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The pharmacology of 5-(2-t-butylamino-1-hydroxyethyl) salicylamide (AH 3474), a β-adrenoreceptor blocking agent

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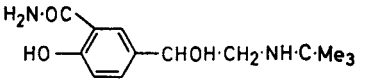
AH 3474 is a specific β-adrenoreceptor antagonist, devoid of stimulant activity. When given by mouth to conscious guinea-pigs and dogs, AH3474 and propranolol are equiactive in antagonizing isoprenaline-induced tachycardia. In anaesthetized animals AH 3474 was 2–4 times less active than propranolol when given intravenously. A similar potency ratio was found in volunteer studies in which the drug was taken orally. On isolated tissues AH 3474 was much less active than propranolol. AH 3474 had 1/10th the activity of propranolol in blocking the inhibitory action of isoprenaline on the rat uterus and was at least 100 times less active in antagonizing the tachycardia induced by adrenaline on the guinea-pig atria. *In vitro*, equilibrium conditions for AH 3474 were obtained in 15 min, whereas 45 min were required for propranolol. AH 3474 antagonized the cardiac arrhythmias induced by ouabain in the anaesthetized dog. The amount required far exceeded the β-adrenoreceptor blocking dose. AH 3474 possessed no "quinidine-like" actions on cardiac muscle of dog or guinea-pig. The local anaesthetic activity of AH 3474 was 400 times less than that of propranolol.

The first drug shown to antagonize the effects of catecholamines at β-adrenoreceptor (β-receptor) sites was dichloroisoprenaline (Powell & Slater 1958). This drug, like its successor pronethalol, possessed some β-stimulant activity (Black & Stephenson, 1962). Later, propranolol, a close analogue of pronethalol, was shown to be a potent β-adrenoreceptor blocker without sympathomimetic activity (Black, Crowther, & others, 1964). Propranolol has also potent quinidine-like and local anaesthetic actions (Morales-Aguilera & Vaughan Williams, 1965).

AH 3474 [I;5-(2-t-butylamino-l-hydroxyethyl)salicylamide] is a β-adrenoreceptor blocking agent devoid of quinidine-like, local anaesthetic and β-stimulant properties. The compound was prepared in the Chemical Research Laboratories of Allen & Hanburys Ltd. Its structure is given below:



I

EXPERIMENTAL

Anaesthetized animals

Cats and dogs. Cats of either sex weighing 2–3 kg were anaesthetized with chloralose (80 mg/kg intravenously) after induction with 3% halothane in nitrous oxide and oxygen (3:1). Beagles of either sex weighing 7–11 kg were anaesthetized with pentobarbitone sodium 30 mg/kg 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. Respiration was recorded via a Magill cuffed endotracheal tube and Statham low pressure transducer. Heart rate was measured by a Nielson instantaneous ratemeter triggered from the pulse pressure sensed by the blood pressure transducer or triggered by the QRS complex of the electrocardiogram. In some experiments the cervical vagus and cardiac accelerans or cervical sympathetic nerves or splanchnic nerves were cut and the peripheral ends stimulated with trains of rectangular pulses of 1 ms duration and supramaximal voltage. Contractions of the cat nictitating membrane were recorded using an isometric strain gauge. Drugs were injected or infused through a cannula in the left femoral vein.

The hind-limb of the anaesthetized dog was perfused as follows: the skin was removed from the right hind-limb and a torniquet applied to the right ankle. A catheter was placed in the right femoral artery so that the tip lay in the abdominal aorta. Ties were placed on the right deep circumflex iliac, external and internal iliac and the deep femoral artery. Blood was sampled from this catheter and pumped (Watson-Marlow peristalic pump) into the right hind limb at a constant rate via a catheter placed in the femoral artery distal to the entry of the sampling catheter. The flow rate was adjusted so that the perfusion pressure approximated to the systemic blood pressure. Perfusion pressure was measured by Devices blood pressure transducer connected to a T-piece proximal to the entry of the catheter into the femoral artery. Drugs were injected into a rubber junction in the sampling catheter.

Rat. Female rats of a weight range 200–300 g were pretreated with 0·1 mg/100 g stilboestrol 24 h before use. Animals were anaesthetized with pentobarbitone sodium, 3–6 mg/100 g intraperitoneally. The trachea was cannulated and the uterus exposed by a mid-line abdominal incision. One horn of the uterus was mobilized and attached to a strain gauge transducer. The external jugular vein was cannulated and oxytocin infused (2 units/kg h^{-1}). Drugs were injected into the venous cannula by a 3-way tap system.

