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Role of α_1- and α_2-adrenoceptors in catecholamine-induced hyperglycaemia, lipolysis and insulin secretion in conscious fasted rabbits

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1 In conscious fasted rabbits an intravenous infusion of clonidine ($2\,\mu g\,kg^{-1}\,min^{-1}$) induced hyperglycaemia. The increase in blood glucose was accompanied by an inhibition of insulin secretion and basal lipolysis.

2 Yohimbine infused at a rate of $20\,\mu g\,kg^{-1}\,min^{-1}$ suppressed clonidine-induced hyperglycaemia and blocked the inhibitory effect on insulin secretion mediated by the α_2-adrenoceptor agonist.

3 The intravenous infusion of amidephrine ($10\,\mu g\,kg^{-1}\,min^{-1}$) induced an increase in insulin secretion in the absence of patent hyperglycaemia. Prazosin, $0.3\,mg\,kg^{-1}$ s.c. selectively antagonized the effect of amidephrine on insulin secretion.

4 Isoprenaline infusion ($4.4\,\mu g\,kg^{-1}\,min^{-1}$) evoked a significant increase in blood glycerol and immunoreactive insulin plasma levels. Both responses were clearly attenuated when α_2-adrenoceptors were simultaneously stimulated by selective (clonidine) and less selective (phenylephrine, $20\,\mu g\,kg^{-1}\,min^{-1}$) agonists.

5 Amidephrine infusion did not induce appreciable changes in blood glycerol nor did it modify, isoprenaline-induced lipolytic response.

6 Simultaneous infusion of isoprenaline and amidephrine induced a remarkable increase in insulin secretion.

7 It is concluded that in normal fasted rabbits stimulation of α_2-adrenoceptors depresses basal and β-adrenoceptor mediated lipolysis and insulin secretion. On the other hand, selective stimulation of α_1-adrenoceptors does not affect lipolysis but induces insulin release. Simultaneous stimulation of α_1- and β-adrenoceptors potentiates the insulin secretory response.

Introduction

The presence of two opposite components in the adrenergic regulation of the lipolytic and insulin secretory processes is now well recognized. Fat and β-cells show both a β-activating and an α-inhibitory response (Malaisse *et al.*, 1967; Loubatieres & Mariani, 1970; Burr *et al.*, 1971; Lafontan & Berlan, 1981). Extensive pharmacological characterization of the α-receptors mediating an antilipolytic effect has been carried out on adipocytes of several species. The presence of α_2-adrenoceptors has been clearly demonstrated by various *in vitro* studies such as direct radioligand binding and metabolic activity measurements (García Sainz *et al.*, 1980; Lafontan & Berlan, 1981; Carpene *et al.*, 1983). Similarly the α-adrenoceptor which mediates the insulin inhibitory response evoked by catecholamines appears to be of the α_2-subtype (Nakaki *et al.* 1981; Langer *et al.*, 1983; Hillaire-Buys *et al.*, 1985). However their effective role *in vivo* has not been fully studied. Recent reports show that the α_2-adrenoceptor agonist clonidine (Clo) reduces basal levels of plasma free fatty acids (FFA) in humans (Thompson *et al.*, 1984) and inhibits the isoprenaline (Iso) induced FFA outflow from bone marrow adipose tissue (Tran *et al.*, 1984). On the other hand, although previous administration of α_2-adrenoceptor antagonists allows for a severe increase in immunoreactive insulin plasma levels (IRI) in the presence of adrenaline (Nakadate

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et al., 1980), phenylephrine (PE) (Moratinos et al., 1986), or glucose (Roach et al., 1985) less consistent results have been found when α_2-adrenoceptors were selectively stimulated.

A reduction (Metz et al., 1978; Hsu & Kummel, 1981; Brown et al., 1985) and no change (Ditullio et al., 1984; Thompson et al., 1984) in basal IRI plasma levels has been reported after the administration of Clo, xylazine and guanfacine.

Therefore using the rabbit as a suitable experimental model (animal adipose tissue and β-cells possess α_2-adrenoceptor receptivity, Lafontan, 1981; Moratinos et al., 1986) we intended to explore in more detail the nature and functional relevance of these α_2-adrenoceptors. The effects of Clo on basal and β-adrenoceptor promoted lipid mobilization and insulin secretion have also been studied.

Finally, considering that lipolysis and insulin secretion are calcium-dependent processes (Allen & Beck, 1986; for review see Malaisse, 1984) and that in a number of biological systems a close association between α_1-adrenoceptor stimulation and calcium mediated effects has been established (liver glycogenolysis, Haylett, 1976; vascular smooth muscle contraction, Cauvin & Malik, 1984) we looked in the present work for the presence of excitatory α_1-adrenoceptors involved in lipolysis and insulin release. Preliminary results in this context have already been published (Carpene et al., 1984).

Methods

Experimental design

The experimental design has been fully described in earlier publications (Moratinos et al., 1975; 1986). Briefly, conscious albino New Zealand male rabbits previously conditioned for restraint were fasted for 24 h before the experiments. Arterial blood was sampled by use of an indwelling cannula placed in the central artery of the ear. Two control samples, separated by an interval of 30 min, were always taken to ensure reliable basal measurements before beginning drug infusions. Drug solutions (unless stated otherwise, see below) were infused for 30 min at a constant rate of $0.2\,ml\,min^{-1}$ through an indwelling cannula in the marginal vein of the contralateral ear. The arterial cannula was maintained functional by a slow constant infusion of physiological saline solution.

Analyses

As an index of lipolytic activity, blood glycerol was measured. Glycerol determination according to the fluorometric micromethod of Laurell & Tibbling (1966) was done with 0.2 ml blood samples. Glycerol levels were quantified enzymatically using glycerokinase and glycerophosphate dehydrogenase and measuring the amount of NADH formed fluorometrically at exciting wavelength of 350 nm and emitted at 458 nm. In the investigation, an Aminco Bowman instrument was used.

Immunoreactive insulin (IRI) was determined by use of a CEA-SORIN radioimmunoassay kit. Human insulin was employed as a standard (International CIS, F-78181 St, Quentin). Plasma glucose was estimated by means of the glucose oxidase procedure using a kit from Boehringer Mannheim Co., West Germany.

Drugs

Fresh stock solutions of (−)-noradrenaline bitartrate, (−)-phenylephrine hydrochloride and (−)-isoprenaline hydrochloride (obtained from Sigma, London) were prepared daily. Catecholamine solutions were made in acidified saline (pH 4.5). Clonidine hydrochloride and amidephrine mesylate were kindly provided from Boehringer Ingelheim and Mead Johnson respectively. Appropriate dilutions were made in saline just before infusion. In some experiments, two adrenoceptor agonists were simultaneously administered by combined infusion of each.

