Brit. J. Pharmacol. (1965), **24**, 449 -454

PRESSOR RESPONSE TO ACETYLCHOLINE AND THE EFFECTS OF *N*-DIETHYLAMINOETHYL-*N*-ISOPENTYL-*N*^{1} *N*^{1}-DI-ISOPROPYLUREA

BY

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(*Received July* 28, 1964)

Although it has been known for many years that the injection of acetylcholine into an atropinized dog results in a biphasic rise in blood pressure, the interpretation of this phenomenon is still a subject of controversy. According to one interpretation, the first component of the biphasic rise is attributed to the nicotinic effect of acetylcholine on sympathetic ganglia while the second component is ascribed to acetylcholine-stimulated release of catechol amines from the adrenal medulla (Goodman & Gilman, 1955). This interpretation has recently been challenged by Gardier, Abreu, Richards & Herrlich (1960) and by Gardier, James, Johnson, Richards & Roesch (1963), who described observations made with acutely adrenalectomized dogs and with dogs treated with P-286 (*N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-di-isopropylurea) which led them to suggest that the adrenal medulla is the only portion of the sympathetic nervous system involved in the rise in pressure. They conclude, " The pressor response was attributed solely to the release of adrenaline from the adrenal medulla," and further, " The pressor response to acetylcholine in the atropinized dog resulted from an increase in cardiac output." The studies reported here resolve this controversy in favour of the original interpretation offered by Goodman & Gilman (1955).

METHODS

Male or female adult mongrel dogs were anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously); small supplementary doses were administered when necessary. Arterial blood pressure was measured by inserting a cannula into either a carotid or a femoral artery and connecting the cannula to a mercury manometer. Blood pressure was recorded on a kymograph. Drugs were injected into a cannula inserted into a femoral vein and flushed in with 6 ml. of heparinized saline. Drug dosages are expressed in terms of the salts used. For intra-aortic injection of acetylcholine bromide, a cannula was inserted through the left femoral artery into the thoracic aorta so that it was located approximately midway between the renal arteries and the top of the aortic arch (the position of the cannula was always checked by *post mortem* examination). For intraventricular injection of acetylcholine, a cannula was inserted through the right carotid artery into the left ventricle. Bilateral adrenalectomy was carried out through a single midline incision and its completeness was checked by examination to ensure that the capsule of the excised adrenal gland was intact. The biphasic pressor response to acetylcholine was elicited in all instances by the following sequence of intravenous drug injections: atropine sulphate (5 mg/kg), physostigmine sulphate (1 mg/kg) and acetylcholine bromide (100 μg/kg).

RESULTS

The effects of sympathetic α- and β-receptor blocking agents

Administration of pronethalol (2.5 mg/kg) resulted in elimination of the primary phase of the biphasic blood pressure response in the atropinized dog and enhancement of the secondary phase both in amplitude and duration (compare Fig. 1,*c* with 1,*b*). Subsequent repeated administration of tolazoline progressively diminished the remaining secondary response so that, after 3 mg/kg, it was nearly obliterated (Fig. 1,*e*). Pronethalol has been

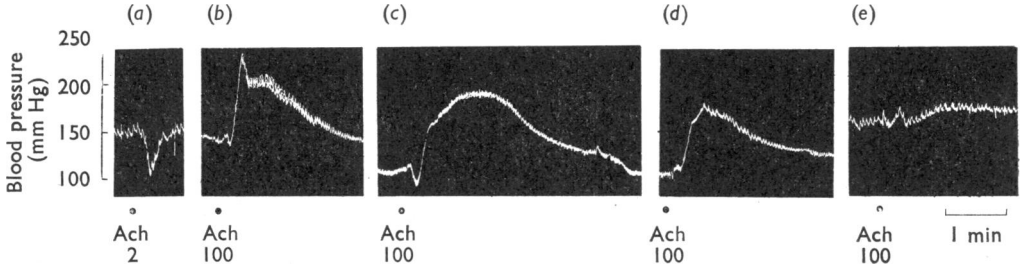

Fig. 1. Blood pressure recorded from the carotid artery of a dog. All injections intravenously. (*a*) At black dot, acetylcholine (Ach, 2 μg/kg). Between (*a*) and (*b*), atropine (5 mg/kg) and physostigmine (1 mg/kg). At all subsequent black dots, acetylcholine (100 μg/kg). Between (*b*) and (*c*), pronethalol (2.5 mg/kg). Just before (*d*), tolazoline hydrochloride (0.5 mg/kg). Between (*d*) and (*e*), tolazoline (2.5 mg/kg).

shown to inhibit actions of catechol amines at β-receptors in the dog (Black & Stephenson, 1962; Donald, Kvale & Shepherd, 1964), while tolazoline and phenoxybenzamine are inhibitors of the actions of catechol amines at α-receptors (Goodman & Gilman, 1955). These results therefore suggest that the primary pressor response is a consequence of actions on β-receptors (for example in the heart) while the secondary pressor response is the result of the algebraic addition of actions on α-pressor and β-depressor receptors. These suggestions were substantiated by further studies in which α-blocking agents were given first.

