 1989; Schimmel, 1973) and thus needs further and deeper consideration. Therefore, it was considered of interest to test the effects of two Ca^{2+} channel blockers, with different cardiac and vascular selectivity, namely verapamil and the dihydropyridine derivative elgodipine, on the in vivo and in vitro metabolic responses induced by a β-adrenoceptor selective agonist, isoprenaline, in rabbits. Ca^{2+} entry blockers are used as therapeutic agents in the treatment of

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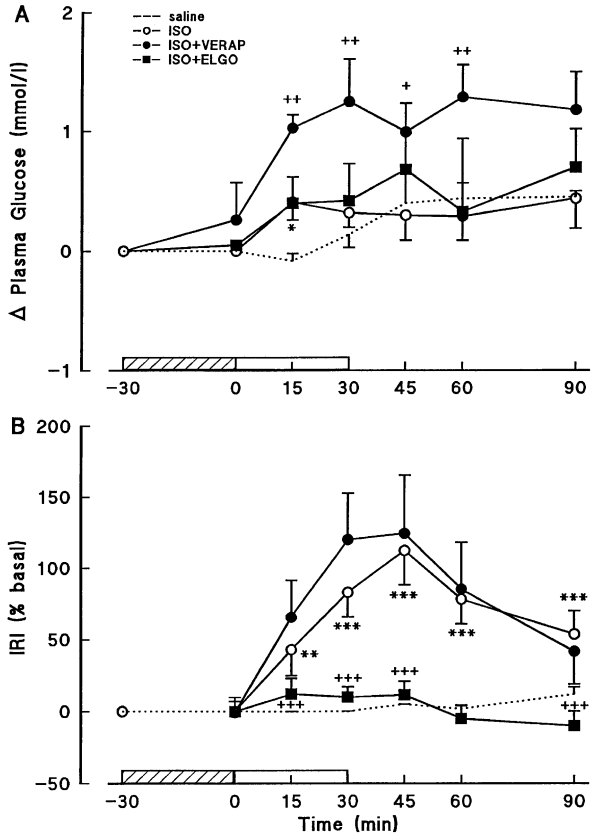

Fig. 1. Changes in plasma glucose (A) and in immunoreactive insulin (IRI) (B) levels induced by isoprenaline (ISO, 0.3 μg kg^{-1} min^{-1}) when infused alone (-○-) or in the presence of either verapamil (0.17 μg kg^{-1} min^{-1}, -●-) or elgodipine (35 ng kg^{-1} min^{-1}, -■-) in fasted rabbits. The Ca^{2+} channel antagonist was infused for 30 min (cross-hatched bar) immediately followed by a 30-min infusion of the agonist (open bar). Ordinate scales, Δmmol/l plasma glucose refers to the variations from control values. IRI levels are expressed as % changes from the control level (control = 100%). Mean responses from at least five rabbits are presented. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, values significantly different from those seen on infusing saline (· · · · · ·). $+P < 0.05$, $++P < 0.01$, $+++P < 0.001$, when the level of significance was calculated between both experimental groups, Ca^{2+} channel blocker treated and non-treated animals.

cardiovascular diseases. As a result of their hypotensive effect, they can evoke a reflex sympathetic discharge with tachycardia (a β-adrenergic-mediated effect). Our study could contribute in this way to understanding the changes in blood glucose homeostasis as a result of the short-term effects derived from this interaction.

A preliminary account of part of this research has been previously published in abstract form (García-Barrado et al., 1998a).

2. Material and methods

2.1. Animals

The experiments were performed in male New Zealand white rabbits aged 7–12 months (body weight between 2.8 and 3.5 kg).

2.2. In vivo experiments

The experimental design carried out in conscious animals fasted for the previous 24 h has been fully described in earlier publications (Moratinos et al., 1988; García-Barrado et al., 1997). Arterial blood was sampled by means of an indwelling cannula placed in the central artery of one ear. Two control samples separated by an interval of 30 min were taken before drug infusion started. Drug solutions 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 (0.07 ml min^{-1}).

2.3. Analyses of glucose, lactate, glycerol and insulin

Plasma glucose was estimated by means of the glucose oxidase procedure using a kit from Boehringer Mannheim (Mannheim, Germany). Blood lactate levels were quantified enzymatically using lactic dehydrogenase and measuring the amount of reduced nicotinamide-adenine dinucleo-

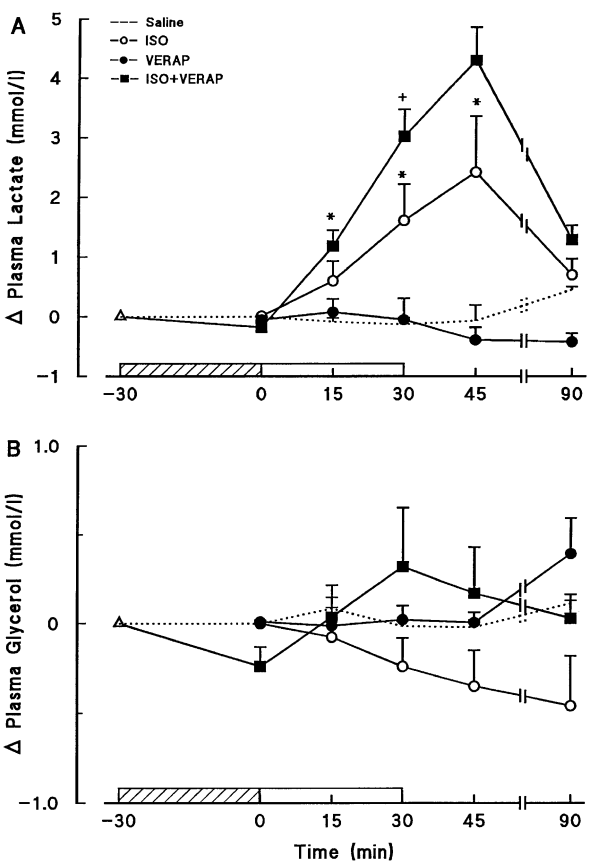

Fig. 2. Changes in plasma lactate (A) and glycerol (B) levels induced by isoprenaline (ISO) when infused alone (-○-) or in the presence of verapamil (-■-) in fasted rabbits. The effects of verapamil are also shown (-●-). The Ca^{2+} channel antagonist was infused for 30 min (cross-hatched bar), followed by a 30-min infusion of either the agonist or saline. For more details see legend for Fig. 1.