Adrenoceptor blocking agents were administered immediately after withdrawal of the second control sample. Yohimbine hydrochloride (Houde) or propranolol hydrochloride (Ayerst) were diluted in saline and infused for 30 min just before beginning agonist administration. Prazosin hydrochloride (Pfizer) was subcutaneously injected also 30 min before the agonist. All drug concentration are expressed in terms of the free base.

Changes in blood glucose, glycerol and plasma IRI were analysed by the one-way analysis of variance (ANOVA). Changes in the last parameter were referred as a % of mean control (see Moratinos et al., 1977; 1986).

Results

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

The i.v. infusion of $2\,\mu g\,kg^{-1}\,min^{-1}$ of the α_2-adrenoceptor agonist clonidine (Clo) reduced significantly below control values (saline-treated rabbits) both blood glycerol and IRI plasma levels (Figure 1) and induced hyperglycaemia (Figure 7a). Maximum inhibition on the basal rate of lipolysis occurred 60 min after Clo infusion (Figure 1a) ($\Delta = -0.080 \pm 0.016\,mmol\,l^{-1}$, $n = 5$, $P < 0.001$,

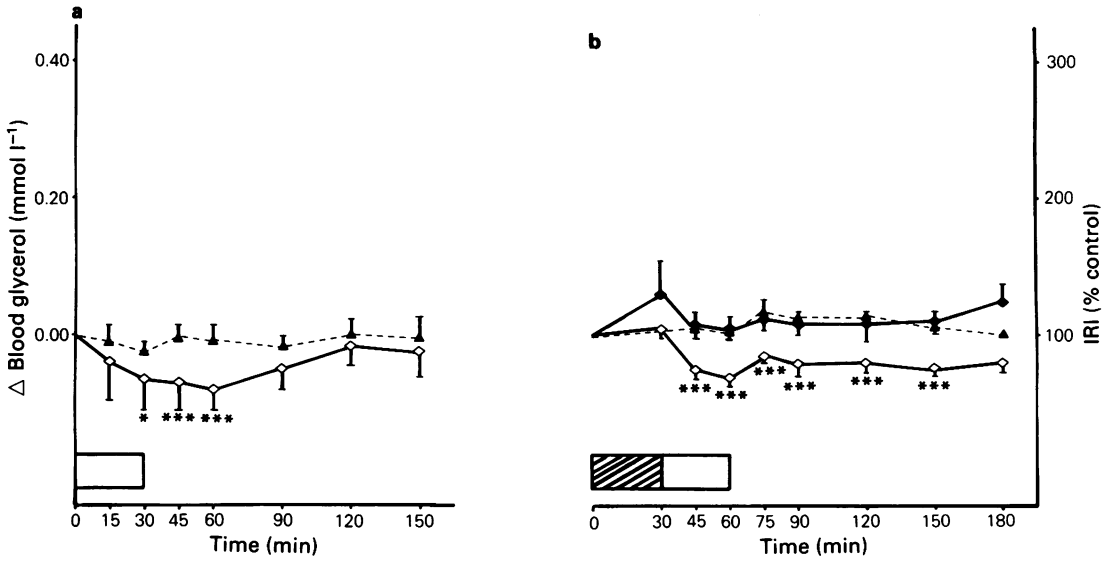

Figure 1 The effects of clonidine (Clo: $2\,\mu g\,kg^{-1}\,min^{-1}$) on blood glycerol (a) and immunoreactive insulin levels (IRI, b) in the absence (◇) and presence (◆) of yohimbine (Yoh; $20\,\mu g\,kg^{-1}\,min^{-1}$) in fasted rabbits. The α_2-adrenoceptor antagonist was infused over a period of 30 min (cross-hatched bar) immediately followed by a 30 min infusion of Clo (open bar). Drugs were administered at a constant rate of $0.2\,ml\,min^{-1}$. Ordinate scales: $mmol\,l^{-1}$ blood glycerol refer to the variations from control values. Values of IRI levels are expressed as a % change from the control level (control level = 100%). Mean responses from at least 5 rabbits are represented. Vertical lines indicate s.e. mean. $^{*}P < 0.05$; $^{***}P < 0.001$, values significantly different from those seen on infusing physiological saline (▲). Clo lowered both parameters, and Yoh significantly blocked Clo-induced inhibition of IRI levels.

versus $\Delta = -0.007 \pm 0.024\,mmol\,l^{-1}$, $n = 5$ in saline treated animals). Blood glycerol absolute values before infusion of either saline or drug were respectively $0.186 \pm 0.029\,mmol\,l^{-1}$ ($n = 5$), and $0.173 \pm 0.021\,mmol\,l^{-1}$ ($n = 5$). In the presence of Clo, plasma IRI levels were lowered throughout the experiment (Figure 1b). Maximal inhibition was observed at the end of drug infusion ($\Delta = -31.4 \pm 6.7\%$, $n = 7$, $P < 0.001$, vs $\Delta = -0.96 \pm 3.30\%$, $n = 7$, in saline control animals). Pre-infusion plasma levels expressed in absolute figures were $10.3 \pm 0.9\,\mu u\,ml^{-1}$ ($n = 10$) and $12.9 \pm 1.6\,\mu u\,ml^{-1}$ ($n = 8$) for drug- and saline-treated animals respectively.

The peak of the mean increase in blood sugar appearing at 45 min was $2.45 \pm 0.47\,mmol\,l^{-1}$, ($n = 6$), $P < 0.001$ in the presence of Clo as compared with $0.36 \pm 0.31\,mmol\,l^{-1}$, ($n = 7$) in saline control. Pre-infusion fasting blood glucose levels for control and experimental animals were very similar ($5.11 \pm 0.25\,mmol\,l^{-1}$, $n = 8$; $4.7 \pm 0.1\,mmol\,l^{-1}$, $n = 7$).

The selectivity of Clo-induced responses was tested in animals previously treated with the α_2-adrenoceptor antagonist yohimbine (Yoh). Pre-infusion with $20\,\mu g\,kg^{-1}\,min^{-1}$ Yoh abolished Clo induced inhibition of IRI plasma levels (Figure 1b) (Δ at 30 min in the presence and absence of the antagonist was: $1.5 \pm 10.5\%$, $n = 6$, $P < 0.001$, and $-31.4 \pm 6.7\%$, $n = 7$). Similarly the hyperglycaemic response was suppressed (Figure 7a) (agonist peak effect at 45 min in the presence and absence of Yoh respectively was: $0.33 \pm 0.35\,mmol\,l^{-1}$, $n = 6$, $P < 0.001$ and $2.45 \pm 0.47\,mmol\,l^{-1}$, $n = 6$).

