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α-Adrenoceptor involvement in catecholamine-induced hyperglycaemia in conscious fasted rabbits

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1 In conscious fasted rabbits an intravenous infusion of phenylephrine (20 μg kg^{-1} min^{-1}) induced hyperglycaemia. The increase in blood glucose was accompanied by a modest increase in insulin secretion and a reduction of liver glycogen. Muscle glycogen and blood lactate levels were not altered by treatment with phenylephrine.

2 Prazosin, 1 mg kg^{-1} s.c., partially attenuated phenylephrine-induced hyperglycaemia.

3 Phenoxybenzamine infusion (16.6 μg kg^{-1} min^{-1}) for 15 min suppressed the increase in blood glucose and the reduction in liver glycogen evoked by phenylephrine. This α-adrenoceptor blocker also clearly attenuated the blood glucose elevation observed on infusing adrenaline at 0.3 μg kg^{-1} min^{-1}.

4 Blockade by phenoxybenzamine of phenylephrine- and adrenaline-induced hyperglycaemia was not accompanied by a significant increase in immunoreactive insulin plasma levels.

5 Yohimbine infused at a rate of 20 μg kg^{-1} min^{-1}, also completely blocked phenylephrine-induced hyperglycaemia. This suppressor effect was accompanied by a marked rebound in insulin secretion.

6 It is concluded that in normal fasted rabbits stimulation of α-adrenoceptors induces hyperglycaemia. The increase in blood glucose depends mainly on liver glycogenolysis and inhibition of insulin secretion. Separate blockade of each component suffices to reduce α-adrenoceptor-mediated hyperglycaemia.

Introduction

It is well established that activation of β-adrenoceptors accelerates hepatic glycogenolysis (Ellis, 1980). In the last few years *in vitro* experiments have provided clear evidence for an additional involvement of α-adrenoceptors in catecholamine-induced liver glycogenolysis. The application of α-adrenoceptor agonists such as amidephrine and phenylephrine (PE) to guinea-pig and rat liver cells and liver slices induces glucose release and phosphorylase activation. The response seems to depend on an increase in the intracellular concentration of ionized calcium mediated by stimulation of α_1-adrenoceptors (see: Haylett & Jenkinson, 1972; Haylett, 1976; Hutson *et al.*, 1976; Assimacopoulos-Jeannet *et al.*, 1977; Osborne, 1978; Burgess *et al.*, 1981; Goodhardt *et al.*, 1984). Thus it is now clear that activation of either type of receptor can accelerate liver glycogenolysis (see Haylett, 1979; Exton, 1982, for reviews). Exton and his collaborators have shown that for this response the sequence of events initiated by stimulation of α- and β-receptors converges at the stage of phosphorylase b kinase (Exton, 1982).

Similarly the hyperglycaemic effect of catecholamines described in intact animals probably involves activation of both α- and β-receptors in most species studied (Nash & Smith, 1972; Al-Jibouri *et al.*, 1980; Ellis, 1980; Lum *et al.*, 1980; Nakadate *et al.*, 1980; Ditullio *et al.*, 1984). However, catecholamine-induced hyperglycaemia should be considered as a complex integrated response including increased liver and muscle glycogenolysis, increased gluconeogenesis, decreased peripheral glucose utilization (Himms-Hagen, 1967; Potter *et al.*, 1977), inhibition of insulin and stimulation of glucagon secretion (Moratinos *et al.*, 1977; Potter *et al.*, 1978; Woodson & Potter, 1979). Therefore all these several components should be kept in mind when analysing the nature of the adrenoceptors involved in the hyperglycaemic effect of catecholamines.

In conscious rabbits indirect experimental data suggest a role for α-adrenoceptors in catecholamine-induced hyperglycaemia. Thus, propranolol failed to antagonize the increase in blood glucose elicited by

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adrenaline (Ad) and noradrenaline (NA), as well as the reduction in liver glycogen evoked by Ad (Potter *et al.*, 1974; Moratinos *et al.*, 1975). Interestingly, yohimbine but not prazosin attenuated the hyperglycaemic effect of Ad in a dose-related manner (Knudtzon, 1984).

The aim of the present work was to study (a) the role of α-adrenoceptors in catecholamine-induced hyperglycaemia in conscious fasted rabbits, (b) the relative contribution of tissue glycogenolysis and insulin secretion to the overall response and, (c) the subtype of α-adrenoceptor mediating these effects.

Methods

Experimental design

Unanaesthetized New Zealand white, male rabbits, aged from 7 to 12 months (body weight between 2.8 and 3.8 kg) were previously conditioned for restraint and fasted for 24 h before the experiments. Sampling of arterial blood was accomplished by using 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 infusion. 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 patency of the arterial cannula was maintained by a slow constant infusion of physiological saline (0.07 ml min^{-1}) throughout the experiments (3.5 h). 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. The total volume of blood sampled did not exceed 15% of the animal's total blood volume.

Analyses

Plasma glucose was estimated by means of the glucose-oxidase procedure using a kit from Boehringer Mannheim Co. West Germany. Blood lactate levels were quantified enzymatically using lactic dehydrogenase and measuring the amount of NADH formed spectrophotometrically at 340 nm (Potter *et al.*, 1977).

Immunoreactive insulin (IRI) was determined by using a CEA-SORIN radioimmunoassay kit. Human insulin was employed as a standard. (International CIS, F-78181 St. Quentin).

