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The action of certain haloalkylamines on some biological activities of bradykinin

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1. Bradykinin and histamine reduced the blood pressure in normotensive anaesthetized rabbits. They produced a pressor or biphasic response when the initial arterial pressure was lowered by acute haemorrhagic shock or mecamylamine blockade. When blood pressure was lowered by pretreatment with reserpine, bradykinin remained depressor and histamine produced a biphasic response.

2. Phenoxybenzamine abolished the pressor responses to bradykinin and histamine, but potentiated and prolonged the depressor response to bradykinin.

3. Phenoxybenzamine-OH and SY28-OH modified neither the pressor nor depressor responses to histamine, nor the pressor response to bradykinin. However, they greatly potentiated and prolonged the hypotensive effect of bradykinin.

4. In the isolated rabbit ear preparation, the initial vascular tone influenced the responses to bradykinin and histamine. In preparations having low vascular tone they were vasoconstrictors, but when the tone was raised by angiotensin or noradrenaline, they were vasodilatators.

5. Phenoxybenzamine blocked the vasconstrictor effect of bradykinin in a rabbit ear preparation having low vascular tone and phenoxybenzamine or its related ethanolamine potentiated the vasodilator response to bradykinin in preparations in which the tone was high.

6. The significance of these findings is discussed.

Rocha e Silva, Corrado & Ramos (1960) showed that phenoxybenzamine prolongs the hypotensive action of bradykinin in the cat, and Rocha e Silva & Leme (1963) further showed that dibenamine and phenoxybenzamine potentiate the stimulant action of this peptide on isolated guinea-pig ileum in low concentrations. Graham & Al-Katib (1966a) attributed the potentiated response of the isolated guinea-pig vas deferens preparation to bradykinin, caused by certain haloalkylamines, to their hydrolysis products the ethanolamines.

In view of the recent advances in the treatment of shock states by certain haloalkylamines (Lillehei, Longerbean, Bloch & Manax, 1964; Rush, Rosenberg & Spencer, 1965; Murphy, Gagnon & Ewald, 1965), and the uncertain role of brady-

kinin in shock states, it was decided to study the effects of certain haloalkylamines on some biological activities of this peptide. Histamine was included in this study because it is closely related to bradykinin in its action and both are known as naturally occurring vasodilators.

Methods

In vivo

Rabbits of either sex weighing between 2 and 3 kg were anaesthetized with pentobarbital sodium (30 mg/kg intravenously). The carotid blood pressure was recorded by means of a polythene cannula connected to a mercury manometer. The response to bradykinin (1 μg/kg) or histamine (10 μg/kg) was recorded every 10 min. In three animals, mecamylamine was injected slowly in doses of 10–40 mg/kg. Bradykinin and histamine tests were repeated after mecamylamine. In five rabbits, catecholamine depletion was induced by pretreating the animals with reserpine (2 mg/kg intraperitoneally, daily for 2 days before the experiment). The response to bradykinin and histamine was studied after treatment with reserpine. In four rabbits acute haemorrhagic shock was induced by withdrawing blood (30–40 ml./kg) from the carotid artery. The effect of the following drugs was studied on the response to bradykinin and histamine under the conditions described above: phenoxybenzamine, phenoxybenzamine-OH, SY28-OH and mepyramine.

Drugs were injected through a two-headed needle in the carotid artery, constructed so that the test dose could be washed in by a following injection of saline. The total volume of each injection was 0·5 ml.

In vitro

Perfused blood vessels of the rabbit ear

The central artery of the rabbit ear was cannulated and perfused with Krebs solution, at 20° C, under a constant head of pressure of 95 mm of water. The venous outflow was monitored continuously with a Gaddum drop recorder. The blood vessels were perfused for at least 1 hr before studying the effects of haloalkylamines on the action of bradykinin and histamine. Three separate experiments were performed for each of the haloalkylamines mentioned in the text. In nine other experiments vascular tone was raised artificially by adding either angiotensin or noradrenaline at a concentration of 0·5 μg/l. to the perfusing fluid before injecting the test drugs. Drugs to be tested were injected into the rubber cannula proximal to the central artery. The volume of injection never exceeded 0·1 ml.

