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THE β-ADRENERGIC BLOCKING AND PRESSOR ACTIONS OF ISOPRENALINE IN THE CAT

BY

K. R. BUTTERWORTH

From the Department of Pharmacology, St. Mary's Hospital Medical School, London, W.2

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In the anaesthetized cat a single, large, intravenous dose of (−)-isoprenaline reversibly blocked the depressor properties of isoprenaline. Further large doses then caused monophasic rises in blood pressure, which were similar in rate of onset and duration to those produced by adrenaline and noradrenaline. This pressor effect was not due to an increase in cardiac output, but the main cause was vasoconstriction in skeletal muscle. The responses to isoprenaline, adrenaline and noradrenaline were compared, both before and after the blocking dose of isoprenaline. Isoprenaline prevented or antagonized the potentiation of the pressor effects of adrenaline and noradrenaline by cocaine. The pressor effect was blocked but not reversed by ergotamine and tolazine, which block sympathetic α-receptors. It is suggested that isoprenaline in large doses has two actions on the vascular system of the cat: a blockade of the sympathetic β-receptors and an excitation of α-receptors.

A reversal of the fall in blood pressure caused by isoprenaline in several species, after the administration of diverse drugs, has been described by a number of authors. For example, this reversal has been obtained after the administration of ergotamine (Hazard, Beauvallet & Guidicelli, 1948), posterior pituitary extract (Hazard *et al.*, 1948; King, 1948) and barium chloride (Woodbury, Braver & Ferguson, 1950). Huidobro, Croxatto, Allende & del Rio (1951) and Walz, Koppanyi & Maengwyn-Davies (1960) have found that a number of sympathomimetic amines reverse the vasomotor response to isoprenaline. One of the most active amines in this respect is phenylephrine (Walz & Maengwyn-Davies, 1960). Such reversals of the effects of catechol amines have been described in other smooth muscle preparations; for example in the isolated aortic strip of the rabbit (Furchgott & Bhadrakom, 1953; Wurzel, Pruss, Koppanyi & Maengwyn-Davies, 1963) and in the isolated uterus of the guinea-pig (Hermansen, 1961).

Coret & van Dyke (1949) showed that large doses of isoprenaline in cats reversibly blocked the depressor response to small doses of isoprenaline. They called this effect "tapenolysis." A similar tapenolytic effect after a slow intravenous infusion of isoprenaline was described by Hermann, Chatonnet & Vial (1954). In 1960, Walz *et al.* gave an intravenous infusion of isoprenaline (about 1 mg/kg) lasting 1 to 2.5 hr into dogs. They found that it not only abolished the depressor effect of isoprenaline but also unmasked a pressor activity to microgram doses of the

drug. Recently Luduena (1962), in a paper on the smooth muscle contracting effects of (−)-isoprenaline, mentioned that he obtained a pure pressor effect from large doses of isoprenaline in two dogs, but he did not investigate this response.

In the course of a study of the relative vasodepressor potencies of the (−)- and (+)-isomers of isoprenaline in cats, it was observed that a pressor response to larger doses of the (−)-isomer could be obtained by the prior administration of a single, large dose of (−)-isoprenaline. This paper describes an investigation of this effect. Part of this work has been summarized elsewhere (Butterworth, 1963).

METHODS

Blood pressure

Seventy-one cats of either sex, weighing between 0.7 and 5.6 kg, were used. The animals were anaesthetized with ether followed by chloralose (60 mg/kg, intravenously) and, except where stated, were premedicated with atropine sulphate (3 mg/kg, subcutaneously). Heparin (1,000 U/kg) was given intravenously. Injections were made into a femoral vein and the blood pressure was recorded by a mercury manometer attached to a cannula in the left carotid artery. A tracheal cannula was inserted and artificial ventilation was given when necessary.

Heart

In some experiments the heart rate was determined by recording the blood pressure on a smoked drum moving at 6 mm/sec and counting the individual beats for 5 sec periods. The amplitude of the beat was recorded by a Cushny myocardiograph which was attached to a Brodie Universal lever.

Blood flow

In the experiments where the blood flow in the right hind-limb was determined, cannulae were inserted into the right femoral vein. The blood flow through the vein was determined by means of a photo-transistor drop-chamber and recorded by a Thorpe impulse counter. Intra-arterial injections were made through fine polyethylene tubing which had been inserted retrogradely into the left femoral artery and passed up to the aortic bifurcation. This ensured the minimal interference with the blood supply to the right hind-limb. All intra-arterial injections of the sympathomimetic amines were made at a constant rate from a micrometer syringe in volumes of 0.01 to 0.05 ml., and control injections of similar volumes of 0.9% saline were made in each experiment.

Chromatography

The isoprenaline samples were examined for contamination by small quantities of other active substances using three paper chromatographic techniques. The first method was that of Shepherd & West (1951), which employs an *n*-butanol:acetic acid:water mixture as the solvent for ascending irrigation and potassium iodate to locate the catechol amines. The second method was that of Crawford & Outschoorn (1951) as modified by Vogt (1952), which employs phenol:hydrochloric acid as the solvent for ascending irrigation. The isoprenaline was applied either as single spots or as a strip along a 15 cm length of the paper (Whatman No. 1). With this method, attempts were made either to reveal other spots on the paper by treatment with potassium ferricyanide or to detect pressor substances after elution of 2.5 cm wide strips along the length of the paper. The eluates were tested biologically on the blood pressure of the cat and fluorimetrically using a semi-automatic method (Burton & Butterworth, 1963). Also, attempts were made to increase the relative concentrations of any contaminant by reapplying regions from a paper which had been run previously, to another paper on to which a second quantity of isoprenaline had been placed. The second paper was run in the usual way and 2.5 cm horizontal strips were eluted and tested as before. The

