Br. J. Pharmac. Chemother. (1968), **31**, 435–446.

THE ANTIHYPERTENSIVE PROPERTIES OF 1-AMINO-4-PHENYL PYRIDINIUM CHLORIDE

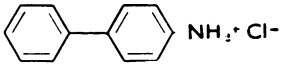
BY

VALERIE A. CULLUM, J. B. FARMER AND SHEILA L. HANDLEY

From the Department of Pharmacology, Allen & Hanburys Ltd., Ware, Herts.

(*Received June* 29, 1967)

This paper describes the effects of an antihypertensive agent (1-amino-4-phenyl pyridinium chloride, AH.2035) on the autonomic nervous and cardiovascular systems, and on some isolated organs of several animal species. Its structure is shown below:



The compound was synthesized in the Chemistry Department, Research Division, Allen & Hanburys Ltd., and has been the subject of a preliminary communication (Brittain, Farmer, Jack, Martin & Ritchie, 1967).

METHODS

Conscious hypertensive dogs

Hypertension was induced in male beagle dogs. All surgical procedures were carried out under aseptic conditions. Anaesthesia was induced with thiopentone sodium and maintained with nitrous oxide and oxygen (3:1 v/v) containing 3% halothane. The left carotid sinus was denervated and the left common carotid artery exteriorized in a tube of skin. At a second operation 3 weeks later both kidneys were mobilized and close-fitting rubber capsules placed around each one in such a way that the renal vessels were not constricted. Three weeks later the capsules were removed leaving the kidney permanently encased in a layer of fibrous tissue.

Two months later dogs with systolic blood pressures maintained at 150 mm Hg or above were selected for experiments. Systolic blood pressure was measured from the exteriorized carotid artery using a sphygmomanometer. Throughout the experiment measurements were taken each day at 10.00, 12.00, 14.00 and 16.00 hr. AH.2035 was given orally in soft gelatin capsules at 11.00 hr.

Anaesthetized cats and dogs

Cats of either sex weighing 2–3 kg were anaesthetized with chloralose 80 mg/kg given intravenously after induction with 3% halothane in nitrous oxide and oxygen (3:1 v/v). Beagles of either sex weighing 8–12.5 kg wre anaesthetized with 30 mg/kg pentobarbitone sodium intravenously. Arterial blood pressure was recorded from a cannula in the right femoral artery by a Devices blood pressure transducer coupled to a Devices polygraph recorder or by a mercury manometer writing on smoked paper. Respiration was recorded *via* a Magill cuffed endotracheal tube by a Statham low-pressure transducer or tambour. On some occasions heart rate was measured by a Devices tachometer triggered from the pulse pressure sensed by the blood pressure transducer. In some experiments the cervical vagus and the greater splanchnic nerve were cut and the peripheral ends stimulated with trains of rectangular pulses of 1 msec duration and supramaximal voltage. Drugs were injected through a cannula in the left femoral vein.

Measurement of cardiac output

Cardiac output was measured by the dye-dilution method. Polyethylene catheters were placed in the external jugular vein and left femoral arteries. The tips of the catheters rested in the right atrium and aortic arch respectively. The dye "coomassie blue" was injected through the jugular catheter, and arterial blood collected in heparinized tubes for colorimetric assay of the dye content.

Isolated spleen of the dog

Spleens were removed from dogs anaesthetized with pentobarbitone and perfused *via* the splenic artery with McEwen's solution (McEwen, 1956), oxygenated with 5% CO_2 in O_2 and maintained at 37° C (Farmer, 1966). A constant perfusion rate was maintained by a peristaltic pump. Inflow pressure was measured by a Devices blood pressure transducer. The splenic nerves were placed across platinum electrodes and stimulated at supramaximal voltages with rectangular pulses 1 msec in duration for 1 min periods every 5 min. Then 1 μg/ml. phenoxybenzamine and 1 μg/ml. cocaine were added to the perfusion fluid. After a period of 30 min the spleen was then stimulated for 2 min periods at the rate of 0.5, 1, 2 and 5 c/s. Each period of stimulation immediately followed the preceding one. The effluent was collected, for the last 30 sec of each stimulation period, into chilled centrifuge tubes containing hydrochloric, ascorbic and edetic acids. Noradrenaline was separated by ion-exchange chromotography (Bertler, Carlsson & Rosengren, 1958) and determined fluorimetrically by a modification of the trihydroxy indole reaction using an auto-analyser (Martin & Harrison, unpublished).

Isolated mesenteric arteries of the dog

The method of Rogers, Atkinson & Long (1965) was used. The branching arterial segments were perfused at constant rate (30 ml./min) with Krebs solution gassed with 95% O_2 and 5% CO_2 and maintained at 37° C. The pressor responses to nerve stimulation and injected catecholamines were recorded with a pressure transducer coupled to a Devices polygraph.

Isolated atria of the cat

Atria were removed, together with the vagus nerve, from anaesthetized cats and suspended in an organ bath containing McEwen solution oxygenated with 5% CO_2 in O_2. Stimulation of the vagus with rectangular pulses 1 msec in duration for 30 sec at varying frequencies resulted in a reduction in rate and amplitude of the spontaneous beat. Contractions of the atria were recorded with an isometric strain gauge and Devices polygraph.

