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COMBINED α- AND β-ADRENOCEPTOR BLOCKING DRUG AH 5158: FURTHER STUDIES ON α-ADRENOCEPTOR BLOCKADE IN ANAESTHETIZED ANIMALS

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1 AH 5158, 5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]salicylamide, competitively antagonised phenylephrine-induced vasopressor responses in anaesthetized dogs, thus confirming that the drug possesses α-adrenoceptor blocking activity.

2 In contrast, AH 5158 was a relatively ineffective antagonist of vasopressor responses to noradrenaline in anaesthetized dogs. Thus, at the lowest dose-level tested (1 mg/kg) AH 5158 abolished the increase in pulse width caused by noradrenaline, but otherwise had little or no blocking effect in doses as high as 10 mg/kg. Propranolol (0.1 mg/kg) also abolished the increase in pulse width caused by noradrenaline. With both drugs this effect is thought to be a consequence of blockade of the β-adrenoceptor-mediated cardiac stimulant action of noradrenaline.

3 The interaction between AH 5158 and noradrenaline in spinal dogs, anaesthetized cats and pithed rats was very similar to that seen in anaesthetized dogs.

4 Noradrenaline pressor responses *were* effectively antagonized by AH 5158 in anaesthetized dogs pretreated with cocaine. The degree of block was similar to that obtained when phenylephrine was the agonist in untreated dogs.

5 These results are consistent with the hypothesis that AH 5158 blocks a cocaine-sensitive inactivation process for noradrenaline in addition to blocking α- and β-adrenoceptors. The resultant increase in the level of circulating noradrenaline would tend to counteract the adrenoceptor blocking action of the drug.

6 The implications of these findings are discussed.

Introduction

AH 5158, 5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl] salicylamide, possesses a unique combination of α- and β-adrenoceptor blocking activity (Farmer, Kennedy, Levy & Marshall, 1972). The α-adrenoceptor blocking action of the drug in the anaesthetized dog was unusual in that vasopressor responses to noradrenaline were blocked to a much lesser extent than those to oxymetazoline. This difference was abolished by pretreating dogs with cocaine. To explain these results it was suggested that AH 5158 blocks a cocaine-sensitive inactivation process for noradrenaline, enabling a larger proportion of the administered dose of noradrenaline to compete with AH 5158 at α-adrenoceptors and thereby reducing the degree of observed antagonism (Farmer *et al.*, 1972).

This paper describes the results of further studies on the interaction between AH 5158 and α-adrenoceptor agonists on the blood pressure and heart rate of anaesthetized animals. These interactions have been re-examined in greater detail in the anaesthetized dog and the investigations have been extended to the spinal dog, anaesthetized cat and the pithed rat.

Methods

Preparation of animals

Beagle dogs of either sex weighing 7-14 kg were anaesthetized with thiopentone sodium, 25 mg/kg intravenously, followed by barbitone sodium, 250 mg/kg intraperitoneally. In some experiments, dogs were anaesthetized with 3% halothane in a nitrous oxide : oxygen (3 : 1) mixture and made spinal by the method of Burn (1952). Artificial respiration with room air was applied through a cuffed endotracheal tube using a stroke volume of

13 ml/kg and a rate of 28/minute. In these experiments and those described below, arterial blood pressure (1 mmHg ≈ 133 Pa) was measured from a cannula in a femoral or carotid artery with a Bell and Howell transducer (type 4-327-L221) and heart rate was obtained from a cardiotachometer triggered by the arterial pressure signal.

Cats of either sex weighing 2.0-3.5 kg were anaesthetized with chloralose, 80 mg/kg intravenously, after induction with 3% halothane in a nitrous oxide : oxygen (3 : 1) mixture. Animals were respired artificially with room air through a tracheal cannula using a stroke volume of 13 ml/kg and a rate of 28/minute.

Male albino rats were anaesthetized with 3% halothane in a nitrous oxide : oxygen (3 : 1) mixture and pithed as described by Shipley & Tilden (1947). The animals were respired artificially with room air through a tracheal cannula using a stroke volume of 1 ml/100 g and a rate of 50/minute.