In another experiment, phenoxybenzamine (5 mg/kg) was administered, followed 30 min later by acetylcholine (100 μg/kg). The first component of the biphasic rise in blood pressure (Fig. 2,*a*) was reduced in intensity and the secondary component was reversed (Fig. 2,*b*);

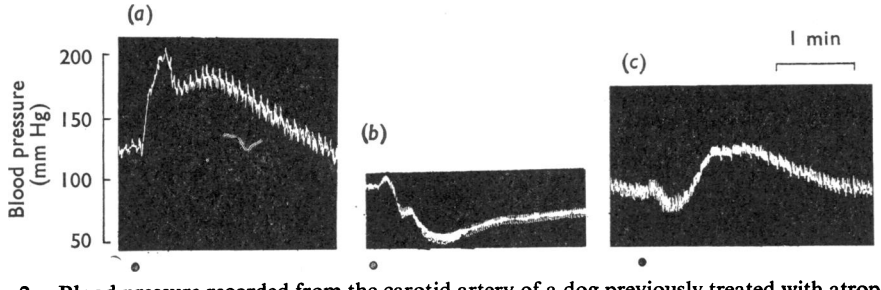

Fig. 2. Blood pressure recorded from the carotid artery of a dog previously treated with atropine sulphate (5 mg/kg) and physostigmine sulphate (1 mg/kg). At each black dot, acetylcholine (100 μg/kg). Between (*a*) and (*b*), phenoxybenzamine (5 mg/kg). Between (*b*) and (*c*), pronethalol (4 mg/kg). All injections intravenously.

a marked reduction in blood pressure resulted. Pronethalol (4 mg/kg) was then administered and followed by acetylcholine (100 μg/kg) (Fig. 2,*c*). The primary response may have been further inhibited while the secondary response was partly restored. Since phenoxybenzamine has quinidine-like depressant effects in the heart (Nickerson & Chan, 1961), it was difficult to decide whether the depression of the primary response following phenoxybenzamine was the result of selective blockade of α-receptors (in contradiction to the results given above) or of nonspecific cardiac depression.

In the next experiment (Fig. 3), tolazoline was substituted for phenoxybenzamine. Increasing doses of tolazoline (*c* and *d*) caused increasing inhibition of the secondary pressor response. Seeming depression of the primary pressor response could be attributed to inhibition of the secondary response which begins before the completion of the primary response. Pronethalol (4 mg/kg) inhibited the residual primary response (Fig. 3,*e*). These results substantiate the previous suggestion that the primary pressor response involves actions at β-receptors, whereas the secondary pressor response involves actions at α-receptors which are opposed by simultaneous depressor actions at β-receptors.

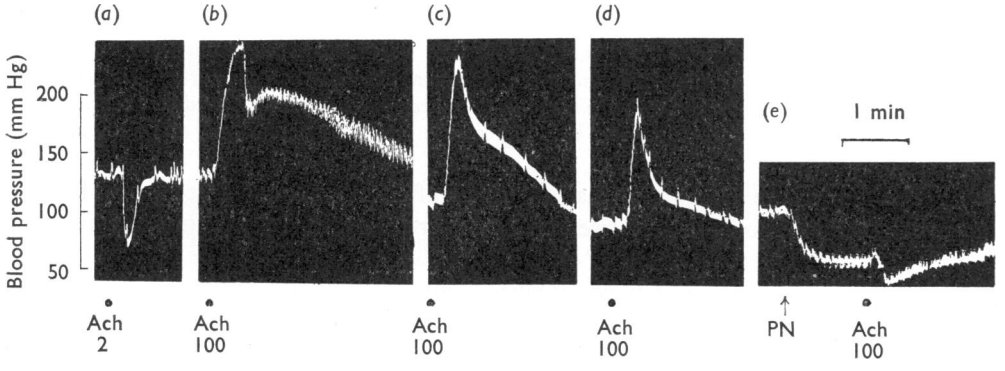

Fig. 3. Blood pressure recorded from the carotid artery of a dog. All injections intravenously. (*a*) At black dot, acetylcholine (Ach, 2 μg/kg). Between (*a*) and (*b*), atropine (5 mg/kg) and physostigmine (1 mg/kg). At all subsequent black dots, acetylcholine (100 μg/kg). Just before (*c*), tolazoline (2 mg/kg). Just before (*d*), tolazoline (4 mg/kg). (*e*) At arrow, pronethalol (PN) (4 mg/kg).