Conscious animals

Dog. Heart rate was determined in normotensive dogs using a Nielson instantaneous ratemeter triggered by the QRS complex of the ECG. The ECG was recorded from plate electrodes attached to the limbs (an area of skin was shaved). The dogs were trained to lie quietly on their right side. Isoprenaline was given intravenously; the β-receptor blocking drugs were given by mouth in hard gelatin capsules.

Hypertensive dogs were prepared as described by Cullum, Farmer & Handley (1967).

Guinea-pig. Heart rate in guinea-pig was determined by the method of Farmer & Levy (1968a). Guinea-pigs, 250–500 g, were trained to stand unrestrained on four plate electrodes, the ECG obtained was used to trigger the instantaneous ratemeter. Drugs were given orally in solution except isoprenaline which was given subcutaneously.

Local anaesthetic activity of the β-receptor blocking drugs was determined in the guinea-pig by the intradermal wheal method of Bülbring & Wajda (1945).

Hypertensive rats. Male Wistar rats, 100–150 g, were made hypertensive by unilateral nephrectomy and subcutaneous implantation of desoxycorticosterone acetate. Heart rate and blood pressure were measured indirectly from the tail (Farmer & Levy, 1968b). Drugs were given orally or subcutaneously in solution.

Isolated tissues

Rat uterus. Uterine horns taken from rats pretreated with 0·1 mg/100 g stilboestrol were suspended in a physiological salt solution at 37° and gassed with 5% carbon dioxide in oxygen. The composition of the salt solution was; g/litre, NaCl 9·0; $NaHCO_3$ 1·0; KCl 0·42; $CaCl_2$ 0·24; glucose 1·0. Contractions were recorded using an isometric strain gauge.

Guinea atria. The hearts of guinea-pigs were removed and placed in chilled McEwen solution (1956). The blood was gently squeezed from the heart and the atria separated, cleared of fat and suspended in McEwen solution maintained at 32° and gassed with 5% carbon dioxide in oxygen. Contractions were measured using an isometric strain gauge. The relative refractory period of the heart muscle was measured by the method of Dawes (1946). The atria were anchored directly to the terminals of a bipolar electrode and were driven with rectangular impulses of 1 ms duration. The maximum rate at which the atria could be driven in absence and presence of a drug was determined. The reciprocal of the maximum rate gives a measure of the relative refractory period. In some experiments the effects of drugs on the force of contraction of spontaneously beating atria were determined.

Drugs. AH 3474 [5-(2-t-butylamino-l-hydroxyethyl)salicylamide], propranolol hydrochloride (ICI), oubain (BDH), isoprenaline sulphate (BW), papaverine hydrochloride (Hopkin & Williams) and quinidine sulphate (BDH). Doses of drugs refer to the bases.

RESULTS

The cardiovascular effects of AH 3474 *and propranolol on the anaesthetized dog Blood pressure, heart rate, carotid occlusion reflex and respiration*

AH 3474, 0·1–5 mg/kg given intravenously, produced a 10–30 mm Hg fall in systolic and diastolic blood pressures and a bradycardia of 10–40 beats/min. The intensity and duration of these effects varied considerably, and graded responses to the drug were not obtained. However, the bradycardia lasted much longer (2–3 h) than the effect on blood pressure (40–80 min). The response to bilateral occlusion of the common carotid arteries was reduced by 30–50% but recovered in parallel with the blood pressure (Fig. 1). Rate and depth of respiration were not affected by AH 3474. Similar effects were observed with 0·05–1·0 mg/kg propranolol.

Isoprenaline-induced tachycardia and depressor responses

AH 3474, 1·6 mg/kg given intravenously, considerably reduced the depressor response and tachycardia caused by intravenous injection of isoprenaline for 2–3 h. Propranolol, 0·4 mg/kg, produced a similar reduction of the responses to isoprenaline. Antagonism of the depressor response was more prolonged than antagonism of the tachycardia. At equiactive doses, the durations of the action of the drugs were similar. In other experiments tachycardia was produced by infusing isoprenaline, with 5 min intervals between infusions. When a reproducible tachycardia was obtained AH 3474 or propranolol were infused at increasing rates. Infusions lasted for 10 min at any given rate and were started 5 min before infusion of isoprenaline. The rate of infusion which caused a 50% reduction of the tachycardia was calculated. For AH 3474, this was 9·5 μg/kg min^{-1} and for propranolol, 7·0 μg/kg min^{-1}.

