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BLOCKADE OF ADRENALINE-INDUCED HYPERGLYCAEMIA IN THE ANAESTHETIZED CAT BY CONTINUOUS INFUSION OF PHENTOLAMINE AND PROPRANOLOL

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1 The effects of adrenoceptor blocking drugs on the metabolic responses to adrenaline infusion (1 μg kg^{-1} min^{-1}) have been studied in the anaesthetized, fasted cat.

2 Propranolol, in doses (0.25 or 1 mg/kg) which prevented completely adrenaline-induced tachycardia, reduced but did not abolish adrenaline-induced hyperglycaemia.

3 Phentolamine infusion, at a rate (15 μg kg^{-1} min^{-1} after a priming dose of 2.5 mg/kg) which reversed the pressor effect of adrenaline, reduced but did not abolish adrenaline-induced hyperglycaemia.

4 The continuous infusion of a combination of phentolamine (15 μg kg^{-1} min^{-1} after a priming dose of 2.5 mg/kg) and propranolol (5 μg kg^{-1} min^{-1} after a priming dose of 0.25 mg/kg) prevented completely the hyperglycaemia response to adrenaline infusion over a 6 h period.

5 The increase in blood lactate concentration produced by adrenaline was prevented completely by the combined infusion of propranolol and phentolamine but was not modified by phentolamine alone.

Introduction

There are many but conflicting reports concerning the type of adrenoceptor mediating the hyperglycaemic response to adrenaline. For example, β-adrenoceptor blocking drugs have been found to be effective in reducing adrenaline hyperglycaemia by some workers (Antonis, Clark, Hodge, Molony & Pilkington, 1967; Lundquist, 1972a; Nash & Smith, 1972; Shikama & Ui, 1975) but not by others (Mennear, Spratto & Miya, 1971; Ablad, Borjesson, Carlsson & Johnsson, 1975; Potter, Moratinos & Ellis, 1977). On the other hand α-adrenoceptor blocking drugs may also reduce the hyperglycaemic effect of adrenaline (Lundquist, 1972b; Shikama & Ui, 1975; Torella, Guigliano, Improta, Giordano, Grazioli & D'Onofrio, 1977). Some studies have shown that combined α- and β-adrenoceptor blockade is necessary for the complete abolition of the response (Antonis *et al.*, 1967; Nash & Smith, 1972; Shikama & Ui, 1975).

Adrenaline-induced hyperglycaemia is a complex response and is the result of several different effects including increased glycogenolysis, increased gluconeogenesis and decreased peripheral glucose utilization (Himms-Hagen, 1967). These effects are brought about partly through direct actions of adrenaline and partly indirectly through impaired insulin release and increased glucagon secretion (Himms-Hagen, 1967; Porte, 1967; Gerich, Lorenzi, Tsalikian & Karam, 1976). The relative contribution of each of these components may differ in different species and under different nutritional conditions (Hornbrook, 1970) thus accounting, at least partly, for the conflicting reports referred to above. In order to examine the possible role of released catecholamines in mediating endotoxin-induced changes in blood glucose, we needed a method for producing a long-lasting inhibition of adrenaline hyperglycaemia in the cat. One previous study in this species has shown that α-adrenoceptor blocking drugs prevent this hyperglycaemic response (Harvey, Wang & Nickerson, 1952) whereas other work (Ellis, Kennedy, Eusebi & Vincent, 1967) has shown the response to be reduced by β-adrenoceptor blocking drugs but *not* by α-adrenoceptor blocking agents. In the present work we have examined the effect, upon adrenaline-induced hyperglycaemia, of α- or β-adrenoceptor blocking drugs administered alone and in combination.

