

## EFFECTS OF ADRENOCEPTOR BLOCKADE ON PLASMA CATECHOLAMINE LEVELS DURING ADRENALINE INFUSION

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- 1 The present experiments investigate the effects of phenoxybenzamine and propranolol, singly and in combination, on plasma catecholamine levels in sheep receiving a three-hour adrenaline infusion.
- 2 Five groups of five anaesthetized sheep were studied for a period of 3 h each. One group acted as a control and received only a saline (0.9% w/v NaCl solution) infusion. A second group received a constant infusion of adrenaline ( $2 \mu\text{g kg body weight}^{-1} \text{ min}^{-1}$ ). A third group received a similar adrenaline infusion, having been premedicated with phenoxybenzamine (1 mg/kg body weight). A fourth group received a similar adrenaline infusion following premedication with ( $\pm$ )-propranolol hydrochloride ( $7 \mu\text{g/kg body weight}$ ). The fifth group received the adrenaline infusion following premedication with both the  $\alpha$ - and  $\beta$ -blocker in the above doses.
- 3 Plasma catecholamines were measured on blood samples taken at seven intervals before, during and following the infusion.
- 4 Control animals receiving only a saline infusion remained physiologically and biochemically stable throughout the experimental period.
- 5 Adrenaline infusion in animals not receiving adrenoceptor blocking drugs caused a rise in plasma adrenaline levels from a low basal value of  $1 \mu\text{g/litre}$  to a maximum level of  $19.8 \mu\text{g/litre}$ . Animals premedicated with phenoxybenzamine exhibited a similar response.
- 6 Animals premedicated with propranolol before the infusion of adrenaline did not demonstrate as marked a rise of plasma adrenaline levels as the two previous groups. The maximum mean plasma adrenaline level recorded in this group was  $6.88 \mu\text{g/litre}$ .
- 7 Animals premedicated with phenoxybenzamine and propranolol before the infusion of adrenaline showed only a small rise in plasma adrenaline levels compared with animals receiving adrenaline infusion alone. The maximum mean plasma adrenaline level in the group was only  $3.43 \mu\text{g/litre}$ .
- 8 The studies demonstrate that by an unknown mechanism  $\beta$ -adrenoceptor blockade with ( $\pm$ )-propranolol, either alone or in combination with phenoxybenzamine, lowers the plasma adrenaline response evoked by adrenaline infusion.

### Introduction

Adrenaline infusion is frequently used in stress experiments as an agonist to simulate the adrenergic response seen in conditions such as haemorrhagic shock (Halmagyi, Irving & Varga, 1968). Investigation of the effects of adrenaline infusion is facilitated by the use of  $\alpha$ - and  $\beta$ -adrenoceptor blocking drugs. It has always been assumed that these drugs do not affect circulating levels of adrenaline and

noradrenaline and only exert their effects by receptor blockade. However, Halmagyi, Kennedy & Varga (1971), using haemorrhage as a stress, have demonstrated that certain combinations of adrenoceptor blockade significantly lower the adrenergic response to blood loss as shown by reduced plasma catecholamine levels in the blocked animals. The present experiments investigate the effects of phenoxybenzamine and propranolol, both singly and in combination, on plasma catecholamine levels in sheep receiving a 3-h adrenaline infusion.

### Methods

Twenty-five female Sussex Border crossbreed sheep of mean weight 60.5 kg were used. Prior to the

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experiments they were sedated with ketamine (Ketalar, Parke-Davis) 20 mg/kg body wt. intramuscularly. This facilitated transport and positioning. Thirty minutes later, anaesthesia was induced with thiopentone (500 mg i.v.), and maintained with intermittent intravenous chloralose, 1% in 0.9% w/v NaCl solution (saline). The trachea was intubated with a cuffed Magill tube and the animals allowed to breathe spontaneously.