In all experiments drugs were administered through a cannula in a femoral vein. Bilateral vagotomy was performed in all anaesthetized animals. Permanent records of experiments were made on a Devices M8 recorder.

Experimental procedure and analysis of results

Unless stated otherwise, the following procedure was used to examine agonist-antagonist interactions in each animal. Vasopressor dose-response curves were obtained by the sequential intravenous injection of increasing doses of either noradrenaline or phenylephrine. Each dose-response curve was made up of four or five doses of agonist administered at intervals of 10-20 min and took about 45 min to complete. Dose-response curves were repeated at intervals of approximately 1 hour. After two control dose-response curves had been obtained, the first dose of AH 5158 was injected and the vasopressor dose-response curve re-determined, beginning 15 min later. Three dose-levels of AH 5158 were examined in each experiment, the doses being given at intervals of approximately 1 hour. A cumulative dosing schedule was used for AH 5158; for example, to increase the dose-level from 3 mg/kg to 10 mg/kg a further dose of 7 mg/kg was administered. Dose-ratios were estimated by comparing the dose-response curves obtained in the presence of AH 5158 with the control dose-response curves. The level of response was selected in each experiment to ensure that comparison was made on the straight-line part of a dose-response curve. When appropriate, the results were expressed graphically by plotting log (agonist dose-ratio − 1) on log antagonist dose (mg/kg i.v.) (Arunlakshana & Schild, 1959).

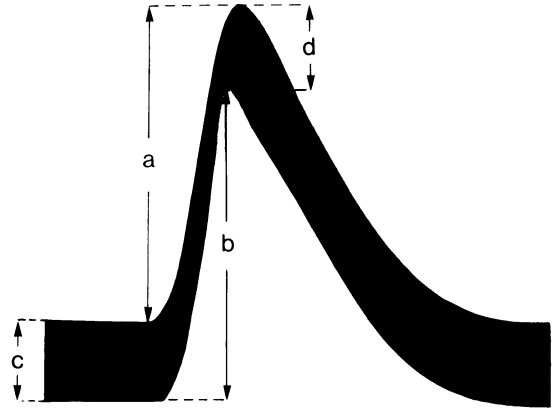

Figure 1 Diagramatic representation of a vasopressor response. The parameters used for analysis were (a) systolic pressor response (b) diastolic pressor response (c) pulse width prior to vasopressor response (d) pulse width at peak of pressor response.

Assessment of the results also involved a detailed analysis of the pressor responses to α-adrenoceptor agonists. The various parameters measured are illustrated diagrammatically in Figure 1.

Drugs

The following drugs were used: AH 5158 hydrochloride (Allen & Hanburys); cocaine hydrochloride (MacFarland Smith); (−)-noradrenaline (BDH); (−)-phenylephrine hydrochloride (Koch-Light); (±)-propranolol hydrochloride (ICI). Solutions were prepared by dissolving drugs in 0.9% w/v NaCl solution. Weights of drugs are expressed in terms of the base.

Results

Anaesthetized dogs

Variation in sensitivity to noradrenaline and phenylephrine In control experiments vasopressor dose-response curves to noradrenaline 0.1-3 μg/kg ($n = 3$) or phenylephrine 1-30 μg/kg ($n = 3$) were obtained at intervals of 1 h over a period of 4-6 hours. Sensitivity to noradrenaline increased by a maximum of 2- to 3-fold over this period; this change was considered to be negligible. There was no consistent change in sensitivity to phenylephrine.