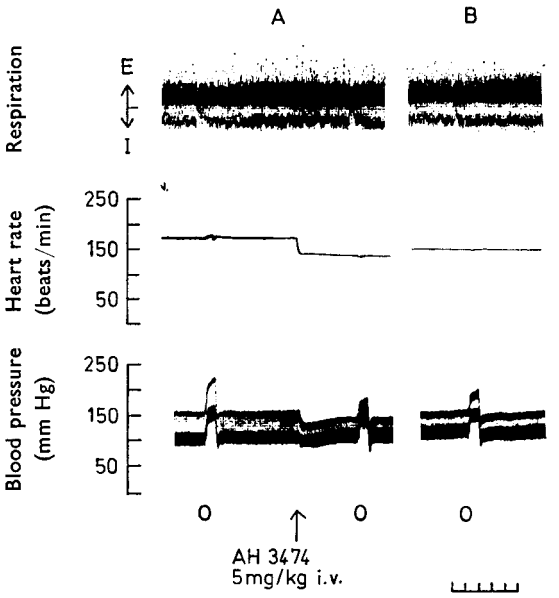


FIG. 1. The effects of AH 3474, 5 mg/kg given intravenously, on respiration, heart rate and blood pressure of anaesthetized dog. O = carotid occlusion for 30 s; 70 min elapsed between panels A and B.

Ouabain-induced cardiac arrhythmia

The antiarrhythmic activities of AH 3474, propranolol and quinidine were compared in anaesthetized dogs. After a control ECG reading was obtained repeated intravenous doses of ouabain were given according to the following schedule; 40 μg/kg immediately, 20 μg/kg after 30 min and 10 μg/kg each 15 min thereafter until cardiac arrhythmia occurred. In control experiments the arrhythmias produced by ouabain usually lasted 2–3 h. In other experiments AH 3474, propranolol or quinidine was infused at increasing concentrations for 5 min periods until the ECG record became normal. The total doses of AH 3474 required to correct the arrhythmias in three separate experiments were 4·5, 11·85 and 11·85 mg/kg. For propranolol the doses were 1·85, 3·85, 3·85 mg/kg and for quinidine the dose was 8·85 mg/kg. Thus AH 3474 under these conditions is approximately equipotent with quinidine but about one-half to one-third as active as propranolol.

Vascular resistance in hind limb

Intra-arterial injection of AH 3474 or propranolol into the perfused hind limb of the dog caused a transient fall in perfusion pressure. This effect was produced by 1–4 mg/kg AH 3474 and by 0·05 to 0·8 mg/kg propranolol. The response to AH 3474 was small and not dose-dependent; that to propranolol was marked and dose-dependent.

In other experiments intra-arterial injections of AH 3474 or propranolol were found to reduce the vasodilation caused by intra-arterial injection of isoprenaline. AH 3474 was 4 times less potent than propranolol.

Acute toxicity of AH 3474 and propranolol

AH 3474 was infused continuously into the femoral vein of the anaesthetized dogs, and blood pressure, heart rate, respiration and ECG were recorded. AH 3474 (1 mg/kg

min^{-1}) reduced the blood pressure and heart rate but did not prove lethal after 100 min of infusion. AH 3474 (4 mg/kg min^{-1}) and propranolol (1 mg/kg min^{-1}) produced slow falls in blood pressure and heart rate which resulted in cardiovascular collapse and death. AH 3474 reduced the depth of respiration but increased the rate, whereas propranolol reduced both depth and rate. The dose infused at the time of death was 200 mg/kg for AH 3474 and 20 mg/kg for propranolol. Infusion of propranolol caused sinus arrhythmia and the ECG showed an increase in the PR and QT intervals and reduction in the QRS complex. These effects were not seen with AH 3474 (Fig. 2).