The effects of intravenous, intra-aortic and intraventricular infusion of acetylcholine

Injection of acetylcholine (100 μg/kg) into the aorta, through a cannula in the femoral artery, produced a monophasic rise in blood pressure (Fig. 4,*a*, right) in contrast to the biphasic rise produced by intravenous injection (Fig. 4,*a*, left). To compensate partially for the dilution of intravenous acetylcholine by blood before it reached the adrenal gland, the acetylcholine solution was diluted four-times with heparinized saline before intra-aortic injection. *Post mortem* examination revealed the end of the intra-arterial cannula to be located well up in the thoracic aorta. Similar results were obtained when this experiment was repeated in other dogs, provided that the end of the cannula was located approximately midway between the renal arteries and the aortic arch. This allowed the administered acetylcholine to mix with the aortic blood before it reaches the adrenal gland.

In another experiment of the same type (Fig. 4,*b*), injection of acetylcholine through a cannula passed through the right carotid artery into the left ventricle produced a biphasic

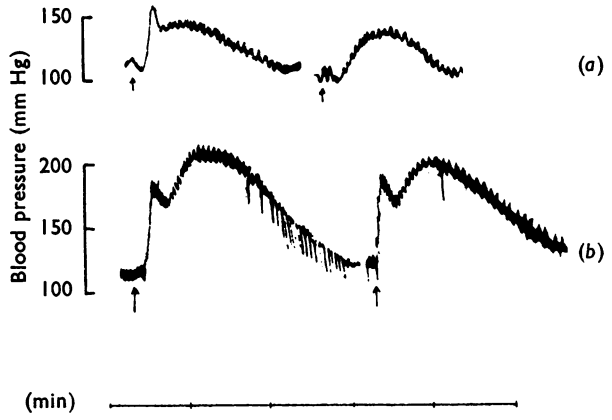

Fig. 4. Blood pressure recorded from the carotid artery in experiment (*a*) and from the femoral artery in experiment (*b*). (*a*) and (*b*), traces from each dog are arranged in a single row. Atropine (5 mg/kg, intravenously) and physostigmine (1 mg/kg, intravenously) had been given to each dog. The left-hand records show the biphasic pressor responses following acetylcholine (100 μg/kg, intravenously). Marked transitory vasodepression during the pressor response in (*b*) was associated with respiratory movements. The right-hand record in (*a*) shows the pressor response to acetylcholine diluted 1 : 4 with heparinized saline and administered through a catheter into the thoracic aorta. The right-hand record in (*b*) shows the response to the same dose of acetylcholine administered through a catheter inserted through the carotid artery into the left ventricle. Injections at arrows, all of 100 μg.

rise in blood pressure similar to that obtained after intravenous injection. The first component of this blood pressure rise began sooner than with intravenous injection.

The blood pressure response in adrenalectomized dogs

After clamping off the blood supply to the adrenal glands, acetylcholine (100 μg/kg) was injected intravenously. The first component of the blood pressure rise was relatively unchanged whereas the second component was greatly reduced in intensity (Fig. 5, top trace, third record). Pronethalol (2 mg/kg) was administered followed by acetylcholine (100 μg/kg); the initial pressor response was now greatly diminished (Fig. 5, top trace, fourth record).

Following bilateral adrenalectomy, acetylcholine (100 μg/kg) was injected intravenously resulting in a monophasic rise in blood pressure (Fig. 5, bottom trace, third record). P-286 (2 mg/kg) was injected and when this was followed by an intravenous injection of acetylcholine (100 μg/kg) a fall in blood pressure was observed (Fig. 5, bottom trace, fourth record).

DISCUSSION

Since ganglionic cells have recently been demonstrated in the heart (Hirsch, Willman, Jellinek & Cooper, 1963) the rapid first component in the blood pressure rise is readily explicable as a consequence of the nicotinic action of acetylcholine on them or on the stellate ganglia. The sensitivity of this component of the response to pronethalol, a β-blocking agent, is thus to be expected. The enhancement of the secondary pressor component by β-blocking agents and its inhibition by α-blocking agents supports the interpretation of