third method was that of Roberts (1962) which uses cellulose phosphate cation-exchange paper. This Whatman P20 paper was cut into 5×45 cm strips and used for descending irrigation. The solvent was an ammonium acetate:acetic acid:isopropanol mixture and the spots were revealed by ammonia vapour after dipping the papers into an ethylenediamine: acetone mixture. The papers were examined under an ultraviolet lamp. Also, two-dimensional chromatograms were run using ascending or descending irrigation of the 46×46 cm sheets. Throughout these chromatographic investigations, adrenaline and noradrenaline were employed as control substances and were run at the same time, either separately from, or mixed with, the isoprenaline.

Drugs

The isoprenaline was obtained from different sources and batches. Six samples of the (−)-isomer and eleven of the racemic form were employed. Except where otherwise stated the (−)-isomer was used. Throughout this paper, "μg doses" implies doses of 5 μg/kg or less and "mg doses" implies doses of 0.5 mg/kg or more.

1% stock solutions of isoprenaline, adrenaline, noradrenaline, ethylnoradrenaline and tyramine were prepared in 0.1 N-hydrochloric acid and stored at 0 to 2° C. Dilutions of these amines were prepared in 0.9% saline and kept in ice throughout the experiment. Only the (−)-isomers of adrenaline and noradrenaline were used. All doses of the catechol amines are expressed in terms of the bases.

The following other drugs were used: acetylcholine chloride, atropine sulphate, bretylium tosylate (Boura & Green, 1959), chloralose, cocaine hydrochloride, dichloroisoprenaline (Powell & Slater, 1958), ergotamine tartrate, heparin, hexamethonium bromide, histamine acid phosphate, nicotine acid tartrate, posterior pituitary extract, pronethalol [1-(2-naphthyl)-2-*iso*-propylaminoethanol, Alderlin; Black & Stephenson, 1962], reserpine (1 mg/ml.) prepared according to the method of Pletscher, Shore & Brodie (1955) and tolazoline hydrochloride. The doses of the drugs are expressed in terms of their bases and, unless otherwise stated, were administered intravenously.

RESULTS

The production of the pressor effect

The repeated intravenous administration of the same dose of isoprenaline (0.05 to 1.0 μg/kg), given at regular intervals of 2 to 5 min depending on the rate of recovery of the blood pressure, produced depressor responses of similar magnitudes. Initially, as is shown in Fig. 1, the depressor response increased as the dose increased, but eventually a dose was reached which produced no greater fall in blood pressure, although the hypotension lasted longer. Subsequently, "mg doses" of isoprenaline produced a monophasic rise in the blood pressure. If the smaller doses were then repeated no fall was produced, but further pressor responses could be obtained from the larger doses. Fig. 1 (second part) shows that the block of the depressor effects of isoprenaline was reversible and that, when further "mg doses" were given, pressor responses could be obtained again. In later experiments it was found that the first dose of 0.5 to 2 mg/kg of isoprenaline always produced a fall in blood pressure. However, if this dose was followed within a few minutes by a second similar dose, a pressor effect was obtained (Fig. 2). It was only after a long period without further dosage that the pressor response could not be repeated. This single dose method of producing a pressor response to isoprenaline was used in later experiments, and such a dose will be referred to as a "priming dose." Throughout the results described in this paper the "priming dose" was administered intravenously, but it was possible to obtain similar results after giving it intraperitoneally.

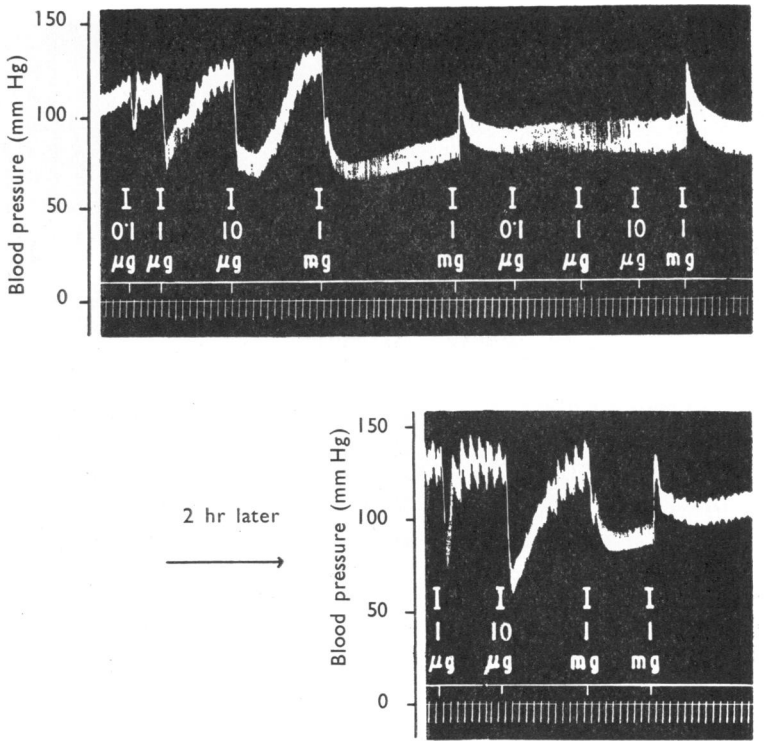

Fig. 1. Cat, 2.0 kg. The effects on the blood pressure (uppermost trace) are shown of successively increasing intravenous doses (values below trace) of isoprenaline (I). Middle trace: Signal mark for injections. Lowest trace: time in 30 sec. The second part of the record was obtained 2 hr after the first and shows that the block of the depressor responses is reversible.