Tissue glycogen

Liver and muscle samples for glycogen analysis were removed under pentobarbitone anaesthesia

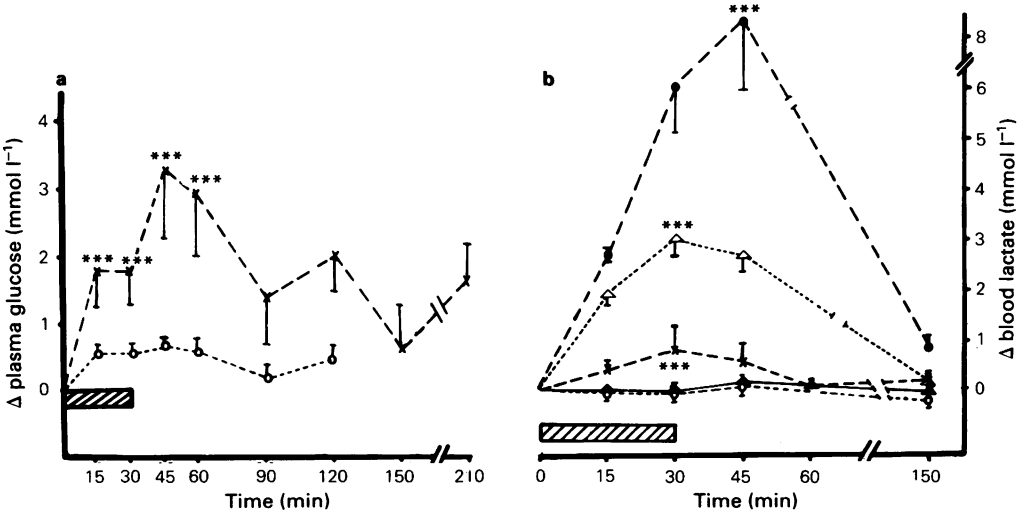

Figure 1 (a) The hyperglycaemic effects of phenylephrine in fasted rabbits and (b) the effects of four sympathomimetic amines on blood lactate. (Equiactive hyperglycaemic doses of these drugs were employed). Drugs were infused at a constant rate (0.2 ml min^{-1}) over a period of 30 min as shown by the cross-hatched horizontal bar. Ordinate scales: Δ mmol l^{-1} plasma glucose and blood lactate refer to the variations from control values. Mean responses (from at least 6 rabbits) after infusion of saline (○), noradrenaline 1 μg kg^{-1} min^{-1} (▲), adrenaline 0.3 μg kg^{-1} min^{-1} (△), isoprenaline 30 μg kg^{-1} min^{-1} (●) and phenylephrine 20 μg kg^{-1} min^{-1} (X) are shown. Vertical lines indicate s.e.mean. ***$P < 0.001$, values significantly different from those seen on infusing physiological saline.

(30 mgkg^{-1}, i.v.) just at the end of drug treatment. The upper left lobe of the liver and the left gastrocnemius muscle were quickly excised and placed in ice-cold saline. The tissues were then prepared in a cold room at a temperature below 5°C; a centre strip of the left lobe of the liver and a piece of gastrocnemius muscle were dissected out. The samples were weighed on a torsion balance (weight of individual tissue samples ranged between 500–700 mg) and then dropped into cold 30% KOH for digestion. The time for the entire process from the removal of the tissue to dropping them into 30% KOH was between 5 and 6 min. Glycogen was isolated and hydrolyzed according to the method of Good *et al.* (1933) and the derived glucose analysed by the glucose-oxidase procedure.

Drugs

Fresh stock solutions of (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, (−)-isoprenaline hydrochloride and (−)-phenylephrine hydrochloride (obtained from Sigma, London) were prepared daily. Catecholamine solutions were made in acidified saline, (pH 4.5). Appropriate dilutions were made in saline just before infusion.

α-Adrenoceptor blocking agents were administered immediately after withdrawal of the second control sample.

Phentolamine mesylate (Ciba), 5 mg kg^{-1} was given as a single s.c. injection (priming dose) of 3.5 mg kg^{-1} followed half an hour later by a continuous intravenous infusion of 50 μg kg^{-1} min^{-1}, for 30 min.

Phenoxybenzamine hydrochloride (a gift from Smith-Kline) was diluted in acidified saline (pH 4) containing 1.5% ethyl alcohol. A total dose of 0.25 mg kg^{-1} was slowly infused (0.05 ml min^{-1}) over a 15 min period; 50 min later the appropriate adrenoceptor agonist was administered. Prazosin hydrochloride (Pfizer) was either injected s.c. or infused i.v., when injected, 1 mg kg^{-1} of this α_1-selective antagonist was administered 30 min before the agonist. Otherwise doses ranging from 0.5 to 10 μg kg^{-1} min^{-1} were infused for 30 min at a constant rate of 0.2 ml min^{-1} followed thereafter by the agonist. Yohimbine hydrochloride (Houde) was diluted in saline containing 1.5% ethyl alcohol: 20 μg kg^{-1} min^{-1} of this α_2-selective antagonist were infused for 30 min just before beginning agonist administration. All drug concentrations are expressed in terms of the free base. Changes in blood glucose, lactate, tissue glycogen and plasma IRI were analysed by analysis of variance. As pre-infusion (control) levels of plasma IRI in fasted rabbits exhibited some degree of variation (Moratinos *et al.*, 1977) changes in this parameter were expressed as a % of mean control.