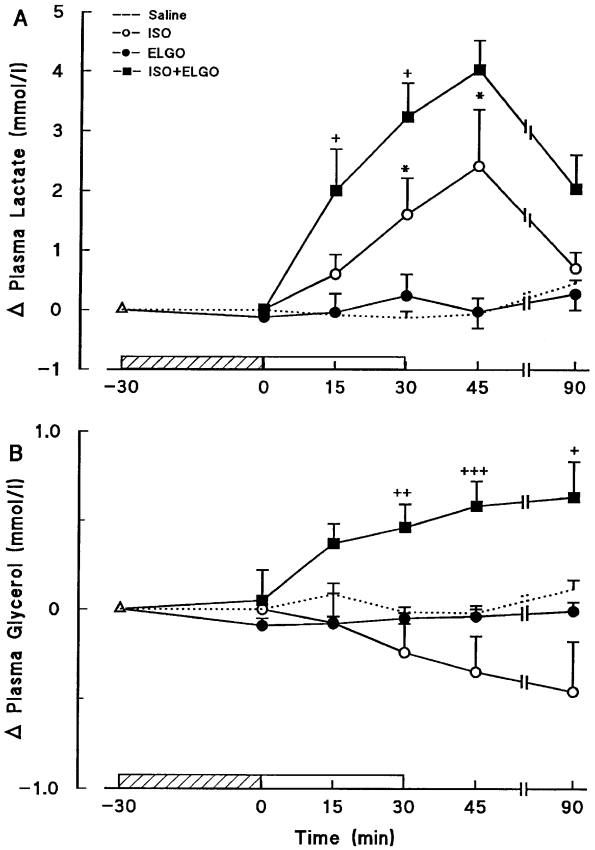

Fig. 3. Changes in plasma lactate (A) and glycerol (B) levels induced by isoprenaline (ISO) when administered alone (-○-) or in the presence of elgodipine (-■-). The effects of elgodipine are also shown (-●-). The Ca^{2+} channel antagonist was infused for 30 min (cross-hatched bar), followed by a 30-min infusion of either isoprenaline, or saline.

tide (NADH) formed spectrophotometrically at 340 nm (Reverte et al., 1991). The determination of glycerol involved the conversion of glycerol to lactate via a three-step enzymatic process involving glycerol kinase, pyruvate kinase and lactate dehydrogenase. The amount of NADH consumed during the conversion of pyruvate to lactate was measured spectrophotometrically at 340 nm. Kits for lactate and glycerol assays were provided by Boehringer Mannheim. Immunoreactive insulin was determined by using a radioimmunoassay kit, with human insulin as standard (CIS-Radioquímica, Spain); the detection limit was 3 μIU ml^{-1}.

2.4. Drugs

Fresh stock solutions of isoprenaline hydrochloride (from Sigma, Madrid, Spain) were prepared daily. Isoprenaline solutions were made in acidified saline (pH = 4.5). Appropriate dilutions were made in saline just before infusion. The Ca^{2+} channel blockers were also diluted in saline and administered immediately after withdrawal of the second control sample. Verapamil hydrochloride (Knoll, Spain) and elgodipine (a gift from Dr. Galiano, Instituto de Investigación y Desarrollo Químico y Biológico, , Spain) were infused for 30 min just before the start of the administration of agonist or saline. All drug concentrations are expressed in terms of free base.

2.5. *In vitro* experiments. Preparation of isolated adipocytes and lipolysis measurements

White adipose tissue of perirenal and epididymal origin was removed from fed rabbits after the induction of general anaesthesia with pentobarbital (30 mg kg^{-1}, i.v.). Isolated fat cells were obtained by collagenase digestion (1.5 mg ml^{-1}) of adipose tissue fragments in Krebs–Ringer–bicarbonate buffer containing albumin (3.5 g 100 ml^{-1}) (KRBA) and glucose (6 mM) adjusted to pH 7.4 just before use. After 30 min of digestion at 37°C under