The following drugs were used: bradykinin (synthetic BRS, 640 Sandoz); phenoxybenzamine hydrochloride (dibenyline, Smith Kline & French) and its synthetic hydrolysis product, phenoxybenzamine-OH; SY28-OH (the ethanolamine of a potent adrenoceptor blocking drug, SY28; this has no adrenoceptor blocking activity but is a powerful potentiator of certain effects of bradykinin (Graham & Al-Katib, 1966a, b); reserpine (Serpasil, Ciba Ltd.); histamine acid phosphate and mepyramine maleate. Doses mentioned in the text are in terms of the salts.

Results

In vivo

In three normotensive rabbits (80–100 mm Hg), bradykinin (1 μg/kg) and histamine (10 μg/kg), injected intra-arterially produced a rapid drop in blood pressure which lasted for 1 or 2 min. As the initial arterial pressure fell during the course of the experiment (40–50 mm Hg), injection of either bradykinin or histamine produced a biphasic response (hypotensive-hypertensive). The pressor phase of the response increased as the arterial pressure decreased. When the arterial pressure was very low (30 mm Hg), the response to bradykinin was always entirely pressor, whereas, that to histamine remained biphasic.

In three other anaesthetized rabbits mecamylamine (10–20 mg/kg) was injected slowly into the carotid artery. This produced a rapid fall in arterial blood pressure (40–60 mm Hg). Higher doses (30–40 mg/kg) reduced the arterial pressure to 30 mm Hg. The response to bradykinin at a pressure of 40–60 mm Hg was biphasic, but at 30 mm Hg it was entirely pressor. The response to histamine was biphasic at all pressures.

Antihistamine (mepyramine) in doses of 5–10 mg/kg abolished the pressor response to histamine and reduced the depressor component. The action of bradykinin was not modified by antihistamine.

Reserpine

Five rabbits were treated with reserpine (2 mg/kg, intraperitoneally) on each of two days before the experiment. The range of the arterial pressure in these animals was 40–50 mm Hg and the injection of bradykinin (1 μg/kg) produced a depressor response only (Fig. 1B). The response to histamine was biphasic.

Acute haemorrhagic shock

After the gradual withdrawal of blood (30–40 ml./kg body weight) from four different rabbits the arterial pressure dropped to 30 mm Hg and remained at that

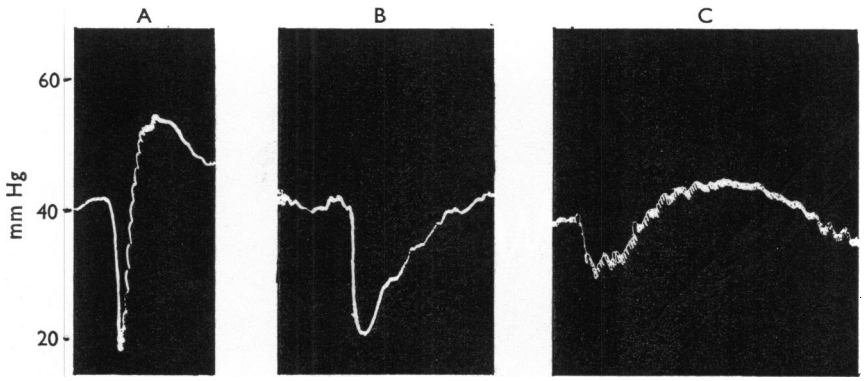

FIG. 1. Effect of bradykinin at low arterial blood pressure in anaesthetized rabbits. In (A) the arterial pressure was maintained at 40 mm Hg by the injection of mecamylamine (30 mg/kg). The response to bradykinin (1 μg/kg) was biphasic (hypotensive–hypertensive). In (B) the arterial pressure of a reserpinized animal was 40 mm Hg and the response to bradykinin was a depressor one. In (C) the arterial pressure was maintained at 40 mm Hg by withdrawal of blood (40 ml./kg). The response to bradykinin was biphasic but the hypotensive component was more prolonged than in (A).

level during the course of the experiment. An hour later, bradykinin (1 μg/kg) produced a biphasic response (hypotensive-hypertensive). The hypertensive component was more prolonged than in mecamylamine-treated animals at the same blood pressure level (see Fig. 1A and C). The response to histamine was always pressor.

Phenoxybenzamine

The intra-arterial injection of a freshly prepared solution of phenoxybenzamine (5 mg/kg) in normotensive rabbits produced a sharp rise in arterial pressure followed by a gradual fall. The mean fall in pressure in four rabbits was 30 mm Hg and remained so during the course of the experiment (3 hr). Bradykinin (1 μg/kg), injected 10 min after phenoxybenzamine, produced a hypotensive response. This hypotension, as well as its duration of action, were greatly increased with time after phenoxybenzamine. The response to histamine after phenoxybenzamine was depressor.