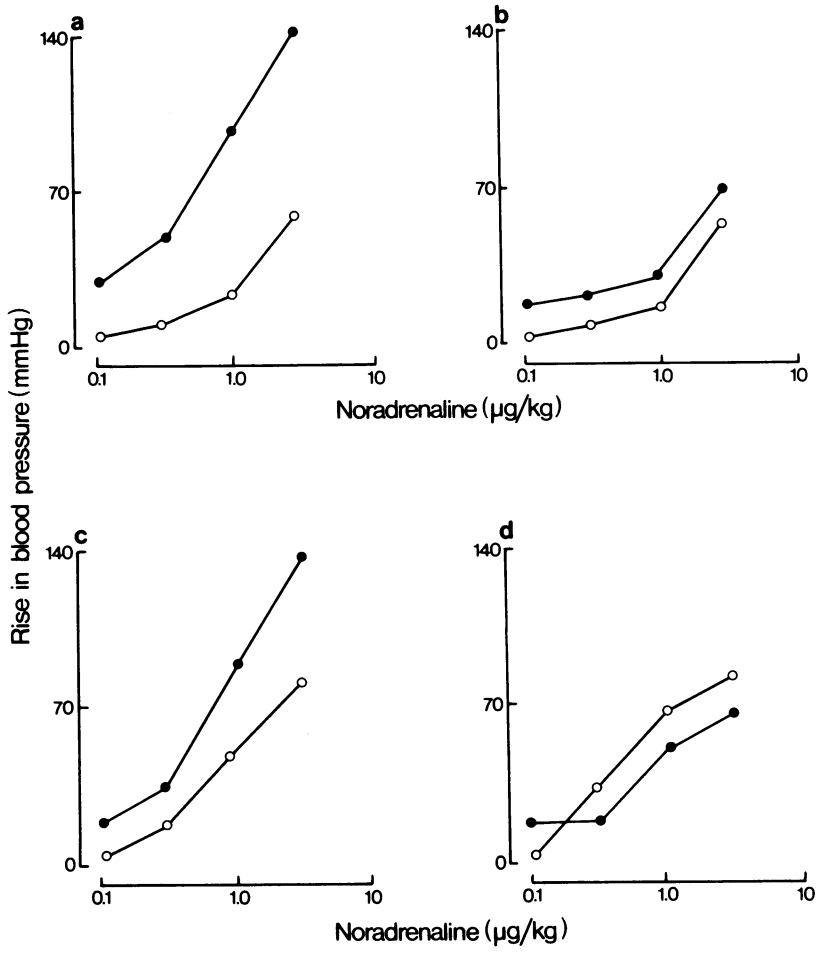

Figure 2 Anaesthetized dog. Pressor responses to noradrenaline; (a) and (c) are control responses; (b) effects of AH 5158 (1 mg/kg) and (d) of propranolol (0.1 mg/kg) on responses to noradrenaline. (ab) and (cd) are the results of single experiments. Systolic pressor response (●), diastolic pressor response (○). All drugs given intravenously. Note that both antagonists reduce systolic pressor responses but have little effect on diastolic pressor responses.

Interaction between noradrenaline and AH 5158 or propranolol The systolic pressor response to noradrenaline was always larger than the diastolic pressor response and this difference increased with increasing dose. Thus, noradrenaline caused dose-dependent increases in pulse width.

In four dogs AH 5158, 1 mg/kg, reduced systolic pressor responses to noradrenaline, but had little effect on diastolic pressor responses. After AH 5158, the systolic and diastolic pressor responses were similar in magnitude so that the increase in pulse width was abolished. The results of one experiment are summarized in Figure 2a, b.

In three experiments propranolol, 0.1 mg/kg, had very similar effects to AH 5158, reducing systolic but not diastolic pressor responses to noradrenaline (Figure 2c,d). Higher doses of AH 5158 (3 and 10 mg/kg) modified systolic and diastolic pressor responses in the same way and so the results obtained with the latter only are described. In two experiments these higher doses of AH 5158 slightly antagonized diastolic pressor responses (maximum noradrenaline dose-ratio, 4-5), but the antagonism was not dose-related. In the other two experiments AH 5158 had no effect on diastolic pressor responses. The results of one experiment