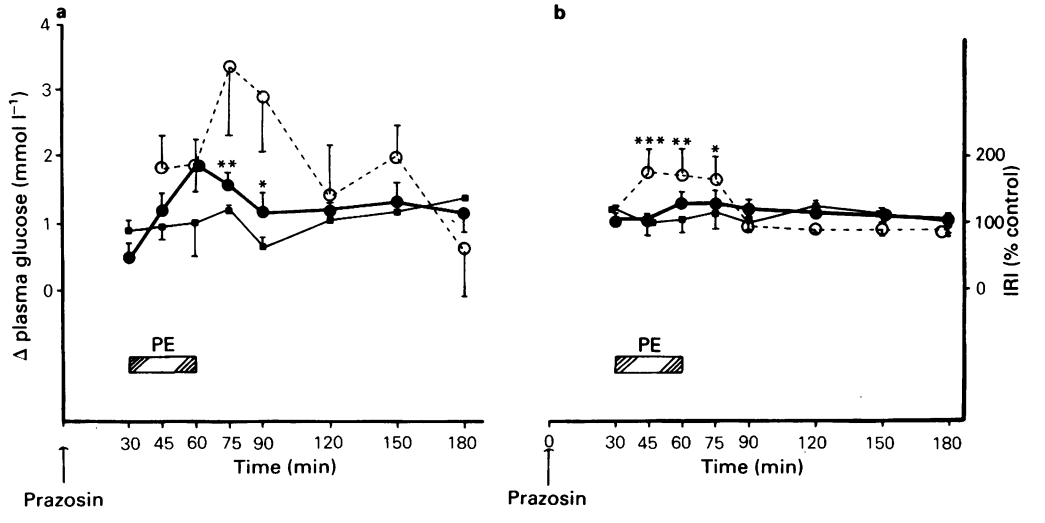

Figure 2 The effects of phenylephrine (PE; 20 μg kg^{-1} min^{-1}) on plasma glucose (a) and immunoreactive insulin levels (IRI, b) in the absence (○) and presence (●) of prazosin (1 mg kg^{-1}). The effects of prazosin (1 mg kg^{-1}) by itself on both parameters are also presented (■). At the arrow prazosin was subcutaneously injected. Thirty minutes later, a blood sample was removed and the saline or phenylephrine infusion started. Values of IRI levels are expressed as a % change from the control level (control level = 100%). Absolute values in μu ml^{-1} are given in the text. *$P<0.05$, **$P<0.01$, ***$P<0.001$, values significantly different from saline (b)- or phenylephrine (a)-treated animals. For more details see text and legend to Figure 1.

Results

Effects of phenylephrine on plasma glucose, insulin and lactate levels

The i.v. infusion of 10 $\mu g\,kg^{-1}$ of the α-adrenoceptor agonist phenylephrine (PE) produced only small increases in plasma glucose (0.53 ± 0.23 and $0.80 \pm 0.38\,mmol\,l^{-1}$ at 15 and 30 min respectively) which were not significantly different from values found in saline treated animals (0.55 ± 0.10, and $0.59 \pm 0.12\,mmol\,l^{-1}$). When the infusion rate was doubled to 20 $\mu g\,kg^{-1}\,min^{-1}$, PE induced a clear hyperglycaemic response (Figure 1); the peak of the mean increase in blood sugar appearing at 45 min was $3.3 \pm 1.1\,mmol\,l^{-1}$ ($n = 7$), $P < 0.001$, in the presence of PE as compared with $0.65 \pm 0.13\,mmol\,l^{-1}$ ($n = 7$) with saline controls (the average pre-infusion arterial glucose value in saline-treated rabbits was $4.6 \pm 0.45\,mmol\,l^{-1}$).

PE at a dose that produced a clear hyperglycaemia induced a small and transient, but significant, increase in blood lactate (at the end of the infusion = $0.81 \pm 0.49\,mmol\,l^{-1}$, $P < 0.001$, versus 0.01 ± 0.11, $n = 7$ after saline). Figure 1 also shows the effects of isoprenaline (Iso), adrenaline and noradrenaline, on blood lactate levels in fasted animals. Equiactive hyperglycaemic doses, (in relation to PE) of these three catecholamines were employed in this experiment. As expected, the greatest response was observed with Iso ($\Delta = 8.30 \pm 1.28\,mmol\,l^{-1}$) followed by Ad ($\Delta = 2.96 \pm 0.25\,mmol\,l^{-1}$). NA failed to modify lactate levels. Pre-infusion (saline- or drug-treated) values for blood lactate ranged between 0.90 and $1.2\,mmol\,l^{-1}$. These values are in agreement with previous results already published (Potter *et al.*, 1977).

In the presence of 20 $\mu g\,kg^{-1}\,min^{-1}$ PE, immunoreactive insulin plasma levels (IRI) were significantly elevated above basal values (Figure 2b). A moderate increase appeared within 15 min and remained for another 30 min (Δ at 30 min $71 \pm 40\%$, $n = 7$, compared with $-8 \pm 20\%$, $n = 7$, with saline; $P < 0.01$). Absolute values for IRI plasma levels were: $24.6 \pm 6.6\,\mu u\,ml^{-1}$ and $44 \pm 22\,\mu u\,ml^{-1}$ (pre-infusion and 30 min after the PE infusion, respectively) versus $28.5 \pm 5.4\,\mu u\,ml^{-1}$ and $26.2 \pm 7.5\,\mu u\,ml^{-1}$ (in saline-treated rabbits).

Effects of phenylephrine in the presence of α-adrenoceptor blocking drugs

Bearing in mind that phenylephrine is an α-adrenoceptor agonist with affinity for α_1- and α_2-adrenoceptors (McGrath, 1982) it was thought necessary to investigate the role of both types of α-receptors in the metabolic responses induced by PE. Therefore the effects of this agonist on blood glucose and plasma insulin were studied again in animals pretreated with selective and non-selective adrenoceptor antagonists.

Prazosin This drug was chosen as an α_1-selective adrenoceptor antagonist. In the rabbit it was found

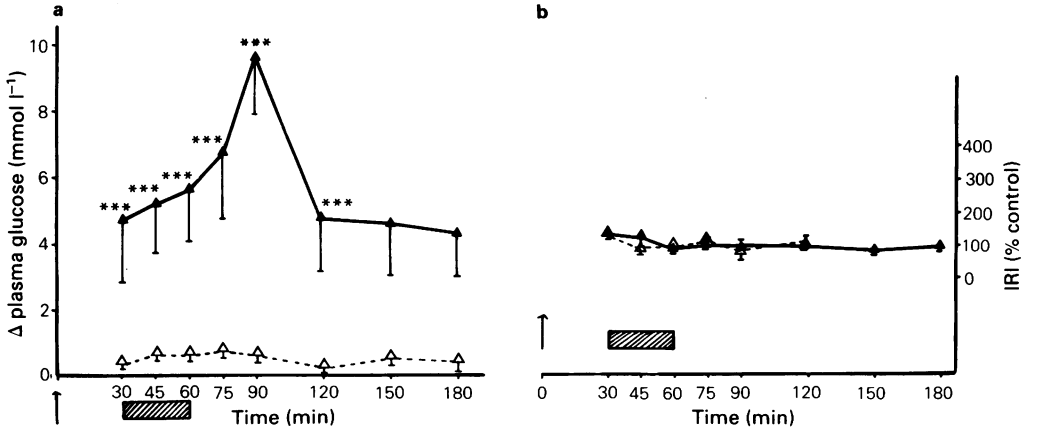

Figure 3 The effects of phentolamine (▲) on (a) plasma glucose and (b) immunoreactive insulin (IRI) plasma levels in fasted rabbits. Phentolamine, 5 mg kg^{-1} total dose was administered as follows: 3.5 mg kg^{-1} at the arrow, followed half an hour later by a continuous i.v. infusion of 50 $\mu g\,kg^{-1}\,min^{-1}$ for an additional 30 min (cross-hatched bar). Just before drug infusion a blood sample was removed. ***$P < 0.001$ values significantly different from saline (Δ). For more detail see legends to Figures 1 and 2.

