RESEARCH PAPERS

EFFECT OF PRONETHALOL ON SOME INHIBITORY ACTIONS OF CATECHOLAMINES

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On the blood pressure of the pithed rat isoprenaline produced a vasodepressor, noradrenaline a pressor, and adrenaline a biphasic response consisting of a rise followed by a fall in the pressure. The magnitude of the responses to increasing doses of adrenaline was not proportional to the doses. An intravenous injection of pronethalol (nethalide) hydrochloride (1 mg./kg.) changed the responses to adrenaline into purely pressor, well-graded responses; they were also increased. The responses to isoprenaline after pronethalol were reduced, but those to noradrenaline remained usually unaltered. Similar results were obtained in anaesthetised rats, rabbits and guinea-pigs, but the augmentation of the pressor effect of adrenaline was not so marked as in pithed rats. The vasodepressor action of dopamine in rabbits and guinea-pigs was not blocked by pronethalol (2·5 to 10 mg./kg.). The inhibitory actions of adrenaline, noradrenaline and isoprenaline on the isolated rat uterus and rabbit colon were not consistently antagonised by concentrations of pronethalol up to 1·0 μg./ml. In experiments on the rabbit duodenum and ileum, pronethalol, 0·2 to 1·0 μg./ml., reduced the relaxations produced by all three catecholamines. These results are discussed in relation to Ahlquist's theory of a dual adrenergic receptor mechanism.

THE report by Powell and Slater (1958) on the pharmacology of dichloroisoprenaline (DCI) aroused considerable interest in the possibility of blocking those actions of catecholamines, notably the cardiac acceleration, vasodepression and inhibition of smooth muscle, which were not effectively antagonised by the classical anti-adrenaline drugs. Recently, Black and Stephenson (1962) have reported that the compound 2-isopropylamino-1-(2-naphthyl) ethanol hydrochloride (pronethalol, nethalide, Alderlin) possessed similar pharmacological properties to those of dichloroisoprenaline, but was superior in lacking intrinsic sympathomimetic activity.

The classical anti-adrenaline drugs such as the ergot alkaloids, and yohimbine and phenoxybenzamine, can block the pressor effect of noradrenaline and reverse the pressor effect of adrenaline into a vasodepressor effect. This phenomenon of "adrenaline-vasomotor-reversal" was interpreted as consequence of blockage of the excitatory (alpha) effect of adrenaline and an unmasking of the vasodilatation (beta-effect), which is overshadowed by the stronger pressor component under normal circumstances.

The present study was made to see the effect of pronethalol on the responses of the blood pressure of rats, rabbits and guinea-pigs to adrenaline, noradrenaline and isoprenaline. Special attention was given to experiments with atropine-treated, pithed rats, since they are particularly

suitable for studying the peripheral action of drugs. In addition, the influence of pronethalol on the vasodepressor effect of dopamine in rabbits and guinea-pigs (Burn and Rand, 1958) was also investigated. Experiments were also made on isolated smooth muscle preparations of rat uterus and rabbit intestine in an attempt to determine whether pronethalol blocks the inhibitory action of catecholamines on these tissues.

Methods

Albino rats of either sex, 150–300 g. weight, were pretreated with atropine sulphate (2 mg. per rat s.c.), anaesthetised with ether and pithed as described by Shipley and Tilden (1947).

In some experiments rats were anaesthetised with urethane, 0·7 ml. of a 25 per cent solution per 100 g. weight, subcutaneously. The blood pressure was recorded from a carotid artery. A cannula was inserted into the trachea to respire the pithed rats artificially, or to aid respiration in the urethane-anaesthetised animals. Drugs were injected into a cannulated femoral vein and washed in with 0·9 per cent solution of sodium chloride. The total volume injected at one time was 0·3 ml.

Rabbits and guinea-pigs were anaesthetised with 25 per cent solution of urethane; the former were injected with urethane intravenously at a slow rate until pain reflexes were abolished; for guinea-pigs the dose of urethane, injected intraperitoneally, was the same as in rats. The carotid blood pressure was recorded with a conventional mercury manometer. Drugs were administered through a cannulated femoral or jugular vein.