Whereas Yoh by itself did not appreciably modify blood glucose or IRI plasma levels (Moratinos *et al.*, 1986) it moderately increased blood glycerol, though significant elevations were found only at 30 and 90 min (Table 1, pool animals). Interestingly when analyzing individual experiments, pool animals could be classified as responsive and unresponsive according to the elevations and temporal course of the response (Table 1). In responsive rabbits the increase in blood glycerol was already evident at the end of Yoh infusion, with a progressive increment throughout the experiment.

Functional role of α_2-adrenoceptors on the lipolytic and insulin secretory responses induced by catecholamines

Significance of inhibitory α_2-adrenoceptors on the responses under investigation has been studied in

Table 1 Effects of yohimbine infusion on blood glycerol in the rabbit

		Blood glycerol (mmol l^{-1}) *Time after the start of the infusion* (min)						
Drug infused	*Controls*	15	30	45	60	90	120	150
Saline	0.186	0.176	0.161	0.181	0.179	0.168	0.185	0.182
0.2 ml min^{-1} (5)	±	±	±	±	±	±	±	±
	0.029	0.023	0.014	0.019	0.024	0.017	0.023	0.029
Responses of all rabbits to								
yohimbine	0.257	0.232	0.312*	0.335	0.364	0.414*	0.392	0.435
20 $\mu g\,kg^{-1}\,min^{-1}$ (10)	±	±	±	±	±	±	±	±
	0.022	0.027	0.035	0.047	0.065	0.095	0.114	0.147
(a) responsive rabbits (4)	0.305	0.249	0.444***	0.510***	0.602***	0.700***	0.686***	0.656**
	±	±	±	±	±	±	±	±
	0.018	0.065	0.048	0.077	0.105	0.180	0.192	0.183
(b) unresponsive rabbits (6)	0.224	0.220	0.212	0.217	0.205	0.238	0.175	0.141
	±	±	±	±	±	±	±	±
	0.027	0.017	0.021	0.021	0.014	0.040	0.025	0.054

Results in absolute values are expressed as mmol l^{-1}.
Controls represent the mean average value of two blood samples removed at 30 min intervals before infusing either saline or drug. Yohimbine-treated animals are classified according to drug response. Number of experiments in parentheses, mean ± s.e. mean.
*$P < 0.05$; **$P < 0.005$, ***$P < 0.001$ values significantly different from saline-treated rabbits.

two ways, as follows: (a) exploring the effects of noradrenaline (NA) on blood glycerol and IRI plasma levels after previous blockade with Yoh (Figure 2) and (b) challenging the stimulatory effects of Iso, on both parameters when an α_2-adrenoceptor agonist is simultaneously administered (Figure 3). An i.v. infusion of 4.4 $\mu g\,kg^{-1}\,min^{-1}$ of NA evoked a minor increase in blood glycerol, (Figure 2a, Δ at 30 min = 0.099 ± 0.028 mmol l^{-1}, $n = 7$, $P < 0.001$, in the presence of the agonist as compared to $\Delta = -0.025 \pm 0.014$ mmol l^{-1}, $n = 5$ in control animals) and a modest but sustained elevation in IRI plasma levels, (Figure 2b; maximum response at 45 min = 134.9 ± 74.6%, $n = 4$, $P < 0.005$ in the presence of NA relative to 17.20 ± 9%, $n = 7$, in saline-treated rabbits).

Blockade of inhibitory α_2-adrenoceptors with Yoh did not clearly enhance the β-mediated lipolytic effect of NA (Figure 2a). A significant potentiation was found only during the first 15 min of NA infusion, $\Delta = 0.19 \pm 0.014$ mmol l^{-1}, ($n = 7$), in Yoh pretreated animals against 0.061 ± 0.022 mmol l^{-1} ($n = 7$) in untreated rabbits $P < 0.05$. However, the insulin secretory response was remarkably accentuated, (Figure 2b, Δ at 15 min in the presence of previous blockade was: 331.15 ± 59.3%, $n = 5$, as compared to 106.9 ± 54.5%, $n = 5$, $P < 0.001$ in NA-treated rabbits).

The excitatory effects of β-adrenoceptor stimulation were challenged in the presence of simultaneous α_2-adrenoceptor activation with Clo. A dose of Iso (4.4 $\mu g\,kg^{-1}\,min^{-1}$) able to induce a modest rise in blood glucose (see Figure 7b) and IRI plasma levels (Moratinos *et al.*, 1977) was selected for these studies.

Peak effects in blood glycerol and IRI after Iso infusion were detected at 45 and 30 min respectively (Figure 3a and b, Δ in blood glycerol = 0.779 ± 0.100 mmol l^{-1}, $n = 8$, $P < 0.001$ as compared to: −0.005 ± 0.019 mmol l^{-1}, $n = 5$ in control rabbits; in IRI, $\Delta = 155 \pm 45.7\%$, $n = 8$, $P < 0.001$ against, −0.96 ± 3.34%, $n = 7$ in control animals). Both responses were significantly lowered by concomitant administration of 2 $\mu g\,kg^{-1}\,min^{-1}$ Clo, (Figure 3, Δ in blood glycerol at 45 min in the presence of Clo was: 0.438 ± 0.006 mmol l^{-1}, $n = 5$, $P < 0.001$, and Δ in IRI plasma levels at 30 min, also in the presence of both agonists 35.5 ± 12.6%, $n = 6$, $P < 0.001$).

Though attenuated these effects were of statistical significance when compared to saline-treated animals.