Isolated central artery of the rabbit ear

The central ear artery was removed from lop-eared rabbits anaesthetized with pentobarbitone sodium, 30 mg/kg intravenously, and prepared for perfusion and periarterial sympathetic nerve stimulation according to the method of de la Lande & Rand (1965). The perfusion fluid, McEwen solution oxygenated with 5% CO_2 in O_2, was circulated by a peristaltic pump. The vasocontrictor response to periarterial nerve stimulation and to injected catecholamines were recorded with a pressure transducer coupled to a Devices polygraph recorder.

Isolated vas deferens of the guinea-pig

The vas deferens was prepared by the method of Huković (1961), bathed in McEwen solution at 32° C, and oxygenated with 5% CO_2 in O_2. The hypogastric nerve was stimulated with rectangular pulses 1 msec in duration. Trains of impulses, sufficient to produce maximal contraction at any given frequency, were applied every 2 min. Isotonic contractions were recorded on smoked paper.

Isolated ileum of the rabbit

Rabbit ileum was prepared for stimulation of the periarterial sympathetic nerves (Finkleman, 1930). The preparation was bathed in McEwen solution at 37° C oxygenated with 5% CO_2 in O_2. The nerves were stimulated with trains of rectangular pulses of 1 msec duration for 30 sec every 5 min. Isotonic contractions were recorded on smoked paper.

Isolated ileum of the guinea-pig

Guinea-pig ileum was prepared as described by Birmingham & Wilson (1965) for stimulation of the parasympathetic and periarterial sympathetic nerves. Isotonic contractions were recorded on smoked paper. The preparation was bathed in Krebs solution and oxygenated with 5% CO_2 in O_2. Stimulation was with trains of rectangular pulses of supramaximal voltage. The parasympathetic stimulation was 0.125 c.p.s., 0.5 msec duration, the sympathetic at different frequencies at 1 msec for 35 sec every 8 min.

Drugs

The doses of drugs given refer to the salt, except for 1-noradrenaline bitartrate and AH.2035 which refer to the base. Drugs used were: acetylcholine chloride; angiotensin (Hypertensin, Ciba); cocaine hydrochloride; hexamethonium bromide; noradrenaline bitrartrate; nicotine hydrogen tartrate; phenoxybenzamine hydrochloride; phentolamine methane sulphonate; propranolol hydrochloride and tyramine hydrochloride.

RESULTS

The effect of AH.2035 on the cardiovascular system of the cat and dog

Conscious hypertensive dogs

The antihypertensive effect of daily administration of AH.2035 was studied in 4 hypertensive dogs. After a 3-day control period AH.2035, 5 mg/kg daily, was given by mouth for 10 days (Fig. 1). On the first day there was a large (80–100 mm Hg) rise in blood pressure accompanied by bradycardia. These effects lasted more than 5 hr but less than 24 hr. During this time the dogs showed pilo-erection. On the second day this rise was small or absent and did not occur thereafter. By the third day the mean daily blood pressure had fallen, and it remained 30–60 mm Hg below control levels without change in heart rate until treatment ceased. At the end of the treatment period the blood pressure of all dogs returned to pretreatment levels within 3 to 6 days.

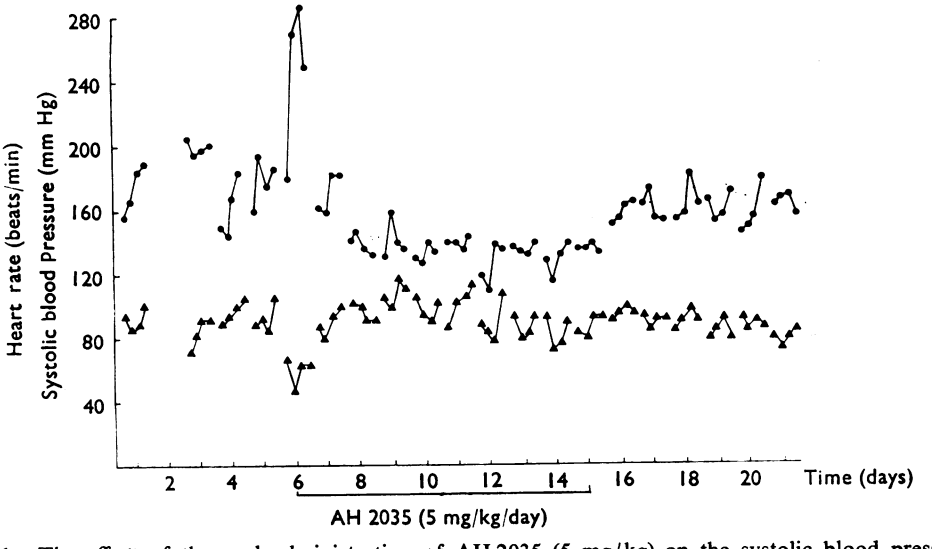


Fig. 1. The effect of the oral administration of AH.2035 (5 mg/kg) on the systolic blood pressure (●) and heart rate (▲) of a hypertensive dog (♂ 12.5 kg). Blood pressure and heart rate readings were taken at 10 a.m., 12 noon, 2 and 4 p.m.