Single horns of the uterus from non-oestrus rats were suspended in De Jalon's solution in a 2 ml. bath. Contractions were induced at regular intervals with carbachol (1 μg./ml.), as described by Gaddum and Lembeck (1949). The inhibitory actions of adrenaline, noradrenaline and isoprenaline were tested by adding each amine to the bath 60 sec. before carbachol. Pronethalol hydrochloride in concentrations of 0·01 to 10·0 μg./ml. was added to the bath 30–60 sec. before catecholamines. In some experiments pronethalol was added to a reservoir of De Jalon's solution, so that the organ could be exposed longer to the action of the drug.

Segments of rabbit duodenum, ileum and terminal colon were suspended in Krebs' solution in a 25 ml. organ bath and gassed with a mixture of 95 per cent O_2 and 5 per cent CO_2. Records were made with a gimbal lever exerting a tension of 0·5 g. and magnifying five times. Pronethalol hydrochloride in concentrations of 0·1 to 10·0 μg./ml. was either added directly to the bath 2–3 min. before adding catecholamines, or was incorporated into the Krebs' solution in the reservoir and allowed to act continuously.

Drugs were dissolved in 0·9 per cent solution of sodium chloride. Stock solutions of (−)-adrenaline base, (−)-noradrenaline bitartrate, (±)-isoprenaline sulphate and dopamine hydrochloride were prepared in 0·01N hydrochloric acid in a concentration of 1 mg./ml. of the base. From these, dilutions were made with 0·9 per cent sodium chloride

solution, containing 10 mg. ascorbic acid per 100 ml. For pithed rats diluted solutions of adrenaline, noradrenaline and isoprenaline as low as 100 ng./ml were prepared, whereas for anaesthetised animals dilutions of 1 μg./ml. or 10 μg./ml. were used. Dopamine was used in concentrations of 100 to 200 μg./ml. The amounts of catecholamines are expressed in terms of their bases. The amounts of pronethalol are given in terms of the hydrochloride.

RESULTS

Observations on Blood Pressure

In pithed rats small doses of adrenaline (1 to 10 ng.) produced usually biphasic responses containing a rapid rise and then a fall in blood pressure (Fig. 1). Occasionally only a fall was observed. The pressor component of the response to adrenaline was not consistently increased with increasing doses. Paradoxically, sometimes a smaller dose provoked a higher rise in blood pressure than did a larger dose (Fig. 1). In contrast, noradrena-

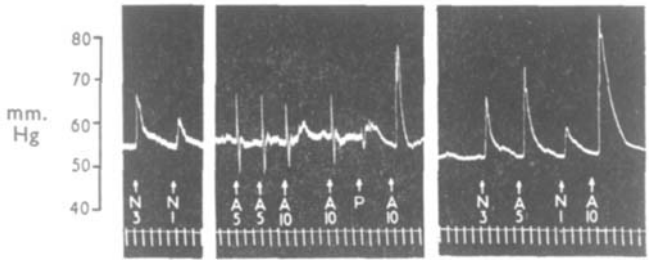

FIG. 1. Carotid blood pressure in mm. Hg of a pithed rat (220 g.) A, adrenaline; N, noradrenaline; doses in ng. P, pronethalol hydrochloride, 1 mg/kg. Between the second and the third record 30 min. elapsed. Time in min.

line always produced a purely pressor response with a good dose-response curve. After repeated injections of adrenaline and noradrenaline, especially after giving larger doses (20 to 30 ng.) the sensitivity of the rat to both amines increased, but the responses to adrenaline remained biphasic and poorly graded.

Isoprenaline caused fall in blood pressure (Fig. 2). The extent and duration of responses were proportional to the doses used.

In anaesthetised rats, rabbits and guinea-pigs, isoprenaline had a depressor effect. Noradrenaline and adrenaline had pressor effects, but with the latter amine, a secondary small fall in the pressure was often seen.

In rabbits and guinea-pigs, dopamine in doses of 10 to 250 μg. always produced depression in blood pressure. Its duration and extent were proportional to the doses used (Fig. 4).

A single injection of pronethalol, 1 mg./kg., in pithed rats caused a transitory small rise (about 10 to 20 mm. Hg) in blood pressure. However, in anaesthetised animals pronethalol always produced a fall of the pressure associated with bradycardia. Pronethalol was administered usually in a single injection following a series of control injections of

adrenaline, noradrenaline, isoprenaline or dopamine. Five or 10 min. after the injection of pronethalol, as soon as the pressure returned to the initial level, the amines were reinjected in the same doses and their effects compared with the controls.