Methods

Cats of either sex weighing between 1.5 and 3.0 kg were used. After an overnight fast the animals were anaesthetized with sodium pentobarbitone (36 mg/kg,

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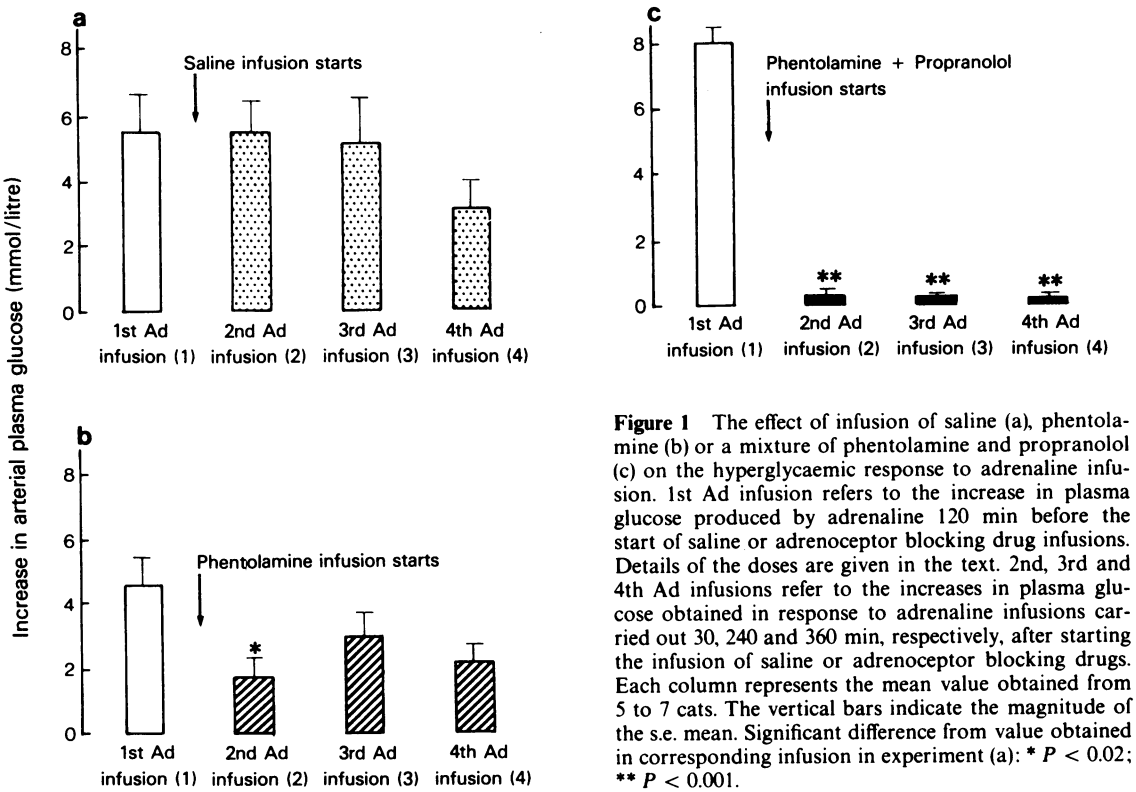

Figure 1 The effect of infusion of saline (a), phentolamine (b) or a mixture of phentolamine and propranolol (c) on the hyperglycaemic response to adrenaline infusion. 1st Ad infusion refers to the increase in plasma glucose produced by adrenaline 120 min before the start of saline or adrenoceptor blocking drug infusions. Details of the doses are given in the text. 2nd, 3rd and 4th Ad infusions refer to the increases in plasma glucose obtained in response to adrenaline infusions carried out 30, 240 and 360 min, respectively, after starting the infusion of saline or adrenoceptor blocking drugs. Each column represents the mean value obtained from 5 to 7 cats. The vertical bars indicate the magnitude of the s.e. mean. Significant difference from value obtained in corresponding infusion in experiment (a): * $P < 0.02$; ** $P < 0.001$.

i.p.). The animals were allowed to breathe spontaneously. Drugs were injected or infused through two cannulae inserted to the level of the right atrium, via the right and left femoral veins. Heparin (100 International units/kg) was injected intravenously. Arterial blood pressure was recorded from the right common carotid artery using a polythene cannula attached to an Elema-Schönander EMT 30 capacitance transducer. This cannula was also used for withdrawal of blood samples. Body temperature was monitored continuously by means of direct reading thermocouples (Ellab, Copenhagen) placed in the mid-oesophagus and the rectum and was maintained in the range 36 to 37.5°C.