Effects of phenylephrine on isoprenaline-induced lipolysis and insulin secretion

When infused at a rate of 20 $\mu g\,kg^{-1}\,min^{-1}$, PE alone induced a small transient increase in blood glycerol (Figure 4a, Δ at 30 min = 0.037 ± 0.029 mmol l^{-1}, $n = 5$, $P < 0.01$ with the agonist as compared to: −0.025 ± 0.014 mmol l^{-1}, $n = 5$ after saline), and a modest rise in IRI plasma levels

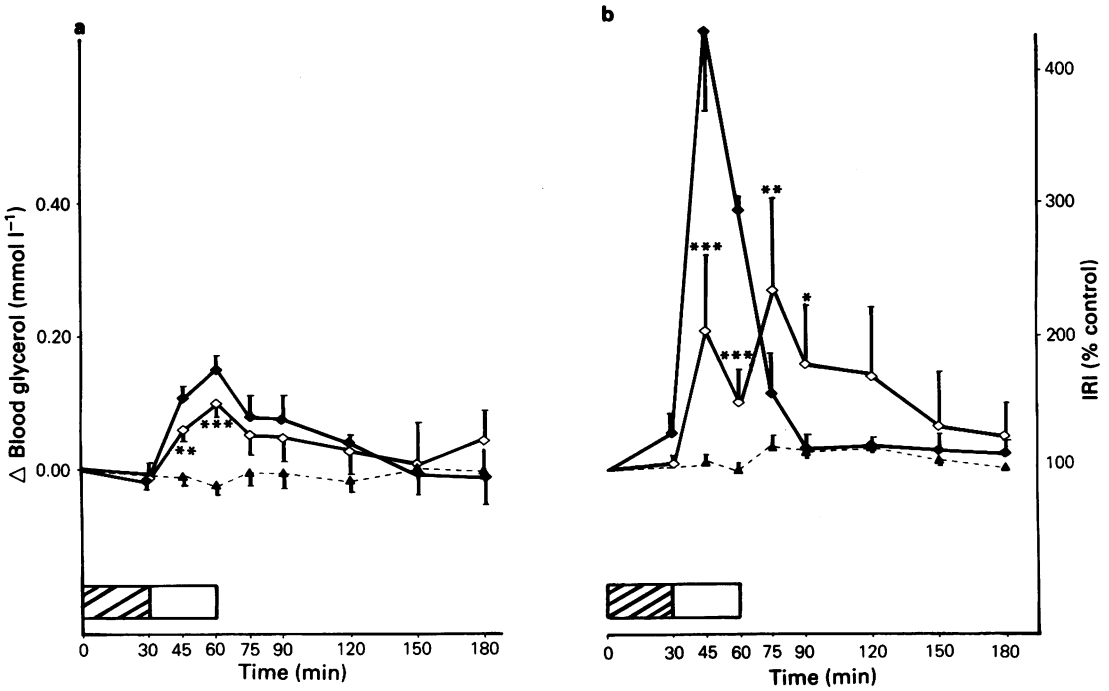

Figure 2 Effects of noradrenaline (NA; 4.4 $\mu g\,kg^{-1}\,min^{-1}$) on blood glycerol (a) and immunoreactive insulin (IRI) levels (b) in the absence (◇) and presence (◆) of yohimbine (Yoh; 20 $\mu g\,kg^{-1}\,min^{-1}$). The effects of infusion of saline (▲) on both parameters are also shown. The experimental design was as described in Figure 1 with NA replacing clonidine. NA induced a modest but significant increase in blood glycerol and a sustained elevation in IRI levels. Only the last response was markedly enhanced by previous treatment with Yoh, (increments at 15 and 30 min were significantly greater, $P < 0.001$, in Yoh-treated as compared to animals treated with the agonist alone) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, values significantly different from saline. For further details see text and legend to Figure 1.

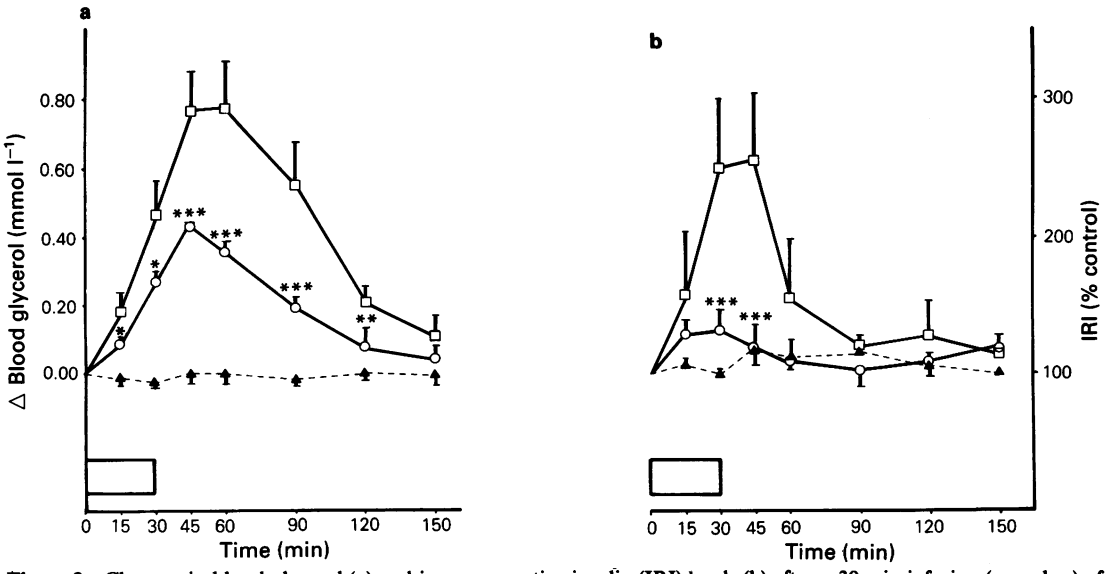

Figure 3 Changes in blood glycerol (a) and immunoreactive insulin (IRI) levels (b) after a 30 min infusion (open bar) of saline (▲), isoprenaline alone 4.4 $\mu g\,kg^{-1}\,min^{-1}$ (Iso, □) and Iso (4.4 $\mu g\,kg^{-1}\,min^{-1}$) plus Clo (2 $\mu g\,kg^{-1}\,min^{-1}$) simultaneously administered (○). Iso induced lipolytic and insulin secretory responses where significantly reduced by concomitant infusion with Clo. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, values significantly different from those seen on infusing Iso. For more details see text and legend to Figure 1.