A plastic sampling cannula (6 F.G. Portex) was inserted into the left femoral vein and advanced into the vena cava to allow sampling of central venous blood. Both antecubital veins were cannulated with fine plastic catheters to allow intravenous infusions of adrenaline, saline and adrenoceptor blocking drugs. Infusions were given by constant volume pumps (Delta, Watson-Marlow) delivering 1 ml/minute. A mercury manometer was connected to a plastic cannula in the right common carotid artery to measure mean arterial pressure.

The animals were allowed to settle for 30 min after the initial surgical procedure before a resting blood sample was taken. By this time the effects of the ketamine had worn off (Baraka, Harrison & Kachachi, 1973).

Five groups of five sheep were investigated, the order of medication being allocated from random number tables. The treatment for the groups was as in Table 1.

Adrenaline was given as adrenaline bitartrate, phenoxybenzamine as phenoxybenzamine hydrochloride (Dibenyline, Smith, Kline & French) and propranolol as ( $\pm$ )-propranolol hydrochloride (Inderal, I.C.I.). All solutions were made up in saline.

Blood samples were taken as follows:

1. Resting control.
2. Resting control after administration of adrenoceptor blocking drugs, where given.
3. Thirty minutes after start of adrenaline infusion, where given.
- 4–6. At 60, 120 and 180 min after start of adrenaline infusion.
7. Sixty minutes after cessation of 3 h adrenaline infusion, where given.

Blood samples (10 ml) were taken from the vena cava into lithium heparin tubes containing 5 mg sodium metabisulphite as antioxidant (Carruthers, Taggart, Conway, Bates & Somerville, 1970). The blood was centrifuged immediately and the plasma transferred to plastic tubes which were placed on dry ice. Plasma samples were kept at  $-76^{\circ}\text{C}$  until assay, within 7 days.

Catecholamines were measured according to the semi-automated method of McCullough (1968), which measures noradrenaline and total catecholamines, adrenaline being determined by difference. Cross reaction of adrenaline in the noradrenaline assay is less than 2%, and interference from catecholamine metabolites is low. The only interference from the catecholamine-like group of compounds in use in medical practice comes from isoprenaline and  $\alpha$ -methylnoradrenaline (formed by the  $\beta$ -hydroxylation of  $\alpha$ -methyldopa) neither of which was used in this study.

## Results

Changes in plasma catecholamine levels in each group were compared with the levels in period 2. This period was chosen as representing the basal value after administration of the adrenoceptor blocking drugs, where given.

In the groups receiving phenoxybenzamine (Group C), propranolol (Group D), and phenoxybenzamine + propranolol (Group E), additional comparisons were made with the corresponding period in the animals given an adrenaline infusion without blockers (Group B). Plasma catecholamine levels are expressed throughout as  $\mu\text{g/litre}$ , and figures quoted unless otherwise stated are mean ( $\pm$  standard deviation). Intragroup comparisons have been made using paired *t*-test, and intergroup comparisons by two-sample *t*-test.

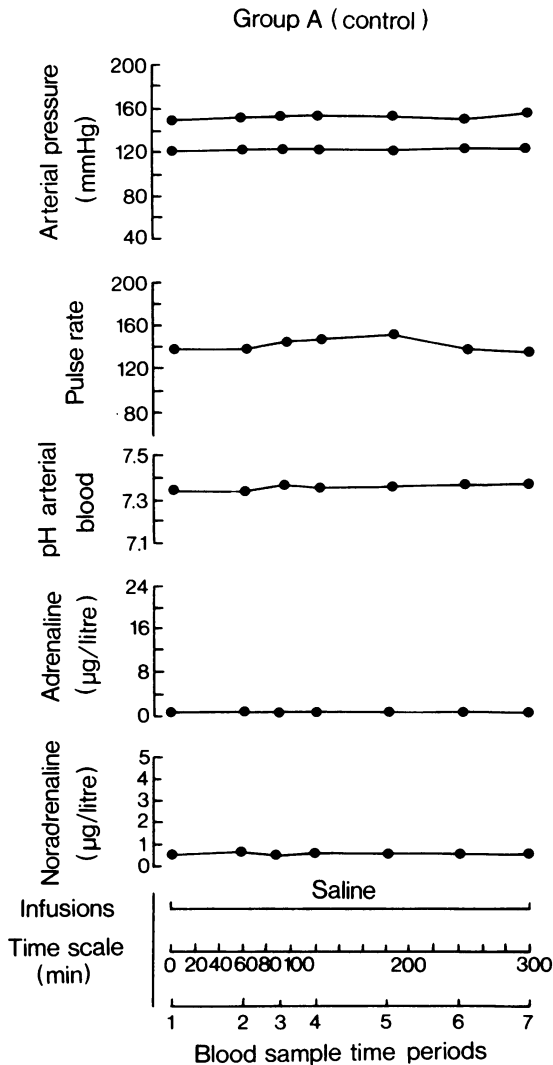
### Group A. Control (Tables 2 & 3, Figure 1)