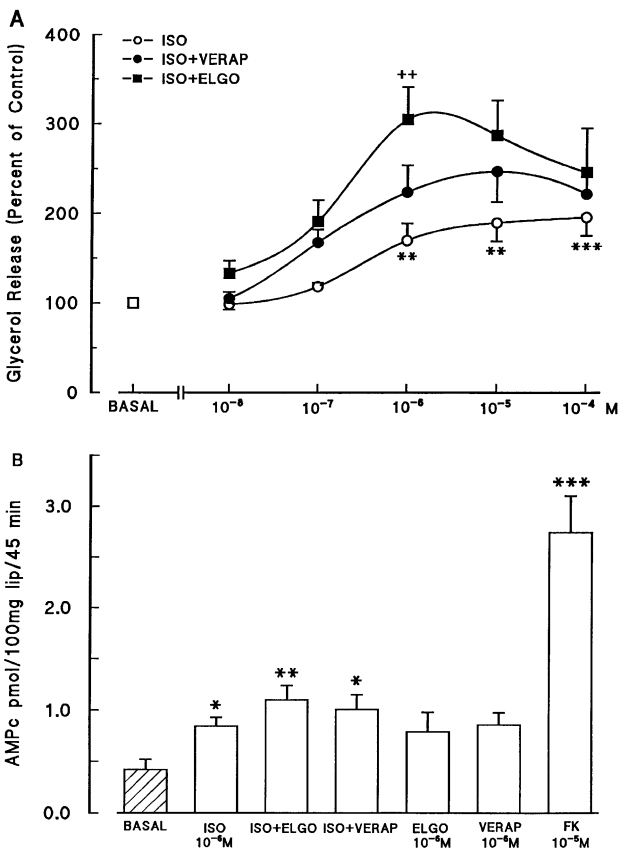

Fig. 4. Effects of increasing concentrations of isoprenaline (ISO) alone (-○-) or in the presence of either verapamil (-●-, 10^{-6} M) or elgodipine (-■-, 10^{-6} M) on glycerol release (A) from isolated rabbit perirenal adipocytes. Values are expressed as percentages of mean control (control = 100%). Concentration–response curves were obtained for at least six individual assays. Basal release was 0.240 ± 0.065 μmol glycerol/100 mg lipid/90 min. In (B), the effect of ISO 10^{-6} M on cAMP accumulation either alone, or in the presence of verapamil (10^{-6} M) or elgodipine (10^{-6} M). Basal values in perirenal fat cells were 0.42 ± 0.10 pmol/100 mg lipid/45 min, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, values significantly different from basal glycerol release or cyclic nucleotide content. $++P < 0.01$, when the level of significance was established between cells incubated with isoprenaline in the absence or presence of elgodipine.

shaking, isolated cells were filtered and washed three times in KRBA buffer to eliminate collagenase. The packed cells were adjusted to a suitable dilution in the same buffer. Fat cells were incubated in plastic vials (1 ml of incubation medium) with gentle shaking at 37°C under an air phase. After a 90-min incubation the tubes were placed on ice and 200-μl aliquots were removed for the determination of glycerol (Wieland, 1957), taken as an index of fat-cell lipolysis. Metabolic activity is expressed as micromoles of glycerol produced per 100 mg of total lipid, determined gravimetrically after extraction according to Dole and Meinertz (1960). Pharmacological agents were added just before the incubation (10 μl dose volume) was started. When isoprenaline was used ascorbic acid (0.1 mM) was included in the medium.

In another set of experiments, mouse adipocytes were used. In this particular case, fat cells were obtained from pooled perirenal and epididymal fat pads removed from fed animals.

2.6. cAMP assay

After incubation, samples were homogenised at 4°C in 1 ml of a solution containing isobutylmethylxanthine (0.4 mM), edetate disodium (EDTA, 40 mM) and NaCl (2.0 mM). Samples were centrifuged 10 min at 0°C ($2000 \times g$) and the fat cake was aspirated and discarded. A sample of the infranatant was added to an equal volume of 10% (w/v) trichloroacetic acid for adenosine 3′,5′-cyclic monophosphate (cAMP) assay. Samples were then extracted with diethyl ether and nucleotide accumulation was estimated by radioimmunoassay (Amersham International TRK 432, UK).

2.7. In vitro insulin release measurements

The experiments were performed with islets isolated by collagenase digestion of rabbit pancreas. After isolation, islets were first pre-incubated for 90 min at 37°C in a bicarbonate buffer medium, pH 7.4, containing 15 mM glucose and supplemented with 1 mg ml^{-1} bovine serum albumin fraction V (Boehringer Mannheim). They were then incubated for 60 min in batches of three, in 1 ml of medium containing appropriate concentrations of glucose and test substances. At the end of the incubation, a portion of the medium was withdrawn and diluted for the insulin assay. Insulin was measured with a radioimmunoassay kit insulin-CT (CIS Bio International, France).

2.8. Statistics and data analysis

Changes in blood glucose, glycerol, lactate and plasma insulin levels from at least five rabbits are expressed as means ± S.E.M. Changes in immunoreactive insulin are expressed as percentages of mean control. When the lipolytic response was investigated in vitro, concentration–response curves were obtained for at least six individual assays.

Values for in vitro insulin secretion are means ± S.E.M. for 15 batches of islets. Changes in glycerol and insulin release are expressed as percentages of mean control. A one-way analysis of variance and the Newman–Keuls test were used for statistical analysis.

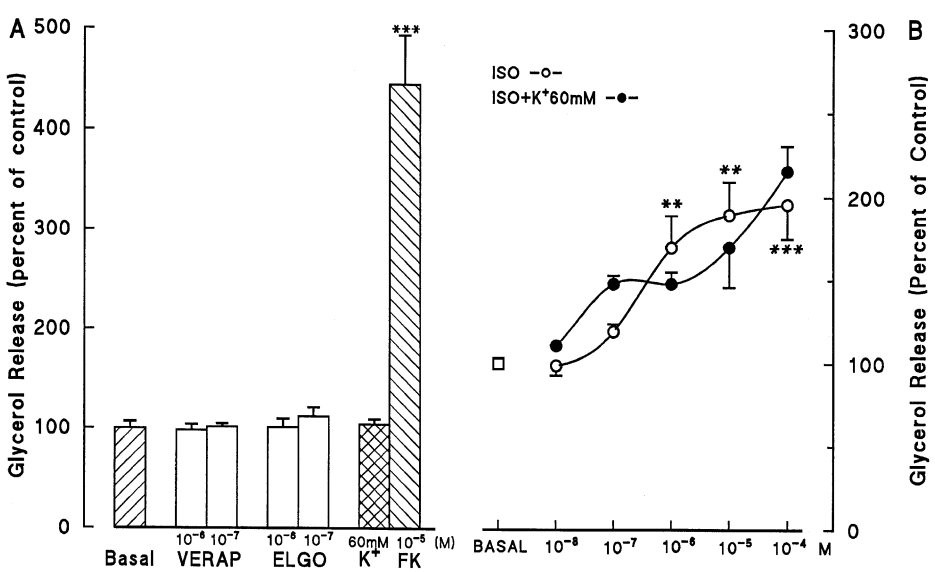

Fig. 5. (A) The effects of Ca^{2+}-channel blocking drugs, high K^+ (60 mM) and forskolin (10^{-5} M) on glycerol release from isolated perirenal fat cells. In (B), the effect of increasing concentrations of isoprenaline (ISO) alone (-○-) or in the presence of 60 mM K^+ (-●-) on glycerol release from cells of the same origin. For more details, see legend for Fig. 4 and text.