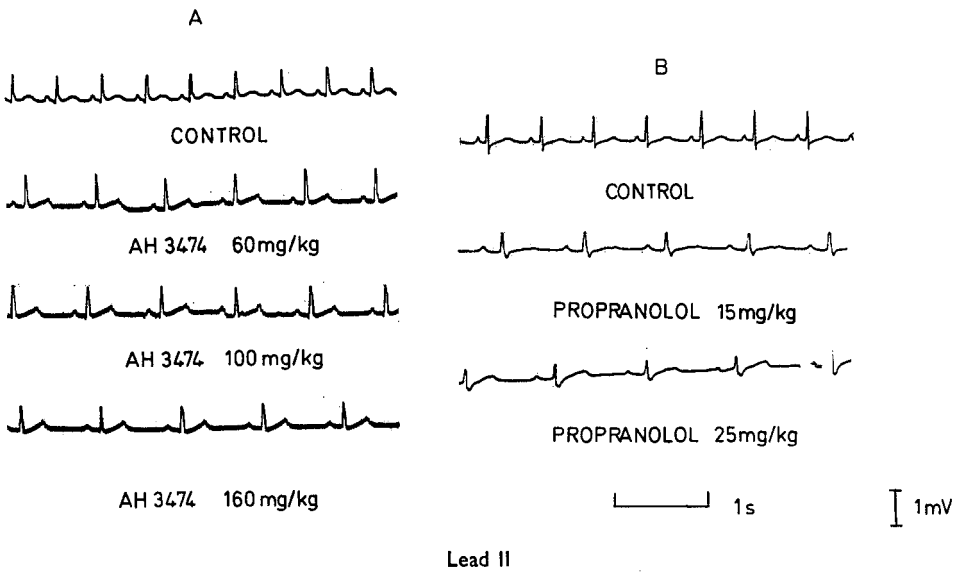


FIG. 2. The effects of AH 3474 (A) and propranolol (B) on the ECG of anaesthetized dog. Both drugs were given as an infusion, AH 3474 at 4 mg/kg min^{-1} and propranolol at 1 mg/kg min^{-1}.

The cardiovascular effects of AH 3474 *in the anaesthetized cat*

(i) *Blood pressure, heart rate and respiration.* AH 3474 in doses of 1, 2 and 5 mg/kg given intravenously, caused a 15–50 mm Hg fall in blood pressure of 10–45 min duration. The heart rate decreased by 5–50 beats/min for a period of 1–2 h. AH 3474 has no effect on heart rate in cats pretreated with reserpine. In the cat, the effect of AH 3474 on blood pressure and heart rate varied considerably and graded responses could not be obtained. The bradycardia lasted much longer than the hypotension. Rate and depth of respiration were not affected by AH 3474.

(ii) *Responses of the blood pressure and heart rate to vasopressor agents and stimulation of peripheral autonomic nervous system.* AH 3474 (5 mg/kg intravenously) augmented the intensity and duration of the pressor responses to noradrenaline and adrenaline. The response to intravenous angiotensin was augmented but that to tyramine was slightly reduced. AH 3474 (1 and 2 mg/kg) had no effect on the biphasic response of the blood pressure to stimulation of the splanchnic nerve.

AH 3474 (1 and 5 mg/kg) had no significant effect on the response of the nictitating membrane to preganglionic stimulation of the cervical sympathetic nerves, but the response to injected adrenaline was slightly potentiated. The response of the heart

rate to accelerans or vagal nerve stimulation was determined before and after infusion of 150 and 750 μg/kg of AH 3474 or propranolol over a 15 min period. The frequencies of nerve stimulation were 1, 2, 5, 10 and 20 Hz. AH 3474 and propranolol reduced the response of the heart rate to accelerans but not vagal nerve stimulation. In some experiments, the response of the heart to vagal stimulation appeared to be enhanced by β-adrenergic blockade. AH 3474 was 2–3 times less active than propranolol in reducing the increase in heart rate caused by stimulating the accelerans nerve.

The effects of AH 3474 *and propranolol on the uterus of the anaesthetized rat*

Intravenous injection of AH 3474, 0·5 mg/kg, or propranolol 0·125, 0·25 and 0·5 mg/kg, had no effect on the spontaneous motor activity of the rat uterus but both drugs reduced the inhibitory response of the uterus to isoprenaline. AH 3474 was 2–4 times less active than propranolol.

