Br. J. Pharmac. Chemother. (1966), **27**, 459–467.

EFFECTS OF PRONETHALOL ON THE CARDIOVASCULAR ACTIONS OF CATECHOLAMINES DURING BLOCKADE BY PHENOXYBENZAMINE

BY

J. GARRETT, A. MALAFAYA-BAPTISTA* AND W. OSSWALD

From the Department of Pharmacology, Medical Faculty, University of Porto, Portugal

(*Received March* 14, 1966)

It has been reported that dichloroisoprenaline abolishes the blockade of sympathetic α-receptors induced by phenoxybenzamine, dihydroergotamine or benzodioxane (Eltherington & Horita, 1960) and chlorpromazine (Gokhale, Gulati & Parikh, 1964). The newer blocking agent pronethalol (Black & Stephenson, 1962) has a similar antiadrenolytic action (Moreira & Osswald, 1965; Gulati, Gokhale & Udwadia, 1965). The mechanism of this interesting phenomenon is largely unknown, although tentative explanations have been offered by the latter authors.

This paper presents the results of experiments in which some cardiovascular parameters of the anaesthetized dog were recorded, in an attempt to elucidate the mechanisms involved.

METHODS

Seventeen mongrel dogs of either sex weighing between 8 and 18.5 kg were anaesthetized with pentobarbitone sodium (30 mg/kg, intravenously) and both vagi were cut in the neck. Blood pressure was measured with a Statham transducer (model P23AA) and a carrier preamplifier from a cannula in the left carotid artery. During artificial ventilation, thoracotomy was performed, a pericardial cradle was prepared and a Walton-Brodie strain gauge arch was sutured to the right ventricle and connected to another carrier preamplifier. After heparinization (5 mg/kg followed by 2 mg/kg at 45 min intervals), blood flow in the femoral artery was measured by means of a Shipley-Wilson type rotameter (Blood Flow Assembly).

All measurements were recorded on a Physiograph Six (E & M Instrument Co.). Heart rate was measured from the original tracings.

After obtaining control responses to intravenous injections of suitable doses of adrenaline, noradrenaline and isoprenaline and to close intra-arterial injections of adrenaline and isoprenaline (0.25 to 16 μg doses), the animals were treated with phenoxybenzamine (5 mg/kg) and these procedures repeated 30 min later. Catecholamine administration was once more repeated some minutes after pronethalol (5 mg/kg) was given. Six dogs received, instead of injections, adrenaline or noradrenaline by intravenous infusion from a constant rate pump (Braun), over a period of 4 min (5 μg/kg/min). In some animals 883 F (diethylaminomethylbenzodioxane hydrochloride) (5 mg/kg) was administered at the end of the experiment.

Drugs. Phenoxybenzamine hydrochloride (Smith, Kline & French) was dissolved to a concentration of 10 mg/ml. in propylene glycol acidified by concentrated hydrochloric acid in order to obtain

* Since deceased.

a 0.01 N solution. This stock solution was diluted five times with 0.9% saline, after addition of a few drops of glacial acetic acid to avoid droplet formation, and infused during a 30-min period. Pronethalol hydrochloride (Alderlin, I.C.I.) was used as a 10 mg/ml. aqueous solution, diluted five to ten times in saline and administered by slow intravenous infusion during 15 to 20 min in order to minimize the fall in blood pressure caused by the drug. (−)-Adrenaline (Hoechst), (−)-noradrenaline (Hoechst) and (±)-isoprenaline (Boehringer, Ingelheim) were used as free bases, dissolved in 0.01 N-hydrochloric acid (1 mg/ml.); these stock solutions were diluted with saline as necessary. For intrafemoral arterial administration, catecholamines were used in freshly prepared isotonic and neutral solutions, and were injected in a constant volume (0.2 ml.) through an indwelling cannula distally to the rotameter and washed in with saline (0.2 ml.). 883 F was used as Prosympal Specia (ampoules containing a 25 mg/ml. solution). Doses are expressed in terms of bases, for catecholamines, and as salts, for other drugs.