A second experiment was undertaken to determine whether the acute rise in blood pressure at the start of treatment could be prevented by gradually increasing the dose of AH.2035 from low levels. A hypertensive dog initially received 0.25 mg/kg twice daily for 2 days. No appreciable pressor response occurred and the dose was increased to 0.5 mg/kg twice daily for a further 2 days. The blood pressure fell by 30 mm Hg during this time. From the fifth to the eighth day the dog was given 1 mg/kg twice daily producing a further 20 mm Hg fall which was not maintained. The dose was therefore increased to 2 mg/kg twice daily producing a further 30 mm Hg fall which was maintained through a further 7 days at this dosage. The blood pressure returned to control levels within 14 days of cessation of treatment. AH.2035 did not effect the heart rate in this experiment.

Throughout the experiments the dogs remained fit and healthy, with no sign of sympathetic impairment such as relaxation of the nictitating membrane or constriction of the pupil.

Anaesthetized cats and dogs

AH.2035 given intravenously to anaesthetized cats or dogs produced an initial pressor effect. In dogs this response occurred with as little as 200 μg/kg, which caused a rise of 20–30 mm Hg for 5–10 min. The effect was dose-dependent and reached a maximum of 80–100 mm Hg rise in blood pressure at 1–2 mg/kg which lasted for up to 45 min. In cats the lowest dose used was 1 mg/kg which caused a 90 mm Hg rise in blood pressure for 20 min ; larger doses did not have greater effects. The shape of the pressor response varied between the two species. In cats the response was monophasic, and accompanied by an intense tachycardia which often lasted for more than 2 hr. In dogs there were two distinct peaks (Fig. 2). The first was of rapid onset and short duration (30 sec). The second phase was slower in onset and longer in duration, the duration was dose-dependent. The secondary phase was accompanied by an intense and long lasting tachycardia. The primary phase was blocked by hexamethonium (10 mg/kg intravenously + 10 mg/kg subcutaneously) and the second was blocked by cocaine 10 mg/kg intravenously) and phentolamine (2 mg/kg intravenously). A depressor phase was sometimes seen after the pressor response had subsided, particularly after doses of 2 mg/kg and above. The fall in blood pressure occurred very slowly, about 10–20 mm Hg/hr, and the fall sometimes continued until the experiment was terminated 2–3 hr later. It is not known whether this fall bears any relation to the antihypertensive effect seen on long-term administration. The pressor response to occlusion of the common carotid arteries for 30 sec (carotid occlusion reflex) was reduced in both species by AH.2035. The reflex could be 90% abolished by 1–2 mg/kg in dogs (Fig. 3) and by 2.5 mg/kg in cats (Fig. 4). The reduction lasted the duration of the experiment (2–6 hr). The rise in blood pressure elicited by stimulation of the greater splanchnic nerve in the cat was not greatly affected. AH.2035 at 2.5–5 mg/kg caused a slight potentiation and 10 mg/kg caused a small (20%) reduction in the response. The fall in blood pressure caused by efferent stimulation of the cervical vagus in the cat was however reduced by AH.2035 ; 1–2.5 mg/kg caused a 50% reduction for 40–120 min.

In both species all doses used (0.5 mg/kg–5 mg/kg in dogs, 2.5–10 mg/kg in cats) caused marked potentiation of the pressor response to intravenous noradrenaline and

angiotensin, and reduced the pressor response to intravenous tyramine (Fig. 4). Small doses of AH.2035 potentiated the response to tyramine in anaesthetized dogs.

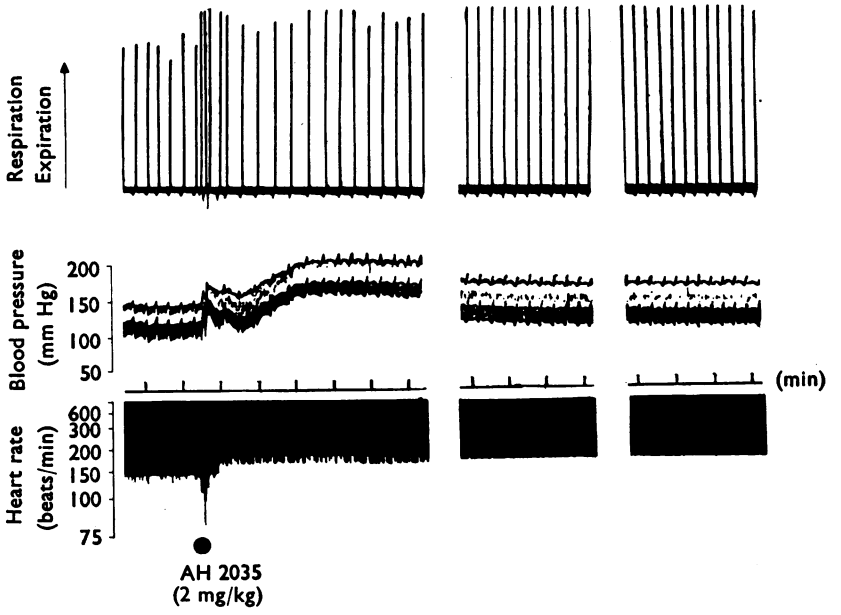


Fig. 2. The effect of intravenous administration of AH.2035 (2 mg/kg) on arterial blood pressure, heart rate and respiration of an anaesthetized dog; 10 min elapsed between panels A and B and B and C.