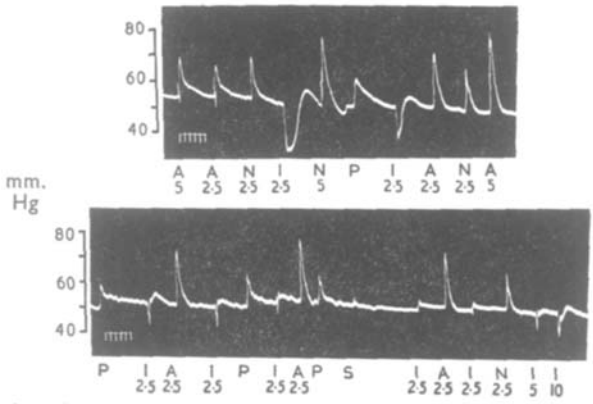

FIG. 2. Carotid blood pressure in mm. Hg. of a pithed rat (245 g.) A, adrenaline; N, noradrenaline; I, isoprenaline; doses in ng. P, pronethalol hydrochloride 1 mg./kg. S, 0·3 ml. of 0·9 per cent solution of sodium chloride. Time in min.

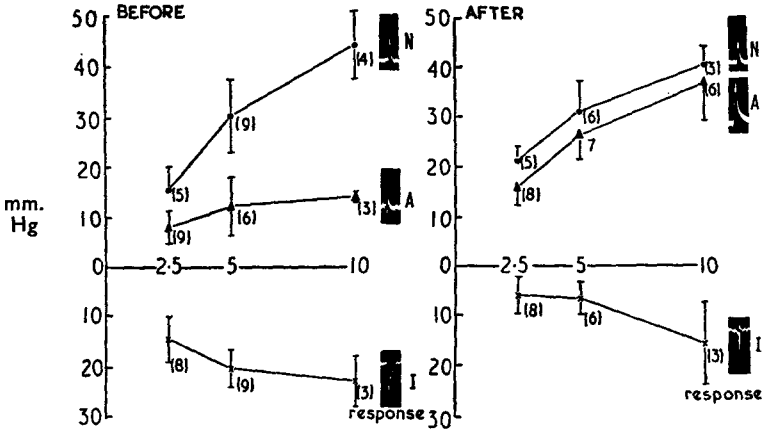

FIG. 3. Dose-response curves of adrenaline (—▲—), noradrenaline (—●—) and isoprenaline (—×—) before and after administration of pronethalol hydrochloride (1 mg./kg.) in 5 rats. Doses on the abscissae (2·5, 5 and 10 ng.); changes in blood pressure are given on the ordinates. Zero-line: initial blood pressure. The type of response to each amine (A, adrenaline, N, noradrenaline, I, isoprenaline) before and after pronethalol is shown on the right hand side of each graph. Note that the flat dose-response curve of adrenaline, on the left, was converted after pronethalol (right) into a steeper curve, parallel with that of noradrenaline. The figures in brackets refer to the number of observations. Each point represents the mean of the observed responses to a given dose. The standard deviations are shown by vertical bars.

Responses after pronethalol. In pithed rats adrenaline produced purely pressor responses, which were much larger than the pressor components of the controls (Fig. 1). A good dose-response relationship was

established and this, together with the increased sensitivity, allowed discrimination of doses differing by 1 or 0·5 ng. The smallest dose of adrenaline to produce a detectable response was often as low as 0·5 ng. This selective potentiation of adrenaline was sometimes so marked that adrenaline became more pressor than an equal dose of noradrenaline.

The responses to noradrenaline were usually unaltered (Fig. 1). In only occasional experiments a slight increase in the responses to this amine was observed. This change, however, may be attributed to the spontaneous increase in the sensitivity of the animal, unrelated to the action of pronethalol.