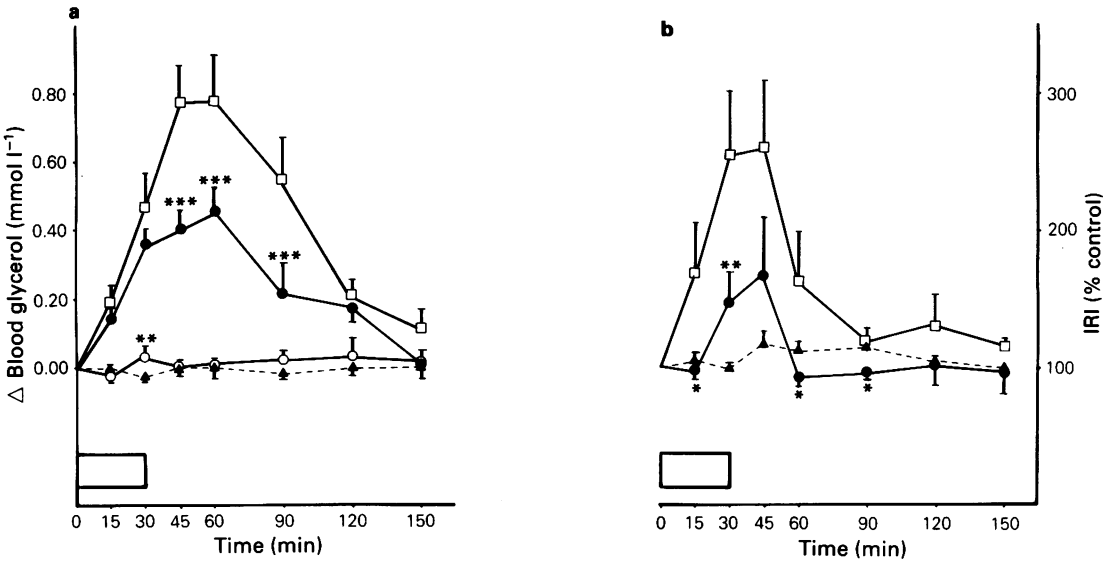

Figure 4 The effects of an i.v. infusion of saline (▲), phenylephrine (PE; 20 μg kg^{-1} min^{-1}, ○), isoprenaline (Iso, 4.4 μg kg^{-1} min^{-1}, □) and Iso plus PE (●) on blood glycerol (a) and immunoreactive insulin plasma levels (IRI, b). PE significantly attenuated the lipolytic and insulin secretory response derived from β-adrenoceptor stimulation. *$P < 0.05$; **$P > 0.01$; ***$P > 0.001$, values significantly different from those observed after Iso infusion. For more details see text and legends to Figures 1 and 3.

(see Moratinos *et al.*, 1986). PE also significantly reduced the metabolic effects derived from β-adrenoceptor stimulation when both agonists were infused together (Figure 4a and b) (Δ in blood glycerol at 45 min after PE + Iso combined infusion was: 0.402 ± 0.057 mmol l^{-1}, $n = 6$, $P < 0.001$ against 0.779 ± 0.100 mmol l^{-1}, $n = 8$ after Iso alone). Interestingly, though the maximum rise in IRI was not significantly different in both Iso and Iso + PE treated rabbits (Δ at 45 min being = $161.1 \pm 47\%$, $n = 8$, $P < 0.2$ versus $69 \pm 40.3\%$, $n = 6$ respectively), the response established more slowly and was of shorter duration in the last situation (see legend to Figure 4b). Again responses detected with PE and Iso together had significant value when compared to changes found in saline-treated rabbits.

Metabolic effects resulting from selective α_1-adrenoceptor stimulation

It has been shown that amidephrine (Amid) behaves as a selective α_1-adrenoceptor agonist in a number of experimental situations such as: rabbit aortic strip (Buchtal & Jenkinson, 1970), rat vas deferens (Vizi & Ludvig, 1983), pithed rat (Flavahan & McGrath, 1980), and cats (Mathy *et al.*, 1983). Consequently this drug was chosen for our experiments.

The i.v. infusion of 10 μg kg^{-1} min^{-1} Amid evoked a minor rise in blood glucose (Figure 5a) which had statistical significance only at 15 min ($\Delta = 0.47 \pm 0.13$ mmol l^{-1}, $n = 10$, $P < 0.005$ as compared to 0.012 ± 0.21 mmol l^{-1}, $n = 8$ with saline). Interestingly, in the absence of patent hyperglycaemia Amid induced a significant increase in IRI plasma levels (Figure 5b), the maximum rise appearing 15 min after the end of drug administration (Δ at 45 min = $126.5 \pm 54\%$, $n = 5$, $P < 0.001$ in the presence of Amid as compared to $17.15 \pm 9\%$, $n = 7$ in saline control animals. A 10% variation in IRI being equivalent to 1.15 μu ml^{-1}). No appreciable lipolytic response was found in the presence of the agonist (Figure 6a; see also for comparison the response to PE in Figure 4a).

As is shown in Figure 5b, 0.3 mg kg^{-1} prazosin (a selective α_1-adrenoceptor antagonist) s.c. injected 30 min before Amid, suppressed the increase in IRI induced by the agonist (Δ found with Amid at 45 min in the presence and absence of prazosin was, respectively, $-9.31 \pm 9.38\%$, $n = 7$, $P < 0.001$ versus 126.5 ± 54, $n = 5$). Smaller doses of prazosin (i.v. infusion of 1.5 μg kg^{-1} min^{-1}) were ineffective. The β-blocker propranolol, at a dose able to antagonize Iso-induced increase in IRI (Moratinos *et al.*, 1977) failed to neutralize the response mediated by Amid (Figure 5b).

