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VASODEPRESSION INDUCED BY ACETYLCHOLINE IN THE ATROPINIZED DOG

BY

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The pressor response to acetylcholine in the atropinized dog resulted from an increase in cardiac output. The pressor response was attributed solely to the release of adrenaline from the adrenal medulla. After giving compound P-286 (*N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-di-isopropylurea) to these dogs, acetylcholine lowered blood pressure, owing to a decrease in total peripheral resistance in the absence of an increase in cardiac output. P-286 presumably blocked the liberation of adrenaline from the adrenal glands by acetylcholine. The blood vessels contributing to the fall in peripheral resistance were not in the intestines. The fall in blood pressure was not blocked by dichloroisoprenaline and it was still present in dogs treated with reserpine. It is suggested that the fall in blood pressure was due to stimulation of ganglion cells subserving vasodilatation.

The pressor action of an intravenous injection of acetylcholine into the atropinized dog is reversed after the release of catechol amines from the adrenal medulla has been blocked by *N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-di-isopropylurea, P-286 (Gardier, Abreu, Richards & Herrlich, 1960). The mechanism suggested by Shaw, Keogh & MacCallum (1948) and Shaw & MacCallum (1949) to explain the reversal, by various drugs, of the pressor response to acetylcholine after atropine is that acetylcholine stimulates sympathetic ganglion cells subserving vasodilator functions, the effect of which is revealed in the absence of pressor activity. In our opinion insufficient evidence has been available to substantiate this hypothesis.

The studies reported here are concerned with the mechanism of the vasodepressor response to acetylcholine after administration of P-286 into the atropinized dog; in addition evidence is given that the pressor response to acetylcholine results from specific stimulation of the adrenal medulla.

METHODS

Adult mongrel dogs, of either sex, were maintained in stage III, plane 3 of general anaesthesia with sodium pentobarbitone (30 mg/kg, intravenously); small supplementary doses (2.5 to 5 mg/kg) were administered when necessary. The usual dose of atropine sulphate was 1 mg/kg,

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given by intravenous injection. Compound P-286 was given usually in a total amount of 6 mg/kg intravenously as three doses of 2 mg/kg over a period of 15 min. Acetylcholine, as the chloride or as the iodide, was given intravenously in doses of 0.001 to 1 mg/kg.

Carotid or femoral arterial pressure was measured in all experiments with either a mercury manometer, a Sanborn 267A or a Statham P23 AC transducer. Recordings were made on kymograph paper, on a Sanborn 150 direct writing oscillograph or on a Grass Model 5 ink writing polygraph, respectively.

Blood flow in a loop of ileum weighing approximately 50 g was used to assess splanchnic vascular resistance. The segment was isolated surgically from the remainder of the intestine, with the blood supply preserved intact. In five experiments the ileum was denervated. In five others innervation was preserved. The principal vein draining this intestinal loop was cannulated and flow was recorded by means of an orifice flowmeter consisting of a differential transducer (Sanborn model 268B) which detected a pressure difference across a constriction in the venous tubing (usual pressure drop about 1 mm Hg). Resistance was calculated as vascular pressure difference (the arterial minus the venous pressure in mm Hg) divided by blood flow (in ml./min/100 g of tissue). The arterial pressure at the level of the gut was recorded from a cannulated side branch of the main artery supplying the segment. Venous pressure was measured upstream from the orifice flowmeter. Both pressures were registered on a Sanborn direct writing oscillograph by means of Sanborn transducers. More detailed descriptions of this technique have been published previously (Johnson & Selkurt, 1958; Selkurt & Johnson, 1958).

Cardiac output was determined by the dye (Evan's blue, T-1824) dilution technique of Hamilton, Moore, Kinsman & Spurling (1932) and total peripheral vascular resistance was calculated as the mean femoral blood pressure (diastolic plus one-third of the pulse pressure in mm Hg) divided by the cardiac output (in ml./min/100 g of body weight). Both femoral blood pressure and lead II of the electrocardiogram were recorded simultaneously on a Grass Model 5 polygraph. Stroke volume was derived from the cardiac output and pulse rate.