Pronethalol reduced the depressor action of isoprenaline. For a complete blockade of isoprenaline, however, higher doses of pronethalol than those which potentiated adrenaline, were required. Fig. 2 illustrates an experiment in which the augmentation of the effect of adrenaline was observed after 1 mg./kg. of pronethalol, which was sufficient only to

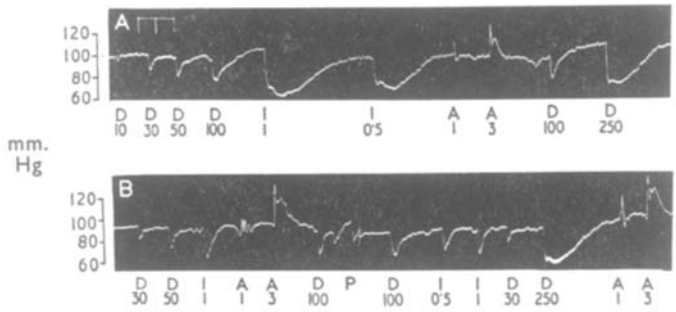

FIG. 4. Carotid blood pressure of a rabbit (2·25 kg.) under urethane-aneasthesia. A, adrenaline; D, dopamine; I, isoprenaline. The numbers denote doses in μg. Between A and B, pronethalol hydrochloride, 2·5 mg./kg., i.v. B taken 10 min. after the injection of pronethalol. At P, an additional dose of pronethalol hydrochloride, 2·5 mg./ kg.,i.v. was given very slowly. At the vertical line the kymograph was stopped for 10 min. Time in min.

reduce the effect of 2·5 ng. of isoprenaline. To block the response to isoprenaline it was necessary to administer a total of 4 mg./kg. of pronethalol. Eventually the effect of isoprenaline was reversed into a small, but definite, increase in blood pressure. Larger doses of isoprenaline (5 and 10 ng.) still caused a slight fall in blood pressure.

Fig. 3 illustrates graphically the responses to adrenaline, noradrenaline and isoprenaline before and after administration of 1 mg./kg. of pronethalol in 5 rats. The dose-response curve and the type of response to adrenaline after pronethalol were changed and became similar to those of noradrenaline. These changes were associated with reduction of the responses to isoprenaline.

Similar observations to those described above were made in anaesthetised animals. Here again, pronethalol in doses of 2·5 to 10 mg./kg. antagonized the effect of isoprenaline. The pressor effect of adrenaline

was increased, although not so much as in pithed rats. Slight enhancement of the responses to noradrenaline was often observed.

The depressor response to dopamine in rabbits and guinea-pigs was not affected by pronethalol in doses of 2·5 to 10 mg./kg. (Fig. 4). The latter dose, however, was always sufficient to reduce or even abolish the responses to equipotent doses of isoprenaline.

Experiments on Isolated Organs

Of eleven uterine horns tested, in only one did pronethalol, in a concentration of 0·1 μg./ml. added to the reservoir, block the effect of adrenaline and isoprenaline (Fig. 5). This block was partially reversible after washing. In all other experiments the concentrations of 0·1 μg./ml. of pronethalol were not sufficient to block adrenaline and isoprenaline. Concentrations of pronethalol above 0·1 μg./ml. caused deterioration of the preparation and the drug itself exerted an inhibitory effect on the carbachol-induced contractions.

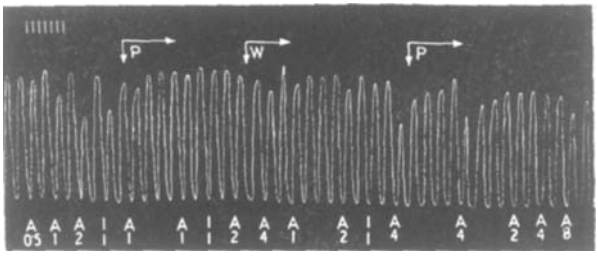

FIG. 5. Contractions of the rat uterus elicited by carbachol (1 μg./ml.). A, adrenaline; I, isoprenaline; the doses are given in ng. P, pronethalol hydrochloride 0·1 μg./ml. from a reservoir with De Jalon's solution. W, washing, change to normal De Jalon's solution. Time in min.

Concentrations up to 1 μg./ml. of pronethalol were usually ineffective in blocking the relaxations of the rabbit terminal colon produced by adrenaline, noradrenaline and isoprenaline. Concentrations of 1 μg./ml. or more usually caused depression of the tone and reduced the spontaneous contractions of the colon. In only one case pronethalol, added to the bath in a concentration of 0·4 μg./ml. and allowed to act for 3 min., reduced the relaxations produced by adrenaline (0·1 μg./ml.) or isoprenaline (0·1 μg./ml.). In another experiment pronethalol (0·1 μg./ml.), together with phenoxybenzamine (1 μg./ml.), partially blocked the relaxations of the colon induced by adrenaline, noradrenaline or isoprenaline (0·1 μg./ml.).