The results in this group of animals demonstrated that the anaesthetized animal lying supine and being

Table 1 Infusion schedules

Group	Pump 1	Pump 2
A	Saline	Saline
B	Adrenaline <sup>1</sup>	Saline
C	Adrenaline <sup>1</sup>	Phenoxybenzamine <sup>2</sup>
D	Adrenaline <sup>1</sup>	Propranolol <sup>3</sup>
E	Adrenaline <sup>1</sup>	Phenoxybenzamine <sup>2</sup> + propranolol <sup>3</sup>

<sup>1</sup> 2  $\mu\text{g}$  body wt.<sup>-1</sup> min.<sup>-1</sup>; <sup>2</sup> 1 mg/kg body wt. in saline over 15 min given 45 min before the infusion of adrenaline, then saline; <sup>3</sup> 7  $\mu\text{g/kg}$  body wt. in saline. Continuous infusion from 45 min before infusion of adrenaline.

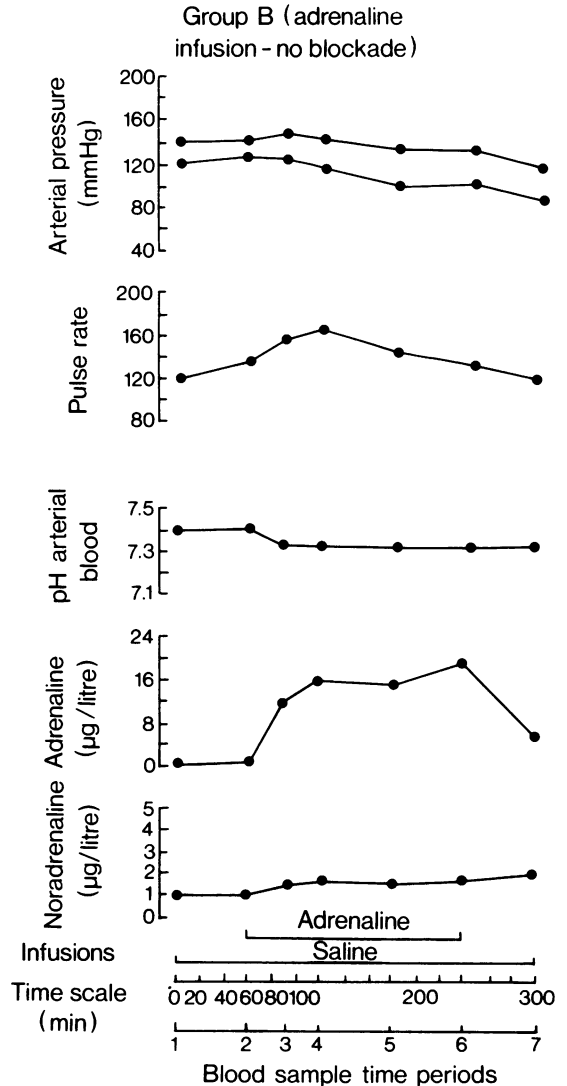


**Figure 1** Pulse, blood pressure, adrenaline and noradrenaline concentrations in saline-infused sheep, Group A (control). Mean values.

infused with saline is physiologically and biochemically stable throughout the experimental period. The maximum adrenaline level recorded in this group was in period 3,  $0.92 (\pm 0.37)$ , and the maximum noradrenaline level in period 2,  $0.75 (\pm 0.13)$ . Throughout the experiment pulse rate, arterial blood pressure and pH remained stable.