3. Results

3.1. Effects of verapamil and elgodipine on the metabolic responses induced by isoprenaline in conscious rabbits

It has been previously reported that verapamil (0.17 μg kg^{-1} min^{-1}) alone does not modify basal values of either plasma glucose or circulating insulin (García-Barrado et al., 1992). However, in the presence of this Ca^{2+} channel antagonist, the weak and transient increase in plasma glucose level evoked by a low dose of isoprenaline (0.3 μg kg^{-1} min^{-1}; Δ at 15 min = 0.41 ± 0.15 mM, $n = 10$ $P < 0.05$ vs. 0.04 ± 0.09 mM, $n = 12$ for saline treated rabbits) was replaced by a moderate and sustained hyperglycaemia (Δ at 30 min for animals infused with isoprenaline in the absence or presence of verapamil was 0.32 ± 0.12 mM, $n = 10$ vs. 1.25 ± 0.3 mM, $n = 6$, respectively, $P < 0.01$, Fig. 1A). Interestingly, verapamil did not reduce the ability of the agonist to increase arterial insulin levels (Δ at 45 min found with the agonist alone or in the presence of the Ca^{2+} channel antagonist was 112.35 ± 24.30%, $n = 6$, vs. 124.35 ± 42%, $n = 8$, Fig. 1B). Elgo-

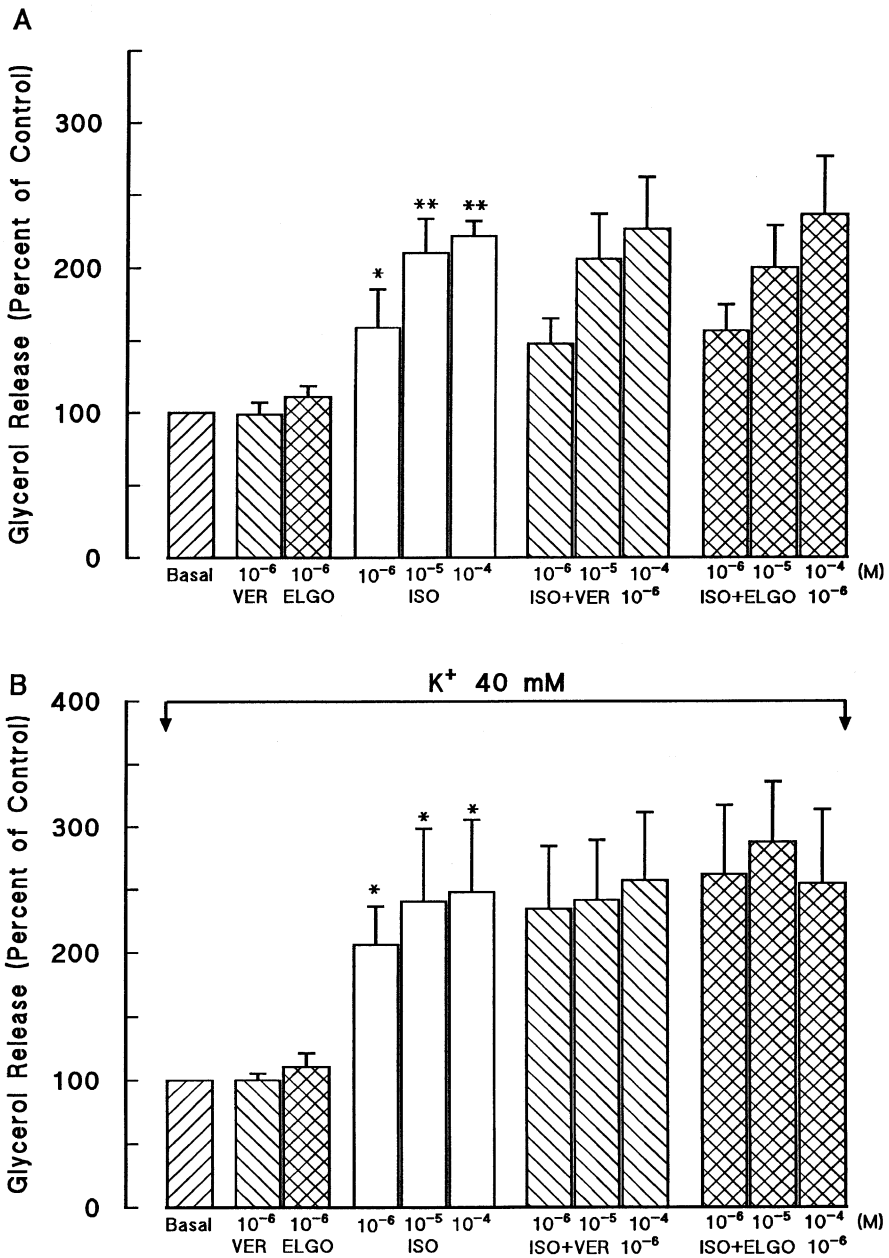

Fig. 6. Effects of increasing concentrations of isoprenaline (ISO) alone (□) or in the presence of either verapamil (▨, 10^{-6} M) or elgodipine (▩, 10^{-6} M) on glycerol release from mouse pooled (perirenal and epididymal) fat cells. Adipocytes were incubated in normal medium (A), or in one containing 40 mM KCl (B). The effects of Ca^{2+}-channel antagonists, alone, are also presented. Basal glycerol release in both normal and enriched K^+ medium was 1.01 ± 0.20 and 1.42 ± 0.34 μmol glycerol/100 mg lipid/90 min ($n = 5$), respectively.

dipine (35 ng kg^{-1} min^{-1}) did not alter the effects of isoprenaline on plasma glucose levels (Δ at 15 min = 0.40 $\pm$ 0.22 mM, $n = 8$) but suppressed its insulin secretory response (Δ at 45 min = 11.45 $\pm$9.40%, $n = 5$ $P < 0.001$, Fig. 1B. It is known that the dyhydropyridine derivative when infused alone induces a mild and transient increase in blood glucose levels; García-Barrado et al., 1997). Pre-infusion absolute values of arterial plasma glucose and circulating insulin for saline and drug-treated animals ranged between 5.40 $\pm$ 0.22 and 5.90 $\pm$ 0.19 mM, and 8.45 $\pm$ 1.60 and 11.1 $\pm$ 1.26 μIU ml^{-1}, respectively.