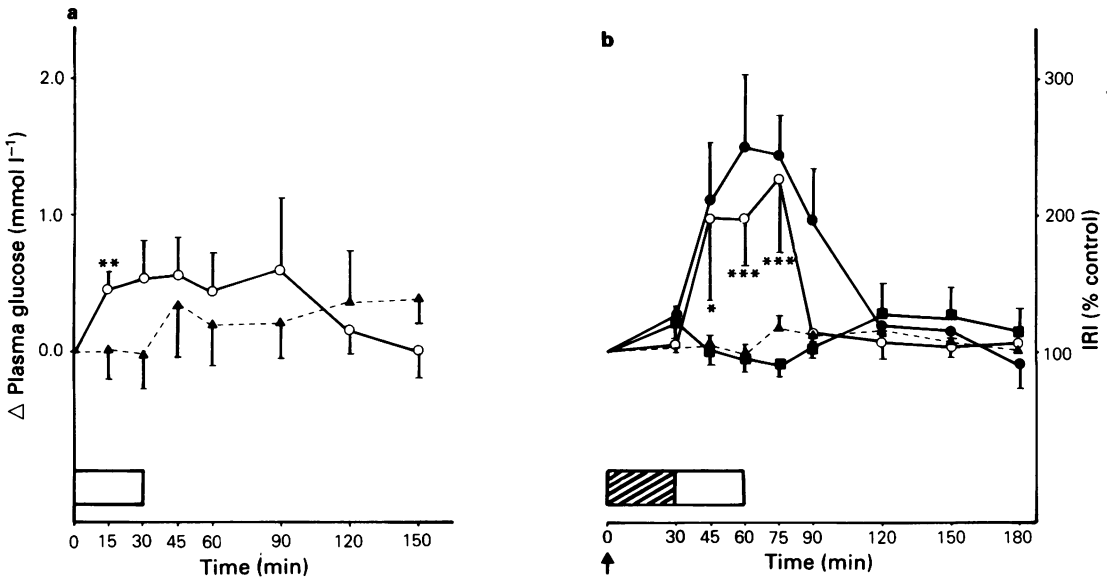

Figure 5 Effects of amidephrine (Amid; $10\,\mu g\,kg^{-1}\,min^{-1}$) on plasma glucose (a) and immunoreactive insulin (IRI) levels (b), in the absence (○) and presence of either propranolol ($0.3\,mg\,kg^{-1}\,min^{-1}$, ●) or $0.3\,mg\,kg^{-1}$ prazosin (■). The β-blocker was infused over a period of 30 min (crosshatched bar) immediately followed by a 30 min infusion of Amid (open bar). At the arrow prazosin was injected subcutaneously. Thirty minutes later Amid infusion started. The effects of infused saline (▲) on both parameters are also shown. Ordinate scale: $\Delta\,mmol\,l^{-1}$ plasma glucose refer to the variations from control values. In the absence of a net hyperglycaemic action amidephrine induced a significant increase in IRI levels which was suppressed by previous treatment with prazosin. $^{*}P > 0.05$; $^{**}P > 0.01$; $^{***}P > 0.001$ values significantly different from saline. For more details see text and legend to Figure 1.

Metabolic effects derived from simultaneous stimulation of α_1- and β-adrenoceptors

The lipopolytic response evoked by Iso was not altered by the simultaneous stimulation of α_1-adrenoceptors with Amid (Figure 6a). On the other hand, the combined simultaneous infusion of Iso and Amid induced a remarkable increase in IRI plasma levels (Figure 6b). Maximum effect was evident earlier than usual (Δ at 15 min = $655.5 \pm 151.4\%$, $n = 5$, $P < 0.001$ when compared to saline control animals) and significant elevated levels were still found for 90 min. In the presence of dual adrenoceptor stimulation, the insulin secretory response was clearly potentiated, since the increases found after combined infusion were significantly greater than those predicted by summation of the individual response to each agonist alone (Δ at 15 min after Iso + Amid infusion = 655.45; Δ at 15 min with Iso + Δ at 15 min in the presence of Amid = 182.17%, $P < 0.001$). This effect was extended for another 45 min (Δ at 60 min after combined infusion = 176.4%; Δ obtained by individual summation = 77.4%, $P < 0.005$).

Plasma glucose rose moderately when both agonists were infused together, (Figure 7b, Δ at 45 min = $1.75 \pm 0.32\,mmol\,l^{-1}$, $n = 7$, $P < 0.001$ as compared to $0.36 \pm 0.31\,mmol\,l^{-1}$, $n = 7$ in control rabbits). The response was very similar to Iso induced hyperglycaemia though slightly higher and more sustained.

Discussion

Since the nature and functional significance of the α_2-adrenoceptors modulating lipolysis and insulin secretion have been clearly established using *in vitro* preparations (Lafontan & Berlan, 1980; Nakaki *et al.*, 1981), we designed this study to examine the same inhibitory systems *in vivo*. The fasted rabbit is suitable for this type of work since it allows for the simultaneous study of both receptor characterization and function as well as for the interplay established between insulin plasma levels and the intensity of the lipolytic effect.

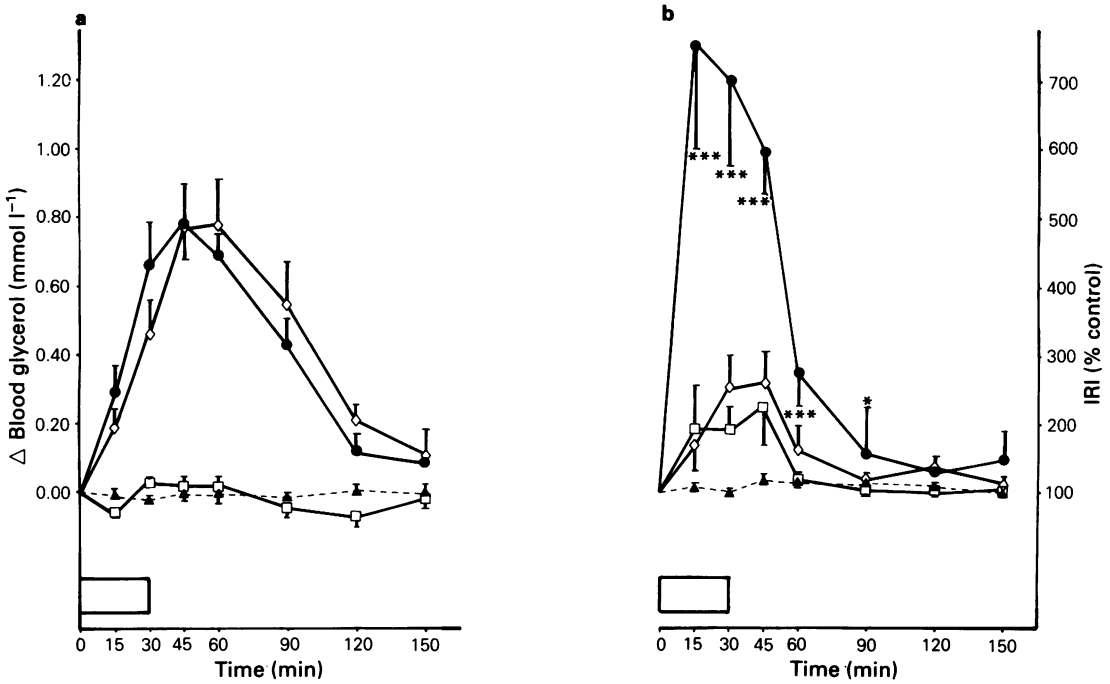

Figure 6 The effects of an i.v. infusion of saline (▲), amidephrine (Amid; $10\,\mu g\,kg^{-1}\,min^{-1}$, □), isoprenaline (Iso; $4.4\,\mu g\,kg^{-1}$, ◇) and Iso + Amid (●) on blood glycerol (a) and immunoreactive insulin (IRI) levels (b). When both agonists were simultaneously administered the insulin secretory response was clearly potentiated. $^{*}P < 0.05$; $^{***}P < 0.001$, values significantly different from saline. For more details see text and legends to Figures 1 and 3.