The effects of AH 3474 *and propranolol on isolated tissues*

Isolated uterus of the rat. AH 3474 (5 and 10 μg/ml) had no effect on the spontaneous contractions of the rat uterus; isoprenaline (1 μg/ml) abolished the contractions. AH 3474 (5 μg/ml) blocked the inhibitory effect of isoprenaline but had no effect on the inhibitory response to papaverine. Similar effects were observed with propranolol at 1/10th the concentration of AH 3474.

Guinea-pig isolated atria. Cumulative dose response curves for adrenaline were determined on spontaneously beating atria by adding geometrically increasing doses without changing the bath fluid, leaving each concentration to exert a maximal effect (60 s) before the addition of the next dose. Dose response curves for increased force of contraction were obtained before and after the addition of AH 3474 or propranolol. The antagonists were allowed 45 min contact with the tissue. This contact time was allowed since it was observed that equilibrium conditions were not obtained earlier with propranolol. Both AH 3474 and propranolol caused dose dependent shifts of the dose response curve to adrenaline. pA_2 values for AH 3474 and propranolol were calculated by the method of Arunlakshana & Schild (1959). Results for each drug were 6·13, 6·02 and 6·5 for AH 3474 and 8·02 and 7·78 for propranolol.

The effects of AH 3474, propranolol and quinidine on the relative refractory period of electrically driven guinea-pig auricles were determined. AH 3474 in concentrations up to 20 μg/ml had no effect but propranolol and quinidine, 0·1 to 10·0 μg/ml, caused concentration-dependent increases in the relative refractory period. At 10 μg/ml the percentage increase in the refractory period was 55 for propranolol and 45 for quinidine (Fig. 3).

AH 3474, propranolol and quinidine also reduced the spontaneous rate of contraction of isolated auricles but only propranolol and quinidine reduced the force of contraction.

Local anaesthetic activity of AH 3474 *and propranolol in the conscious guinea-pig*

Graded dose response curves for local anaesthetic activity were obtained for AH 3474, propranolol and procaine by the intradermal wheal test in the guinea-pig. Propranolol was 10 times more active and AH 3474 was 40 times less active than procaine.

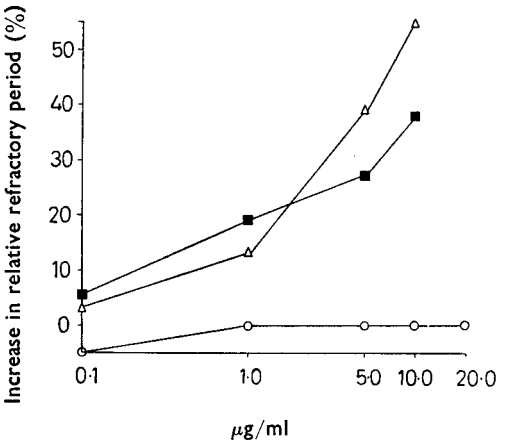


FIG. 3. The effects of AH 3474 (○—○), propranolol (△—△) and quinidine (■—■) on the relative refractory period (R.R.P.) of guinea-pig cardiac muscle *in vitro*, % increase in R.R.P. is plotted against log concentration of drug. Each point is the mean of two experiments.

The effect of AH 3474 *and propranolol on isoprenaline-induced tachycardia in conscious animals*

Guinea-pig. After two half-hourly heart rate determinations, groups of 4 animals were orally dosed with AH 3474 (50 mg/kg), propranolol (50 mg/kg) or saline. The effects of subcutaneous injections of isoprenaline (30 μg/kg) on heart rate were determined for each animal, 1, 3 and 5 h after administration of the β-receptor blockers. AH 3474 and propranolol had similar potencies and durations of action in blocking isoprenaline tachycardia (Fig. 4).

Dog. Propranolol or AH 3474, given orally, antagonized the tachycardia produced by repeated intravenous injection of isoprenaline 0·3 μg/kg. Typical results in the same dog are shown in Fig. 5. AH 3474 and propranolol (0·25 or 0·5 mg/kg.) were about equipotent in this test with maximal actions after 1 h. Both drugs at 0·5 mg/kg acted for longer than 4 h.