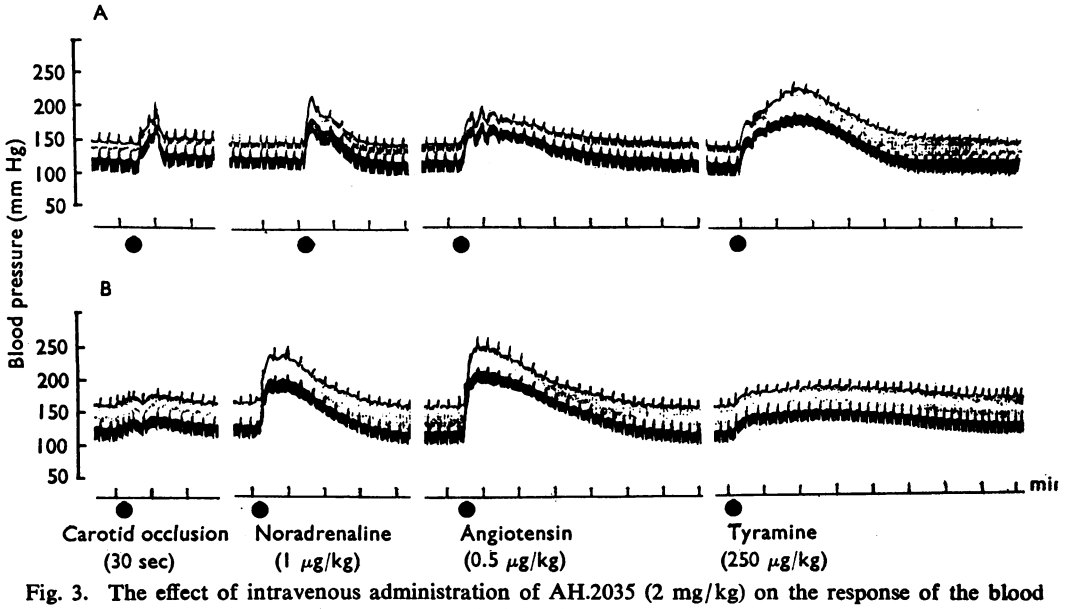


Fig. 3. The effect of intravenous administration of AH.2035 (2 mg/kg) on the response of the blood pressure of an anaesthetized dog to occlusion of the common carotid arteries, injection of noradrenaline, angiotensin and tyramine; 30 min elapsed between panels A and B.

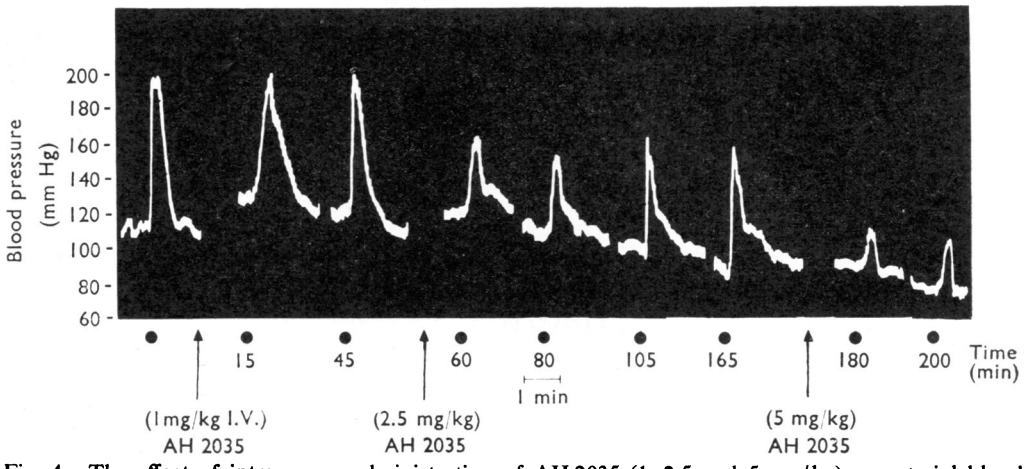


Fig. 4. The effect of intravenous administration of AH.2035 (1, 2.5 and 5 mg/kg) on arterial blood pressure and carotid occlusion reflex (0.30 sec) of an anaesthetized cat.

In dogs the pressor response to nicotine was potentiated by 0.5 mg/kg AH.2035, but slightly reduced by 5 mg/kg, whereas in cats 2.5 mg/kg had no effect and 5 mg/kg caused some potentiation.

Cardiac output was measured in 4 dogs. Two dogs received 2.5 mg/kg and 2.1 mg/kg AH.2035 intravenously. Cardiac output was measured before and 1 hr after the drug. In 1 dog given 2.5 mg/kg the cardiac output increased by 25% after AH.2035, whereas in the other it decreased by 17%. In the 2 dogs given 1 mg/kg the cardiac output increased by 8% and 50%. Blood pressures at the time of the second cardiac output measurement were near control levels, except for the dog with reduced cardiac output in which the blood pressure fell from 150 to 80 mm Hg. All 4 dogs showed pronounced tachycardia. Although variable results were obtained from cardiac output measurements, no marked reduction of cardiac output occurred from a direct action of AH.2035 on the myocardium.