Reserpine was dissolved in a solution of 20% ascorbic acid at a concentration of 10 mg/ml. Intraperitoneal injections were of 0.5 mg/kg for 3 successive days or of 0.1 mg/kg for 5 successive days.

Analysis of variance was used for statistical evaluation, P values of 0.05 or less being considered significant (Snedecor, 1956).

RESULTS

Changes in splanchnic vascular resistance. In the atropinized dog acetylcholine caused a marked rise in mean systemic blood pressure before administration of P-286 and a fall in blood pressure after P-286 (Table 1). This reversal of response to acetylcholine could be explained if P-286 prevented the vasoconstriction in the mesenteric blood vessels caused by adrenaline. Pertinent results on vascular resistance in the ileum are presented in Table 1. Acetylcholine produced a small increase in vascular resistance in the ileum before administration of P-286 and a small decrease after P-286; however, this change was not statistically significant.

To determine whether blockade by P-286 of sympathetic ganglia would explain the small change in resistance that did occur, experiments were carried out with the ileum after section of the postganglionic sympathetic nerves. In this preparation acetylcholine significantly increased both ileal vascular resistance and systemic arterial blood pressure. After administration of P-286, acetylcholine did not

TABLE 1

EFFECT OF P-286 ON CARDIOVASCULAR RESPONSES TO ACETYLCHOLINE IN ATROPINIZED DOGS

The values are means with standard errors. Five experiments each were done with the innervated and denervated ilea. *Value significantly different ($P<0.05$) from control. † For units see Methods

	Innervated ileum		Denervated ileum	
	Control	Acetylcholine (1 mg/kg)	Control	Acetylcholine (1 mg/kg)
Before P-286				
Arterial blood pressure (mm Hg)	116±7·6	*180±11·5	84·4±5·65	*141±2·71
Ileal vascular resistance†	6·20±1·41	7·72±2·17	4·58±0·75	*8·50±0·77
After P-286				
Arterial blood pressure (mm Hg)	111±2·81	*77±3·42	60·7±2·02	*33·2±5·26
Ileal vascular resistance†	5·33±0·53	4·77±0·68	3·22±0·22	3·73±0·43

significantly alter resistance, and there was a fall in blood pressure (Table 1). Therefore it was concluded that stimulation of sympathetic ganglion cells did not contribute to the ileal vasoconstriction caused by acetylcholine.

Cardiac output and total peripheral vascular resistance. Measurements of these parameters were necessary to elucidate the effector sites responsible for the pressor and depressor responses to acetylcholine. The results are presented in Table 2.

The cardiac output during the rise in blood pressure produced by acetylcholine was approximately twice the control value. This increase was due, mainly, to an increase in heart rate. Concomitant with the change in cardiac output there was a slight decrease in peripheral resistance. However, in the presence of P-286, the fall in blood pressure caused by acetylcholine was associated with a 58% decrease in peripheral resistance, which was statistically significant.

β-Receptors. That stimulation of β-receptors (Ahlquist, 1948) could not account for the hypotension induced by acetylcholine is indicated by experiments illustrated in Fig. 1. Dichloroisoprenaline failed to block the hypotensive response to acetylcholine after P-286, whereas an equivalent depressor response to isoprenaline was completely blocked.