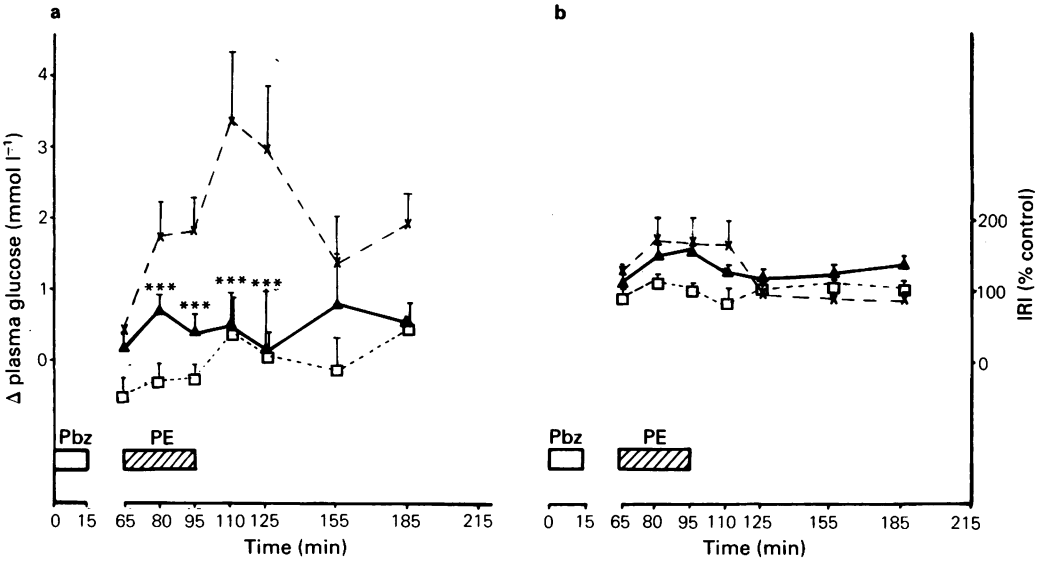

Figure 4 The effects of α-adrenoreceptor blockade with phenoxybenzamine (Pbz) on phenylephrine-induced hyperglycaemia (a) and changes in immunoreactive insulin (IRI) levels (b). The effects of Pbz alone (□) on both parameters are also shown. A total dose of 0.25 mg kg^{-1} Pbz was slowly infused at a rate of 0.05 ml min^{-1} for 15 min (open bar). Fifty minutes later a blood sample was removed and then saline or phenylephrine (PE) infusion begun (cross-hatched bar). The increase in blood glucose induced by PE was completely blocked, in the absence of any rebound in IRI. Mean responses to phenylephrine (20 μg kg^{-1} min^{-1}) in the absence (X) and presence (▲) of Pbz are shown. For more details see legends to Figures 1 and 2.

that 1 mg kg^{-1} prazosin exerted a very good degree of postsynaptic α_1-adrenoceptor blockade (Hamilton *et al.*, 1982). However, 1 mg kg^{-1} prazosin, when injected s.c. 30 min before the agonist, attenuated the effect of PE on blood glucose (Figure 2a). Thus the increase in glucose induced by PE was 3.3 ± 1.1 mmol l^{-1} ($n = 7$) as compared to 1.58 ± 0.23 mmol l^{-1} ($n = 7$), $P < 0.01$, in animals previously treated with the antagonist. Prazosin itself induced small fluctuations in plasma glucose which were only significant at 45 min (Δ 1.1 ± 0.1 mmol l^{-1}, $P < 0.001$).

Since prazosin at the dose tested in this study could certainly elevate circulating levels of plasma NA (Hamilton *et al.*, 1982), which in turn might interfere with the response to be blocked, smaller doses of this α_1-selective antagonist were also examined. Again an i.v. infusion of prazosin at 0.5 to 10 μg kg^{-1} min^{-1} administered for 30 min just before PE, reduced the hyperglycaemic effect of this agonist in much the same way as did the larger dose (data not presented). These smaller doses of prazosin should not induce any appreciable increase in NA plasma levels (Hamilton *et al.*, 1982).

Prazosin tends to reduce the effects of PE on IRI plasma levels (Figure 2b) though a significant decrease was only observed at 15 min (Δ = 5.25 ± 5%, $n = 6$, $P < 0.01$). Values of IRI plasma levels in the presence of 1 mg kg^{-1} prazosin did not differ from those in saline control rabbits (Figures 2 and 3).

Phentolamine In cats and mice phentolamine antagonized Ad-induced hyperglycaemia (Al-Jibouri *et al.*, 1980; Nakadate *et al.*, 1980). It was interesting to check whether this antagonist, with affinity for both α_1- and α_2-adrenoceptors, could also interfere with the increase in blood glucose evoked by PE in the rabbit.

Unexpectedly, phentolamine provoked a very marked and long-lasting hyperglycaemia (Figure 3) reaching a peak at 30 min after the end of phentolamine infusion (Δ = 9.57 ± 1.72 mmol l^{-1}, $n = 6$, $P < 0.001$, versus 0.21 ± 0.23 mmol l^{-1}, $n = 7$, with saline). Two hours after the priming dose of the blocker blood glucose levels were still significantly elevated (Δ = 4.74 ± 1.63 mmol l^{-1}, $n = 6$, $P < 0.001$, versus 0.51 ± 0.23 mmol l^{-1}, $n = 7$). Insulin secretion was inhibited throughout the experiment since in the presence of such a severe hyperglycaemia IRI plasma levels fluctuated within the same range in both phentolamine- and saline-treated rabbits (Figure 3b).