The duration of time for which a pressor response could be obtained after the initial " priming dose " of isoprenaline depended upon the total amount of isoprenaline that had been given after the " priming dose." After only one pressor response, a depressor response to a " mg dose " could be obtained about 30 min later. However, when a number of pressor doses had been given then pressor responses to " mg doses " could be elicited after a rest of several hours without the necessity of giving a further " priming dose." After the " priming dose " of isoprenaline, doses of 2 to 5 μg/kg of ethylnoradrenaline, which previously had been causing depressor responses, gave pressor responses. A pressor response to " mg doses " of isoprenaline could be produced without a " priming dose " being given if large doses of pronethalol (up to 40 mg/kg) were administered. In such experiments the initial pronounced depressor response to isoprenaline was blocked. The amplitudes of the response to acetylcholine, histamine, nicotine and posterior pituitary extract were not modified by the " priming dose " of isoprenaline.

Once the " priming dose " of isoprenaline had been given and the blood pressure had returned to its resting level, a dose/response relationship existed for the subsequent pressor responses to isoprenaline (Fig. 2). This relationship was similar to

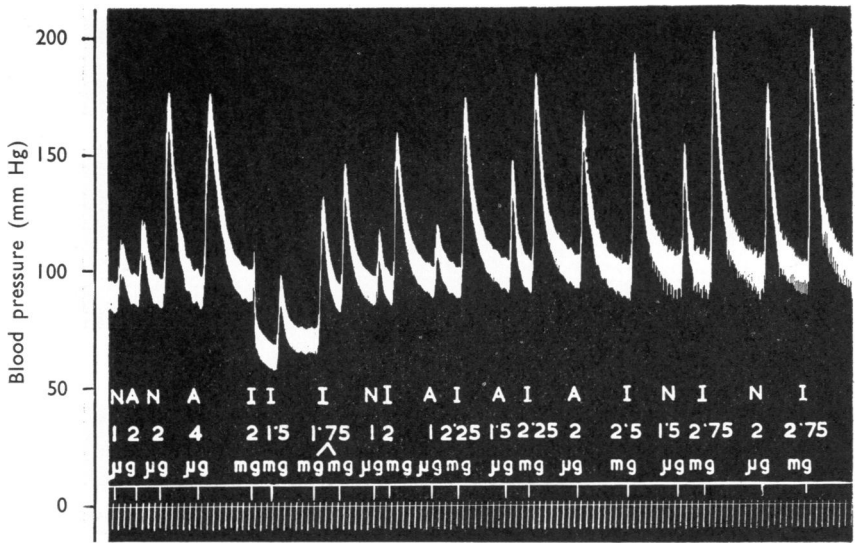

Fig. 2. Cat, 2.2 kg. Dose/response relationships are shown for isoprenaline (I), adrenaline (A) and noradrenaline (N) on the blood pressure after the " priming dose " of isoprenaline (2 mg). Traces as in Fig. 1. The similarity in the general shape of the responses to the three catechol amines may be seen.

that for either adrenaline or noradrenaline, but about 1,000-times the dose of isoprenaline was required to produce a response of a similar magnitude. If the responses of Fig. 2 are plotted graphically, it is found that initially adrenaline was half as active as noradrenaline, but after the " priming dose " of isoprenaline, adrenaline was more active than before, while the noradrenaline sensitivity remained unchanged. Thus the activities of the two drugs became equal. This result has been confirmed in more detailed experiments where the doses were injected randomly according to a Latin Square design. Also, it was found that the dose/response line for the pressor responses to isoprenaline was parallel to those obtained for adrenaline and for noradrenaline, but was different from the line for the depressor responses to isoprenaline. The rate of onset of each pressor response to isoprenaline was as rapid as that for the depressor response. The general shape of the pressor response was similar in rate of onset and duration to those for adrenaline and for noradrenaline. In most experiments there was little or no alteration in the amplitude of the pressor response to repeated doses of isoprenaline. As many as forty successive doses were given. When any variation was seen it was accompanied by a similar alteration in the sensitivities to adrenaline and to noradrenaline. In those few experiments where there was a gradual reduction in the pressor response to isoprenaline, an infusion of noradrenaline did not increase the amplitude of the response.

Attempts were made to obtain pressor responses to doses of isoprenaline of about 0.5 μg/kg, as reported by Walz *et al.* (1960) in dogs. Ten attempts were made using slow infusions of up to 31 mg/kg of isoprenaline, taking up to 4 hr and