#### Group B. Adrenaline infusion (Tables 2 & 3, Figure 2)

In this group, the physiological response to adrenaline infusion was similar to that described previously.



**Figure 2** Pulse, blood pressure, adrenaline and noradrenaline concentrations in sheep infused with adrenaline alone, Group B. Mean values.

There was an increase in pulse rate and a gradual fall in arterial pressure (Irving, Britton, Wood, Padgham & Carruthers, 1968). Infusion of adrenaline caused a rise in plasma adrenaline levels from a low basal value of  $1.00 (\pm 0.58)$  to maximum levels in period 6 of  $19.8 (\pm 9.27)$ . The adrenaline levels in periods 3–7 were all significantly raised from period 2 (3.  $P < 0.02$ ; 4.  $P = 0.01$ ; 5.  $P < 0.01$ ; 6.  $P > 0.01$ ; 7.  $P < 0.02$ ). There were no significant changes in the plasma noradrenaline levels throughout the observation period.

*Group C. Phenoxybenzamine plus adrenaline (Tables 2 & 3, Figure 3)*

In these animals, phenoxybenzamine premedication caused a fall in arterial pressure which became more marked after the start of the adrenaline infusion. The rise in plasma levels of adrenaline during the course of the experiment showed similar trends to Group B. Maximum levels of adrenaline were seen in period 5 of 21.56 ( $\pm 15.36$ ) and plasma adrenaline was significantly raised above period 2 in periods 3–6 (3.  $P < 0.01$ ; 4.  $P < 0.01$ ; 5.  $P < 0.01$ ; 6.  $P < 0.05$ ). Noradrenaline levels were significantly higher in period 7 ( $P > 0.02$ ). At no time were adrenaline or noradrenaline levels different from Group B.

*Group D. Propranolol plus adrenaline (Tables 2 & 3, Figure 4)*

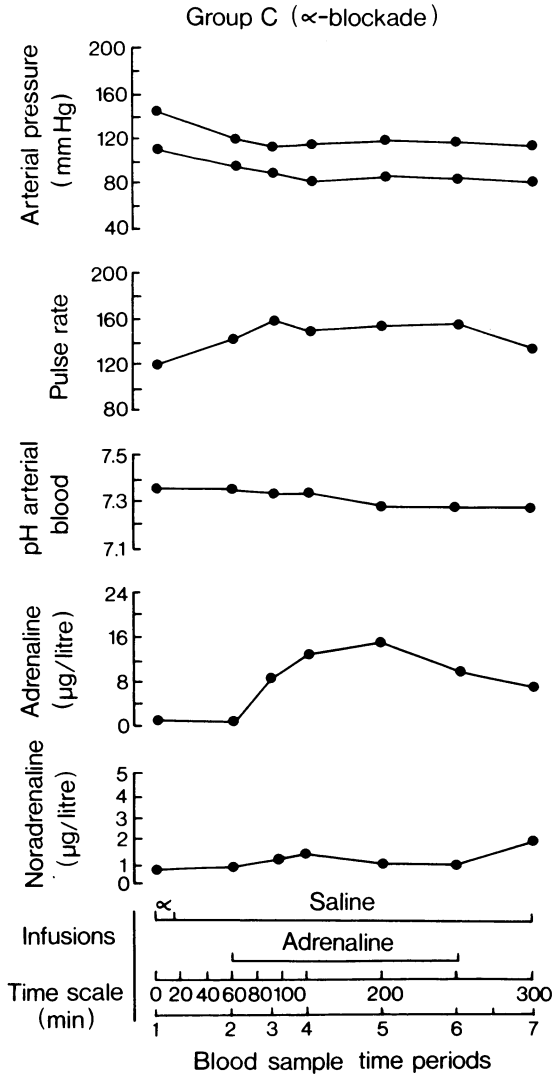
Propranolol prevented the tachycardia which accompanies adrenaline infusion. An initial hypertensive response to the adrenaline infusion was soon followed by a gradual fall in arterial blood pressure. Plasma adrenaline levels during the infusion of adrenaline again rose, but were only significant in periods 5–7 when compared with period 2 (5.  $P > 0.01$ ; 6.  $P > 0.02$ ; 7.  $P = 0.05$ ). The maximum plasma adrenaline level recorded in this group was 6.88 ( $\pm 2.47$ ). Plasma adrenaline levels were significantly lower than in corresponding periods in the adrenaline infusion group (Group B) in periods