Pretreatment with AH.2035 on anaesthetized cats and dogs

Cats were given AH.2035 subcutaneously. Two animals had 25 mg/kg daily for 2 days, and 2 had 10 mg/kg daily for 4 days. The cats were anaesthetized 24 hr after the last injection. In treated animals the pressor responses to intravenous noradrenaline, angiotensin and nicotine were potentiated when compared with untreated controls. The pressor effect of tyramine was markedly reduced. The initial blood pressure was lower than in control animals, especially in the 2 animals which were given 25 mg/kg AH.2035. These cats had blood pressures of 60 and 90 mm Hg compared with a mean $\pm$S.E. of 126 ± 4.7 mm Hg in 10 control cats.

Six beagle dogs were given 5 mg/kg AH.2035 orally for 2 days, and were anaesthetized 24 hr after the last dose. Two responses to intravenous noradrenaline, angiotensin and tyramine, and to bilateral occlusion of the common carotid arteries, were obtained in each animal (Fig. 5). The pressor responses to carotid occlusion and intravenous tyramine were significantly reduced ($P<0.01$), whereas the response to noradrenaline was

significantly potentiated ($P<0.001$). The response to angiotensin was not significantly altered. The initial blood pressure (mean $\pm$ S.E.) of the treated animals was significantly lower than that of the controls (105 ± 5.9 mm Hg against 130 ± 8.9 mm Hg) and the heart rate was slower (130 ± 8.7 beats/min against 150 ± 6.0 in the controls).

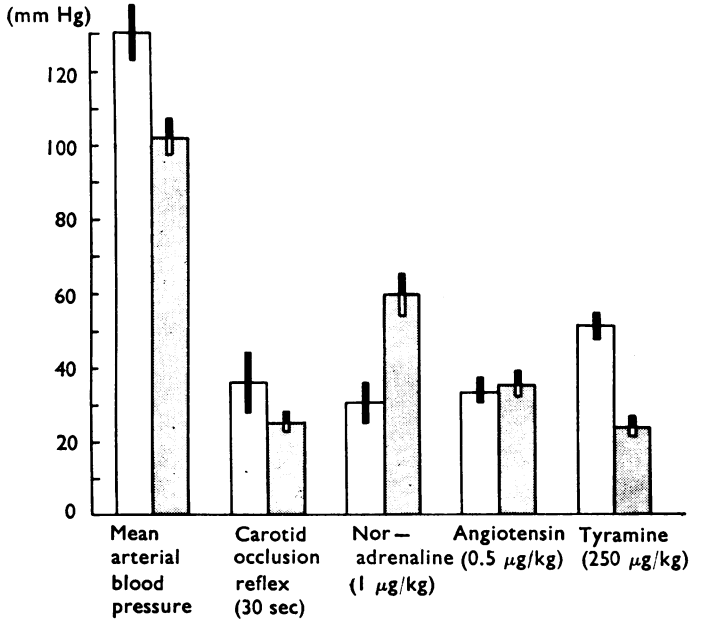


Fig. 5. The effect of pretreatment with AH.2035 on the blood pressure and vasopressor responses of an anaesthetized dog. Open columns=control dogs (n=4); shaded columns=dogs pretreated with 2 doses of 5 mg/kg AH.2035 orally 24 and 48 hr previously (n=6).

The effect of AH.2035 on isolated tissue preparations

Central ear artery of the rabbit

AH.2035 caused an increase in tone of this preparation when added to the perfusion fluid in concentrations of 1 μg/ml. upward. The response to periarterial nerve stimulation of 1 and 2 c.p.s. was reduced by 0.1 μg/ml. AH.2035, leaving stimulation at 5 and 10 c.p.s. unaffected. Higher concentrations (1–10 μg/ml.) caused potentiation, at all frequencies of nerve stimulation, of the response to injected noradrenaline and of the initial phase of the tyramine response while reducing the secondary phase.

Rabbits were pretreated with AH.2035, 25 mg/kg subcutaneously twice daily for 2, 3 or 4 days. Arteries were prepared 18–20 hr after the last injection. Responses to all frequencies of nerve stimulation were reduced after 3–4 days. There was no change in the response to noradrenaline or the primary phase of the response to tyramine, while the secondary phase of the response to tyramine was virtually absent (Fig. 6).

Dog spleen

Two dogs received 5 mg/kg AH.2035 for 2 days and on the 3rd day the spleens were removed and perfused with McEwen solution. Initially the effect of splenic nerve stimulation

on perfusion pressure was investigated. Figure 7A and B shows the response to sympathetic nerve stimulation of a normal spleen and a spleen taken from a dog pretreated with AH.2035. The responses of the spleen to all frequencies of nerve stimulation were reduced by pretreatment with the compound. Similarly after addition of phenoxybenzamine and cocaine to the perfusion fluid the output of noradrenaline during sympathetic nerve stimulation was much reduced in spleens from pretreated dogs (Fig. 7C).