In view of this intrinsic effect exhibited by phentolamine, smaller doses of the antagonist were tried. Even 1 mg kg^{-1} s.c. caused hyperglycaemia (blood

glucose rose 2.2 mmol l^{-1} above basal values for almost 3 h mean response in two animals). Finally in two other rabbits phentolamine was slowly infused at a very low dose, 15 $\mu g\,kg^{-1}\,min^{-1}$ for 30 min. The same degree of increase in blood sugar was again seen (data not presented).

The unexpected behaviour of phentolamine in the rabbit prompted us to study the action of other α-adrenoceptor blocking drugs.

Phenoxybenzamine When slowly infused (16.6 $\mu g\,kg^{-1}\,min^{-1}$) over a 15 min period, Pbz (total dose 0.25 mg kg^{-1}) evoked small fluctuations in blood glucose and IRI plasma levels (Figure 4). Changes in arterial glucose which varied between -0.48 ± 0.26 and 0.45 ± 0.40 mmol l^{-1} throughout the experiment were significantly different from saline controls at 0, 15 and 30 min ($P < 0.001$). Changes in plasma IRI levels remained within -18.41 ± 20 and $15.71 \pm 0.8\%$ (the pre-infusion level of IRI expressed in absolute values was 8 ± 0.2 $\mu u\,ml^{-1}$ and changes in this parameter after drug infusion varied between 6.6 ± 0.7 and 9.2 ± 1 $\mu u\,ml^{-1}$, respectively).

In cats Pbz markedly reduced PE hyperglycaemia, but did not modify the response to Ad (Kuo *et al.*, 1977). In mice Pbz also fails to antagonize Ad-induced hyperglycaemia (Nakadate *et al.*, 1980). Therefore, it was most interesting to test the effect of Pbz against equiactive hyperglycaemic doses of PE and Ad.

When given 50 min before PE the hyperglycaemia was completely suppressed (Figure 4) (PE peak effects at 45 min in the absence and presence of Pbz were respectively 3.34 ± 1.07 mmol l^{-1}, $n = 7$, versus 0.50 ± 0.40 mmol l^{-1}, $n = 12$, $P < 0.001$). Pbz also clearly attenuated the increase in blood glucose elicited by Ad (Figure 5). The maximum increase of 4.3 ± 1 mmol l^{-1}, $n = 5$, found at the end of the agonist infusion was reduced to 2.35 ± 0.25 mmol l^{-1}, $n = 9$, ($P < 0.001$) in those rabbits previously treated with Pbz. The response to Ad was partially but significantly lowered for an additional 30 min, though a residual hyperglycaemia was still observed in the presence of the α-antagonist. It is interesting to note that the pattern of IRI plasma levels found after PE was not altered by Pbz (Figure 4b).

Insulin secretion was similarly inhibited in the presence of Ad (Figure 5). (Modest increases detected with the agonist lacked statistical significance; at 30 min, $61.5 \pm 54\%$, $n = 5$, with Ad versus $-8 \pm 20\%$, $n = 7$, with saline, $P < 0.1$; absolute values at those times for both experimental designs were: 32.5 ± 4.5 $\mu u\,ml^{-1}$ with Ad versus 25.2 ± 7.5 $\mu u\,ml^{-1}$ with saline). The inhibitory response to Ad remained unchanged after treatment with the α-blocker (Figure 5).

Yohimbine When this selective α_2-adrenoceptor antagonist was infused intravenously at a rate of 20 $\mu g\,kg^{-1}\,min^{-1}$, for 30 min (Figure 6), changes in arterial glucose fluctuated between -0.37 ± 0.30 and 0.35 ± 0.47 mmol l^{-1}, ($n = 5$) throughout the ex-

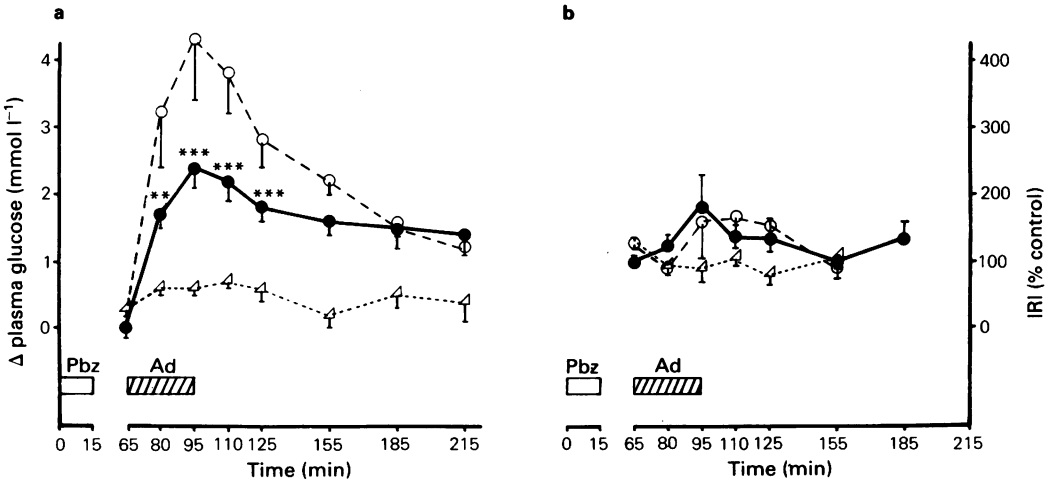

Figure 5 Effects of adrenaline (Ad, 0.3 $\mu g\,kg^{-1}\,min^{-1}$) on plasma glucose (a) and immunoreactive insulin (IRI) plasma levels (b) in the absence (○) and presence (●) of phenoxybenzamine (Pbz, 0.25 mg kg^{-1}). The effects of infusions of saline (△) on both parameters are also shown. The experimental designed was as described in Figure 4 with Ad replacing phenylephrine. Ad-induced hyperglycaemia was significantly reduced ($P < 0.001$) in the presence of Pbz. No insulin secretory response was evident. For more detail see legend to Figure 2.