Plasma glucose was determined with the Beckman Glucose Analyser and blood lactate was measured by the Hohorst enzymatic method (using a Boehringer test combination). Blood gases and pH were monitored by appropriate Radiometer electrode systems and did not change significantly from the control values of P_{O_2}, 106 mmHg, P_{CO_2} 25 mmHg and pH 7.4.

Adrenaline was administered by infusion at a dose of 1 μg kg^{-1} min^{-1} for 10 min via one of the femoral venous catheters and blood pressure and the electrocardiogram monitored continuously. At the end of the 10 min period a blood sample was taken for glucose or lactate analysis. The infusions were repeated at intervals of not less than 2 h. Propranolol was administered (intravenously) as a single injection of either 0.25 or 1.0 mg/kg. Phentolamine was administered as a single injection (priming dose) of 2.5 mg/kg followed by a continuous intravenous infusion (15 μg kg^{-1} min^{-1}). When both drugs were employed (combined α- and β-adrenoceptor blockade) injection of propranolol (0.25 mg/kg) and phentolamine (2.5 mg/kg) slowly over 5 min was followed by continuous intravenous infusion of both drugs at doses of 5 μg kg^{-1} min^{-1} and 15 μg kg^{-1} min^{-1}, respectively. Saline (0.9% w/v NaCl solution) was infused continuously in control animals at a rate similar to that used for the combined adrenoceptor blocking infusion (2 ml/h).

Values were expressed as means ± s.e. mean and significance was tested by Student's t tests for paired or unpaired data as appropriate. Mean values were assumed to be significantly different where $P < 0.05$.

Results

Effects of repeated infusions of adrenaline

In order to investigate the duration and effectiveness of the various adrenoceptor blocking regimens within