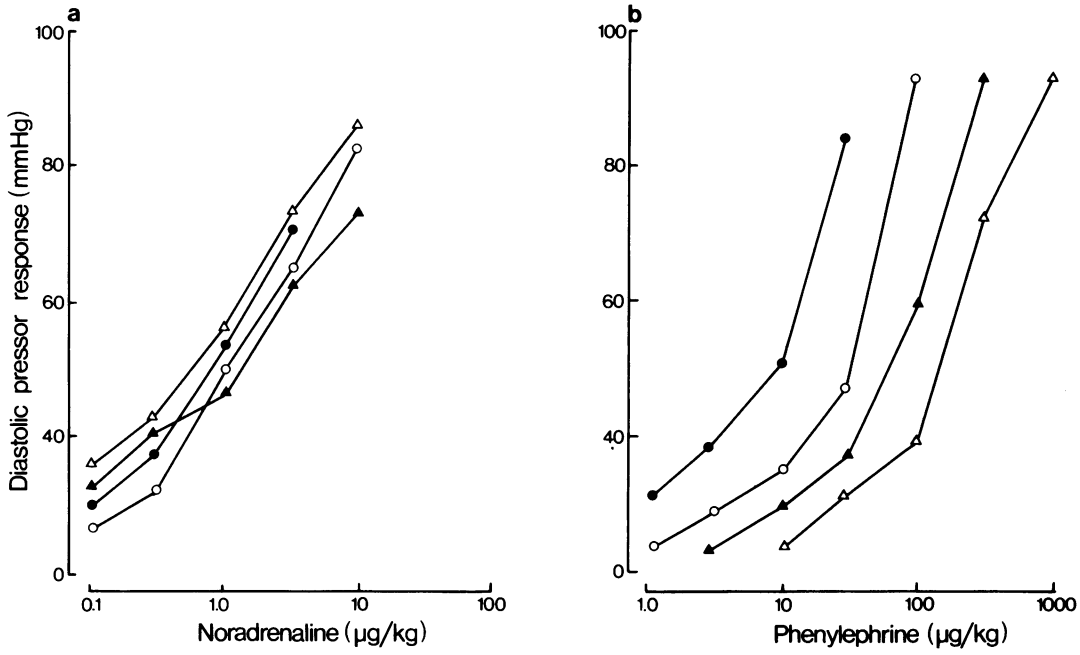

Figure 3 Anaesthetized dog. Comparison of the effect of AH 5158 on diastolic pressor responses to noradrenaline (a) and phenylephrine (b). (a) and (b) are results from individual experiments. Control (●) and after AH 5158, 1 mg/kg (○), 3 mg/kg (▲), 10 mg/kg (△). All drugs given intravenously.

illustrating the interaction between noradrenaline diastolic pressor responses and AH 5158, 1, 3 and 10 mg/kg, are shown in Figure 3a.

The pressor responses to noradrenaline were accompanied by decreases in heart rate of up to 20 beats/min, but these were not dose-related. In some dogs small increases in heart rate preceded the decreases. Both were abolished by AH 5158, 1 mg/kg, or by propranolol, 0.1 mg/kg.

Interaction between phenylephrine and AH 5158 The vasopressor responses to phenylephrine differed from those to noradrenaline in that systolic and diastolic pressor responses were similar in size. Consequently, pulse width was not increased by phenylephrine.

The effect of AH 5158, 1, 3 and 10 mg/kg, on pressor responses to phenylephrine was examined in three dogs. Systolic and diastolic pressor responses were blocked to a similar extent and so only results for the latter are described. AH 5158 caused dose-related parallel shifts to the right of the phenylephrine dose-response curve. In Figure 3b the results of a typical experiment are contrasted with those obtained with noradrenaline (Figure 3a). The combined results of the three experiments with phenylephrine are expressed in the form of an Arunlakshana & Schild (1959) plot in Figure 4a.

Pressor responses to phenylephrine were accompanied by decreases in heart rate of 10-30 beats/minute. These decreases were abolished by AH 5158, 1 mg/kg.

Interaction between noradrenaline and AH 5158 in cocaine-pretreated dogs Preliminary experiments were directed towards determining the dose of cocaine required for maximum potentiation of noradrenaline pressor responses. The degree of potentiation increased with doses of cocaine up to 10 mg/kg. At 30 mg/kg, cocaine caused pronounced cardiac arrhythmias and hypotension, resulting in death. Therefore, cocaine was used at the 10 mg/kg dose-level in these experiments. This dose, infused over a period of 15-20 min, increased diastolic blood pressure by 15-40 mmHg and heart rate by 10-40 beats/minute. On termination of the infusion, blood pressure and heart rate stabilized at levels slightly below control within 10-15 minutes. The potentiating action of cocaine began to wane after 2 h and so the effect of only one dose of AH 5158 was examined in each dog. The