RESULTS

Experiments with adrenaline and noradrenaline

Blood pressure. During the control period, the usual pressor responses to injections of adrenaline were observed (Fig. 1). Intravenous infusion of adrenaline or noradrenaline increased blood pressure during the duration of the infusion (Table 1). It is apparent from Table 1, as was to be expected, that adrenaline caused the systolic blood pressure

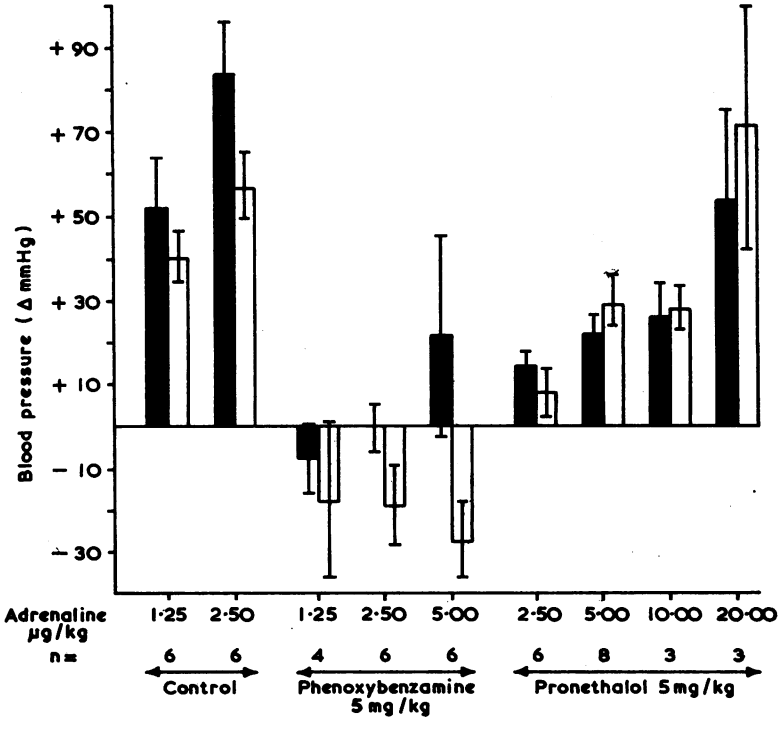

Fig. 1. The effect of intravenous adrenaline on the blood pressure of anaesthetized dogs, during the control period and after successive administration of phenoxybenzamine and pronethalol. The columns give the means and the standard errors of the increases or decreases in systolic pressure (shaded columns) and diastolic pressure (open columns); the doses used and the number of experiments (n) are also shown.

TABLE 1

EFFECTS OF THE INTRAVENOUS INFUSION OF ADRENALINE AND NORADRENALINE ON THE BLOOD PRESSURE OF DOGS, BEFORE AND DURING ADRENERGIC BLOCKADE

Values are increases or decreases in blood pressure expressed as mean increase in systolic pressure ± standard error over mean increase in diastolic pressure ± standard error. The numbers of animals are given in parentheses. Infusions were given during 4 min

Drug	Blood pressure change (mm Hg)		
	Control	After phenoxybenzamine (5 mg/kg)	After pronethalol (5 mg/kg)
Adrenaline, 5 μg/kg/min (3)	+122·3± 8·4	+10 ±17·8	+40·0±2·5
	+ 56·0±11·7	− 19·6± 5·5	+48·3±4·2
Noradrenaline, 5 μg/kg/min (3)	+ 87·5± 3·3	+17·5± 2·4	+25·0±5·1
	+ 82·5± 2·5	+ 3·5±11·1	+31·0±9·1

to increase more and the diastolic blood pressure to increase less than did noradrenaline; in fact, after noradrenaline was administered rises in systolic and diastolic blood pressure were almost identical. After phenoxybenzamine (5 mg/kg) adrenaline decreased blood pressure, especially diastolic pressure, while the response to noradrenaline was greatly reduced. With the highest dose (5 μg/kg) of adrenaline systolic pressure showed a small rise, although diastolic pressure fell greatly (Fig. 1 and Table 1). β-Receptor blockade produced by pronethalol (5 mg/kg), during the action of phenoxybenzamine, restored the pressor responses to both adrenaline and noradrenaline. However, these catecholamines never reacquired their full pressor activity, the values of the hypertensions observed being much lower than those of the control responses (Fig. 1).