TABLE 2

CARDIOVASCULAR RESPONSES TO ACETYLCHOLINE IN ATROPINIZED DOGS BEFORE AND AFTER P-286

The values are means with standard errors for five experiments. * Value significantly different ($P<0.05$) from control. † For units see Methods

	Control	Acetylcholine (1 mg/kg)
Before P-286		
Arterial blood pressure (mm Hg)	140±9·22	*185·6±7·28
Peripheral vascular resistance†	6·43±0·64	5·27±1·14
Cardiac output (l./min)	2·56±0·14	*4·70±0·90
Cardiac stroke volume (ml.)	14·96±1·02	20·62±3·61
After P-286		
Arterial blood pressure (mm Hg)	110±10·26	*56·8±8·89
Peripheral vascular resistance†	5·28±0·55	*2·20±0·32
Cardiac output (l./min)	2·44±0·14	2·95±0·41
Cardiac stroke volume (ml.)	18·34±1·06	20·08±1·26

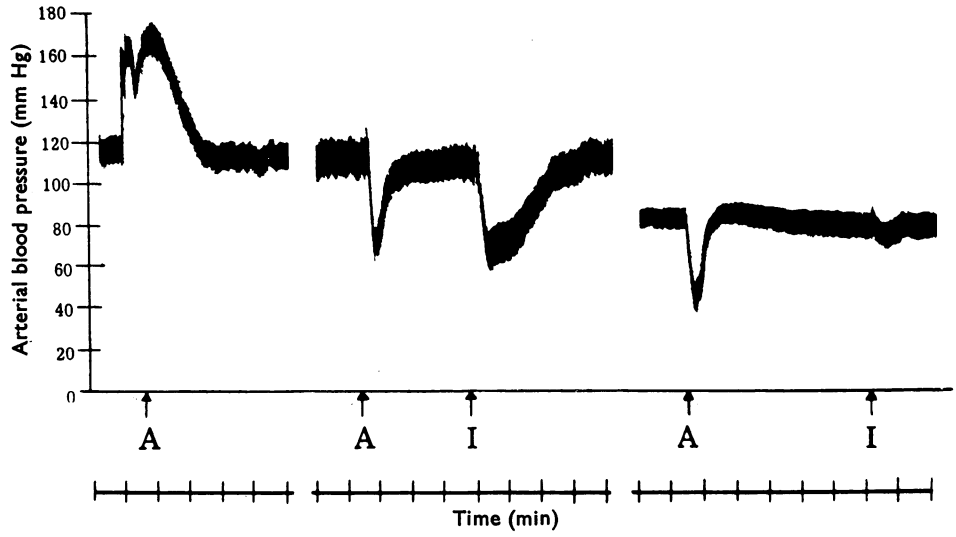

Fig. 1. Experimental evidence indicating that the depressor response to acetylcholine is not a result of stimulation of β-receptors. Arterial blood pressure of a dog which had received atropine sulphate (1 mg/kg intravenously). At A, acetylcholine chloride (1 mg/kg intravenously); at I, isoprenaline (1 μg/kg intravenously). Between first A and second A, P-286 (4 mg/kg) was injected intravenously; between I and third A, dichloroisoprenaline (10 mg/kg intravenously). Time marks, 1 min.