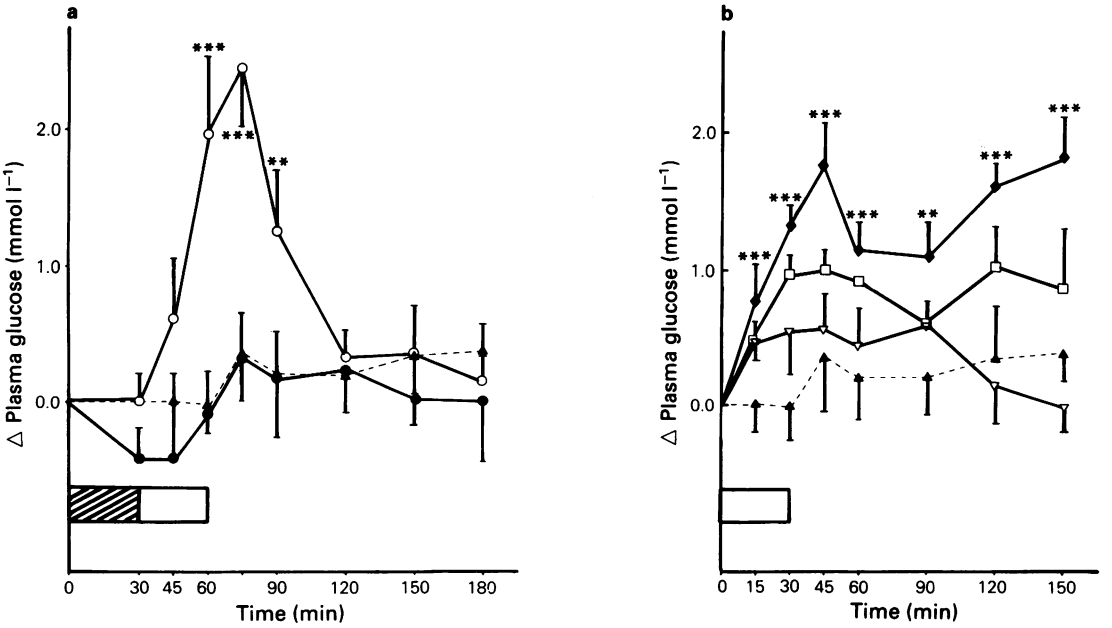

Figure 7 Changes in plasma glucose after the i.v. infusion of clonidine, $2\,\mu g\,kg^{-1}\,min^{-1}$ (a, ○), amidephrine, $10\,\mu g\,kg^{-1}\,min^{-1}$ (▽), isoprenaline, $4.4\,\mu g\,kg^{-1}\,min^{-1}$ (□), and isoprenaline plus amidephrine (◆) in (b). Blockade of clonidine-induced hyperglycaemia by previous infusion of $20\,\mu g\,kg^{-1}\,min^{-1}$ yohimbine (●, crosshatched bar) is represented (the response being significantly suppressed at 30 min, $P < 0.001$, 45 min $P < 0.001$ and 60 min $P < 0.025$) $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, values significantly different from saline (▲). For more details see text and legend to Figures 1 and 5.

As the whole animal has been used in this research, some previous considerations are required. We believe that the estimation of circulating levels of glycerol and IRI are reliable indices for lipolysis and insulin secretion, even accepting that plasma levels of both are influenced by tissue perfusion and clearance (Macdonald *et al.*, 1985). Our present studies show a lack of correlation between circulating substrate levels and the expected state of tissue perfusion. For instance in the presence of a powerful vasoconstrictor drug like Amid, the insulin secretory response to Iso was enhanced, although the β-lipolytic effect remained unaltered. (Figure 6a, b; see also Figure 3a). On the other hand, it has been confirmed that the insulin inhibitory effect of adrenoceptor agonists is not related to vasoconstriction (Hillaire-Buys *et al.*, 1985).

It is also interesting that in the present investigation changes in blood glycerol were relatively independent of the insulin secretory process. Thus, Clo simultaneously reduced basal levels of blood glycerol and IRI (Figure 1) as well as the lipolytic and insulin secretory responses induced by Iso (Figure 3; see also Figure 6) (basal or pre-infusion levels of IRI in the different groups of animals fall within the same range; 9 ± 0.53 to $13 \pm 1.62\,\mu u\,ml^{-1}$). A lack of correlation between insulin and glycerol levels was also found when the lipolytic response to Iso was compared in normal and diabetic animals (Potter *et al.*, 1977).

In conscious fasted rabbits the intravenous infusion of Clo reduced basal levels of blood glycerol and IRI (Figure 1) and increased plasma glucose (Figure 7a). A similar reduction in FFA plasma levels has been described in man (Thompson *et al.*, 1984). Also Clo produced hyperglycaemia in the dog (Humphreys & Reid, 1979) and a simultaneous inhibition of insulin secretion in the rat (Ditullio *et al.*, 1984). In man, reduced basal levels of IRI in the presence of hyperglycaemia has also been described after the administration of either Clo or guanfacine (Metz *et al.*, 1978; Brown *et al.*, 1985). Though stimulation of central α_2-adrenoceptors by Clo could explain some of these results (Humphreys & Reid 1978; Ditullio *et al.*, 1984), we have reason to believe that Clo exerts significant peripheral effects on blood glycerol, IRI and plasma glucose levels. Thus in cord-sectioned patients this α_2-agonist evoked hyperglycaemia and inhibited insulin release (Metz *et al.*, 1978). Our present findings show that PE, an agonist with affinity for α_2-adrenoceptors but with minimal central action, reproduced the inhibitory responses mediated by Clo (Figure 4). We propose therefore that the effects of Clo on blood glycerol and IRI levels are the result of a direct peripheral stimulation of inhibitory α_2-adrenoceptors located on adipocytes and the pancreatic β-cell, confirming in this way previous *in vitro* data (Lafontan & Berlan, 1980; Nakaki *et al.*, 1981; Langer *et al.*, 1983; Hillaire-Buys *et al.*, 1985).

More difficult to understand is the hyperglycaemic effect resulting from α_2-adrenoceptor stimulation considering that this receptor subtype is not directly involved in liver glycogenolysis stimulation. Glucagon release might well mediate the glycogenolytic response since feeding enhances the hyperglycaemic effect of Clo (Moratinos, unpublished observations) and guanfacine administration has been reported to increase circulating glucagon in man (Brown *et al.*, 1985). Suppression by Yoh of Clo-induced hyperglycaemia (Figure 7a) and inhibition of insulin secretion (Figure 1b) confirms as α_2 the nature of the adrenoceptors involved in the responses under investigation.

The effects of Yoh on blood glycerol requires comment. According to circulating substrate levels, rabbits could be classified into two groups (Table 1). The larger one showed no change in glycerol levels thus confirming lack of effect of Yoh, as had already been described by Moratinos *et al.* (1986). In the smaller group, Yoh did raise blood glycerol. These results could be explained by animals differing in their sensitivity to the central lipolytic effect of Yoh, with subsequent adrenaline release (Kumon *et al.*, 1976; 1977).