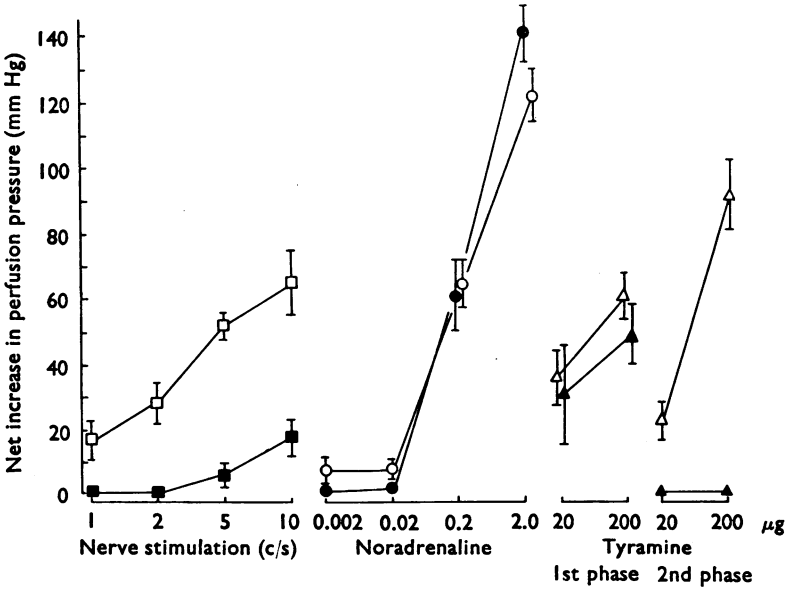


Fig. 6. The effect of pretreatment of rabbits with AH.2035 on the response of the isolated central ear artery to periarterial nerve stimulation, noradrenaline and tyramine. Open symbols represent responses from control rabbits (n=12) and closed symbols responses from rabbits pretreated by 3 days with 25 mg/kg AH.2035 twice a day by subcutaneous injection (n=4).

Vas deferens of the guinea-pig

The effect of AH.2035 on the response of the vas deferens to preganglionic nerve stimulation was complex. The responses depended upon the frequency of stimulation and the concentration of AH.2035 in the bathing fluid. AH.2035, 0.5 and 2.5 μg/ml., reduced and delayed the response of the vas deferens to stimulation at 2 c/s; higher concentrations of AH.2035 reduced or increased the response. Concentrations of AH.2035 up to 5 μg/ml. usually increased the response to nerve stimulation at 5 c/s, whereas higher concentrations reduced it. AH.2035 up to 10 μg/ml. increased the responses at 10 and 20 c/s. The response to noradrenaline was increased by concentrations of AH.2035 up to 25 μg/ml.

AH.2035, 0.5 to 10.0 μg/ml., prevented the adrenergic neurone blocking action of guanethidine, but did not completely reverse the blocking action of guanethidine once established.

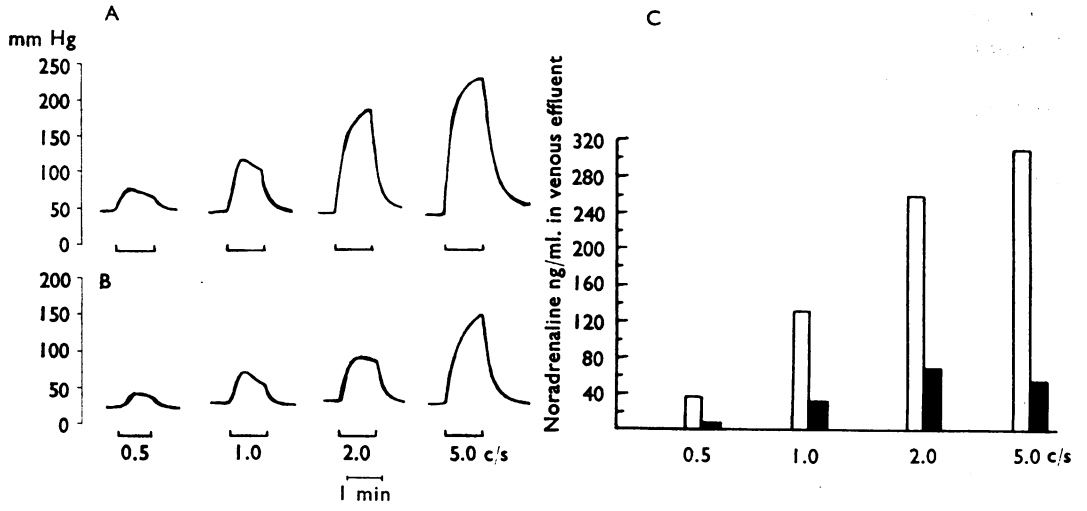


Fig. 7. The effect of pretreatment with AH.2035 (5 mg/kg 24 and 48 hr previously) on the response of the isolated dog spleen to sympathetic nerve stimulation. Panel A shows the vasoconstrictor response of a normal spleen and panel B the response of a spleen taken from a pretreated dog. Panel C shows the output of noradrenaline from the spleen during the last 30 sec of a period of 2 min stimulation. Open columns show mean results for 3 untreated dogs; closed columns for 2 treated dogs. Phenoxybenzamine and cocaine (1 μg/ml. each) were present in the perfusion fluid (for details see text).

Vasa deferentia removed from guinea-pigs pretreated with AH.2035 (10 mg/kg for 10 days; 20 mg/kg for 4 days and 50 mg/kg for 4 days) showed reduced responses to hypogastric nerve stimulation at 2, 5, 10 and 20 c/s. The addition of dexamphetamine to the bathing fluid failed to reverse this effect.

Ileum of the guinea-pig

AH.2035 at 0.1 μg/ml. had no effect on responses of the ileum to co-axial and periarterial nerve stimulation, or added noradrenaline. At 1 μg/ml. responses to sympathetic periarterial nerve stimulation at 5 and 10 c/s were reduced, as was the noradrenaline response, but parasympathetic co-axial nerve stimulation was unaffected.