It was also evident that while during the control period adrenaline caused greater rises in systolic than in diastolic blood pressure, after phenoxybenzamine and pronethalol the increases in diastolic blood pressure roughly equalled those observed in systolic blood pressure.

Heart rate and contractile force. The positive inotropic responses to adrenaline, observed during the control period (+145±26 g and +156±25 g for the 1.25 and 2.50 μg/kg doses, respectively) were not significantly modified by the injection of the α-receptor blocking drug phenoxybenzamine. Pronethalol injected after phenoxybenzamine abolished the inotropic action of these doses of adrenaline, and allowed even very high doses (10 to 20 μg/kg) to cause only moderate changes in contractile force.

The influences exerted by the blocking drugs on the chronotropic effects of adrenaline were complicated by their own actions on heart rate. In spite of considerable tachycardia caused by phenoxybenzamine, adrenaline retained its accelerator action, the highest rates being observed under these circumstances; however, the absolute increases in rate were smaller than during the control period. Similar observations on isolated heart preparations have been reported by Nickerson & Chan (1961). After pronethalol, which by itself induced a reduction of the basal heart rate to values less than those observed formerly, the smaller doses of adrenaline no longer had any accelerator effect; a moderate increase in heart rate was observed only after the higher doses. Figure 2 summarizes these changes.

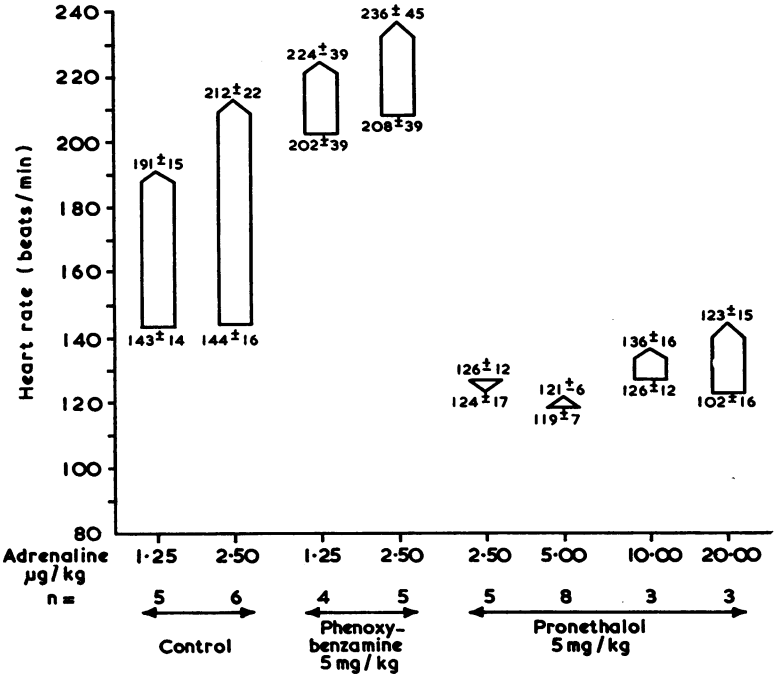

Fig. 2. The effect of adrenaline on the heart rate of anaesthetized dogs. The bars represent the magnitude and the direction of the changes recorded. Numerals indicate the means and standard errors of the means. The doses used and the number of experiments (n) are also shown.

Femoral arterial blood flow

The following results were obtained in the experiments designed to investigate the action of intra-arterial adrenaline (Fig. 3): the vasoconstrictor effect caused by adrenaline was proportional to the dose injected and was converted to a large vasodilatation by phenoxybenzamine. This classic sign of blockade of α-receptors was changed by pronethalol administration to a vasoconstriction, which however did not reach the intensity exhibited during the control period. Figure 4 illustrates a representative experiment.