The intravenous infusion of rather a high dose of NA (producing a maximal hyperglycaemic effect, Potter *et al.*, 1974) induced a mild rise in blood glycerol (Figure 2a). The simultaneous increase found in IRI (Figure 2b) cannot alone explain the weak lipolytic effect since similar elevations in plasma IRI induced by NA and Iso (pre-infusion levels for both groups of animals were respectively; 9.5 ± 1.1 and $9 \pm 0.53\,\mu u\,ml^{-1}$) were accompanied by a much greater lipolytic-response to Iso (Figures 2 and 3). Smaller doses of NA which did not change basal IRI produced a minor increase in blood glycerol in spite of marked hyperglycaemia (Moratinos, unpublished observations). In fasted rabbits the lipolytic order of potency for the three catecholamines was: Iso > Ad > NA (Potter *et al.*, 1974). On the other hand when these α_2-adrenoceptors were blocked by Yoh the lipolytic response to NA was not clearly enhanced (Figure 2a). Though a clear rebound in IRI took place in the presence of the antagonist (Figure 2b), this insulin increase could not be the only explanation for the still attenuated lipolytic response (see also Figure 6). The weaker affinity of NA (as compared to Ad) for α_2-adrenoceptors and poor ability to increase circulating glycerol could thus explain our present findings.

Functional relevance for these α_2-adrenoceptors

was clearly shown when the stimulatory effects of Iso were attenuated by simultaneous administration of Clo or PE (Figures 3 and 4). A similar inhibition of Iso-induced FFA outflow from bone marrow adipose tissue in the presence of Clo has already been reported (Tran *et al.*, 1984). Also in human adipocytes, Clo reduced the lipolytic effect of theophylline (Lafontan & Berlan, 1980) whereas glucagon-mediated increase in cyclic AMP accumulation from rat pancreatic islets was prevented in the presence of Clo (Nakaki *et al.*, 1981). Since lipolysis and insulin secretion are two responses dependent on membrane adenylate cyclase activation and cyclic AMP generation, simultaneous inhibition of the same system by α_2-adrenoceptor agonists could easily explain our results (Lafontan & Berlan, 1985). However, an inhibitory step at a later stage than cyclic AMP generation cannot be ruled out since insulin release induced by β_2-adrenoceptor-mediated cyclic AMP from rat pancreatic islets was blocked by Clo (Nakaki *et al.*, 1983). Reduction of Iso-effects on blood glycerol in the presence of PE confirms former *in vitro* results with rabbit isolated adipocytes (Lafontan, 1979) and adds further support for the well-known ability of PE to stimulate α_2-adrenoceptors (McGrath, 1982). Interestingly the insulin secretory response to Iso was not so clearly attenuated by PE (Figure 4b). Probably the small PE β-component (Moratinos *et al.*, 1986) and the stimulation of excitatory α_1-adrenoceptors (see below) could counteract the inhibitory effect.

It is now well established that α_1-adrenoceptor stimulation is involved in a number of calcium-mediated responses (such as liver glycogenolysis: see Reinhart *et al.*, 1984; Williamson *et al.*, 1986 for more recent reviews; or vascular smooth muscle contraction: Cauvin & Malik, 1984). As adipose tissue lypolysis and insulin secretion are also calcium-dependent processes (Allen & Beck, 1986; Malaisse, 1984 for a general review), we considered it important to test the role of α_1-adrenoceptors on plasma glucose, lipolysis and insulin secretion in our *in vivo* model using Amid as a very selective agonist (McGrath, 1982).

The intravenous infusion of Amid did not induce significant changes in blood glycerol (Figures 4a and 6a) nor did the α_1-agonist alter the increase and the time course of Iso-induced lipolytic response (Figure 6a). These results ruled out an involvement of α_1-adrenoceptors on tissue lipolysis confirming in this way previous *in vitro* data (Lafontan & Berlan, 1985). Interestingly, Amid did not induce noticeable changes in plasma glucose but did increase IRI plasma levels (Figure 5). A lack of a patent hyperglycaemic effect is surprising since it has been clearly shown that Amid increases glucose release, and activates glycogen phosphorylase from guinea-pig and rabbit liver slices (Haylett & Jenkinson, 1972; Haylett 1976; Osborn 1978). It appears that, at the dose employed in the present work, Amid exerted a more marked and persistent effect on insulin secretion (note that the response was maintained over 45 min) than on liver glycogenolysis, thereby masking any subsequent increase in circulating glucose. Therefore the increase in IRI detected in the presence of Amid could be related to a direct drug stimulation of the β-cell. This interpretation is supported by the selective blockade observed in the presence of prazosin (Figure 5b). It is also interesting to mention in this context that the β-adrenoceptor antagonist propranolol consistently attenuated NA hyperglycaemia whereas it failed to antagonize the stimulatory effect of the agonist on IRI plasma levels (Moratinos, unpublished observations).

Recent work (Malaisse & Moratinos, 1986) has shown that Amid failed to evoke insulin secretion from rat pancreatic islets. This could be explained by species differences since Amid was also unable to induce contractile responses in rat isolated thoracic aorta (Chiu *et al.*, 1986). When Amid and Iso were infused simultaneously a remarkable increase in insulin secretion occurred (Figure 6b). Comparison of responses to single drug infusion and combined drug administration show that (a) the insulin releasing effect was clearly potentiated when both agonists were infused together, (b) the time-course of the response was altered since the peak effect established earlier in the presence of dual stimulation. Such a large rise in IRI levels could not be ascribed to parallel hyperglycaemia since maximum increase in IRI ($\Delta = 655.5 \pm 151\%$) occurred at the time of a very modest increase in plasma glucose ($\Delta = 0.77 \pm 0.26\,\text{mmol}\,l^{-1}$; Figure 7b). Certainly hyperglycaemia encountered after combined agonist infusion (Figure 7b) cannot really explain by itself the insulin secretory response (Moratinos *et al.*, 1977). If we assume that insulin secretion in the presence of α_1-adrenoceptor stimulation is a calcium-dependent process involving the calmodulin branch (as has been reported for other tissue: liver cell, Exton, 1985; Manalan & Klee, 1984) the increase in cyclic AMP found with Iso would produce a much greater insulin secretory response (Rasmussen, 1986).

The results indicate a physiological relevance for inhibitory α_2-adrenoceptors. Stimulation of these receptors should reduce the β-mediated response but even more important, would restrain potentiation derived from α_1-β interplay.

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