The β-adrenoceptor agonist induced a significant increase in plasma lactate levels (Δ at 45 min = 2.42 $\pm$ 0.9 mM, $n = 8$ $P < 0.05$ vs. 0.07 $\pm$ 0.25 mM, $n = 8$ for saline control rabbits) and evoked a minor but non-significant reduction in the levels of circulating glycerol (Δ at 45 min = -0.35 ± 0.20 mM, $n = 8$, vs. -0.020 ± 0.04 mM, $n = 6$, for control animals, Fig. 2). Plasma levels of lactate and glycerol were not significantly modified after verapamil administration. The Ca^{2+} entry blocker did not alter the effect of isoprenaline on plasma glycerol levels (Δ at 45 min = 0.17 $\pm$ 0.26 mM, $n = 5$, n.s.) but the ability of this agonist to increase the levels of lactate was significantly enhanced (Δ at 30 min in the absence and presence of verapamil was 1.60 $\pm$ 0.70 mM, $n = 8$ vs. 3.02 $\pm$ 0.45 mM, $n = 8$ $P < 0.05$, Fig. 2). Elgodipine by itself induced very minor changes in the plasma levels of both substrates but the dihydropyridine derivative significantly potentiated the lipolytic effect of isoprenaline (Δ at 45 min in the absence and presence of elgodipine was -0.35 ± 0.20 mM, $n = 8$ vs. 0.58 $\pm$ 0.14 mM, $n = 7$ $P < 0.001$, Fig. 3B), as well as the rise in plasma lactate level due to β-adrenoceptor stimulation (Δ at 30 min in the presence of elgodipine = 3.24 $\pm$ 0.57 mM, $n = 7$, $P < 0.05$, Fig. 3A).

In these experimental designs, pre-infusion levels of plasma lactate fluctuated between 1.05 $\pm$ 0.19 and 1.77 $\pm$ 0.34 mM and those for glycerol ranged from 0.26 $\pm$ 0.01 to 0.84 $\pm$ 0.29 mM.

3.2. Effects of verapamil and elgodipine on the lipolytic response to isoprenaline in isolated fat cells

Fat cells were incubated in the absence of adenosine deaminase, since its inclusion is unable to increase basal lipolysis in rabbits (Castan et al., 1994). Isoprenaline induced a moderate lipolytic response (Fig. 4A). In cells of perirenal origin, a significant increase in glycerol release was already observed with a dose of 10^{-6} M ($\Delta = 170 \pm$ 19.2%, $n = 10$, $P < 0.001$, basal release being 0.240 $\pm$ 0.065 μmol glycerol/100 mg lipid/90 min). A higher concentration of the agonist was required to obtained a significant effect in fat cells from epididymal tissue (10^{-4} M $\Delta = 257.8 \pm 83\%$, $n = 5$, $P < 0.05$; basal release = 0.200 $\pm$ 0.06 μmol glycerol/100 mg lipid/90 min. Data not shown.). Verapamil (10^{-6} M) did not modify the lipolytic effect of isoprenaline in epididymal cells though it slightly, but not significantly, enhanced the response to the agonist in perirenal fat cells. Elgodipine, also 10^{-6} M, potentiated the response to the β-adrenoceptor agonist in both perirenal (isoprenaline 10^{-6} M, Δ in the absence and presence of the drug was = 170 $\pm$ 19.24%, $n = 10$; 304.7 $\pm$ 36.6%, $n = 10$, $P < 0.01$, Fig. 4A) and epididymal fat cells (10^{-6} M, Δ in the absence and presence of the Ca^{2+} channel antagonist = 198 $\pm$ 55.6%, $n = 5$, vs. 282.4 $\pm$ 76.3%, $n = 5$, $P < 0.01$. Data not shown.). The basal release of glycerol was not affected by the addition of any of the Ca^{2+} entry blockers (Fig. 5A).

In perirenal fat cells, the lipolytic response to β-adrenoceptor stimulation in the presence of both Ca^{2+} channel-blocking drugs was correlated with the accumulation of