**Table 2** Adrenaline concentration ( $\mu\text{g/litres}$ ) in central venous blood. Mean  $\pm$  s.d. during the 7 sampling periods in the saline infusion group (A), adrenaline infusion group (B), phenoxybenzamine premedicated group (C), propranolol premedicated group (D), and phenoxybenzamine and propranolol premedicated group (E).

Group	Period 1	2	3	4	5	6	7
A	0.91 $\pm 0.36$	0.81 $\pm 0.09$	0.92 $\pm 0.37$	0.66 $\pm 0.25$	0.74 $\pm 0.41$	0.52 $\pm 0.33$	0.65 $\pm 0.24$
B	0.57 $\pm 0.44$	0.67 $\pm 0.43$	12.80 $\pm 5.96$	16.40 $\pm 6.82$	15.60 $\pm 5.93$	19.80 $\pm 9.27$	6.55 $\pm 2.97$
C	1.30 $\pm 1.42$	1.44 $\pm 1.31$	10.20 $\pm 4.13$	18.50 $\pm 17.90$	21.60 $\pm 15.40$	14.20 $\pm 10.60$	9.99 $\pm 7.47$
D	0.85 $\pm 0.63$	0.74 $\pm 0.34$	5.96 $\pm 1.38$	5.72 $\pm 2.32$	6.88 $\pm 2.47$	6.79 $\pm 5.40$	2.96 $\pm 2.21$
E	0.38 $\pm 0.23$	1.04 $\pm 0.87$	3.43 $\pm 2.48$	3.31 $\pm 2.54$	2.11 $\pm 0.83$	1.78 $\pm 0.92$	2.66 $\pm 2.71$

**Table 3** Noradrenaline concentration ( $\mu\text{g/litre}$ ) in central venous blood. Mean  $\pm$  s.d. during the 7 sampling periods in the saline infusion group (A), adrenaline infusion group (B), phenoxybenzamine premedicated group (C), propranolol premedicated group (D), and phenoxybenzamine and propranolol premedicated group (E)

Group	Period 1	2	3	4	5	6	7
A	0.68 $\pm 0.11$	0.75 $\pm 0.13$	0.57 $\pm 0.30$	0.68 $\pm 0.30$	0.60 $\pm 0.28$	0.68 $\pm 0.39$	0.71 $\pm 0.25$
B	0.98 $\pm 0.54$	1.00 $\pm 0.58$	1.28 $\pm 0.67$	1.50 $\pm 0.72$	1.23 $\pm 0.51$	1.83 $\pm 0.83$	2.39 $\pm 2.07$
C	0.70 $\pm 0.29$	1.00 $\pm 0.48$	1.20 $\pm 1.09$	1.71 $\pm 1.30$	1.06 $\pm 0.44$	0.96 $\pm 0.40$	2.44 $\pm 1.49$
D	1.03 $\pm 0.59$	1.27 $\pm 0.59$	1.68 $\pm 1.65$	1.39 $\pm 0.79$	1.41 $\pm 0.59$	1.91 $\pm 1.28$	2.38 $\pm 1.32$
E	0.85 $\pm 0.16$	0.86 $\pm 0.19$	0.58 $\pm 0.19$	0.93 $\pm 0.88$	0.85 $\pm 0.32$	0.80 $\pm 0.16$	0.95 $\pm 0.44$