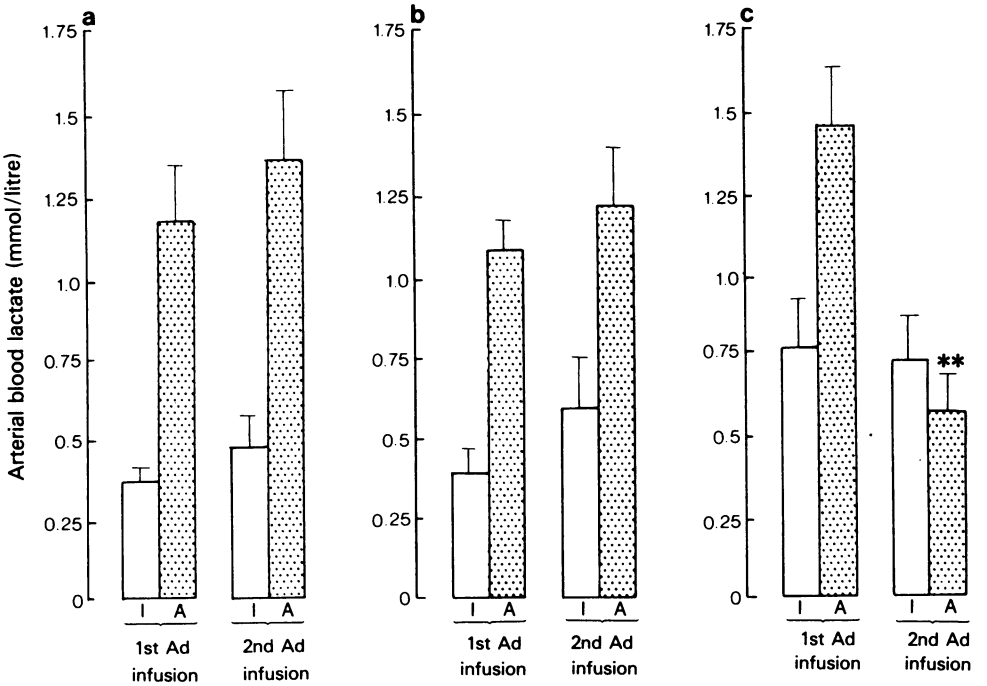

Figure 2 The effect of infusion of saline (a), phentolamine (b) or a mixture of phentolamine and propranolol (c) on the elevation in blood lactate produced by infusion of adrenaline. Details of the doses are given in the text. I refers to values obtained before adrenaline infusion and A refers to values obtained at the end of a 10 min adrenaline infusion. 1st Ad infusion refers to values obtained before the start of the infusion of saline or adrenoceptor blocking drugs. 2nd Ad infusion refers to values obtained at the end of a 10 min adrenaline infusion started 240 min after starting the infusion of saline or adrenoceptor blocking drugs. Each column represents the mean value obtained from 5 to 7 cats. The vertical bars indicate the magnitude of the s.e. mean. Significant difference from the appropriate control adrenaline infusion: ** $P < 0.001$.

the same animal it was necessary to establish the reproducibility of the hyperglycaemic effect of adrenaline infusions. Figure 1 shows the effect on plasma glucose of four successive 10 min adrenaline infusions administered with at least 2 h between each. The times of the infusions are given in the figure legend. The hyperglycaemic response to adrenaline infusion was reproducible for the first three infusions. Although there was a clear hyperglycaemic response to the fourth infusion it was considerably reduced in magnitude. The hyperglycaemic response was accompanied by small increases in heart rate and in systemic arterial blood pressure. The increases in heart rate were fairly reproducible over the four infusions (i.e. during infusion I from 185 ± 6 to 218 ± 11 beats/min; infusion II from 190 ± 14 to 211 ± 12 beats/min; infusion III from 182 ± 13 to 211 ± 8 beats/min and infusion IV from 187 ± 12 to 214 ± 10 beats/min). However, the magnitude of the hypertensive response tended to decrease with successive infusions +33 ± 11; +33 ± 5; +19 ± 6; +19 ± 6 mmHg, respectively (systolic) and +24 ± 9; +16 ± 6; +3 ± 7 and +9 ± 4 mmHg (diastolic) from resting values of 103 ± 7 mmHg (systolic) and 78 ± 7 mmHg (diastolic).

The blood lactate concentration was measured in the first and third adrenaline infusions only and the increases were of similar magnitude in both infusions (Figure 2).

The effect of propranolol on the responses to adrenaline infusions

Propranolol (0.25 mg/kg or 1.0 mg/kg) was administered intravenously 15 min before the second adrenaline infusion. Propranolol itself decreased systemic arterial blood pressure by −3 ± 2 mmHg (systolic) and −4 ± 2 mmHg (diastolic) with the smaller dose and by −14 ± 3 mmHg (systolic) and −21 ± 3 mmHg (diastolic) with the 1.0 mg/kg dose. Heart rate was markedly decreased (−27 ± 14 and −48 ± 5 beats/min, respectively). The effects of propranolol on

the hyperglycaemic response to adrenaline are shown in Table 1. Propranolol in either dose did not change significantly the basal glucose concentration (6.7 ± 0.9 mmol/litre and 9.4 ± 1.3 mmol/litre respectively in the experiments with 0.25 and 1 mg/kg propranolol) and reduced significantly the hyperglycaemic response to adrenaline but failed to abolish it completely. The adrenaline-induced pressor response appeared to be augmented by propranolol. The pressor responses to adrenaline after 0.25 mg/kg propranolol were +47 ± 6 mmHg (systolic) and +44 ± 5 mmHg (diastolic) and, after 1.0 mg/kg propranolol, +46 ± 13 mmHg (systolic) and +43 ± 10 mmHg (diastolic). The adrenaline-induced tachycardia was abolished by the smaller dose of propranolol and converted to a bradycardia (−31 ± 16 beats/min) by the larger dose.