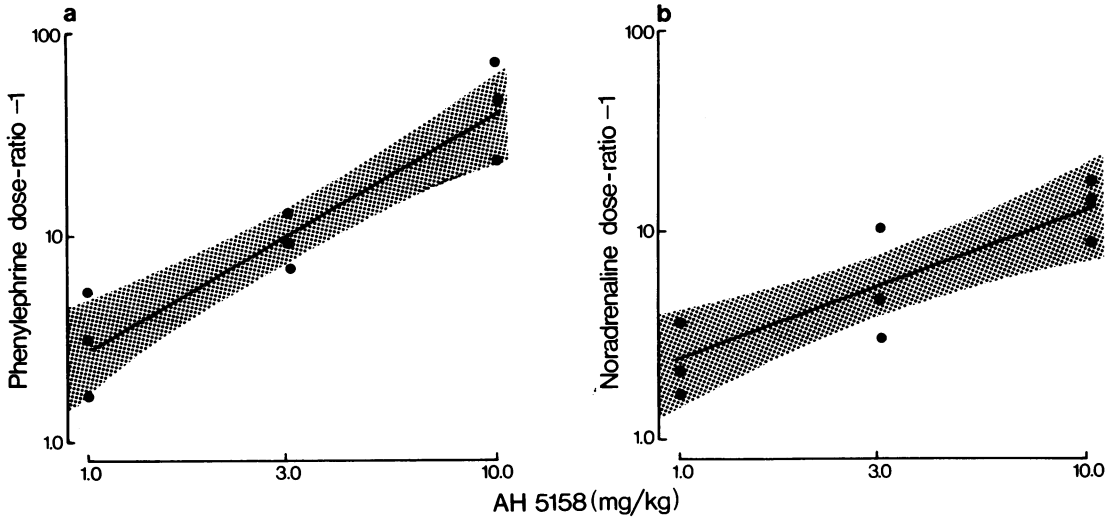

Figure 4 Arunlakshana & Schild (1959) plots computed for the interaction between (a) AH 5158 and phenylephrine in anaesthetized dogs and (b) AH 5158 and noradrenaline in anaesthetized dogs pretreated with cocaine, 10 mg/kg i.v. Each point represents a single estimate of dose-ratio −1. In (a) AH 5158 was administered at three dose-levels in each of three dogs. In (b) only one dose of AH 5158 was administered to each dog. The shaded envelopes enclose the 95% confidence limits of the regression lines. The limits for the two regression lines overlap. All drugs given intravenously.

following procedure was used: a pressor dose-response curve was obtained for noradrenaline, cocaine was infused over a period of 15-20 min to give a total dose of 10 mg/kg, a further 20 min was allowed to elapse and a second noradrenaline dose-response curve was obtained; AH 5158, 1, 3 or 10 mg/kg, was injected and 15 min later a third noradrenaline dose-response curve was obtained. The potentiating effect of cocaine was estimated by comparing the first and second noradrenaline dose-response curves; the blocking effect of AH 5158 was estimated by comparing the second and third noradrenaline dose-response curves. Diastolic pressor responses were used to construct dose-response curves. Each dose-level of AH 5158 was tested in three dogs.

Sensitivity to noradrenaline was greatly increased by cocaine, the dose-response curve being displaced to the left by a factor of 15 (95% confidence limits 8-27, $n = 9$). In these animals the subsequent administration of AH 5158, 1, 3 or 10 mg/kg, produced parallel dose-related displacements to the right of the noradrenaline dose-response curve. This contrasts with the results obtained in the absence of cocaine (see above). Results for these experiments are summarized in the form of an Arunlakshana & Schild (1959) plot in Figure 4b. Comparison of Figure 4a and b shows that the degree of α-adrenoceptor blockade produced by AH 5158 is similar when phenylephrine is the agonist in untreated dogs and when noradrenaline is the agonist in cocaine-treated dogs.

In two experiments cocaine, 10 mg/kg, had no effect on sensitivity to phenylephrine pressor responses.