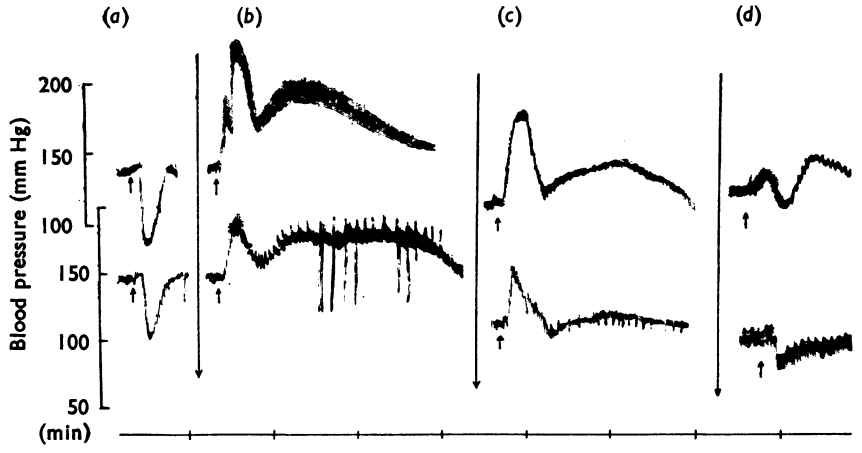

Fig. 5. Experiments on two dogs, blood pressure traces arranged in two rows. (*a*) Responses to acetylcholine (2 μg/kg, intravenously). (*b*) Responses to acetylcholine (100 μg/kg, intravenously) after atropine and physostigmine administered in usual dosage. (*c*) Responses to the same dose of acetylcholine after the adrenal gland blood supply had been clamped (top) or the adrenal glands had been removed (bottom). (*d*) Responses to the same dose of acetylcholine after pronethalol (2 mg/kg, intravenously) (top) or P-286 (2 mg/kg, intravenously) (bottom). All acetylcholine injections at arrows.

Goodman & Gilman (1955) that release of an adrenaline-like substance from the adrenal medulla was the cause of this component. It is clearly not consistent with the proposal (Gardier *et al.*, 1963) that the pressor response was due to an increase in cardiac output.

The intra-aortic injection of acetylcholine leads to a monophasic pressor response and this result receives a ready explanation if the interpretation offered by Goodman & Gilman (1955) is accepted. Thus, according to this view the intra-aortic injection of acetylcholine results initially in a rapid stimulation of the adrenal medulla, producing a protracted rise in blood pressure, and while the pressor response is still in progress the acetylcholine, much diluted by blood, will reach the heart and only weakly stimulate the heart or stellate ganglia. As a consequence, the second component of the blood pressure rise will not be observed and a monophasic rise in blood pressure will be observed. Furthermore, the rapid biphasic pressor response observed following the intraventricular injection of acetylcholine is in accord with the interpretation of Goodman & Gilman (1955).

Injection of acetylcholine into the atropinized, adrenalectomized dog resulted in a rapid brief monophasic rise in blood pressure which could be blocked by the β-blocking agent, pronethalol. Clearly, if the adrenal medulla is the only component of the sympathetic system involved in the biphasic rise in blood pressure, acetylcholine should elicit no blood pressure rise in atropinized, adrenalectomized dogs. Moreover, the monophasic rise in blood pressure produced by acetylcholine in atropinized, adrenalectomized dogs is reversed when the acetylcholine injection is preceded by 2 mg/kg of P-286, demonstrating that P-286 in the doses used by Gardier *et al.* (1960, 1963) does not produce selective blockade of the adrenal medulla as claimed by these authors. It also blocked the nicotinic action of acetylcholine on cardiac or stellate ganglia, thus accounting for the results obtained by Gardier *et al.* (1960) namely complete blockade of the pressor response to acetylcholine.

SUMMARY

1. The mechanisms responsible for the biphasic pressor response to acetylcholine in the atropinized dog have been analysed. The studies were carried out on atropinized dogs using sympathetic α- and β-receptor blocking agents, intravenous and intra-aortic injections of acetylcholine and adrenalectomy.

2. The results strongly support the classical view that the primary short pressor effect of acetylcholine results from stimulation of sympathetic ganglia, while the secondary prolonged pressor effect results from the release of an adrenaline-like material from the adrenal medulla which acts on both α- and β-receptors in the vasculature.

3. P-286 blocked the pressor effects of acetylcholine in adrenalectomized dogs in doses (2 mg/kg) similar to those required to block adrenal medullary stimulation.

The authors wish to acknowledge the assistance in this project of the following students in this university: M. Akpata, M. Davidman, G. Hunter, F. Harris, L. Kulak, D. Lake, G. McBride, K. Rentiers, C. A. Sheppard and P. Whidden. Acknowledgment is expressed also to Dr D. Secord for assistance in carrying out the adrenalectomies, and to Dr Leighton Smith, Vice-President-Medical Director, Ayerst, McKenna & Harrison (Montreal) for a gift of pronethalol.

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