Phenoxybenzamine (5 mg/kg) abolished the pressor response to bradykinin when the arterial pressure was lowered by the ganglion blocker mecamylamine.

In shocked animals, phenoxybenzamine was injected slowly and at the same time normal saline was infused to counteract the further hypotension induced by phenoxybenzamine. In this way, the arterial pressure was maintained at 30–40 mm Hg for 3 hr. The biphasic response to bradykinin was changed to a depressor response, and the duration of the hypotension was greatly prolonged (Fig. 2 and Table 1). The pressor response to histamine in shocked animals was altered to a depressor one after phenoxybenzamine.

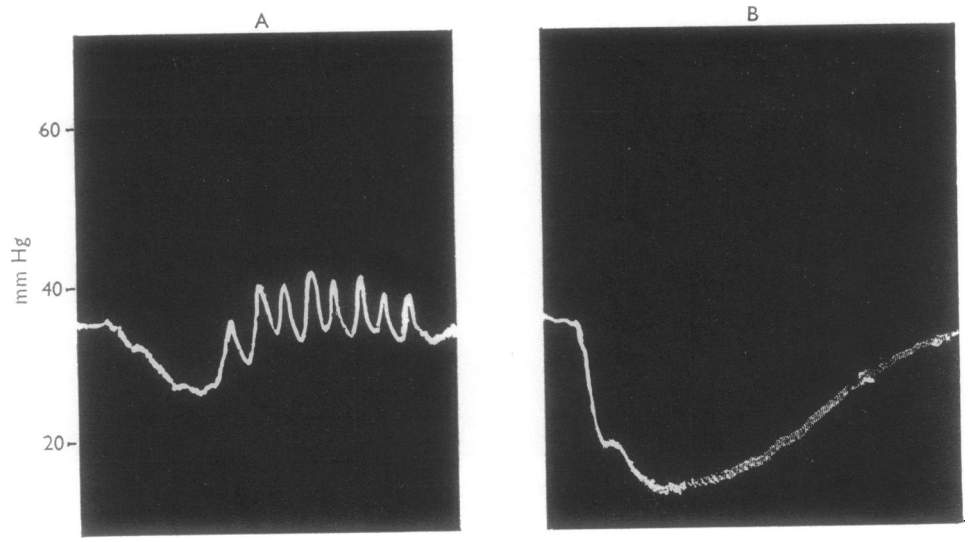

FIG. 2. Action of bradykinin (1 μg/kg) on the arterial pressure of anaesthetized rabbit in haemorrhagic shock, (A) before and (B) after treatment with phenoxybenzamine (5 mg/kg).

Phenoxybenzamine-OH and SY28-OH

Injection of either of the synthetic products of hydrolysis of the potent adrenoceptor blockers phenoxybenzamine or SY28 in a dose of 5 mg/kg produced a transient fall in arterial pressure. The biphasic, or alternatively, pressor response to bradykinin in mecamylamine-treated rabbits, and the pressor response to this peptide in shocked animals were unaffected by treatment with either of the haloethanolamines. The depressor responses to bradykinin were potentiated and prolonged with time after the haloethanolamine in all instances. The response to histamine was unaffected by the haloethanolamine.

In vitro

Perfused rabbit ear

Injection of 0·1–100 ng of either bradykinin or histamine into the cannula in the central artery, decreased the flow. This effect increased with increasing concentrations of either of the compounds and was reversible within 5 min. The addition of mepyramine (10^{-7} g/ml.) to the perfusate prevented the effect of histamine but not that of bradykinin. The addition of phenoxybenzamine (10^{-7} g/ml.) to the perfusate blocked the effect of both histamine and bradykinin. Phenoxybenzamine-OH (10^{-7} g/ml.) was unable to modify the action of either histamine or bradykinin.

When angiotensin was used (0·5 μg/l.) in the perfusing solution, the flow was reduced down to about 20% of the control level and was maintained so for 2–3 hr. Injection of either bradykinin or histamine in doses of 0·05–100 ng increased the flow. This effect was not reversible for 1 hr and more angiotensin was needed to achieve the original flow. Injection of 100–200 ng of noradrenaline reversed the vasodilator effects of bradykinin and histamine. Mepyramine (10^{-7} g/ml.) blocked the dilator effect of histamine but not that of bradykinin.