In the experiments on rabbit duodenum and ileum, pronethalol in concentrations of 0·2 to 1·0 μg./ml. only partially blocked the relaxations induced by adrenaline, noradrenaline or isoprenaline (Fig. 6). Higher concentrations than 1 μg./ml. caused depression of the tone and reduced the frequency of the rhythmic movements of the intestine.

Discussion

The present experiments show that pronethalol selectively potentiates the pressor response to adrenaline presumably by blocking the vasodilator component of its action. Powell and Slater (1958) working with cats, and Outschoorn and Jacob (1960) working with rats, reported blockade of the vasodilator action of adrenaline by dichloroisoprenaline, a drug which has similar pharmacological properties to those of pronethalol. In both species, the pressor activities of adrenaline and noradrenaline after dichloroisoprenaline were enhanced, but in neither a selective potentiation for adrenaline was observed. In experiments on man, Bharadway and Shanks (1962) found that dichloroisoprenaline blocked the vasodilatation in the arm caused by adrenaline. Larger doses of dichloroisoprenaline abolished not only the dilator, but also the constrictor action of adrenaline.

The characteristic effect of pronethalol observed in pithed rats, seems to be explicable in terms of the dual receptor mechanism, proposed by Ahlquist (1948). According to this theory the sympathomimetic amines

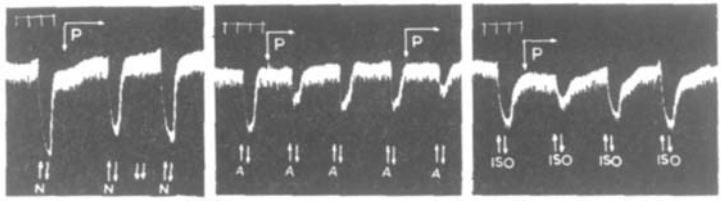

FIG. 6. Rabbit duodenum. First record: effect of pronethalol (P, 0·2 μg./ml.) on the relaxations caused by noradrenaline (N, 0·1 μg./ml.) Middle record: effect of pronethalol (P) on the relaxations caused by adrenaline (A, 0·04 μg./ml.) Left P, pronethalol 0·2 μg./ml.; Right P, pronethalol 0·4 μg./ml. Last record: effect of pronethalol (P, 1 μg./ml.) on the relaxations caused by isoprenaline (ISO, 0·04 μg./ml.) Upward arrows indicate the addition of the amines to the bath; downward arrows indicate washings. Time in min.

exert their action by combining either with alpha- or with beta-receptors, or with both. In agreement with this conception, the purely pressor response to noradrenaline, due to a predominant vasoconstriction, is an alpha-effect, while the depressor action of isoprenaline, a result of vasodilatation, is a beta-effect. Adrenaline appears to possess both effects. If pronethalol blocked the beta-receptors and consequently abolished the vasodilatation, this in turn would enhance the constrictor action, since it is no longer counteracted by the dilator action. Therefore with pronethalol we have another type of "adrenaline-reversal", which is the opposite of that obtained with the classical anti-adrenaline drugs.

The depressor action of dopamine in rabbits and guinea-pigs (Burn and Rand, 1958) does not appear to be a beta-effect, since it was not blocked by pronethalol.

The question of the blockade of the actions of catecholamines on the rabbit intestine and rat uterus is more complex. According to Ahlquist (1962) the rat uterus contains beta-receptors, whereas in the intestine

there are both alpha- and beta-receptors. Furchgott (1959), however, suggested that the receptors in the intestinal smooth muscle were neither alpha nor beta. The present inconsistent and mostly negative results on the isolated preparations of the rat uterus and rabbit intestine with pronethalol, neither support nor contradict the above-mentioned conceptions. One of the difficulties in the assessment of the blocking activity of pronethalol was the fact that it itself exerted an inhibitory action on the uterine and intestinal tissues. Whether it was a genuine sympathomimetic beta-effect or only papaverine-like action, it was not determined. Therefore, for practical reasons, pronethalol is not a suitable agent for blocking the actions of catecholamines on the intestine and uterus.

However, pronethalol appears to be a useful pharmacological tool in the study of other effects of sympathomimetic amines. Vanov and Vogt (1963) have found it useful in the bioassay of adrenaline on pithed rat blood pressure. Black and Stephenson (1962) have considered even the possibility for the therapeutic use of pronethalol in some heart diseases.

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