Spinal dogs

In four spinal dogs pressor responses to noradrenaline, 0.1-3 μg/kg, were very similar in character to those obtained in anaesthetized dogs. Systolic pressor responses were larger than diastolic pressor responses and, consequently, pulse width was increased. The effect of AH 5158 on noradrenaline pressor responses was also very similar to that seen in anaesthetized dogs. Thus, at 1 mg/kg AH 5158 reduced systolic but not diastolic pressor responses and abolished the increases in pulse width. Larger doses of AH 5158 (3 and 10 mg/kg) had no further blocking effect in three dogs. In the fourth dog the diastolic pressor dose-response curve was displaced to the right by a maximum of 4-fold, but this antagonism was not dose-related.

Pressor responses to noradrenaline were accompanied by dose-related increases in heart rate of up

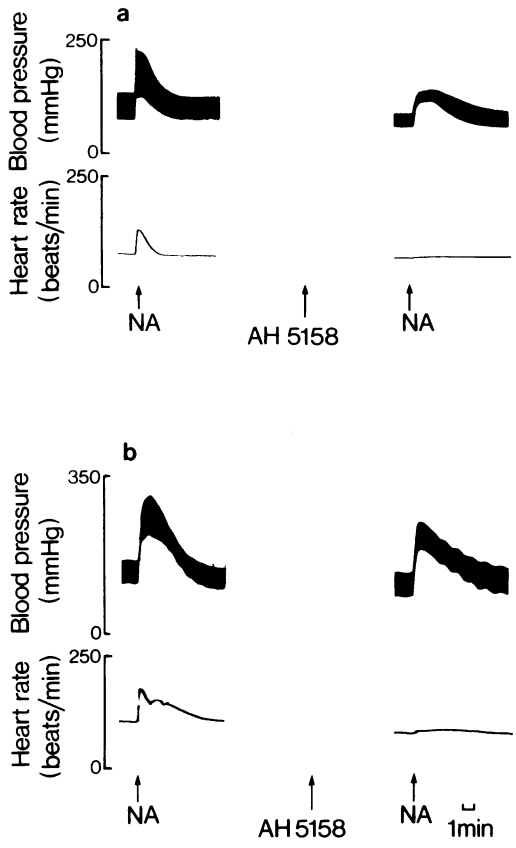

Figure 5 Comparison of the effects of AH 5158, 3 mg/kg, on responses of blood pressure and heart rate to noradrenaline, 3 μg/kg (NA), in (a) the spinal dog and (b) the anaesthetized cat. Drugs given intravenously.

to 100 beats/min, in contrast to the decreases or biphasic responses seen in the anaesthetized dog. These heart rate increases were abolished by AH 5158, 1 mg/kg.

The effects of AH 5158, 1 mg/kg, on the blood pressure and heart rate responses to noradrenaline, 3 μg/kg, are illustrated in Figure 5a.

Anaesthetized cats

In three anaesthetized cats the responses to noradrenaline, 0.1-3 μg/kg and the interaction between noradrenaline and AH 5158, 1-10 mg/kg, on blood pressure and heart rate were almost identical to those obtained in anaesthetized and spinal dogs. Records from one experiment in an anaesthetized cat are shown in Figure 5b.

Pithed rats

In three pithed rats the responses to noradrenaline, 0.1-3 μg/kg, and the interaction between noradrenaline and AH 5158, 1-10 mg/kg, on blood pressure and heart rate were almost identical to those obtained in anaesthetized and spinal dogs and anaesthetized cats.

Discussion

In a previous study in the anaesthetized dog, AH 5158 exhibited α-adrenoceptor blocking activity when oxymetazoline was the agonist used (Farmer *et al.*, 1972). This activity has been confirmed in the present study using phenylephrine. Comparison of the two series of experiments shows that vasopressor responses to these agonists were antagonized to a similar degree by AH 5158.

In contrast, AH 5158 was a relatively ineffective antagonist of vasopressor responses to noradrenaline, which confirms the previous finding (Farmer *et al.*, 1972). The more detailed analysis reported here gives rise to two questions. First, why does AH 5158, at the lowest dose-level tested (1 mg/kg), abolish the increases in pulse width caused by noradrenaline, and second, why does AH 5158 otherwise have little or no blocking effect in doses as high as 10 mg/kg?