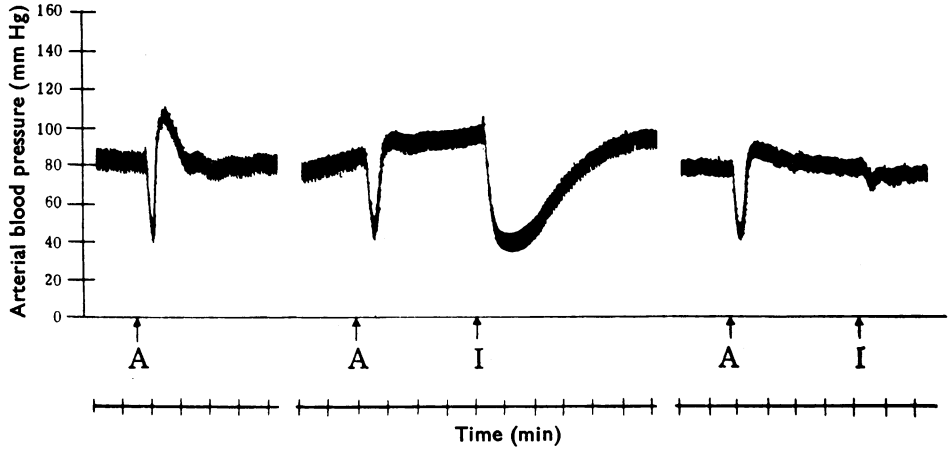

Fig. 2. Experimental evidence against the possibility of a contribution by catechol amines to the hypotensive response to acetylcholine. Arterial blood pressure of a dog, previously treated with reserpine and given atropine sulphate (1 mg/kg intravenously). At A, acetylcholine chloride (1 mg/kg intravenously); at I, isoprenaline (1 μg/kg intravenously). Between first A and second A, P-286 (6 mg/kg) was injected intravenously; between I and third A, dichloroisoprenaline (15 mg/kg intravenously). Time marks, 1 min.

Animals treated with reserpine. Dogs were depleted of catechol amines by treatment with reserpine to test the possibility that the hypotensive response to acetylcholine was mediated by adrenaline. Although, in these conditions, the pressor response to acetylcholine was not present (a biphasic response was usually seen), to reverse the pressor response completely the same dose of P-286 was necessary as was needed in the animals not treated with reserpine. Dichloroisoprenaline had no effect on this vasodepression in doses that abolished an equivalent hypotensive response to isoprenaline (Fig. 2).

Cholinergic vasodilatation. Evidence that acetylcholine is not competitively displacing atropine from receptor sites for muscarinic drugs is necessary if the hypothesis of Shaw *et al.* (1948, see Introduction) is to be accepted. Earlier studies have indicated that this depressor response was not prevented by doses of atropine as large as 20 mg/kg (Gardier *et al.*, 1960). To study the possibility of competitive displacement more fully, dose/response curves for acetylcholine after administration of different doses of atropine were determined in three experiments (Fig. 3). The largest dose of acetylcholine used in the absence of atropine was that which caused