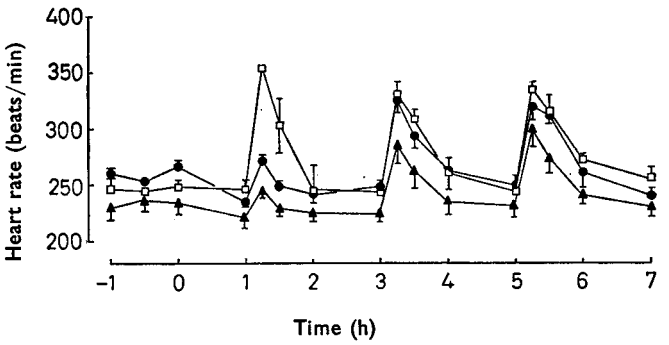


FIG. 4. The effects of AH 3474 (●—●) and propranolol (▲—▲), 50 mg/kg, given orally at time O, on isoprenaline induced tachycardia in guinea-pigs. Isoprenaline, 30 μg/kg, was given subcutaneously at 1, 3 and 5 h after drug administration. Each point is the mean response ± s.e. for a group of 4 guinea-pigs. □—□ saline control group.

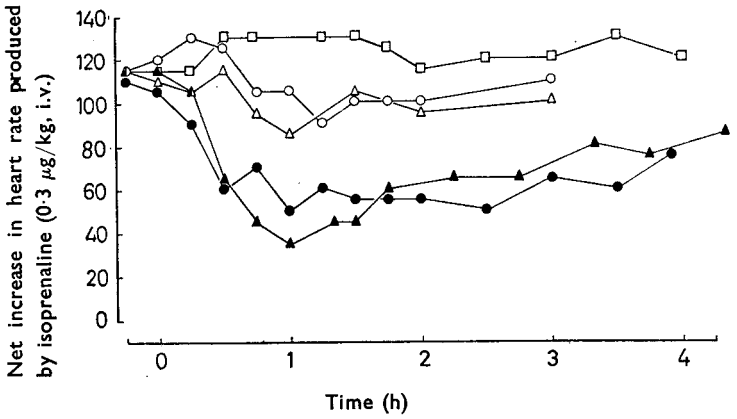


FIG. 5. The effects of AH 3474, 0·25 mg/kg (○—○) and 0·5 mg/kg (●—●), and propranolol, 0·25 mg/kg (△—△) and 0·5 mg/kg (▲—▲), on isoprenaline induced tachycardia in the conscious dog. □—□ saline control group. Drugs were given orally at time O. Isoprenaline, 0·3 μg/kg was given intravenously.

The effects of AH 3474 on systolic blood pressure and heart rate of the conscious hypertensive rat

The systolic blood pressures and heart rates of a group of hypertensive rats were determined daily. After control readings had been established supramaximal β-blocking doses of AH 3474 (50 mg/kg) were given subcutaneously twice daily for 2 days, 2 h before and 4 h after the blood pressure and heart rate determinations. The results are shown in Fig. 6. AH 3474 produced a fall in heart rate of some 50 beats/min without changing the systolic blood pressure. The heart rate took several days to recover after the last dose of AH 3474.

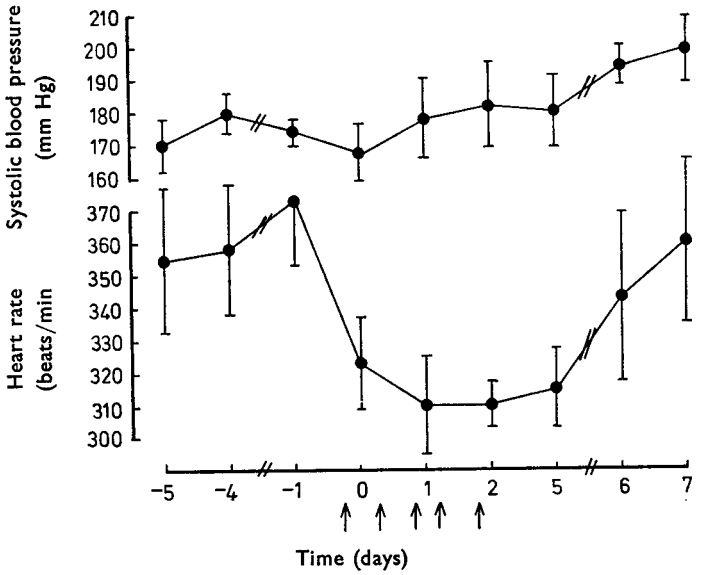


FIG. 6. The effects of AH 3474 on systolic blood pressure and heart rate of conscious hypertensive rats. AH 3474, 50 mg/kg, was given subcutaneously at ↑ 2 h before and 4 h after blood pressure and heart rate determinations.