The answer to the first question probably lies in the dual origin of the noradrenaline pressor response. This consists of a peripheral vasoconstrictor component mediated *via* α-adrenoceptors and a cardiac component (positive chronotropic and/or positive inotropic) mediated *via* β-adrenoceptors. The cardiac component appears to be responsible for the increase in pulse width; thus, phenylephrine, which has much less cardiac stimulant action than noradrenaline (Trendelenburg, Muskus, Flemming & Gomez Alonso de la Sierra, 1962; Shanks, 1966) caused no increase in pulse width. AH 5158 exerts a pronounced block of cardiac β-adrenoceptors at 1 mg/kg (Farmer *et al.*, 1972; this study) and this action is probably responsible for preventing the increases in pulse width to noradrenaline. The almost identical results obtained for the interaction between noradrenaline and propranolol provide strong support for this idea.

The second question can be answered by referring to the experiments carried out in dogs treated with cocaine. In these animals noradrenaline pressor responses *were* effectively antagonized by AH 5158, thus confirming our previous finding (Farmer *et al.*, 1972). In the experiments reported here, care was taken to minimize the influence of

cocaine-sensitive uptake mechanisms. The block of noradrenaline pressor responses by AH 5158 was then similar in degree to the block of phenylephrine responses in untreated dogs. These findings are consistent with the hypothesis that the α-adrenoceptor blocking action of AH 5158 is normally counteracted when noradrenaline is the agonist, because AH 5158 also blocks a cocaine-sensitive inactivation mechanism for noradrenaline. At each dose-level the increase in the level of circulating noradrenaline is apparently just sufficient to counteract the α-adrenoceptor blockade. This situation does not arise when phenylephrine is the agonist because it is not inactivated to any significant degree by these mechanisms, as indicated by the lack of potentiation of pressor responses by cocaine. We intend to test the hypothesis of a 'cocaine-like' action of AH 5158 more directly by comparing the blood levels of intravenously administered noradrenaline before and after administration of AH 5158.

Blockade of noradrenaline inactivation mechanisms by AH 5158 would also be expected to influence its β-adrenoceptor blocking action when noradrenaline is the agonist. However, because AH 5158 is some 4 to 5 times more potent as an antagonist at β-adrenoceptors than at α-adrenoceptors (Farmer *et al.*, 1972) the increases in the levels of circulating noradrenaline are probably not sufficient to overcome the β-adrenoceptor blockade. The present study bears this out since AH 5158 effectively antagonized the cardiac stimulant actions of noradrenaline.

The present experiments do not indicate the location of the cocaine-sensitive inactivation process thought to be blocked by AH 5158. There are two likely sites; first, the peripheral vascular beds, where noradrenaline is removed from the circulation into sympathetic nerve terminals by the uptake$_1$ process (Whitby, Hertting & Axelrod, 1960; Whitby, Axelrod & Weil-Malherbe, 1961; Gryglewski & Vane, 1970; Iversen, 1971), and second, the lungs, where noradrenaline is removed from the circulation by an uptake process sensitive to cocaine, but which otherwise differs from uptake$_1$ (Alabaster & Bakhle, 1973; Iwasawa & Gillis, 1974).

The lack of antagonism of noradrenaline pressor responses by AH 5158 is not restricted to the anaesthetized dog; it was also demonstrated in the spinal dog, anaesthetized cat and pithed rat. It may also explain differences in the pharmacological results obtained with AH 5158 in man. Thus, in human volunteers Boakes, Knight & Prichard (1971) found AH 5158 to be about 4 times less potent as an antagonist at α-adrenoceptors than at β-adrenoceptors, whereas Collier, Dawnay, Nachev & Robinson (1972) found it to be 40 to 200 times less potent. It may be relevant that the former group used phenylephrine as the α-adrenoceptor agonist whereas the latter group used noradrenaline.

The ineffectiveness of AH 5158 against systemically administered noradrenaline raises a doubt about its ability to block responses to noradrenaline released from sympathetic nerves. This question has obvious clinical implications. We have shown that AH 5158 *does* effectively block responses to sympathetic nerve stimulation in the heart and peripheral vasculature of the dog (unpublished observations). It has also been shown to antagonize responses to cardiac sympathetic nerve stimulation in man (Collier *et al.*, 1972). This difference in the ability of AH 5158 to antagonize systemically administered and neuronally released noradrenaline remains to be explained.

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