Once again, increase in dosage of adrenaline did not result in proportionate increases in the size of blood flow reductions (Fig. 3).

Experiments with isoprenaline. In some animals the effects of isoprenaline were also studied. As was expected, the fall in diastolic blood pressure accounted essentially for the decrease in mean pressure observed during the control administrations. Phenoxybenzamine had no influence on this diastolic blood pressure fall due to isoprenaline, which however was abolished by pronethalol. The actions of isoprenaline on heart rate and contractile force were modified by phenoxybenzamine and pronethalol in exactly the same pattern as described above for adrenaline. The decrease of femoral bed resistance caused by the intra-arterial injection of isoprenaline was not modified by phenoxybenzamine but blockade of β-receptors by pronethalol abolished this vasodilator effect.

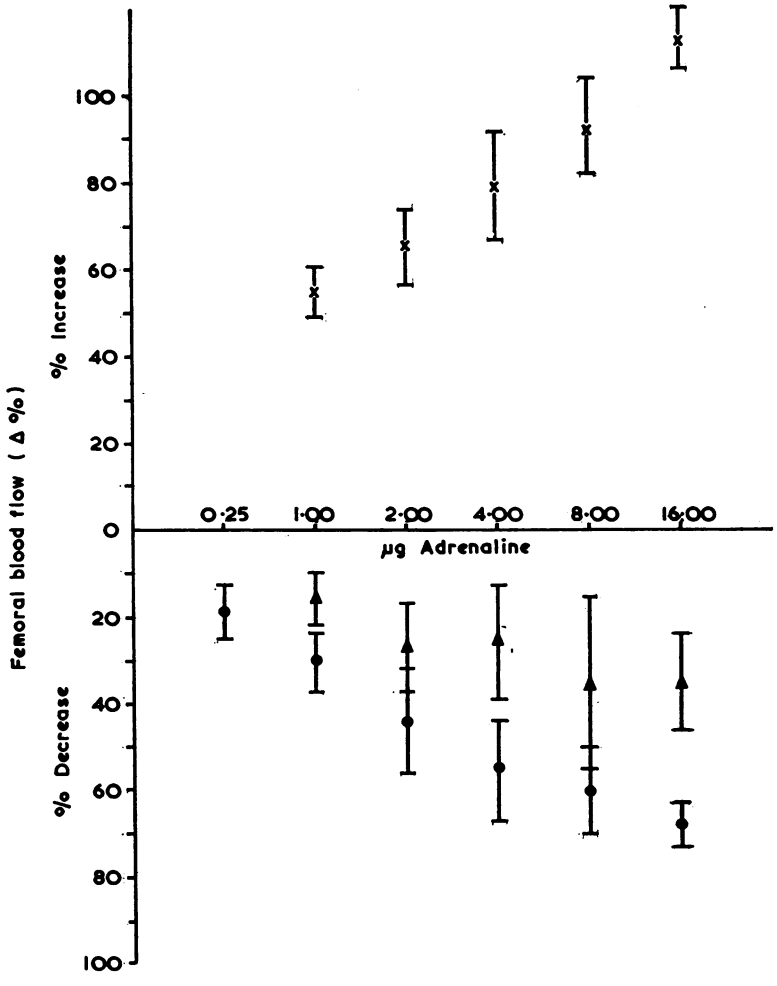

Fig. 3. The effect of intra-arterial adrenaline on femoral blood flow; decreases or increases are indicated in percentages of the control values, recorded immediately before injections. Effects of adrenaline during the control period ●●; after phenoxybenzamine (5 mg/kg) ××; and after phenoxybenzamine + pronethalol (5 mg/kg) ▲▲.