The effects of AH 3474 *on systolic blood pressure and heart rate in the conscious hypertensive dog*

AH 3474 was given orally to 3 dogs on sixteen consecutive days. The animals received 1 mg/kg for 1 day, 2 mg/kg for 7 days and 20 mg/kg for 3 days. Doses of 2 to 5 mg/kg AH 3474 produced a bradycardia of 10–20 beats/min. At 10 and 20 mg/kg a small, probably insignificant fall in systolic blood pressure (10–15 mm Hg) was observed in 2 dogs.

DISCUSSION

AH 3474 has been shown to have β-receptor blocking activity in conscious and anaesthetized animals and on isolated tissues. In conscious dogs and guinea-pigs AH 3474 and propranolol, given orally, were about equipotent and had similar durations of action in antagonizing the tachycardia produced by isoprenaline. However, in anaesthetized animals the β-receptor blocking activity of AH 3474 was 2–4 times less than that of propranolol when assessed against the tachycardia produced by isoprenaline or accelerans nerve stimulation, but equiactive doses had similar durations of action. The second estimate of relative potency is close to that found in volunteer studies. Single oral doses of AH 3474, 100 mg, or propranolol, 40 mg, were about equally effective in suppressing exercise-induced tachycardia. AH 3474 acted for 3 to 4 h and propranolol slightly longer (W.T. Simpson, Personal communication).

AH 3474 was much less active than propranolol on isolated tissues. The β-blocking potency of AH 3474 on rat isolated uterus was 1/10th that of propranolol whilst on isolated atria, the activity was some 100 times less. It was also noted that AH 3474 took about 15 min to produce a constant reduction in the response of the isolated atria to adrenaline whereas propranolol took 45 min. There is an obvious discrepancy between the *in vivo* and *in vitro* potency ratios for the two drugs. The most likely explanation is that with propranolol the *in vitro* tests do not only measure β-receptor blocking activity, but also an intracellular quinidine-like action on the contractile mechanism. The latter action is clearly shown with higher concentrations of propranolol by the increase of the relative refractory period and the decrease in force of contraction of the atria. Propranolol, like most potent quinidine-like drugs, is easily lipid soluble and would therefore be expected to penetrate cell membranes and, given the right structural requirements, firmly associate with nonpolar receptors in the cells. perhaps in the contractile protein itself. AH 3474 did not increase the refractory period or decrease the force of contraction of heart muscle. It is probably significant that AH 3474 is much more polar than propranolol (the partition coefficient between water buffered to pH 7·2 and ethylene dichloride is 21·0 for AH 3474 and 0·18 for propranolol) and would not be expected to enter cells freely and there associate with nonpolar structures. The same properties may account for the lack of local anaesthetic activity in AH 3474.

AH 3474 given intravenously to anaesthetized animals caused bradycardia and hypotension. The bradycardia was attributed to blockade of resting sympathetic tone since AH 3474 did not affect the heart rates of cats pretreated with reserpine. The latter result also shows that AH 3474 is devoid of intrinsic β-receptor stimulant activity. This conclusion is confirmed by the lack of response of the rat uterus and guinea-pig atria to AH 3474. The fall in blood pressure with AH 3474 in anaesthetized

animals may be due to decreased cardiac ouput without change in peripheral resistance as suggested by Shanks (1966) for propranolol. Effective β-receptor blocking doses of AH 3474, like MJ 1999 and propranolol, failed to lower the blood pressure in conscious hypertensive rats and dogs. The much higher doses of propranolol which lower blood pressure in hypertensive rats do so by impairing cardiac function (Farmer & Levy, 1968b).

Propranolol is used in man as an antifibrillatory drug. From animal data, its effect might be mainly due to its β-blocking action or its quinidine like action or both (Morales-Aguilera & Williams, 1965; Howe & Shanks, 1966). In the present experiments AH 3474 was clearly shown to be devoid of a quinidine like action but it did correct ouabain-induced cardiac arrhythmias at about 12 times its β-blocking dose. Propranolol corrected these arrythmias at 4 times its β-blocking dose, a dose which did not greatly effect the force of contraction of heart muscle in anaesthetized dogs. These results indicate that ouabain reversal, at least in part, is mediated through β-receptor blockade.

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