When noradrenaline was used (0·5 μg/l.) to increase vascular tone, artificially, the flow was reduced to 10–20%. Such a flow was also sustained for more than 2 hr. Bradykinin and histamine in doses of 1–100 ng produced vasodilatation. This effect was always submaximal and was quickly reversible. Mepyramine (10^{-7} g/ml.) blocked the effect of histamine but not that of bradykinin. Phenoxybenzamine (10^{-7} g/ml.) abolished the artificial tone induced by noradrenaline. Phenoxybenzamine-OH (10^{-7} g/ml.) in the perfusate did not influence noradrenaline tone ; it potentiated the vasodilator effect of bradykinin but not that of histamine.

TABLE 1. *Effect of certain haloalkylamines on the arterial pressure responses of anaesthetized rabbit to bradykinin*

Haloalkylamines	Action on blood pressure* (a)	(b)	Dose	H† (a)	(b)	R (a)	(b)	Me (a)	(b)
Phenoxybenzamine	r	f	5 mg/kg	bi	d	d	—	p, bi	d
Phenoxybenzamine-OH	f	0	5 mg/kg	bi	bi	d	—	p, bi	p, bi
SY28-OH	f	0	5 mg/kg	bi	bi	d	—	p, bi	p, bi

* Column 2 describes the arterial pressure response to the injection of the haloalkylamine; (*a*) is the initial response, observed within 20 sec of the injection of the drug; (*b*) is the subsequent response. r, Rise in pressure; f, fall in pressure; 0, no effect.

† Columns 4, 5 and 6 describe the arterial pressure responses to bradykinin (*a*) before and (*b*) after treatment with the haloalkylamine in (H) haemorrhagic shock, (R) after reserpine pretreatment and, (Me) after mecamylamine blockade of ganglia. p, Pressor response; d, depressor response; bi, biphasic response; —, not tested. The depressor response to bradykinin after the haloethanolamine was greatly potentiated.

Discussion

Bradykinin and histamine are known to produce vasodilatation. However, bradykinin has recently been shown to produce a pressor response (Lang & Pearson, 1968) and vasoconstriction *in vitro* (Weigershausen & Hennighausen, 1966). In the present experiments in rabbits it was demonstrated that both bradykinin and histamine increased the blood pressure or produced a biphasic (hypotensive-hypertensive) response after the blood pressure was reduced by: (*a*) administration of mecamylamine, (*b*) withdrawal of blood to induce haemorrhagic shock or (*c*) during the course of experiments with repeated administration of bradykinin and histamine. The pressor response to both drugs was dependent on the initial level of the blood pressure. The lower the arterial pressure the more marked was the pressor response to both substances. In *in vitro* experiments, the initial vascular tone was the important factor affecting the response to bradykinin and histamine. In an atonic preparation the response was vasoconstriction. If the tone was raised artificially by angiotensin or noradrenaline the response was altered to vasodilatation.

Bradykinin has been shown to release catecholamines from the suprarenal medulla (Feldberg & Lewis, 1964), and from the superior cervical ganglion of the cat (Lewis & Reit, 1965). The release of catecholamines by bradykinin might explain in part the mechanism of its pressor response, because it was absent in the reserpine-treated rabbits and was abolished by the adrenoceptor blocking agent phenoxybenzamine. Phenoxybenzamine also blocked the action of bradykinin *in vitro*. The potentiation of the depressor response to bradykinin as well as the prolongation of this effect by phenoxybenzamine may well be due to the hydrolysis product ethanolamine, which is readily formed in buffered media, (Graham & Al-Katib, 1966a). The ethanolamines potentiated and prolonged the hypotensive effect of bradykinin *in vivo* and its dilator action *in vitro*. The pressor response to histamine *in vivo* as well as its vasoconstrictor action *in vitro* was abolished by both mepyramine and phenoxybenzamine. Phenoxybenzamine has been shown to have antihistaminic activity (Graham, 1962).

It is interesting to find that phenoxybenzamine in haemorrhagic shock alters the vasoconstrictor actions of both histamine and bradykinin to a prolonged vasodilatation. This might be of significance in explaining the mode of action of phenoxybenzamine as a peripheral vasodilator in haemorrhagic shock in man and experimental animals.

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