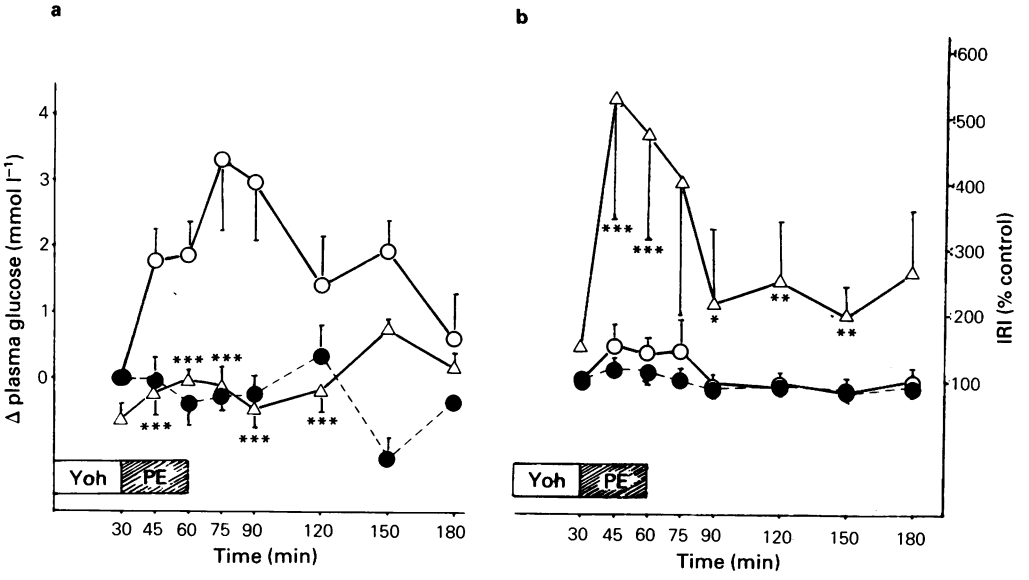

Figure 6 Effects of phenylephrine (PE, $20\,\mu g\,kg^{-1}\,min^{-1}$) on plasma glucose (a) and immunoreactive insulin (IRI) plasma levels (b) in the absence (○) and presence (△) of the α_2-adrenoceptor antagonist yohimbine (Yoh, $20\,\mu g\,kg^{-1}\,min^{-1}$). The open bar represents a 30 min infusion with yohimbine, immediately followed by a 30 min infusion of either saline (●) or PE (△ cross-hatched bar). In the presence of yohimbine, PE-induced hyperglycaemia was suppressed in the face of a severe increase in IRI levels, $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

periment. These changes were significant at 30, 45 and 60 min ($P<0.001$). Changes in IRI plasma levels varied between $-11.7 \pm 14.7\%$ and $20.7 \pm 10.1\%$ ($n = 10$). When the antagonist was infused just before PE, the hyperglycaemic response evoked was completely suppressed (Figure 6). (PE peak effect at 45 min in the absence and presence of yohimbine was respectively $3.34 \pm 1.07\,mmol\,l^{-1}$, $n = 7$, versus $-0.11 \pm 0.33\,mmol\,l^{-1}$, $n = 6$; $P<0.001$). This effect on blood glucose was accompanied by a pronounced rebound in insulin secretion (Figure 6).

In the presence of yohimbine, PE produced a very marked elevation in IRI plasma levels. Maximum responses were observed 15 min after the start of the agonist infusion, and at its finish (responses to PE at 15 and 30 min were: $428 \pm 183\%$ and $373 \pm 170\%$, $n = 5$; $P<0.001$) although a residual increase persisted through to the end of the experiment.

Effects of phenylephrine on liver and muscle glycogen

In an attempt to understand the origin of PE-induced hyperglycaemia, changes in the glycogen content of liver and muscle were studied in fasted rabbits infused with the α-adrenoceptor agonist. The rabbits were anaesthetized with pentobarbitone just at the end of drug administration, and blood as well as tissue samples were then quickly removed for analyses.

At the end of the 30 min i.v. infusion of $20\,\mu g\,kg^{-1}\,min^{-1}$ PE, liver glycogen was significantly reduced from $5.88 \pm 0.96\,mg\,kg^{-1}$ ($n = 6$, saline control) to $3.17 \pm 0.76\,mg\,g^{-1}$ wet weight tissue, $n = 4$; $P<0.001$ (Figure 7). Muscle glycogen did not change ($4.51 \pm 0.52\,mg\,g^{-1}$ wet weight tissue, $n = 10$, after PE, versus $5 \pm 0.7\,mg\,g^{-1}$ after saline). Blood glucose increased by $2.2 \pm 0.45\,mmol\,l^{-1}$.

Pbz alone did not significantly affect the blood glucose concentration or tissue glycogen content. However when slowly infused 50 min before PE it completely antagonized the effects of this agonist on liver glycogen and blood glucose. At the end of PE administration, liver glycogen was $6.6 \pm 1.2\,mg\,g^{-1}$ wet weight tissue, $n = 7$, similar therefore to the value found in saline control animals. Muscle glycogen content remained unchanged.

Discussion

The i.v. infusion of PE, an α-adrenoceptor agonist, induces hyperglycaemia in normal fasted rabbits (Figure 1). At the dose employed in this work the response evoked by PE was very similar both in magnitude and time course to that seen in fasted rabbits after an i.v. infusion of either Ad (Figure 5) or NA, (Potter *et al.*, 1974; Moratinos *et al.*, 1975).