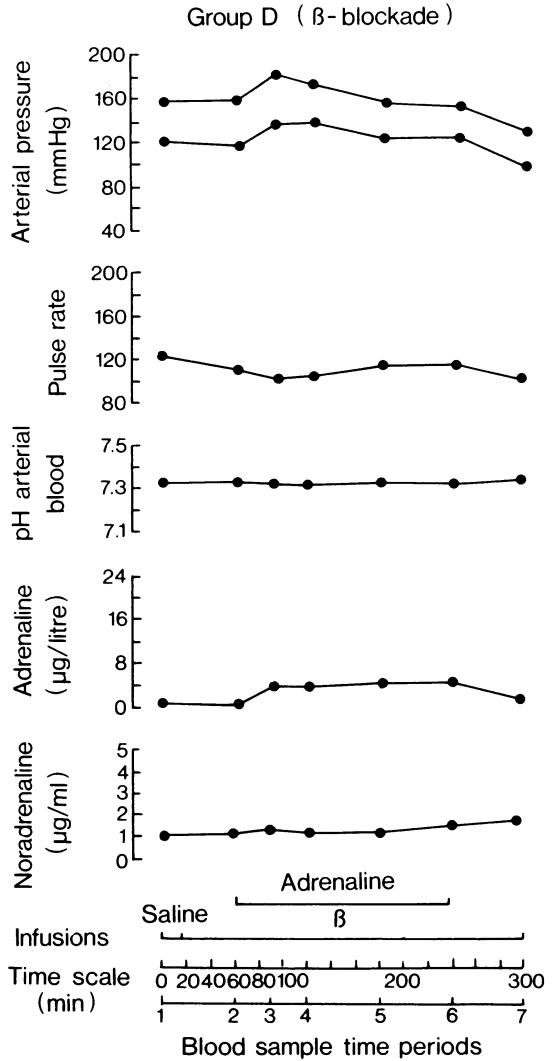


**Figure 3** Pulse, blood pressure, adrenaline and noradrenaline concentrations in sheep infused with adrenaline after pretreatment with phenoxybenzamine, Group C. Mean values.

3–6 (3.  $P < 0.05$ ; 4.  $P > 0.02$ ; 5.  $P > 0.02$ ; 6.  $P < 0.05$ ). Noradrenaline failed to show any significant changes either when compared with period 2 or when compared with Group B.

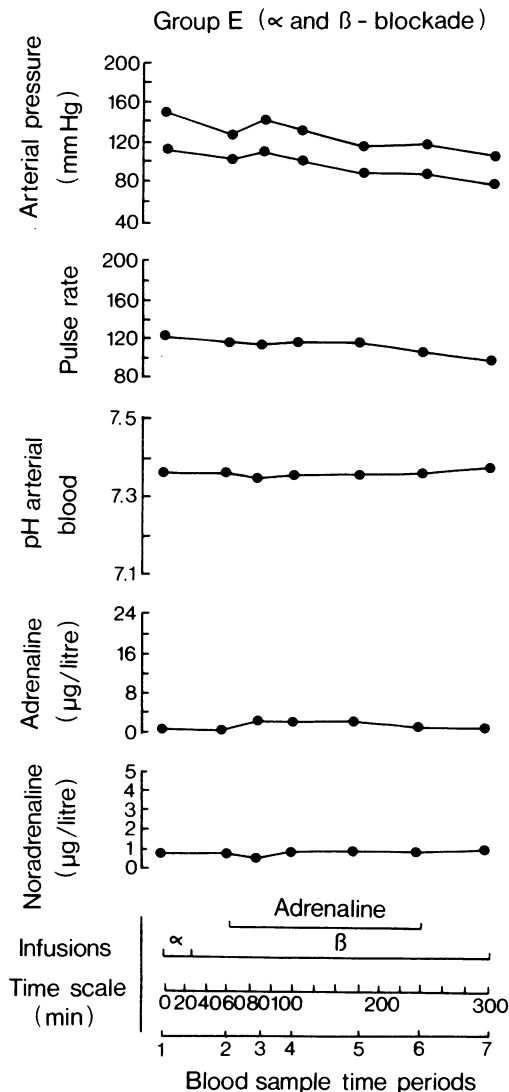
**Group E. Phenoxybenzamine plus propranolol plus adrenaline (Tables 2 & 3, Figure 5)**