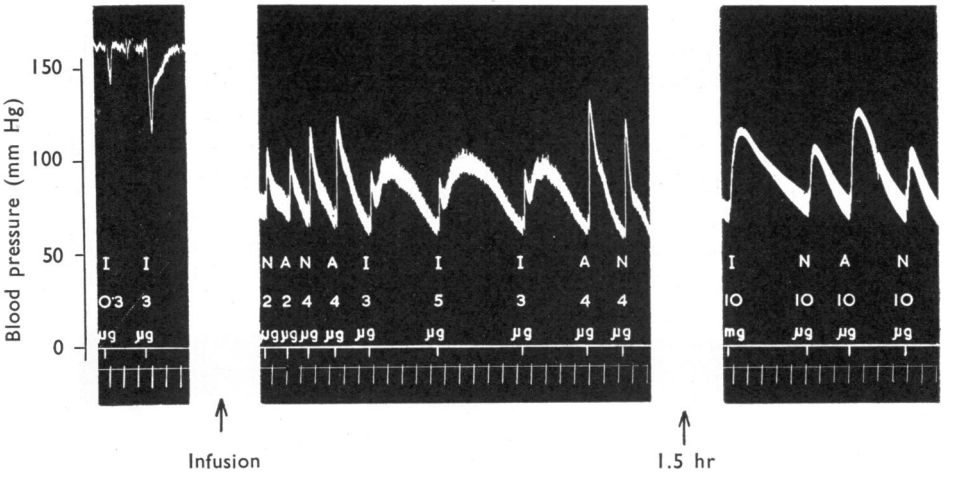

Fig. 3. Cat, 1.2 kg. Traces as in Fig. 1, except that time marks are 1 min. An experiment in which pressor responses were obtained to small doses of isoprenaline (I) after the intravenous infusion (at arrow) of 1.2 mg of isoprenaline (10 μg/ml.) lasting 2 hr. These biphasic responses to isoprenaline differed in shape from the monophasic responses obtained from the intravenous injection of adrenaline (A), noradrenaline (N) and the large doses of isoprenaline. The third part of the record shows the typical pressor response to a large dose of isoprenaline following a gradual loss in sensitivity to "μg doses" of this substance. 1.5 hr between second and third records.

waiting up to 150 min after completion of the infusion, but a pressor response to "μg doses" was obtained in only one experiment. Fig. 3 shows an initial depressor response to 3 μg of isoprenaline. Following the infusion of 1.2 mg of isoprenaline, which took 2 hr, the resting blood pressure was halved. The same dose of isoprenaline now produced a pressor response, but there was a conspicuous difference in the duration and amplitude of this response from those due to adrenaline and to noradrenaline. There was a gradual loss in sensitivity to the small doses of isoprenaline and eventually they became ineffective. When the dose of isoprenaline was increased to the "mg range" then pressor responses, which were different in general shape from the previous responses to isoprenaline, were obtained in the usual way. After eliciting pressor responses to "mg doses" of isoprenaline, attempts were made to obtain responses to doses of isoprenaline in the "μg range," but without success. An alternative method of trying to obtain pressor responses to "μg doses" was to give 0.5 mg/kg as a single rapid injection and then to give "μg doses" of isoprenaline 2 hr later, but no pressor response to these doses was obtained. It was only when "mg doses" were given that pressor responses were obtained.

Modifications of the pressor effect

Making the cats "spinal," bilateral adrenalectomy, hepatectomy, evisceration and artificial ventilation did not modify the pressor response. Block of autonomic ganglionic transmission by hexamethonium (50 to 100 mg/kg), of parasympathetic nervous pathways by atropine (3 mg/kg, subcutaneously) and of the release of the mediator from sympathetic nerves by bretylium (1 to 10 mg/kg) neither blocked

the depressor response to reveal the pressor effect nor modified the pressor response, except possibly to increase it. This increased response was observed with all treatments which tended to lower the resting blood pressure and was due probably to this cause alone.

If cocaine (5 mg/kg, intramuscularly) was administered before the " priming dose " of isoprenaline, it potentiated adrenaline and noradrenaline in the usual way, but on the subsequent administration of the " priming dose " of isoprenaline the amplitudes of the responses to adrenaline and to noradrenaline were reduced, and that to noradrenaline returned to the control level before cocaine (Fig. 4). At this stage

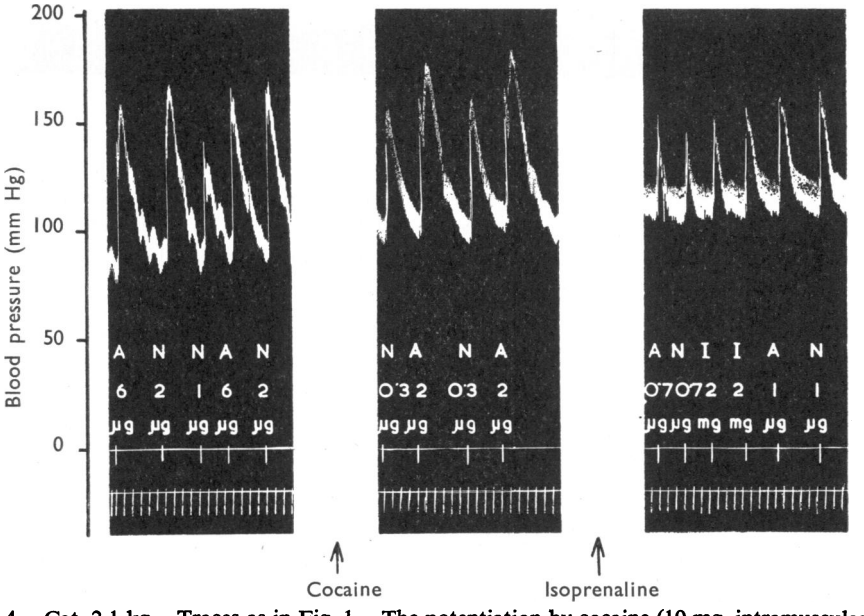

Fig. 4. Cat, 2.1 kg. Traces as in Fig. 1. The potentiation by cocaine (10 mg, intramuscularly, at first arrow) of the effects of adrenaline (A) and noradrenaline (N) on the blood pressure, and the subsequent antagonism of this increased effect by the " priming dose " (3 mg intravenously, at second arrow) of isoprenaline (I) are shown.

of the experiment the ratio of the equipressor doses of adrenaline and noradrenaline was nearer to one than before the administration of cocaine. If cocaine was given after the " priming dose " it did not increase the pressor responses to isoprenaline, adrenaline and noradrenaline (Fig. 5). Sensitivity to tyramine (0.2 to 2.0 mg/kg) was not modified by the " priming dose " of isoprenaline, and the subsequent administration of cocaine blocked the effect of tyramine (Fig. 5). Once the response to tyramine had been blocked by cocaine it could not be restored by further large doses of isoprenaline.