The effect of phentolamine on the responses to adrenaline infusions

Phentolamine was administered by a single bolus injection followed by a continuous slow intravenous infusion throughout the whole experimental period. This treatment resulted in a decrease in blood pressure (from 121 ± 4 to 104 ± 5 mmHg (systolic); $P < 0.02$ and from 92 ± 3 to 75 ± 4 mmHg (diastolic); $P < 0.01$) but there was no significant change in heart rate (193 ± 12 to 197 ± 11 beats/min) or plasma glucose (6.6 ± 0.44 to 7.4 ± 0.61 mmol/litre). However, the blood lactate concentration was increased significantly (0.37 ± 0.08 to 0.66 ± 0.13 mmol/litre; $P < 0.005$). These are 'steady-state' values obtained 30 min after the start of the infusion.

Adrenaline was infused 0.5, 4 and 6 h after the start of the phentolamine infusion and resulted in a reduction in systemic arterial pressure (e.g. −24 ± 11 mmHg (systolic) and −42 ± 9 mmHg (diastolic) compared to +26 ± 9 mmHg (systolic) and +4 ± 5 mmHg (diastolic) *before* phentolamine; $P < 0.01$). This 'adrenaline-reversal' occurred without a modification of the heart rate response (+24 ± 8 beats/min before phentolamine and, for example, +21 ± 9 beats/min after it). The blood lactate concentration was increased by adrenaline in phentolamine-treated cats (Figure 2). The hyperglycaemic response was reduced by phentolamine, although this effect was significant only in the case of the first post-phentolamine adrenaline administration (Figure 1).

The effects of combined α- and β-adrenoceptor blockade

The doses used and the method of administration have been outlined in the Methods section. The important point is that, after the initial 'priming' injections, the infusion of propranolol and phentolamine was continued throughout the whole experimental period (7 h). This resulted in a stable haemodynamic situation over the entire experiment. For example, 0.5 h after starting the infusion the arterial pressure had fallen to 92 ± 9 mmHg (systolic) and 74 ± 9 mmHg (diastolic) from the pre-drug levels of 122 ± 12 mmHg and 100 ± 11 mmHg respectively ($P < 0.01$); the heart rate had decreased from 180 ± 16 to 138 ± 10 beats/min ($P < 0.05$). The comparative values at 6 h after starting the infusion were 88 ± 7 mmHg, 67 ± 7 mmHg and 146 ± 9 beat/min, respectively. The adrenoceptor blocking drug combination had no significant effects, at any time during the infusion period, on the plasma glucose concentration (7.3 ± 1.6 mmol/litre pre-drug infusion and 7.3 ± 1.8, 6.9 ± 1.2 and 6.3 ± 0.9 mmol/litre after 0.5, 4 and 6 h of infusion respectively).

The haemodynamic and metabolic effects of adrenaline were reduced markedly by this drug combination. There was no adrenaline-induced change in heart rate, arterial blood lactate concentration (Figure 2) or arterial plasma glucose concentration (Figure 1). However, there was a hypertensive response, although this was reduced, being +34 ± 7 mmHg (systolic) and +16 ± 5 mmHg (diastolic) before the combined adrenoceptor blockade and +10 ± 4 and +9 ± 3

Table 1 Effect of propranolol on the hyperglycaemia produced by infusions of adrenaline (1 μg kg^{-1} min^{-1} for 10 min)

		Increase in plasma glucose (mmol/litre)		
Treatment	n	*1st adrenaline infusion*	*2nd adrenaline infusion*	*Level of significance*
Control	4	9.5 ± 1.7	8.1 ± 1.6	
Propranolol 0.25 mg/kg	6	5.7 ± 1.1	2.5 ± 0.44	$P < 0.05$
Propranolol 1 mg/kg	4	10.0 ± 1.3	3.4 ± 1.1	$P < 0.025$

Control refers to cats receiving acid saline instead of propranolol. 1st adrenaline infusion refers to the effect of adrenaline infusion before the administration of saline or propranolol. 2nd adrenaline infusion refers to the effects of adrenaline infusion after the administration of saline or propranolol. Values are means ± s.e. mean.

mmHg respectively at 6 h ($P < 0.05$ and $P < 0.01$, respectively).