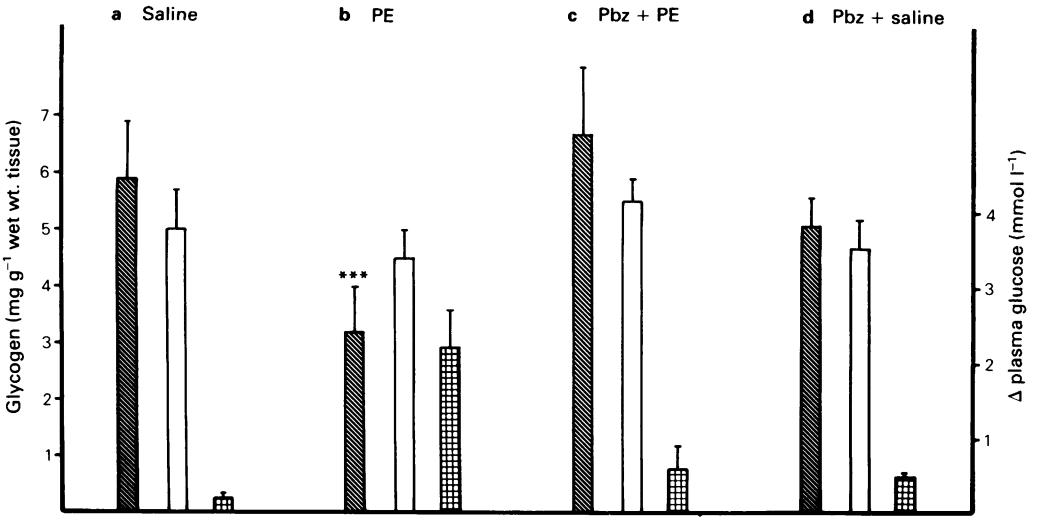

Figure 7 The effects of (b) phenylephrine (PE, $20\,\mu g\,kg^{-1}$), (c) PE in the presence of phenoxybenzamine (Pbz, $0.25\,mg\,kg^{-1}$), and (d) Pbz by itself on tissue glycogen levels in the liver (hatched columns) and muscle (open columns) and blood glucose levels (cross-hatched columns) of fasted rabbits. Tissue and blood samples were taken at the end of drug treatment (for more details see Methods). Each column and vertical line represents the mean and s.e.mean of results from at least six animals. ***$P<0.001$, values significantly different from controls, infusion of physiological saline $1\,ml\,kg^{-1}$ (a).

However, PE was less potent than Ad or NA (the order was Ad > NA > PE) and the peak effect occurred later. A similar hyperglycaemic effect of PE has also been described in dogs (Privitera & Rosenblum, 1966) and cats (Kuo *et al.*, 1977). PE effects on blood glucose were accompanied by small elevations in IRI plasma levels (Figure 2). As a similar increase in blood glucose should elicit by itself a greater insulin secretory response (Moratinos *et al.*, 1977) we propose that PE, like Ad and NA, is able to inhibit insulin release in the presence of patent hyperglycaemia. This inhibitory effect (perhaps mediated by stimulation of α_2-adrenoceptors – see Nakaki *et al.*, 1981) should contribute to the overall hyperglycaemic action of PE. Our *in vivo* results are confirmed by results from *in vitro* experiments which show that PE has an inhibitory effect on glucose-stimulated insulin release in the isolated perfused pancreas of the rat (Hillaire-Buys *et al.*, 1985) as well as the rat and mouse pancreatic islets (Nakaki *et al.*, 1981; Ismail *et al.*, 1983). Again PE was less potent than Ad and NA (Moratinos *et al.*, 1977).

Interestingly, in fasted rabbits stimulation of α-adrenoceptors also induced activation of liver glycogenolysis. At the end of the PE infusion liver glycogen stores had been notably reduced (Figure 7). These *in vivo* results agree with previous experimental data showing an increase in liver glycogen phosphorylase activity as well as glucose release, from an isolated perfused liver preparation of the cat, in response to PE (Kuo *et al.*, 1977; Lum *et al.*, 1980). Thus, experiments with whole animals support current ideas on the involvement of α-adrenoceptors in liver glycogenolysis derived from *in vitro* work using rat, guinea-pig, and rabbit isolated liver cells and/or liver slices (Haylett & Jenkinson, 1972; Haylett, 1976; Hutson *et al.*, 1976; Ven de Verve *et al.*, 1977; Blackmore *et al.*, 1978; Osborn, 1978; Aggerbeck *et al.*, 1980a,b; Althaus-Salzmann *et al.*, 1980).

It was thought that the hypoxia due to α-adrenergically-induced vasoconstriction, and hence diminished hepatic blood flow, could stimulate glycogenolysis (Levine, 1965). However, in our experiments ischaemia cannot entirely explain the activation of phosphorylase (phosphorylase activation being detected in isolated cells – see above references). Besides, ischaemia appears to cause glycogenolysis for disposal of glucose phosphates within the liver cell, rather than for release as glucose to blood (Hems *et al.*, 1976).

The increase in blood glucose evoked by PE could reflect increased glycogenolysis and/or increased gluconeogenesis. However, the latter is unlikely to be the cause of the hyperglycaemic response to PE in the intact rabbit since PE neither reduced muscle glycogen content (Figure 7), nor induced appreciable increases in blood lactate (Figure 1) or glycerol levels (Moratinos, unpublished observations). On the other

hand experiments carried out in fasted rabbits as well as in hepatocytes from 48 h fasted rabbits have shown that gluconeogenesis activation seems to be more dependent on β- than α-receptor stimulation (Potter *et al.*, 1977; Yorek *et al.*, 1980; see also Sacca *et al.*, 1983; Cherrington *et al.*, 1984). Finally a direct inhibitory effect of PE on peripheral glucose uptake should contribute to the overall response (Rizza *et al.*, 1980).

In fasted rabbits pretreatment with prazosin attenuated PE effects on blood glucose but the rise in IRI plasma levels was transiently inhibited (Figure 2). There is no clear explanation for the failure of prazosin to produce a complete block, since the drug behaves as a very potent antagonist of Ad-induced activation of glycogen phosphorylase in rat isolated hepatocytes (Aggerbeck *et al.*, 1980a,b). Also prazosin at a dose of 1 mg kg^{-1} should certainly block postsynaptic α_1-adrenoceptors in the rabbit, though this blockade would have been accompanied by a simultaneous increase in circulating NA plasma levels (Hamilton *et al.*, 1982). This increase could in theory interfere with the response to be blocked. However, when smaller doses of the α_1-antagonist, which are unable to elevate circulating NA (Hamilton *et al.*, 1982), were used, PE-induced hyperglycaemia was blocked to the same extent as with the larger dose (Moratinos, unpublished observations). Similarly in mice and female rabbits prazosin was unable to antagonize Ad-induced hyperglycaemia (Nakadate *et al.*, 1980; Knudtzon, 1984). Hence, at the present time the discrepancies between *in vitro* and *in vivo* data can only be explained in terms of species differences, perhaps reflecting variations in the inactivation of prazosin by the liver.