Cats, which had received two intraperitoneal doses of 3 mg/kg of reserpine with a 24 hr interval between the doses, were anaesthetized as described in Methods 24 hr after the second injection. In these animals the pressor response to tyramine was absent, but the depressor and pressor responses to isoprenaline were the same

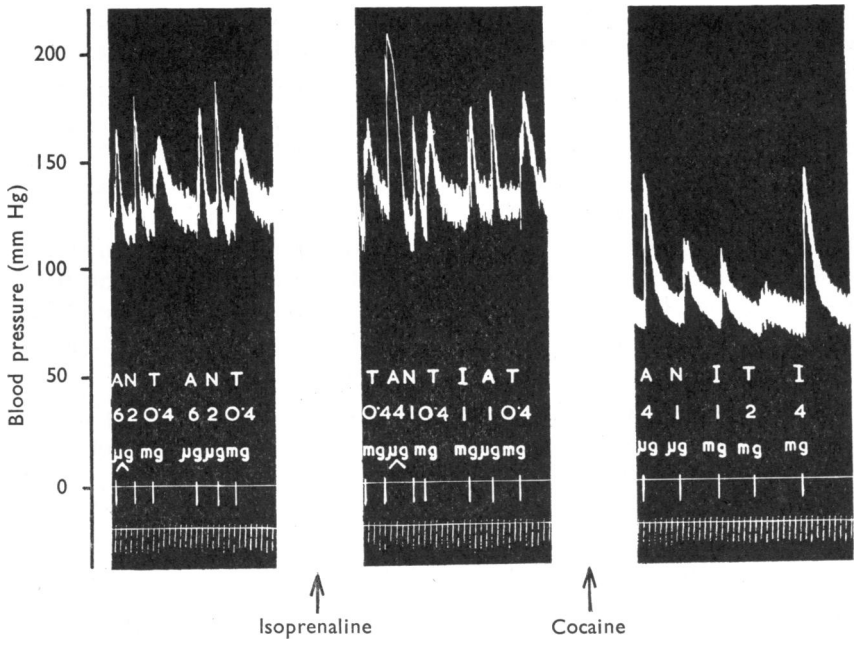

Fig. 5. Cat, 3.0 kg. Traces as in Fig. 1. The effects of the " priming dose " (2 mg, intravenously, at first arrow) of isoprenaline (I) and the subsequent administration of cocaine (10 mg intramuscularly, at second arrow) on the pressor responses to isoprenaline, adrenaline (A), noradrenaline (N) and tyramine (T) are shown.

as those obtained in cats not treated with reserpine. Subsequent doses of adrenaline and noradrenaline gave typical pressor responses.

The pressor responses to isoprenaline, adrenaline and noradrenaline were blocked by ergotamine (0.5 to 1.5 mg/kg) and by tolazoline (1.5 to 15 mg/kg), but, as was expected, no depressor effect from isoprenaline or from adrenaline reappeared. The block due to ergotamine lasted for several hours but that due to tolazoline was of shorter duration. As the block due to tolazoline wore off, the pressor effects of isoprenaline, adrenaline and noradrenaline returned at similar rates. After the " priming dose " of isoprenaline, dichloroisoprenaline (up to 20 mg/kg) or pronethanol (up to 10 mg/kg) did not modify the pressor responses to isoprenaline, adrenaline, noradrenaline or ethylnoradrenaline.

The effect on the heart

In the cat premedicated with atropine, " µg doses " of isoprenaline, adrenaline and noradrenaline caused increases in the heart rate. When the " priming dose " of isoprenaline was given there was an increase in heart rate of as much as 50%. This faster rate was maintained for several hours if further pressor doses of isoprenaline were given. After the " priming dose " the administration of " mg doses " of isoprenaline or of " µg doses " of adrenaline or noradrenaline caused the usual pressor responses but no further change in the heart rate. At this stage no increase in the amplitude of the response, as recorded by the Cushny myocardiograph, was produced

by pressor doses of isoprenaline, adrenaline or noradrenaline. The administration of hexamethonium (50 to 100 mg/kg) did not modify the increased heart rate.

Pronethalol, in doses up to 40 mg/kg, although preventing the increases in rate produced by "μg doses" of isoprenaline, adrenaline or noradrenaline, did not prevent the large increase which was produced by the "priming dose" of isoprenaline. Dichloroisoprenaline itself was able to increase the heart rate. After the "priming dose," dichloroisoprenaline (2.5 to 10 mg/kg) and pronethalol (2.5 to 10 mg/kg) caused small reductions in the elevated heart rate each time a dose of either of these substances was administered. If too large a dose of pronethanol was given the heart fibrillated. Thus pronethalol was given in small, subdivided doses. Although the "priming dose" of isoprenaline had a great stimulant effect on the heart, no toxic effects such as fibrillation were ever seen.

In the cat not treated with atropine the responses were similar to those in the atropinized animal except that the heart rate was slowed at the peak of the pressor responses by vagal inhibition.