Discussion

The present results suggest strongly that both α- and β-adrenoceptors are involved in adrenaline-induced hyperglycaemia in the fasted cat. β-Adrenoceptor blockade with propranolol, whilst preventing completely adrenaline-induced tachycardia, failed to produce a complete blockade of the hyperglycaemic response. On the other hand α-adrenoceptor blockade with phentolamine also reduced adrenaline hyperglycaemia, suggesting the additional involvement of α-adrenoceptors. The complete antagonism of the adrenaline response by a combination of α- and β-adrenoceptor blocking drugs, in a dose regimen that completely prevented adrenaline-induced tachycardia and markedly reduced the pressor response to adrenaline, appeared to confirm the involvement of both adrenoceptor types in mediating adrenaline-induced hyperglycaemia. Some caution is necessary in interpreting the effect of α-adrenoceptor blocking drugs on the hyperglycaemic response to adrenaline. Phentolamine has been found to increase plasma insulin concentrations in various species (Lundquist, 1972b; Furman & Tayo, 1974). This effect is probably due to the removal of an α-adrenoceptor-mediated inhibition of insulin secretion, together with the unmasking of β-adrenoceptor-mediated stimulation. The main consequence of this effect in the present context is likely to be a physiological antagonism of the hepatic components of the hyperglycaemic effect of adrenaline through the well documented inhibitory effects of insulin on glycogenolysis and gluconeogenesis (Jefferson, Exton, Butcher, Sutherland & Park, 1968; Mortimore, King, Mondon & Glinsmann, 1968). Thus antagonism of adrenaline hyperglycaemia by phentolamine need not necessarily implicate the direct involvement of α-adrenoceptors in the response. Unfortunately, we have been unable to measure plasma immunoreactive insulin concentrations in the cat in view of the apparently low cross-reactivity of cat insulin with available antisera (unpublished observations).

Our findings are rather different from those presented in a paper published whilst the present work was in progress (Kuo, Kamaka & Lum, 1977). These authors could not demonstrate any significant blockade of adrenaline-induced hyperglycaemia in the fasted cat when propranolol or phenoxybenzamine were administered separately. Moreover, they were unable to block completely the hyperglycaemic effect of adrenaline (50 to 70% blockade) when a combination of the two adrenoceptor blocking drugs was used, although phenoxybenzamine and propranolol were shown to prevent completely the hyperglycaemic responses to phenylephrine and isoprenaline respectively. The increase in blood glucose produced by adrenaline infusion (1 $\mu g\ kg^{-1}\ min^{-1}$) in their experiments was very similar to that obtained in the present work. There is no obvious explanation for the different results obtained by these authors. Differences may result from the different α-adrenoceptor blocking drug used (phenoxybenzamine compared with phentolamine in the present work) and from the different dose regimen (bolus doses of blocking drugs compared with continuous infusion after priming doses in the present work). Moreover Kuo *et al.* (1977) did not report the effect of their blocking drugs on the cardiovascular responses to adrenaline infusion and thus had no supporting evidence that other α- and β-adrenoceptor-mediated responses to adrenaline were prevented. We also agree with Kuo *et al.* (1977) in implicating β-adrenoceptors in the adrenaline-induced increase in lactate. This was completely unaffected by phentolamine (in doses that caused reversal of the hypertensive effect) but abolished by a combination of phentolamine and propranolol (Figure 2).

From the present work it appears that it is unnecessary to implicate receptors other than α- and β-adrenoceptors in mediating the hyperglycaemic effect of adrenaline in the fasted cat. Moreover we have shown that it is possible to produce a long lasting (at least 7 h) blockade of adrenaline-induced hyperglycaemia in the fasted cat by the continuous infusion of a combination of propranolol and phentolamine at a rate which does not modify the fasting plasma glucose concentration. This drug combination can therefore be used to investigate the possible role of released adrenaline in mediating changes in plasma glucose and lactate following the administration of endotoxin.

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