Combined adrenoceptor blockade produced a stable physiological model in which adrenaline infusion



**Figure 4** Pulse, blood pressure, adrenaline and noradrenaline concentrations in sheep infused with adrenaline after pretreatment with propranolol, Group D. Mean values.

caused little change in pulse rate or pH. There was a slight fall in arterial pressure. The rise in plasma adrenaline levels during adrenaline infusion was small when compared with the animals receiving adrenaline infusion alone (Group B), or after phenoxybenzamine administration (Group C). The maximum plasma adrenaline levels were in period 3,  $3.43 (\pm 2.48)$  and were significantly higher than in period 2 in periods 3–6 (3.  $P < 0.05$ ; 4.  $P = 0.05$ ; 5.  $P > 0.01$ ; 6.  $P = 0.01$ ). When compared with the animals receiving adrenaline



**Figure 5** Pulse, blood pressure, adrenaline and noradrenaline concentrations in sheep infused with adrenaline after phenoxybenzamine and propranolol pretreatment, Group E. Mean values.

infusion alone (Group B), the levels were significantly lower in periods 3–6 (3.  $P > 0.02$ ; 4.  $P = 0.02$ ; 5.  $P = 0.01$ ; 6.  $P > 0.01$ ).

In periods 5 & 6, adrenaline levels were significantly lower than in the corresponding period in the animals receiving propranolol (Group D) (5.  $P < 0.01$ ; 6.  $P = 0.05$ ). Again there were no significant changes in noradrenaline levels, either within or between this group and the others.

## Discussion

Infusion of adrenaline or other sympathomimetic amines has been widely used to investigate their physiological and biochemical effects and to assess the influence of adrenergic antagonists. It has been assumed in most of these investigations that the circulating concentrations of catecholamines are the same in both the control and the adrenoceptor blockade experiments. Recent studies have cast doubt on the accuracy of this assumption (Irving *et al.*, 1974).

The present studies clearly demonstrate that  $\beta$ -adrenoceptor blockade either alone or in combination with  $\alpha$ -blockade decreases the rise in plasma adrenaline produced by adrenaline infusion. This observation does not appear to have been made before, although Halmagyi *et al.* (1971) noticed that dogs premedicated with phenoxybenzamine and propranolol prior to haemorrhage did not produce the high levels of adrenaline and noradrenaline observable in non-medicated dogs with an equivalent degree of blood loss and hypotension. However, in their studies,  $\beta$ -blockade alone did not show a similar effect.

Previous studies have shown that combined adrenoceptor blockade improves the tolerance of sheep and dogs to severe haemorrhagic hypotension and prolonged adrenaline infusion (Halmagyi *et al.*, 1968; Halmagyi *et al.*, 1971). It is known that sustained high circulating levels of catecholamines are deleterious and it may be that the beneficial effect of combined adrenoceptor-blockade results not from receptor blockade but from the lowering of the circulating catecholamine concentrations produced by the drugs.

The explanation for the differences in adrenaline concentration between the four groups of animals in our study is not at the present time clear. Fundamentally, three processes might be altered by adrenoceptor blockade, namely, endogenous secretion of catecholamines in response to adrenaline infusion, uptake of catecholamines into tissues and, thirdly, enzymatic breakdown of the hormones.

It is known that infusion of sympathomimetic amines induces an additional endogenous release of adrenaline from the adrenal gland (Walker, Zileli, Reutter, Shoemaker, Friend & Moore, 1959). However, it would seem unlikely that adrenoceptor blockade, either  $\alpha$ - or  $\beta$ -, would influence this release because the neurogenic synapse within the adrenal medulla is cholinergic.