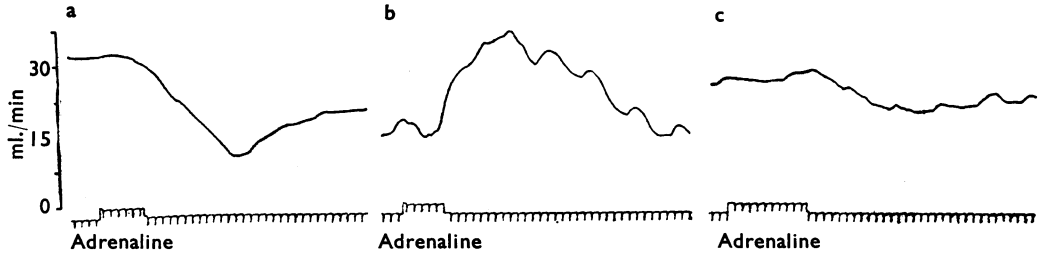

Fig. 4. Dog, 16 kg, pentobarbitone sodium 30 mg/kg, intravenously, bilateral vagotomy, artificial ventilation. Effects of intra-arterial adrenaline (Ad, 0.5 μg/kg) on mean femoral blood flow, during the control period *a*; after phenoxybenzamine (5 mg/kg) *b*; and again after pronethalol (5 mg/kg) *c*; time marks in seconds (speed of the paper, 0.25 cm/sec).

Experiments with 883 F. In some experiments 883 F (5 mg/kg) was injected after establishment of "complete" adrenergic blockade by administration of phenoxybenzamine (5 mg/kg) and pronethalol (5 mg/kg); in all cases the administration of 883 F resulted in a suppression of the anti-adrenolytic action of pronethalol that has already been described. This means that after administration of 883 F adrenaline had no pressor effect on the blood pressure or even elicited a hypotension, and that the intra-arterial injection of adrenaline no longer provoked any vasoconstriction.

Duration of the effect of pronethalol. The above described antagonism by pronethalol of adrenergic blockade caused by phenoxybenzamine, although established immediately after injection, attained maximum intensity about 15 to 20 min thereafter and then slowly declined; 60 to 90 min after injection there was no evidence of any remaining antagonistic effect.

DISCUSSION

The experiments reported in this paper amply confirm the results of Gulati *et al.* (1965) and Moreira & Osswald (1965); in a short report Tuttle (1965) refers to similar results. While these authors state that pronethalol causes the reappearance of the pressor effects of adrenaline (and noradrenaline) after phenoxybenzamine administration, Karim (1964) reported that pronethalol abolishes the vasodepressor response to adrenaline and noradrenaline observed after phenoxybenzamine. However, he used doses of these amines which are too low, in our experience, to induce the reappearance of pressor effects.

This previous work has well established the phenomenon described, but information about the underlying mechanism is still missing. Since we have measured the factors mainly responsible for blood pressure responses to drugs—i.e., heart rate and contractile force and peripheral resistances (in a selected vascular bed)—our results appear to offer an explanation for the observed antagonism between phenoxybenzamine and pronethalol. First of all, the return of the pressor response to adrenaline could be mediated through an increased cardiac action working against an increased peripheral resistance, as has been suggested by Levy & Ahlquist (1957), Sutherland, Ahlquist & Ogden (1964) and Levy (1964) when discussing similar effects of other drugs, mainly vasoconstrictors. However, it is well known that pronethalol effectively antagonizes the inotropic and chronotropic actions of catecholamines (see for example Black & Stephenson, 1962; Koch-Weser, 1964; Wislicki, 1964), and our results show that previous administration of phenoxybenzamine does not interfere with this blocking action of pronethalol. Only very high doses of adrenaline (20 μg/kg) could partially break through the blockade established by pronethalol, causing moderate increases in heart rate and contractile force, and for the hypertension induced by these high doses changes in cardiac activity cannot be excluded. On the other hand, pronethalol exhibits a vasodilator and hypotensive action and in no instance did it, in our experience, increase peripheral resistances, even when administered after phenoxybenzamine; in this connexion we cannot confirm the statement of Gulati *et al.* (1965) that pronethalol causes the blood pressure, lowered by phenoxybenzamine, to rise to values only slightly below the original level.