The blood flow through the hind-limb

The effects of intra-arterial and intravenous injections of isoprenaline, adrenaline and noradrenaline on the blood flow through the hind-limb were studied in sixteen cats. As has been shown by Bowman (1959a, b) and by others, these effects depended upon the dose of amine administered, the route of injection, the systemic arterial blood pressure and the vasomotor tone.

In Fig. 6, which was from a typical experiment, the intra-arterial administration of 0.4 μg of adrenaline caused weak vasodilatation followed by vasoconstriction, 0.4 μg of noradrenaline caused vasoconstriction and 2 μg of isoprenaline caused a pronounced vasodilatation. The 0.9% saline given as a control had no effect. The volume of this saline was at least as large as the volumes of the other substances administered. After the "priming dose" of 0.5 mg/kg of isoprenaline given intravenously, the intra-arterial administration of 0.4 μg of adrenaline and 0.4 μg of noradrenaline caused only vasoconstriction, 2 μg of isoprenaline and the control saline had no effect, but when larger doses of isoprenaline (0.2 mg) were given vasoconstriction was seen. Vasodilatation could still be produced by histamine. The "priming dose" of isoprenaline was given intravenously since its intra-arterial administration caused some deterioration in the condition of the animal. When the effect of the "priming dose" was wearing off, it was possible to produce first a prolonged dilatation by isoprenaline and then vasoconstriction from subsequent doses (Fig. 7). The results from the intravenous administration of the amines were similar to those obtained using the intra-arterial route, but the results before the "priming dose" were less readily reproducible and seemed to depend particularly upon the variations in blood pressure. As is shown in Fig. 7, cutting the sciatic and femoral nerves after the "priming dose" of isoprenaline increased the resting blood flow. An increase in the effects of all three sympathomimetic amines was then seen, but the pattern of their responses was not altered. The results from experiments where the skin of the limb had been removed did not differ from those with the intact limb.

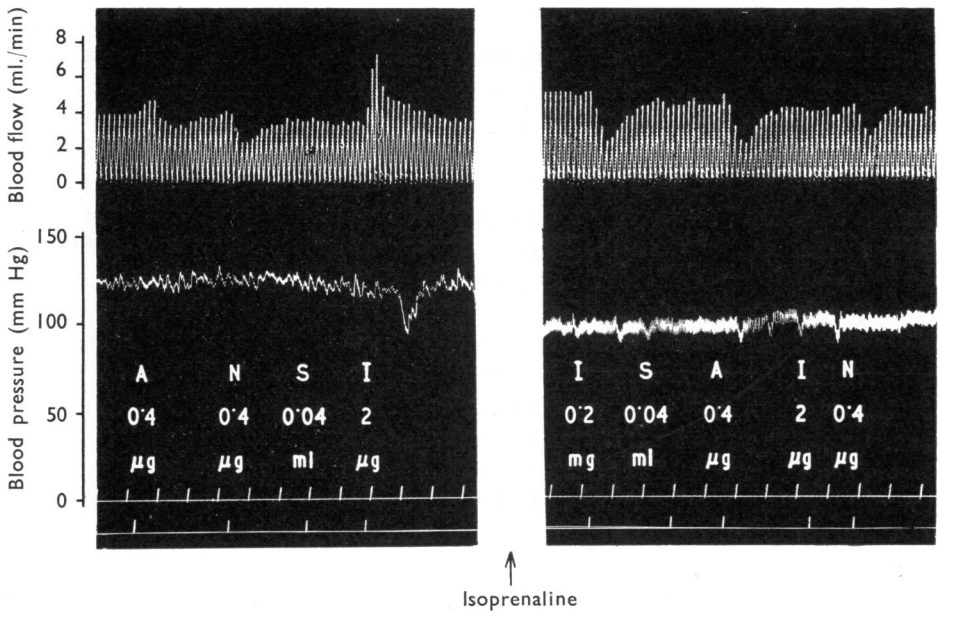

Fig. 6. Cat, 1.2 kg. Traces from above down: hind-limb blood flow, arterial blood pressure, time in 1 min and signal for injections. To show the effect of intra-arterially administered isoprenaline (I), adrenaline (A), noradrenaline (N) and a control of 0.9% saline (S) on the blood flow through the hind-limb and the systemic blood pressure before and after the " priming dose " of isoprenaline (0.6 mg, intravenously, at arrow).