Uptake mechanisms are the principal means for the inactivation of catecholamines *in vivo*. There are two types of uptake. The first is the return of catecholamines to the sympathetic neurone from which they were released, known as Uptake<sub>1</sub>. The second is Uptake<sub>2</sub>, where the catecholamines are taken up into other cells; such as smooth muscle. Uptake<sub>1</sub> is

saturated at low external catecholamine concentrations and shows a preference for noradrenaline. Uptake<sub>2</sub> predominates at high external catecholamine concentrations and has a higher affinity for adrenaline than for noradrenaline. The capacity of Uptake<sub>1</sub> is low whilst that of Uptake<sub>2</sub> is high (Lightman & Iversen, 1969).

Phenoxybenzamine is known to inhibit both Uptake<sub>1</sub> and Uptake<sub>2</sub> (Iversen, 1973; Starke, 1973) and it is also known that phenoxybenzamine will increase the stimulation-induced overflow of noradrenaline from nerve endings. Clearly this could mean that in the Group C animals the adrenaline concentrations are increased as a result of increased overflow. However, it would be expected that noradrenaline would be increased as well, which it was not. Also this does not explain the reduced concentrations of adrenaline in Group E.

Propranolol inhibits Uptake<sub>1</sub> but its action on Uptake<sub>2</sub> is unknown (Euler & Lishajko, 1968) although it is known that the closely related pronetholol inhibits Uptake<sub>2</sub> (Eisenfeld, Axelrod & Krakoff, 1967) and that propranolol itself will inhibit noradrenaline uptake into platelets (Bygdeman & Johnsen, 1969). However, in order to reduce the adrenaline concentration by this mechanism it is necessary to postulate that propranolol facilitates uptake even in the presence of phenoxybenzamine. Facilitation of uptake has never been described.

Catabolism of catecholamines occurs as a result of the action of two main enzyme systems. Catechol-O-methyltransferase (COMT) occurs principally in the liver and normally selectively methylates the 3-OH group on the catechol ring. COMT converts adrenaline and noradrenaline to their respective metanephrines. Jarrott (1973) has shown that up to 60% of COMT activity remains after sympathectomy showing the majority to be extraneuronal. In considering the effect of adrenoceptor blocking drugs this extraneuronal COMT could play an active part in inactivating excess adrenaline and noradrenaline, particularly as the patterns of blood flow to various organs, including the liver, will be altered by adrenoceptor blockade. Bohuon & Assicot (1973) have extensively studied COMT activity in both liver and erythrocytes. Amongst inhibitors of COMT *in*

*vitro* phenoxybenzamine and propranolol were studied. Membrane bound COMT of erythrocytes was inhibited by low concentrations of propranolol. In erythrocyte ghost fractions a 17% inhibition of COMT was achieved at  $10^{-10}$  M, whereas in the solubilized membrane fraction 4% inhibition was seen at propranolol concentrations of  $10^{-4}$  M. In the soluble fraction of the cell no inhibition by propranolol was seen at this concentration. Phenoxybenzamine showed no inhibition of COMT activity. It has also been shown that high circulating catecholamine levels may induce COMT activity reflected in the urinary output of metanephrines after periods of catecholamine activity and infusion during open heart surgery (Hine, Wood, Mainwaring-Burton, Butler & Irving, 1975). As the animals in our study were not catheterized and plasma metanephrine concentration could not be measured, no estimate of COMT activity is available.

The second major enzyme system in the degradation of catecholamines is monoamine oxidase (MAO). This enzyme is not inhibited by  $\alpha$ - or  $\beta$ -blockers in the doses used in these experiments.

A third degradation system (see Erwin, 1973) consists of two enzymes, an NAD-dependent aldehyde dehydrogenase and an NADP-dependent aldehyde reductase. These enzymes convert the aldehydes resulting from MAO activity to their corresponding acid or alcohol respectively. It is not known whether they are inhibited by adrenoceptor blocking drugs and they only have an effect after the catecholamines have been inactivated by MAO. In conclusion, therefore, it would seem unlikely that interference with the degradation enzymes for catecholamines would explain our findings.

Finally, these studies demonstrate that investigation of a sympathetic response using  $\beta$ -blockade may result in different plasma catecholamine levels in the control and the test experiments. Interpretation of the results must therefore be undertaken with care and in the light of the actual catecholamine concentrations.

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