The other pertinent point of action of the drugs, the peripheral vascular smooth muscle, suggested by the shape of the pressor response to adrenaline, observed after both phenoxybenzamine and pronethalol (gradual increase in systolic and diastolic pressures

with reduction of the pulse pressure), has been confirmed by the experiments in which intra-femoral arterial injections of adrenaline were given. The vasodilatation due to adrenaline after phenoxybenzamine was converted by pronethalol to a vasoconstriction. These results are evidence for the vascular site of action suggested by Moreira & Osswald (1965).

The way in which pronethalol is able to cause vascular smooth muscles to contract in response to adrenaline, although phenoxybenzamine (or its active metabolites) is present, is obviously open to discussion. A direct deblocking effect of pronethalol on α-receptors occupied by phenoxybenzamine (or its metabolites) with removal of the α-blocking drug from its reactive sites would make the receptors once more available to adrenaline. Such a mechanism has been postulated by Gulati *et al.* (1965) on the basis of experiments with the rabbit aortic strip. However, the adrenergic blocking agent used in their experiments was phentolamine, and we think it unwise to extend results with this competitive antagonist of adrenaline to phenoxybenzamine, a drug causing a non-equilibrium (*unsurmontable*) blockade of α-receptors. Moreover, if such a deblocking action is general, adrenaline should regain entirely or almost entirely its pressor and vasoconstrictor effects; as described above, this never happened. To explain this discrepancy, Gulati *et al.* (1965) invoked a weak α-blocking effect of pronethalol, a possibility not ruled out by our experiments but in disagreement with previous results with pronethalol. Waelen, Sonneville, Ariëns & Simonis (1964) have shown that pronethalol does not modify the vasoconstrictor effects of intra-arterially injected adrenaline and noradrenaline, and Honkomp & Buckley (1964) found that in the strongly concentrated nictitating membrane adrenaline causes a relaxation which is converted to a contraction by pronethalol.

An alternative explanation, which seems to fit the facts better, rests upon the hypothesis presented some years ago by Nickerson, Henry & Nomaguchi (1953). These authors concluded from their results that reversal of the actions of adrenaline occurs when up to 50% of the α-receptors in vascular smooth muscles are blocked by β-haloakylamines. The fact that "complete" blockade by dibenamine or phenoxybenzamine can be reinforced by ergot alkaloids, phentolamine, piperoxane, benzodioxane and other adrenergic blocking drugs (Osswald, 1960) represents further evidence for this assumption. If this is correct, the action of adrenaline is reversed because the β-receptors are more numerous than the spare α-receptors and/or because the amine has a lower affinity for the latter. Under these circumstances, pronethalol would effectively block the β-receptors, and the injected adrenaline could only react with the α-receptors not occupied by phenoxybenzamine; only a limited number of α-receptors would be available for interaction with adrenaline. Our results seem to support this speculation. Firstly, catecholamines never reacquired their full pressor or vasoconstrictor effects; secondly, and this point seems most important, the benzodioxane compound 883 F regularly abolished the reversal induced by pronethalol, which strongly suggests that it occupied the α-receptors spared by phenoxybenzamine.

SUMMARY

1. The mechanism of the reversal by pronethalol of the pressor response to adrenaline after phenoxybenzamine has been investigated in the anaesthetized dog by recording

simultaneously changes in blood pressure, heart rate and contractile force and femoral arterial blood flow during the interaction of adrenaline, phenoxybenzamine, pronethalol and benzodioxane.

2. Phenoxybenzamine reversed the blood pressure response, abolished the decrease in femoral blood flow and had no blocking effect on the increase in heart rate and contractile force due to adrenaline.

3. Pronethalol, injected during blockade by phenoxybenzamine, caused adrenaline to reacquire partially its pressor effects and abolished the actions on the heart.

4. The vasoconstriction provoked by intra-arterial injection of adrenaline was converted to vasodilatation by phenoxybenzamine and again to vasoconstriction by pronethalol, although without reacquiring its former magnitude.

5. Pronethalol-induced return of the pressor and vasoconstrictor effects of adrenaline was abolished by benzodioxane (883 F).

6. The influence of phenoxybenzamine and pronethalol on the cardiovascular actions of noradrenaline and isoprenaline is also described.