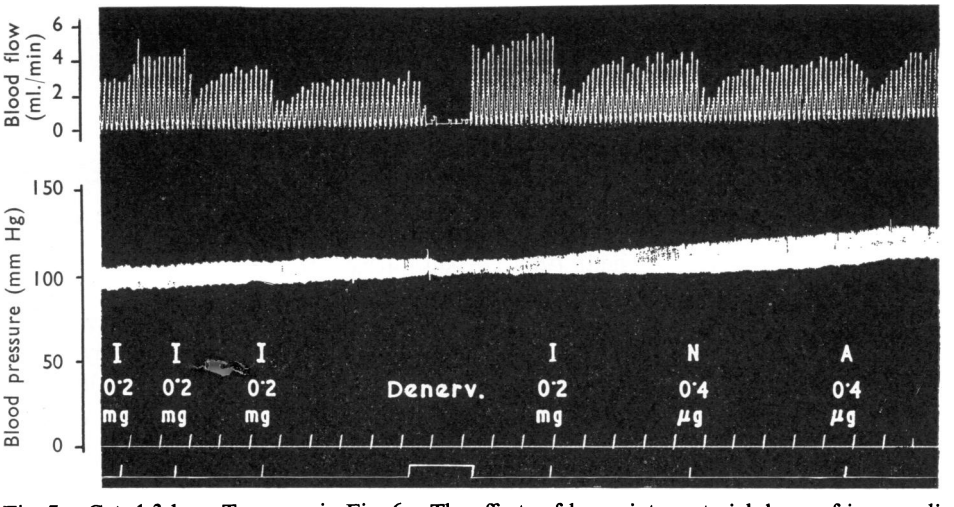

Fig. 7. Cat, 1.3 kg. Traces as in Fig. 6. The effects of large, intra-arterial doses of isoprenaline (I) on the blood flow through the hind-limb are shown in an experiment in which the block due to the " priming dose " of isoprenaline was wearing off. Also, the effects of isoprenaline, adrenaline (A) and noradrenaline (N) after cutting the sciatic and femoral nerves (Denerv.) are shown.

Chromatography

In an attempt to find any contaminant present in the samples which could have been responsible for the pressor activity, three types of chromatographic examination were employed. Quantities of isoprenaline up to 60 mg were placed on the papers. Since no pressor substance was detected either on the papers or after elution of strips of the papers, only the results from the most sensitive method are described. Control samples of adrenaline or noradrenaline in quantities as low as 0.1 μg could be detected on the cellulose phosphate paper, but when adrenaline or noradrenaline was added to the isoprenaline slightly larger concentrations were needed for definite detection. When allowance is made for the minimum sensitivity for detection in particular experiments, then it may be concluded that adrenaline or noradrenaline, if present, occurred in concentrations of less than 0.0008%. From the fluorimetric examination of the eluted samples it may be concluded that if adrenaline or noradrenaline was present it would have had a concentration of less than 0.0001%. To produce the pressor effect described in this paper they needed to be present in about 0.1%. If any other substance of this type were present as a contaminant, it would have to be present in a greater concentration.

DISCUSSION

After the intravenous administration of a large dose of isoprenaline, the depressor effects of small doses of this amine are blocked and further large doses of isoprenaline produce a pressor response. These results may be interpreted in terms of the sympathetic α- and β-receptor theory as postulated by Ahlquist (1948). Usually, when a substance blocks the responses to larger doses of itself it is considered that there is a non-specific desensitization. However, the large doses of isoprenaline also blocked the depressor effects of adrenaline and ethylnoradrenaline. Levy & Ahlquist (1960) have proposed that if a substance blocks the depressor response to isoprenaline and converts the depressor response to ethylnoradrenaline to a pressor one, then it blocks β-receptors. Isoprenaline fulfils these requirements while not preventing the depressor activity of drugs, such as acetylcholine and histamine, which act in other ways. The results described in this paper also indicate that isoprenaline is as active as its dichloro derivative, dichloroisoprenaline, and its naphthyl derivative, pronethalol, in this respect. When such a β-receptor block of responses to isoprenaline had been produced, either by isoprenaline or by pronethalol, it was found that " mg doses " of isoprenaline caused a pressor effect. This could be due to an action on the α-receptors. Such a suggestion is substantiated by the block of the pressor responses to isoprenaline by drugs that block α-receptors in doses which also block the responses to adrenaline and noradrenaline.

It was thought that any modification of the response to isoprenaline by the administration of cocaine might help in the elucidation of the mechanism of the pressor effect of isoprenaline. The " priming dose " of isoprenaline either prevented or caused the disappearance of the potentiation of adrenaline and noradrenaline by cocaine, and thus it was reasoned that the isoprenaline may be competing with cocaine at the site of action of cocaine. Therefore attempts were made to restore

the response to tyramine, which had been blocked by cocaine, by the administration of isoprenaline. However, no pressor response to tyramine returned. On the administration of the " priming dose " of isoprenaline after cocaine, the amplitudes of the increased responses to adrenaline and to noradrenaline were reduced, and that to noradrenaline returned to the control level before cocaine. The degree of reduction in the response to adrenaline was variable, possibly depending upon the initial degree of activity of adrenaline on the β-receptors, which now had been blocked by the large dose of isoprenaline. From these experiments it may be inferred that isoprenaline is interfering with the potentiating properties of cocaine and also that the mechanism by which it produces a pressor response could be similar to that for adrenaline and noradrenaline.

The evidence for concluding that the pressor response to isoprenaline cannot be due to small quantities of an active impurity such as noradrenaline is as follows. Firstly, the presence of another substance could not be demonstrated chromatographically. Secondly, when allowance is made for the difference in potency between the racemic drug and the (−)-isomer, then seventeen samples of isoprenaline from different sources and batches obtained over a period of 6 years produced the same effect in the same dose. Thirdly, no sample failed to produce the pressor effect. Fourthly, the route of chemical synthesis known to have been employed for the majority of samples was such that the only impurities that could theoretically be present are substances which are pharmacologically inactive in the concentrations employed. Fifthly, the values for the melting points and optical rotations of the (−)-isomers were within the ranges given by Lands, Ludena & Tullar (1954) for a chemically pure sample of isoprenaline. For the above reasons it is concluded that the pressor activity was due to the isoprenaline itself and not to any impurity. If any impurity were present, it occurred in such a low concentration that it would be necessary for it to have a degree of activity on the cat blood pressure far in excess of that of adrenaline or noradrenaline.