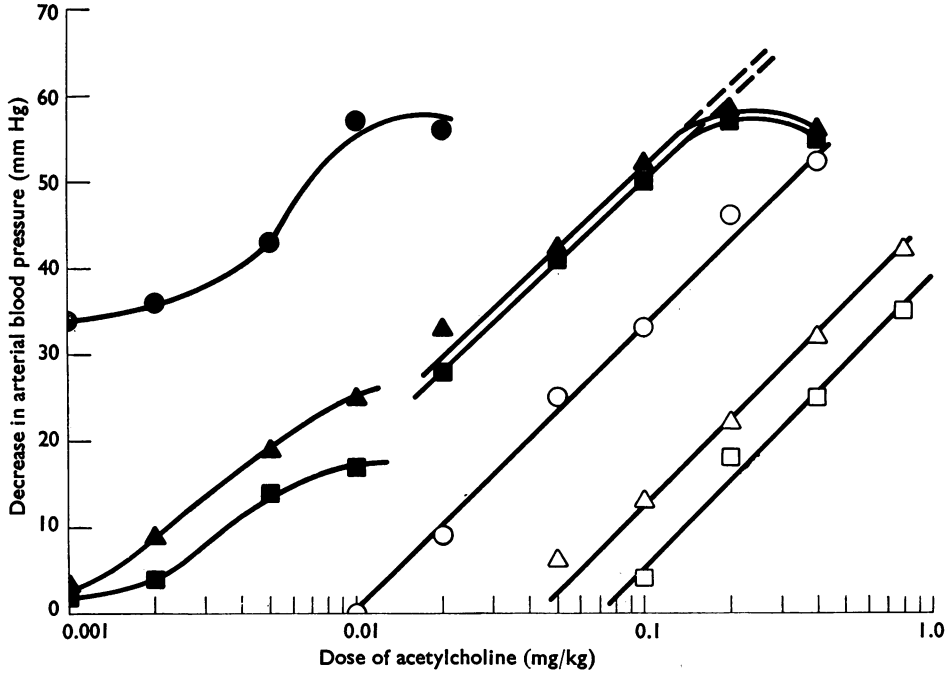

Fig. 3. Mean depressor responses (ordinate, mm Hg) to acetylcholine iodide (abscissa, log scale, mg/kg intravenously) in the dog before and after atropine sulphate and P-286 (three experiments). ●— Responses to acetylcholine before atropine. ○— Responses to acetylcholine after 0·1 mg/kg of atropine intravenously. ■— Second determination of responses to acetylcholine without additional atropine. ▲ — Responses to acetylcholine after 6 mg/kg of P-286 intravenously and without additional atropine. □— Responses to acetylcholine after a further 1 mg/kg of atropine intravenously, but without additional P-286. △— Second determination of responses to acetylcholine without additional atropine or P-286. Time between ○ and ■ 26 min, between ■ and ▲ 22 min, and between □ and △ 16 min.

transient cardiac arrest. In the presence of the smaller dose of atropine the depressor response to the larger doses of acetylcholine was somewhat reduced and a pressor response appeared.

The following conclusions were drawn from these experiments. (1) A dose of 0.1 mg/kg of atropine sulphate completely blocked the depressor responses to doses of 0.001 to 0.01 mg/kg of acetylcholine iodide. (2) Increasing the doses of acetylcholine in the presence of 0.1 mg/kg of atropine again resulted in depressor responses the size of which was linearly related to the logarithms of the dose of acetylcholine (Fig. 3, O). (3) When the doses of acetylcholine were repeated without additional atropine, the dose/response curve differed in form (Fig. 3, ■); it had an initial sigmoid portion and then a linear part. The initial sigmoid portion may be due to competitive displacement of atropine from receptors for muscarinic drugs ; the linear part may be due to stimulation of ganglia subserving vasodilator functions, the threshold dose for such a stimulation being 20 μg/kg of acetylcholine. An increase in the dose of atropine to 1 mg/kg reduced, but did not abolish, the responses to acetylcholine. (4) The only observed effect of P-286 was to decrease the rate at which the blocking action of atropine wore off (Fig. 3, ▲). This effect accords with a previous observation that P-286 has a weak atropine-like activity (Gardier, 1961).

DISCUSSION

Stimulation of sympathetic ganglion cells and of the adrenal medulla is the usually accepted explanation for the pressor response to large doses of acetylcholine in atropinized animals (Goodman & Gilman, 1955). However, observations made with acutely adrenalectomized animals provided evidence that the adrenal medulla was the only portion of the sympathetic system involved in the rise in pressure (Gardier *et al.*, 1960). This conclusion is confirmed by the results reported here. The pressor response was entirely due to an increase in cardiac output, the peripheral vascular resistance being slightly decreased. The pressor response to adrenaline is also due to increased cardiac output (Goldenberg, Pines, Baldwin, Greene & Roh, 1948). Since the storage site for adrenaline in the dog is the adrenal medulla (Hagen, 1959) it seems probable that acetylcholine specifically stimulates the gland and that P-286 blocks this action. However, this conclusion does not explain the hypotensive action of acetylcholine in the presence of atropine and P-286.

The hypotensive response is a result of decreased peripheral resistance. Our evidence suggests that sufficient atropine was present to block any muscarinic action of acetylcholine. It is possible that the effect of stimulation of sympathetic ganglion cells subserving vasodilatation was revealed, once the release of adrenaline from the adrenal medulla had been blocked.

The effect of acetylcholine after atropine in increasing vascular resistance more in the denervated than in the innervated ileum may be explained by sensitization of the ileal blood vessels to the adrenaline liberated from the adrenal medulla. However, the time elapsed after denervating the tissue was less than 12 hr, which is said to be the minimum period for the appearance of increased sensitivity (Brown, 1961).

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