Ilea taken from guinea-pigs pretreated with AH.2035 50 mg/kg subcutaneously daily for 1–4 days showed reduced responses to periarterial nerve stimulation at 2, 5, 10, 20 and 50 c/s. The reduction in responses was statistically significant after 2–4 days pretreatment. Responses to co-axial nerve stimulation were normal in ilea from pretreated animals.

Ileum of the rabbit

The inhibitory effect of periarterial nerve stimulation of the pendular movements of the rabbit ileum was potentiated by AH.2035, 1.0–2.0 μg/ml., whereas the response to noradrenaline was unaffected. At 10 μg/ml. the responses to nerve stimulation and noradrenaline were reduced as were the spontaneous movements of the tissue. AH.2035,

2.5 μg/ml. prevented and reversed the adrenergic neurone blocking action of guanethidine (0.5 μg/ml.) on this preparation.

Atria of the cat

The response of the atria to acetylcholine and vagal stimulation was reduced by AH.2035, 1 μg/ml. This decrease did not occur in the presence of 0.5 μg/ml. of the β-blocking agent propranolol.

Mesenteric artery of the dog

AH.2035, 0.1, 1 and 10 μg/ml. added to the perfusion fluid potentiated the vasoconstrictor response of the mesenteric artery to sympathetic nerve stimulation at 5, 10 and 20 c/s and to noradrenaline. The potentiation was most marked at 1 μg/ml. AH.2035.

DISCUSSION

AH.2035 has been shown to have some properties which are similar to those of guanethidine. Both AH.2035 and guanethidine administered intravenously to anaesthetized animals produce a dose-dependent vasopressor response which can be attributed to mobilization of endogenous stores of noradrenaline. However, AH.2035 would seem to be considerably more active in this respect since 15 mg/kg guanethidine produced a pressor response of 45 min duration in the dog (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960), whereas a similar rise in blood pressure was produced by 1–2 mg/kg AH.2035. The above authors observed that a transient fall in blood pressure preceded the period of hypertension with guanethidine, but when AH.2035 was injected there was a transient increase in blood pressure accompanied by a bradycardia and respiratory stimulation. Since these effects were blocked by hexamethonium the increase in blood pressure is probably due to stimulation of autonomic ganglia, the bradycardia to baroreceptor reflex and the effect on respiration to chemoreceptor stimulation. As with guanethidine, when the period of hypertension had subsided the resting blood pressure declined to below pre-injection level and at this time the response of the blood pressure to noradrenaline was enhanced, while that to tyramine and occlusion of the common carotid arteries was reduced. Similar effects were seen in dogs pretreated with AH.2035.

On isolated tissues AH.2035, unlike guanethidine, had little or no adrenergic neurone blocking activity. Blockade of the response to low frequency sympathetic nerve stimulation was observed with low concentrations (0.1 μg/ml.) of AH.2035 on isolated ear arteries of the rabbit and vas deferens of the guinea-pig. Higher concentrations of AH.2035 potentiated the responses to all frequencies of nerve stimulation and potentiated responses of the tissues to added noradrenaline. This effect can be attributed to indirect sympathomimetic activity or blockade of noradrenaline uptake by AH.2035. Evidence of indirect sympathomimetic activity of AH.2035 *in vitro* is given by the finding that AH.2035 prevented and reversed the neurone blocking action of guanethidine on the Finkleman preparation. Since AH.2035 reduced the carotid occlusion reflex in the anaesthetized dog, the compound was tested on an isolated artery preparation from the same species. AH.2035 did not block the responses of the isolated mesenteric artery preparation to sympathetic nerve stimulation. However this preparation only responds

in vitro to relatively high rates of stimulation, 5 c/s and above, and the possibility of neurone blockade at lower rates of activity *in vivo* cannot be entirely ruled out.

Since the antihypertensive activity of AH.2035 in the conscious dog was gradual in onset, it was decided to test for adrenergic neurone blocking activity in tissues taken from animals pretreated with AH.2035. Responses of isolated tissues to pre- and post-ganglionic sympathetic nerve stimulation were reduced after 2–3 days pretreatment with AH.2035, and addition of dexamphetamine did not reverse the blockade. The reduced responses of the isolated spleen from a pretreated dog were correlated with a decrease in the output of noradrenaline in the splenic vein during post-ganglionic nerve stimulation.

AH.2035 does not appear to affect parasympathetic transmission. AH.2035 reduced the response of the blood pressure to efferent vagal stimulation in anaesthetized cats. However, since the main component of a vagal depressor response is bradycardia, and since injections of AH.2035 produced a long lasting tachycardia, reduction of the response to vagal stimulation would be expected. AH.2035 in the presence of propranolol had no effect on responses of the isolated cat atria to vagal stimulation or added acetylcholine. In addition the responses of isolated guinea-pig ileum to co-axial parasympathetic nerve stimulation were normal in guinea-pigs pretreated with AH.2035.

Some of the pharmacological properties of AH.2035 resemble those of 3-phenoxy-propylguanidine. However, this compound did not potentiate responses of isolated tissues to sympathetic nerve stimulation in acute experiments (Bartlet, 1962), and no impairment of responses to sympathetic nerve stimulation was shown in animals pretreated with 3-phenoxy-propylguanidine (Chen, Ensor, McCarthy, McLean & Campbell, 1964).