7. It is concluded that pronethalol reduces the effects of the α-receptor blockade due to phenoxybenzamine by an action on vascular smooth muscle.

8. It is suggested that pronethalol by blocking β-receptors makes available for interaction with adrenaline (and noradrenaline) the α-receptors assumed to have been spared by phenoxybenzamine.

This investigation was supported by a grant (No. 178) from the Research Council of the Scientific Affairs Division of the North Atlantic Treaty Organization. We are grateful to Dr. J. C. A. Langtry, Medical Department, Imperial Chemical Industries, Ltd., Alderley Park, Macclesfield, Cheshire, for the pronethalol (Alderlin), and to C. H. Boehringer Sohn, Ingelheim, Germany, for the isoprenaline used for this study.

REFERENCES

BLACK, J. W. & STEPHENSON, J. S. (1962). Pharmacology of a new adrenergic beta-receptor-blocking compound (Nethalide). *Lancet*, **ii**, 311–314.

ELTHERINGTON, L. G. & HORITA, A. (1960). Interactions of dibenzyline and DCI on the cardiovascular system and nictitating membrane. *Fedn Proc.*, **19**, 122.

GOKHALE, S. D., GULATI, O. D. & PARIKH, H. M. (1964). An investigation of the adrenergic blocking action of chlorpromazine. *Br. J. Pharmac. Chemother.*, **23**, 508–520.

GULATI, O. D., GOKHALE, S. D. & UDWADIA, B. P. (1965). Antagonism of adrenergic blockade by pronethalol. *Archs int. Pharmacodyn. Thér.*, **156**, 389–397.

HONKOMP, L. J. & BUCKLEY, J. P. (1964). Further evidence for beta-receptors in the cat nictitating membrane. *Fedn Proc.*, **23**, 125.

KARIM, S. M. M. (1964). The mechanism of the depressor action of noradrenaline in the cat. *Br. J. Pharmac. Chemother.*, **23**, 592–599.

KOCH-WESER, J. (1964). Direct and beta adrenergic receptor blocking actions of nethalide on isolated heart muscle. *J. Pharmac. exp. Ther.*, **146**, 318–326.

LEVY, B. (1964). Alterations of adrenergic responses by N-isopropyl-methoxamine. *J. Pharmac. exp. Ther.*, **146**, 129–138.

LEVY, B. & AHLQUIST, R. P. (1957). Inhibition of the adrenergic depressor response. *J. Pharmac. exp. Ther.*, **121**, 414–420.

MOREIRA, M. G. & OSSWALD, W. (1965). Pronethalol-induced reversal of adrenergic vasodepression. *Nature, Lond.*, **208**, 1006–1007.

NICKERSON, M. & CHAN, G. C.-M. (1961). Blockade of responses of isolated myocardium to epinephrine. *J. Pharmac. exp. Ther.*, **133**, 186–191.

NICKERSON, M., HENRY, J. W. & NOMAGUCHI, G. M. (1953). Blockade of responses to epinephrine and norepinephrine by dibenamine congeners. *J. Pharmac. exp. Ther.*, **107**, 300–309.

OSSWALD, W. (1960). Reversal of adrenergic vasodepression. *Archs int. Pharmacodyn Thér.*, **126**, 346–358.

SUTHERLAND, J. H. R., AHLQUIST, R. P. & OGDEN, W. S. (1964). The mechanism of the cardiovascular action of isopropylmethoxamine. *Fedn Proc.*, **23**, 124.

TUTTLE, R. S. (1965). Reciprocal antagonism of dibenzyline and nethalide. *Fedn Proc.*, **24**, 712.

WAFLEN, M. J. G. A., SONNEVILLE, P. F., ARIËNS, E. J. & SIMONIS, A. M. (1964). The pharmacology of catecholamines and their derivatives. Part II: An analysis of the actions on blood flow and oxygen exchange in skin and muscle. *Arzneim.-Forsch.*, **14**, 11–19.

WISLICKI, L. (1964). The effect of pronethalol on rabbit auricles and its interactions with sympathomimetic amines. *Archs int. Pharmacodyn. Thér.*, **152**, 69–78.