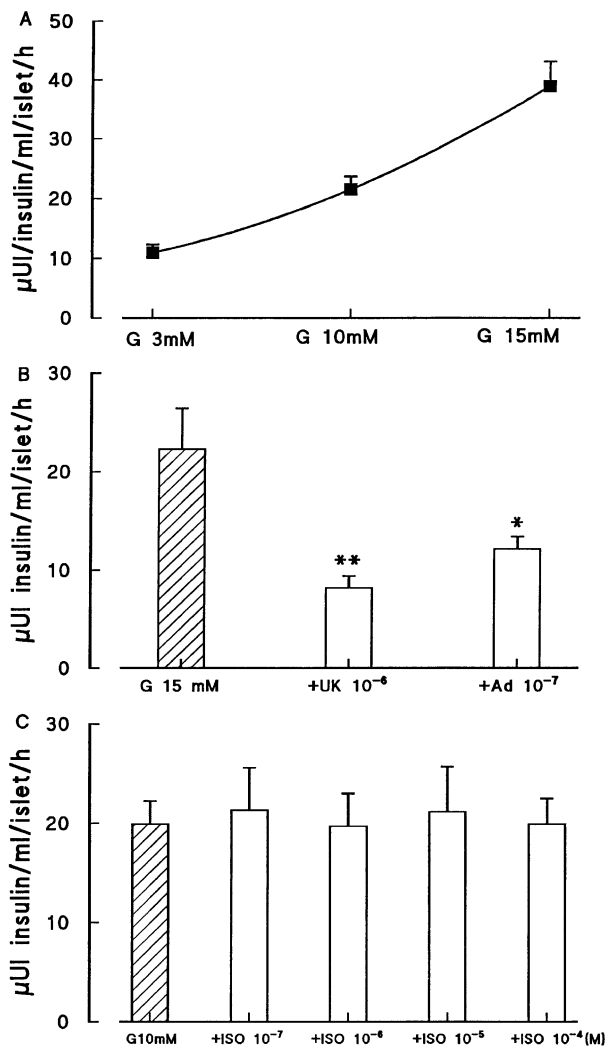

Fig. 7. Insulin release from rabbit isolated islets. Islets were incubated for 60 min in 1 ml of medium containing different glucose concentrations (A). The inhibitory effect of either adrenaline (10^{-7} M) or UK14,304 (brimonidine) (10^{-6} M) on glucose (15 mM)-mediated insulin release are shown in (B). The effects of different concentrations of isoprenaline (ISO) when the islets were bathed in 10 mM glucose are shown in (C). Values are means $\pm$ S.E.M. for 15 batches of islets. $^{*}P < 0.05$, $^{**}P <$ 0.01, values significantly different from the insulin secretory response to 15-mM glucose.

cAMP. Again, isoprenaline 10^{-6} M evoked a significant increase in the accumulation of this nucleotide (from 0.42 $\pm$ 0.10, $n = 6$ to 0.85 $\pm$ 0.08 pmol/100 mg lipid/45 min, $n = 6$, $P < 0.05$, Fig. 4B). In the presence of elgodipine, a further increase was detected after the addition of the β-adrenoceptor agonist (1.10 $\pm$ 0.14 pmol/100 mg lipid/45 min, $n = 6$, $P < 0.01$, Fig. 4B). Elgodipine and verapamil increased cAMP accumulation (0.78 $\pm$ 0.20; 0.83 $\pm$ 0.14 pmol/100 mg lipid/45 min, respectively $n = 6$), but this increase was not statistically significant. As expected, forskolin 10^{-5} M induced a very marked increase (2.74 $\pm$ 0.36 pmol/100 mg lipid/45 min, $n = 5$. $P < 0.001$, Fig. 4B).

3.3. The lipolytic response to isoprenaline in the presence of high K^+

The experiments were carried out in perirenal fat cells. Basal release of glycerol was not altered by the addition of 60 mM KCl to the incubation medium (0.235 $\pm$ 0.020 vs. 0.220 $\pm$ 0.035 μmol glycerol/100 mg lipid/90 min in normal Krebs, Fig. 5A). However, cells responded with a very large increase in glycerol release when forskolin 10^{-5} M was added (normal medium, $\Delta = 444.25 \pm 49\%$, $n = 5$. $P < 0.001$, Fig. 5A). Similarly, neither verapamil (10^{-6} M) nor elgodipine (10^{-6} M) modified glycerol release in the presence of 60 mM K^+ (data not included). K^+ excess did not enhance the lipolytic effect of isoprenaline (Fig. 5B).

The lipolytic response mediated by the β-adrenoceptor agonist in the presence of both Ca^{2+} channel antagonists, either in normal medium or in high K^+, was also investigated in mouse adipocytes. In normal medium (basal release = 1.01 $\pm$ 0.20 μmol glycerol/100 mg lipid/90 min, $n = 5$), the response to isoprenaline was not affected by the addition of 10^{-6} M of either Ca^{2+} channel antagonist (maximum increase in glycerol release was found with the agonist 10^{-5} M, $\Delta = 210.25 \pm 23.3\%$, $n = 5$, $P < 0.01$, Fig. 6A). In 40 mM KCl (basal release = 1.42 $\pm$ 0.34 μmol glycerol/100 mg lipid/90 min, $n = 5$), the dose–response curve for isoprenaline did not differ from that recorded in normal medium. The Ca^{2+} channel antagonists did not alter the effect of isoprenaline or basal glycerol release (Fig. 6B).

3.4. Effect of Ca^{2+} channel antagonists on insulin secretion from isolated islets

In the presence of 10 mM glucose, isoprenaline (10^{-7}–10^{-4} M) did not elicit insulin release (Fig. 7C). Hormone secretion did not differ from basal release (19.91 $\pm$ 2.32 μIU/ml/islet/h, $n = 28$). As expected, rabbit islets responded to glucose in a concentration-dependent manner. Similarly, a significant inhibitory response induced by either UK 14,304 (brimonidine) or adrenaline (60.20 $\pm$ 5.6%, $n = 22$ $P < 0.01$, and 45.40 $\pm$ 5.7%, $n = 10$ $P < 0.05$, Fig. 7A and B) in the presence of 15-mM glucose was obtained. Therefore, for further studies, forskolin instead of isoprenaline was used. Verapamil and elgopine (both 10^{-6} M) blocked insulin release in the presence of 15 mM glucose (62.63 $\pm$ 5.79%, $n = 18$ $P < 0.05$ and 68.25 $\pm$ 5.70%, $n = 19$ $P < 0.05$, respectively; basal secre-