The vasopressor effect of isoprenaline cannot be due to the release of catechol amines from chromaffin stores for the following reasons. In a cat treated with reserpine where the stores had been depleted, the response to tyramine was absent but the response to isoprenaline was normal. The pressor response to isoprenaline was not blocked by cocaine as was the response to tyramine. On those occasions when the sensitivity to isoprenaline was decreasing, an infusion of noradrenaline did not increase the depressed response. The general shape of the pressor response to isoprenaline was similar in rate of onset and duration to that of injected adrenaline or noradrenaline, rather than to the slower and more prolonged response due to release from catechol amine stores.

The " priming dose " of isoprenaline caused an increase of as much as 50% in the resting heart rate and, after this " priming dose," no changes in the elevated rate were produced by isoprenaline, adrenaline or noradrenaline. Also these drugs did not increase the amplitude of the heart contractions as recorded by the myocardiograph. These latter two facts are consistent with the idea that the pressor responses to isoprenaline were not due to an increase in the cardiac output. It is probable that after the " priming dose " of isoprenaline, the heart was beating at

the maximal rate of which it was capable and that this effect was not reduced by parasympathetic control. This latter point is confirmed by the pronounced vagal inhibition seen in the non-atropinized animal when there was a rise in blood pressure caused by the subsequent administration of the sympathomimetic amines. Since hexamethonium did not modify the heart rate, it is unlikely that the increase in heart rate and amplitude was central in origin. The high rate may be due to the isoprenaline first stimulating the sympathetic β-receptors of the heart before blocking them to the catechol amines. In the cat premedicated with atropine it may be presumed that the heart has been chemically denervated after the " priming dose " of isoprenaline.

It is considered that the effect described in this paper is different from that reported by Walz *et al.* (1960) for the following reasons. There is at least a 1,000-fold difference in the doses required to produce a depressor and a pressor response, whereas Walz *et al.* (1960) employed the same " μg dose " for both types of responses. There was no increase in the heart rate when the pressor " mg doses " were given, but according to these authors the effect of " μg doses " is accompanied by an increase in the heart rate, although they do not consider this to be the main cause of the pressor effect. Levy & Ahlquist (1961) performed similar experiments and considered that the pressor response to isoprenaline following phenylephrine is due to cardiac stimulation in the presence of a constricted vascular bed. They also found that the effect was blocked by dichloroisoprenaline, in disagreement with Walz *et al.* (1960), and that it could not be produced after the prior administration of this drug. The pressor effect from " mg doses " of isoprenaline described in this paper is not blocked by dichloroisoprenaline and can be produced after the previous administration of dichloroisoprenaline. It is blocked, although not reversed, by the drugs, ergotamine and tolazoline, which block α-receptors. Another piece of evidence is that the effect of " mg doses " is not increased by cocaine, in contrast to the effect of the " μg doses " described by Walz *et al.* (1960).

Attempts were made to obtain a pressor response to " μg doses " of isoprenaline, as has been obtained by Walz *et al.* (1960) in dogs, but in only one experiment did this occur. Normally the general shape of the response to " mg doses " of isoprenaline is similar to those of adrenaline and of noradrenaline, but in this experiment the pressor response to " μg doses " was biphasic and it is possible that in some way the secondary rise in pressure was due to stimulation of the sympathetic nervous system, in particular the adrenal medullae. Later in the experiment, " mg doses " of isoprenaline were given and the shape of these pressor responses was similar to those due to adrenaline and noradrenaline. Since this pressor response to " μg doses " could not be obtained in other experiments it was not possible to study it further.

It is obvious that the pressor effect of isoprenaline described in this paper is the same as that seen by Luduena (1962) in dogs. He found that a dose of 10 mg/kg produced a fall of pressure in one dog, a rise followed by a fall in two and only a rise in two others. If he had investigated this effect it is probable that by repeating his doses several times he would have obtained pure pressor responses in each of the dogs.

The " priming dose " of isoprenaline increased the blood pressure response to adrenaline when the ratio of the equipressor doses of adrenaline to noradrenaline was greater than one, thus bringing this ratio towards one. Usually such an effect can be seen in a cat that has been anaesthetized for some hours, particularly in those experiments where the resting blood pressure gradually falls, but this isoprenaline effect was immediate. This result may be interpreted as due to the block of the effects of adrenaline on β-receptors, leaving only its actions on α-receptors. After the " priming dose " of isoprenaline, the dose/response curves for the pressor responses to isoprenaline, adrenaline and noradrenaline were parallel, which may imply that the three amines were acting on the same receptors.

Experiments were performed to determine the site of action and the mode of production of the vasopressor reversal due to isoprenaline. The effect was not due to an action on the heart, as has been discussed previously. Thus it is likely to be due to an action on various vascular beds. Since the effect is still present after evisceration, the blood flow through the skinned and unskinned hind-limb was investigated and it was shown that the main site of action is probably the blood vessels of skeletal muscle. Neither making the cats " spinal," nor hepatectomy, nor adrenalectomy modified the response and, as has been discussed previously, the response was not due to the release of catechol amines from chromaffin stores or to the contamination of the isoprenaline by small quantities of catechol amines. The vasoconstriction produced by the large doses of isoprenaline was still present in the hind-limb when the femoral and sciatic nerves were cut high up in the limb. Also the response was still present after the administration of hexamethonium, atropine or dichloroisoprenaline. The administration of tolazoline, which blocks α-receptors, blocked the vasoconstrictor effects but no vasodilator component was revealed. However, the latter effect was not expected because of the prior administration of a dose of isoprenaline which would block β-receptors. Posterior pituitary extract was still able to cause vasoconstriction. Thus it is probable that the pressor response to large intravenous doses of isoprenaline in the cat is produced mainly by a vasoconstriction of the blood vessels of skeletal muscle, due to an action on sympathetic α-receptors.

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