In contrast to prazosin, Pbz, an antagonist of both α_1- and α_2-adrenoceptors, suppressed PE-induced hyperglycaemia and liver glycogenolysis (Figures 4 and 7) as well as significantly reducing the effect of Ad on blood glucose (Figure 5). It is interesting that these effects were seen in the absence of any alteration in the pattern of IRI plasma levels evoked by either agonist (Figure 4 and 5), suggesting that the reduction in the hyperglycaemic response was a direct consequence of a blocking action of Pbz on the liver cells. PE-induced hyperglycaemia has also been found to be blocked by Pbz in the intact cat. In the same species Pbz antagonized glucose release induced by PE in an isolated liver perfusion preparation (Kuo *et al.*, 1977; Lum *et al.*, 1978; Aggerbeck *et al.*, 1980a,b). Could the blocking effects of Pbz be explained on the basis of its affinity for both α_1- and α_2-adrenoceptors? Although clonidine, an α_2-selective adrenoceptor agonist, has been shown to increase blood glucose in several species (Metz *et al.*, 1977, Ditullio *et al.*, 1984), Pbz was unable to antagonize clonidine-induced hyperglycaemia in fasted rabbits (Moratinos, unpublished observations). Therefore, it is reasonable to exclude an involvement of α_2-adrenoceptors in the blocking action of Pbz, which in any case preferentially blocks α_1-adrenoceptors (Dubocovich & Langer, 1974; Hamilton *et al.*, 1983).

As already mentioned Pbz clearly reduced Ad-induced hyperglycaemia. This is of importance since in the rabbit β-adrenoceptor blockade with propranolol failed to antagonize the increase in blood glucose caused by either NA or Ad (Potter *et al.*, 1974; Moratinos *et al.*, 1975) nor did it prevent the reduction in liver glycogen produced by Ad. Taken together, these experiments point towards a greater participation of α_1-adrenoceptors in liver glycogenolysis and a β-adrenoceptor involvement in gluconeogenic substrate mobilization.

The slow intravenous infusion of yohimbine slightly lowered blood glucose though no significant changes in basal IRI were detected (Figure 6). In rats, both an increase (Ahren *et al.*, 1984) and no change (Ditullio *et al.*, 1984) in insulin plasma levels have been described. In the rabbit, the failure of yohimbine to modify appreciably either parameter tends to discount any important role for endogenous circulating catecholamines (which can be expected to be somewhat increased after blockade of α_2-presynaptic receptors).

The hyperglycaemic response to PE was completely blocked by yohimbine (Figure 6), and this blockade was accompanied by a severe and maintained rebound in IRI plasma levels (Figure 6). Our own results and those from *in vitro* experiments, do not support a role for α_2-adrenoceptors in liver glycogenolysis (Aggerbeck *et al.*, 1980a,b; Kunos, 1984). Thus, the inhibition by yohimbine of PE-induced hyperglycaemia may not have been the result of an antagonism at the hepatic level but rather a consequence of the occupancy of pancreatic α_2-inhibitory adrenoceptors. The subsequent increase in insulin secretion should counteract liver glycogenolysis (Van de Werve *et al.*, 1977). In mice and female rabbits yohimbine similarly inhibited Ad-induced hyperglycaemia with a concomitant increase in plasma insulin (Nakadate *et al.*, 1980; Knudtzon, 1984). Also, in rats and mice isolated pancreatic islets yohimbine was effective in antagonizing the inhibitory effect of Ad on insulin secretion (Nakaki *et al.*, 1981; Ismail *et al.*, 1983).

The rebound in insulin secretion found in our experiments could partially be explained by a β-adrenoceptor-mediated effect of PE. However, PE neither activated muscle glycogenolysis (only a tiny and transient increase in blood lactate was observed) nor increased blood glycerol (unpublished observations), nor was the insulin secretory response seen in the presence of Pbz (Figure 4) or phentolamine (Moratinos, unpublished observations). On the other hand activation of pancreatic α_1-adrenoceptors by PE could be a contributory factor responsible for insulin release. (Experiments in progress in our laboratory add further support to this theory, since the very

selective α_1-adrenoceptor agonist, amidephrine, also increases IRI plasma levels in fasted animals).

More difficult to understand is the complex behaviour of phentolamine, since this α-adrenoceptor blocker evoked in the rabbit a dose-dependent hyperglycaemia and an inhibition in insulin secretion (Figure 3). The anomalous effects seen with phentolamine could perhaps be explained in terms of several experimental findings: phentolamine may increase circulating levels of endogeneous catecholamines and these raised levels of catecholamines could increase plasma cyclic AMP (Kunitade & Ui, 1978) and plasma glucagon (Miller & Horton, 1979). In any case phentolamine has been recently described as an unexpected agonist in the rabbit (Angus & Lew, 1984) making this drug unsuitable as an α-adrenoceptor antagonist in this species.

The role of glucagon on Ad-induced hyperglycaemia is not yet well defined. At least in rabbits plasma glucagon levels were only increased when larger doses of Ad than those infused in our experiments were administered (Potter *et al.*, 1978; Knudtzon, 1984). Whereas other data have suggested that glucagon secretion seems to be mediated by β- and perhaps α_2-adrenoceptors (Samols & Weir, 1979; Knudtzon, 1984). As plasma glucagon levels were not measured in the present work we cannot negate its influence on the overall response. However, in the light of our results the contribution of glucagon to α_1-adrenoceptor-mediated effects does seem to be negligible.

We conclude that α-adrenoceptors are involved in the hyperglycaemic action of catecholamines in fasted rabbits. This α-adrenoceptor-mediated blood glucose increase is thought to be mainly the result of liver glycogenolysis stimulation via α_1-adrenoceptors and inhibition of insulin secretion dependent on α_2-adrenoceptor activation. Hence, selective adrenoceptor blockade at hepatic (Pbz) or pancreatic (yohimbine) sites reduces the response.

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