Biochemical studies carried out in our laboratories show that AH.2035 inhibits noradrenaline uptake and depletes catecholamines from peripheral stores to a maximum of 66% (Martin and Harrison, to be published). The pharmacological evidence suggests that pretreatment with AH.2035 leads to impairment of post-ganglionic sympathetic transmission by interfering with release of noradrenaline, perhaps because of selective depletion of the catecholamine stores. The resulting " neurone blockade " is qualitatively different from that produced by guanethidine in that it is not reversed by dexamphetamine (Day & Rand, 1963). The antihypertensive effect of AH.2035 in the conscious hypertensive dog probably results from a decrease in peripheral resistance, although an effect on cardiac output cannot be entirely excluded despite the observation that the drug did not significantly reduce cardiac output acutely in anaesthetized dogs.

SUMMARY

1. AH.2035 produced a vasopressor response in anaesthetized animals due to release of endogenous catecholamines. After the period of hypertension (approximately 15 min), hypotension developed accompanied by a decrease in the carotid occlusion reflex and a decrease in the response to tyramine. The response of the blood pressure to noradrenaline was enhanced.

2. The response of isolated tissues to pre- and post-ganglionic sympathetic nerve stimulation was generally enhanced by AH.2035, although low concentrations reduced the response to low frequency stimulation in some tissues, such as the vas deferens of the guinea-pig and the central artery of the rabbit ear. The responses to parasympathetic stimulation were not blocked by AH.2035.

3. Anaesthetized cats and dogs pre-treated with AH.2035 demonstrated a reduced mean arterial blood pressure, carotid occlusion reflex and vasopressor response to tyramine. The vasopressor activity of noradrenaline was enhanced. Tissues removed from animals pretreated for several days with AH.2035 showed an impaired response to sympathetic nerve stimulation. The output of noradrenaline from the dog spleen during stimulation of sympathetic nerves was reduced in dogs pretreated with AH.2035. The impairment of the responses to sympathetic nerve stimulation was not reversed by dexamphetamine.

4. The oral administration of AH.2035, 5 mg/kg, to conscious renal hypertensive dogs caused a reduction in systolic blood pressure without any change in heart rate. On the first day of administration a large and sustained rise in systolic blood pressure occurred but not on subsequent days. On the second and third day of administration there was gradual reduction in systolic blood pressure. The antihypertensive effect was maintained for 10 days without any marked sign of tolerance. There were no visible signs of sympathetic impairment. The initial hypertensive effect could be avoided by approaching the antihypertensive dose level gradually.

The authors would like to thank Mr. C. Harrison and Mr. L. E. Martin for carrying out biochemical studies with this compound. We are grateful to Mrs. M. Tribe, Mr. C. T. Alabaster and Mr. R. A. Coleman for technical assistance.

REFERENCES

BARTLET, A. L. (1962). Some cocaine-like actions of 3-phenoxypropylguanidine. *Br. J. Pharmac. Chemother.*, **18**, 475–489.

BERTLER, A., CARLSSON, A. & ROSENGREN, E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta. physiol. scand.*, **44**, 273–292.

BIRMINGHAM, A. T. & WILSON, A. B. (1965). An analysis of the blocking action of dimethylphenyl piperazinium iodide on the inhibition of isolated small intestine produced by stimulation of the sympathetic nerves. *Br. J. Pharmac. Chemother.*, **24**, 375–386.

BRITTAIN, R. T., FARMER, J. B., JACK, D., MARTIN, L. E. & RITCHIE, A. C. (1967). 1-Amino-4-phenyl pyridinium chloride, a potential antihypertensive agent. *Nature, Lond.*, **213**, 731–732.

CHEN, G., ENSOR, C. R., MCCARTHY, D. A., MCLEAN, J. R. & CAMPBELL, A. (1964). The pharmacology of 3-phenoxypropylguanidine. *J. Pharmac. exp. Ther.*, **143**, 374–382.

DAY, M. D. & RAND, M. J. (1963). Evidence for a competitive antagonism of guanethidine by dexamphetamine. *Br. J. Pharmac. Chemother.*, **20**, 17–28.

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.

FARMER, J. B. (1966). Liberation of noradrenaline from the dog spleen. *J. Pharm. Pharmac.*, **18**, 765–767.

FINKLEMAN, B. (1930). On the nature of inhibition in the intestine. *J. Physiol., Lond.*, **70**, 145–157.

HUKOVIĆ, S. (1961). Responses of the isolated sympathetic nerve–ductus deferens preparation of the guinea-pig. *Br. J. Pharmac. Chemother.*, **16**, 188–194.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

MAXWELL, R. A., PLUMMER, A. J., SCHNEIDER, F., POVALSKI, H. & DANIEL, A. I. (1960). Pharmacology of [2-(octahydro-1-azocinyl)-ethyl]-guanidine sulphate (Su-5864). *J. Pharmac. exp. Ther.*, **128**, 22–29.

ROGERS, L. A., ATKINSON, R. A. & LONG, J. P. (1965). Responses of isolated perfused arteries to catecholamines and nerve stimulation. *Am. J. Physiol.*, **209**, 376–382.