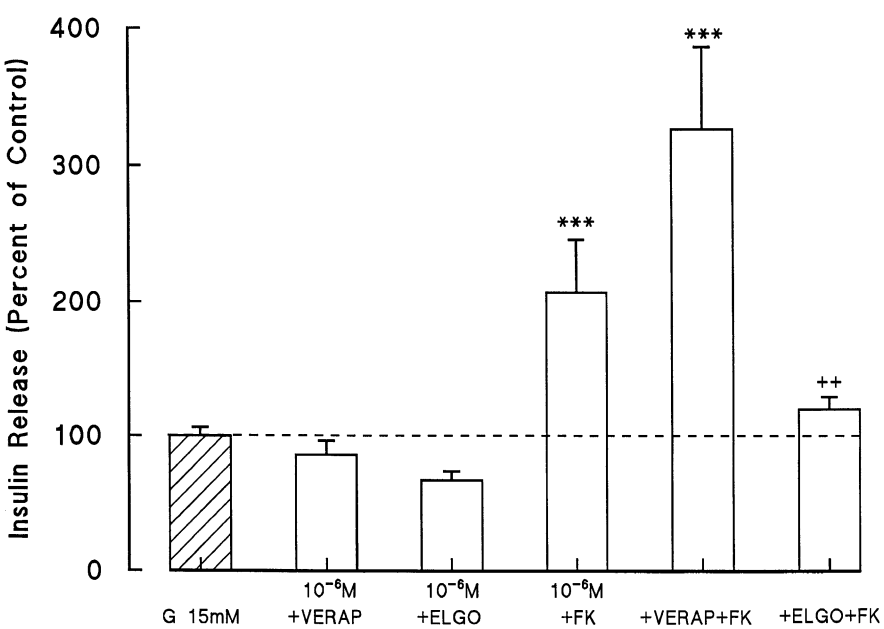

Fig. 8. Effect of forskolin (10^{-6} M) on glucose (15 mM)-induced insulin release from rabbit isolated islets, either in the absence or presence of verapamil (10^{-6} M) or elgodipine (10^{-6} M). Values are expressed as percentages of mean control (control = 100%). Insulin release in the presence of glucose was 19.14 $\pm$ 3.5 μIU/ml/islet/1 h, $n = 22$. $^{*}P < 0.05$, $^{***}P < 0.001$, values significantly different from the insulin secretory response to glucose. $+P < 0.05$, when the level of significance was established between islets treated with forskolin in the absence or presence of elgodipine.

tion = 19.14 ± 3.50 μIU/ml/islet/h, $n = 22$, Fig. 8). In very good agreement with in vivo data, verapamil seemed to increase the insulin secretory response to 10^{-6} M forskolin, though the response was not statistically significant (Δ in the absence and presence of the Ca^{2+} channel antagonist was 62.48 ± 19.02%, $n = 16$; 88.90 ± 18.82%, $n = 18$). Elgodipine suppressed the forskolin effect (Fig. 8).

4. Discussion

The present in vivo study was carried out using a rather small dose of isoprenaline. At this particular concentration, the β-adrenoceptor agonist already induces significant haemodynamic changes (García-Barrado et al., 1998b), which are accompanied by an increase in the levels of plasma lactate and insulin. The lack of a lipolytic response and the very weak effect of isoprenaline on plasma glucose levels derive from its ability to stimulate insulin secretion (Potter et al., 1972).

At the doses used in the present work, basal levels of plasma glucose, insulin (García-Barrado et al., 1997), lactate and glycerol were not changed after either verapamil or elgodipine administration. However, the effects of both drugs on the metabolic responses elicited by isoprenaline were complex and at first sight contradictory. Surprisingly, verapamil enhanced the weak hyperglycaemic effect of the agonist even if this Ca^{2+} channel antagonist failed to block the increase in insulin secretion mediated by the β-adrenoceptor agonist. Interestingly, a similar response pattern was found when verapamil was infused in the presence of a higher concentration of isoprenaline, though a greater increase in blood glucose was then reported (García-Barrado et al., 1992). Elgodipine clearly suppressed this response. It is known that insulin release triggered by a rise in cAMP in the β-cell partly results from Ca^{2+} influx through dihydropyridine-sensitive L-type voltage-dependent Ca^{2+} channels (Leiser and Fleischer, 1996; Suga et al., 1997). Our results with elgodipine certainly support this notion. The failure of verapamil could be explained by additional actions of this Ca^{2+} channel antagonist that mask its own blocking properties (Lebrun et al., 1997: see below for further discussion).

A greater rise in plasma lactate levels was found when isoprenaline was administered in the presence of either verapamil or elgodipine. This increase in circulating lactate levels could be due to a reduction in lactate uptake/utilisation by the liver and a larger release of the substrate from muscle. Stimulation of glucose production from lactate seems to be mediated by β-adrenoceptor agonists, being dependent on cAMP accumulation and an increase in cytosolic Ca^{2+} (Sánchez-Gutiérrez et al., 1997; García-Barrado et al., 1998b; Hers and Hue, 1983). Therefore, the effect of the agonist on liver gluconeogenesis activation would be attenuated by both compounds, considering that isoprenaline-mediated liver glycogenolysis is also dependent of extracellular Ca^{2+} and can be antagonised by Ca^{2+} channel-blocking agents (Maroto et al., 1992). However, both Ca^{2+} entry blockers could enhance the well-established effect of isoprenaline on muscle glycogenolysis and lactate release (Moratinos et al., 1975) by different mechanisms. Verapamil could increase cytosolic Ca^{2+}, and consequently, glycogenolysis, as the result of its own inhibitory effect on the sarcoplasmic reticulum Ca^{2+}-ATPase, reducing Ca^{2+} sequestration (Ortega et al., 1997). Elgodipine, like other dihydropyridines, could directly increase glucose uptake and lactate formation (Foot and Leighton, 1994). Therefore, more lactate would be available for release. Only elgodipine mediated an increase in plasma glycerol levels after isoprenaline administration. Suppression of the insulin secretory rise induced by the β-adrenoceptor agonist would help to facilitate its own lipolytic activity. In addition, elgodipine directly potentiated the lipolytic response to isoprenaline in fat cells of different origin (perirenal and epididymal), an effect that was accompanied by an increased accumulation on cAMP in perirenal adipocytes. Since dihidropyridines acting as phosphodiesterase inhibitors can potentiate the effects of β-adrenoceptor agonists on different tissues (Sarriá et al., 1994), it is reasonable to assume that elgodipine working in a similar way could enhance the lipolytic effect of isoprenaline.

Muscle and fat glucose uptake is reduced by the β-adrenoceptor agonist, even during insulin stimulation, as a result of phosphorylation and inactivation of the glucose transporter (Clark et al., 1995; Jensen et al., 1997; Lawrence et al., 1990; Nishimura et al., 1991; Faintrenie and Géloën, 1992). This inhibitory effect could be counteracted by elgodipine, which, like nifedipine, might activate glucose transport and lactate formation (Giugliano et al., 1980). By contrast, verapamil, by increasing muscle cytosolic Ca^{2+}, could reduce glucose uptake (Foot and Leighton, 1994), thus reinforcing the inhibitory effect of isoprenaline. This also explains the modest but sustained increase in plasma glucose levels when both the β-adrenoceptor agonist and verapamil were administered together (see also García-Barrado et al., 1992).

In rabbit isolated islets, isoprenaline did not induce insulin release in the presence of 10-mM glucose. These in vitro results are in clear contradiction to previous in vivo data (Moratinos et al., 1977) and the present work. The islets also failed to respond to isoprenaline and the selective β_2-adrenoceptor agonist terbutaline when the glucose concentration was raised to 15 mM (data not shown). Interestingly, forskolin-stimulated insulin release in response to glucose was dose-dependent, and the functional inhibitory response mediated by the α_2-adrenoceptor agonist adrenaline and brimonidine was clearly shown. The results concerning the direct effects of β-adrenoceptor agonists on isolated islets are controversial. Human pan-

creatic β-cells are equipped with β_2-adrenoceptors since an insulin secretory response is found after clenbuterol administration (Lacey et al., 1993). However, the rabbit behaves like the rat and the mouse, species where the in vitro secretory response to isoprenaline is hard to evoke (Lacey et al., 1991). We do not have a clear explanation for these conflicting results. There is an abundance of data showing good parallelism between islet blood flow and plasma insulin levels. Therefore the increase islet blood flow induced by β-adrenoceptor stimulation could be one of the mechanisms involved in the secretory response described in conscious rabbits (Atef et al., 1996).

Interestingly, irrespective of whether the insulin secretory response was elicited by isoprenaline or forskolin, both Ca^{2+} channel-blocking drugs behaved in a similar way in vivo and in vitro. The underlying mechanism for cAMP enhancement of insulin secretion is not easily understood. cAMP modulates the activity of L-type voltage-dependent Ca^{2+} channels and non-selective cation channels, and, more importantly, it seems to sensitise the exocytotic machinery to Ca^{2+} through a cAMP/protein-kinase A pathway (Leiser and Fleischer, 1996; Ding and Gromada, 1997; Renström et al., 1997; Yajima et al., 1999). Our results with elgodipine support the primary role played by the influx of extracellular Ca^{2+} through L-type Ca^{2+} channels, and the cAMP-mediated excitatory effect. Verapamil, however, at a concentration able to block the insulin secretory response to a glucose load (García-Barrado et al., 1997), did not modify the effect of any of the used excitatory agents. It has recently been reported that verapamil and other phenylalkylamines, but not nifedipine, decrease the activity of ATP-sensitive K^+ channels (K_{ATP} channels) in the β-cell in a concentration-dependent manner (Lebrun et al., 1997). In this way, verapamil could enhance the ability of cAMP to block K_{ATP} channel activity (Holz et al., 1993; Gromada et al., 1997), thus restraining its own inhibitory properties.

The role played by external Ca^{2+} and Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels in lipolysis is certainly controversial. Changes in the Ca^{2+} concentration of the incubation medium did not alter the lipolytic response induced by isoprenaline (Ebert et al., 1974) or attenuate the effect of adrenaline or noradrenaline (Emami and Perry, 1986; Izawa and Komabayashi, 1994). More recent experiments showed that Ca^{2+} entry through dihydropyridine-sensitive channels could be implicated in mediating the increase in lipolysis seen in ethanol withdrawal (Jelic and Taberner, 1996). However, no evidence for inward currents carried by Ca^{2+} have been reported (Ramírez-Ponce et al., 1996), and no increase in cytosolic/intracellular calcium $[Ca^{2+}]_i$ was detected in rat fat cells incubated in the presence of isoprenaline (Izawa et al., 1994). Our results for both rabbit and mouse fat cells do not support the involvement of an increase in cytosolic Ca^{2+} through these channels in the lipolytic effect of this agonist.

K^+ excess, which is known to depolarise and to evoke a sustained increase in intracellular Ca^{2+} (Pershadsingh et al., 1989), did not increase glycerol release, or potentiate the effect of the β-adrenoceptor agonist. Ca^{2+} channel antagonists can block the K^+-mediated increase in $[Ca^{2+}]_i$ (Pershadsingh et al., 1989) in fat cells but did not alter the response to K^+ or reduce the effect of isoprenaline. It has been shown that lipolysis is affected to different extents by dihydropyridines (Jelic and Taberner, 1997). In rabbit adipocytes, both verapamil and elgodipine increased, though not significantly, cAMP accumulation, and elgodipine enhanced in a parallel way the increase in glycerol release and cyclic nucleotide content in fat cells in the presence of the β-adrenoceptor agonist. Neither agent modified the effect of isoprenaline in mouse cells. Hormone-sensitive lipase stimulation by agonist mainly relies on enzyme phosphorylation by the cAMP/protein-kinase A pathway because a large part of lipase activity is under the strict control of intracellular cAMP levels. Thus, phosphodiesterase inhibition by elgodipine could explain our data. However, other kinases besides protein-kinase A may be involved in lipase activation by isoprenaline, and the Ca^{2+}-calmodulin complex may be necessary for full activation of the response in trained animals (Izawa and Komabayashi, 1994).

In summary, the modest but sustained increase in blood glucose levels found with the β-adrenoceptor agonist when infused in the presence of verapamil seems to result from the inhibitory effects of both compounds on peripheral glucose uptake. Both Ca^{2+} channel antagonists attenuated the effect of isoprenaline on liver glucose production from lactate. In addition to their ability to block Ca^{2+} channels, these drugs also exhibit several other complex and different properties (inhibition of muscle sarcoplasmic reticulum Ca^{2+} pump, K_{ATP} channels, phosphodiesterase) if specific metabolic responses are investigated. The different behaviour shown by verapamil and elgodipine may help us to understand the difficulties encountered when discussing the deleterious effects of Ca^{2+} channel-blocking drugs on glucose homeostasis. Under our experimental conditions, the metabolic alterations observed in the presence of verapamil and elgodipine, though sustained and statistically significant, were rather modest and probably devoid of serious pathological complications.

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