EFFECTS OF RESERPINE AND HYDRALLAZINE ON ISOLATED STRIPS OF CAROTID ARTERIES

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Spirally cut sections of horse carotid artery have been found suitable for the qualitative testing of vasoconstrictors and their antagonists. With this preparation reserpine and hydrallazine non-specifically antagonise the contractions induced by a number of stimulant drugs, including adrenaline, noradrenaline, histamine, 5-hydroxytryptamine, acetylcholine, barium chloride and potassium chloride. Measurements of "self protection"[3] by means of Furchgott's method but using strips of horse carotid arteries have shown that hydrallazine may possess an affinity for adrenergic receptors.

The hypotensive action of hydrallazine appears to be predominantly peripheral. That of reserpine seems to consist of two effects, one on the central nervous system and the other directly on the blood vessels. Of the two effects it is difficult to assess precisely which is the more important.

Hydrallazine, dihydrallazine and some related compounds cause vasodilatation in the rat isolated perfused hindquarters and the rabbit ear and antagonise the vasoconstriction produced in these preparations by adrenaline, noradrenaline, histamine, barium chloride and 5-hydroxytryptamine.[1] Both hydrallazine and dihydrallazine relax isolated aortic strips from cats and rabbits, and antagonise the contractions caused by adrenaline, noradrenaline, histamine and 5-hydroxytryptamine but not those caused by barium chloride[1]. Recently, reserpine has also been shown to have a direct action upon arterial smooth muscle[2].

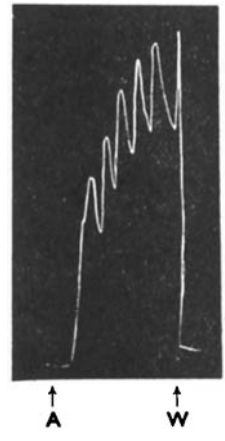

Fig. 1. Spontaneous rhythmic activity in a strip of horse carotid artery: At A, 0·66 μg. Hm for 6 minutes. At W, wash out.

Preparations from the aortae of cats and rabbits were unsatisfactory because the magnitude of the contraction or relaxation was small. Furthermore, the response was slow and recovery prolonged, so that the time interval between doses was often as much as two hours, and many of the strips were refractory to stimulant drugs, including acetylcholine, 5-hydroxytryptamine and histamine. We have now used spirally cut strips of horse carotid artery to study the effects of hydrallazine and reserpine on arterial smooth muscle.

Materials and Methods

The composition of the Tyrode's solution was as follows (g./litre). NaCl 8·0, KCl 0·198, $CaCl_2$ 0·2, $MgCl_2$ 0·1, NaH_2PO_4 0·05, $NaHCO_3$ 1·0, glucose 1·0.

In studying drug antagonisms, the following drugs were used, dissolved in Tyrode's solution. Acetylcholine chloride (ACh), (−)-adrenaline hydrochloride (Ad), (−)-noradrenaline bitartrate (NA), 5-hydroxytryptamine creatinine sulphate (5-HT), histamine acid phosphate (Hm), barium chloride ($BaCl_2$), potassium chloride (KCl), atropine sulphate (atropine), mepyramine maleate (mepyramine), phentolamine, (+)-lysergic acid diethylamide tartrate (LSD), 1-hydrazinophthalazine hydrochloride (hydrallazine), and reserpine in the form of a buffered solution in ascorbic acid-sodium ascorbate.

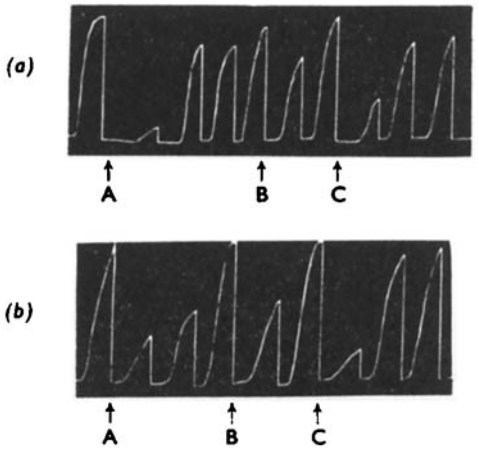

FIG. 2. (*a*) Noradrenaline-phentolamine antagonism in horse carotid artery strips. All contractions due to 0·5 μg. NA. At A, 0·03 μg. phentolamine. At B, 0·01 μg. phentolamine. At C, 0·02 μg. phentolamine. (*b*) Acetylcholine-atropine antagonism in horse carotid artery strips. All contractions due to 0·2 μg. of ACh. At A, 0·02 ng. atropine. At B, 0·01 ng. atropine. At C, 0·04 ng. atropine.

Lengths of common carotid artery were removed from horses immediately after death and placed in Tyrode's solution. 10 cm. lengths were cut spirally (Furchgott and Bhadrakom[3]), and pieces about 2 cm. long were set up in oxygenated Tyrode's solution at 36°; bath volumes varied from 10 to 75 ml. Before drugs were added to the bath a tension of 10 g. was applied for 1 hour. The drugs were then added and left in contact with the tissue for 5 minutes. In some experiments the contraction was complete in 4 minutes, in others, 6 minutes. When this happened the period of contact between the drug and the tissue was decreased or increased. Some strips showed rhythmic activity in the presence of Ad or Hm and these were rejected. An example is shown in Figure 1. After the drug was washed out, the lever returned slowly to the base-line. With ACh contractions, relaxation on washing took place in 5 to 20 minutes, but with the other drugs the relaxation took 30 minutes or more. Standard reproducible submaximal responses were obtained to ACh, NA, Ad, Hm, 5-HT, KCl and $BaCl_2$. Once these had been obtained, reserpine or hydrallazine was added 20 minutes before the next addition. Total time of contact of reserpine or hydrallazine with the tissue was 25 minutes.

The artery strips always contracted after the addition of ACh but were less sensitive to the other drugs, and sometimes did not respond unless high doses were used. Stored at 4°, artery strips retained their sensitivity to drugs for about 3 days. In one or two cases sensitivity was maintained for much longer.

Drug concentrations are expressed as the final concentration per ml. of the bath fluid.

RESULTS

ACh (0·1 ng. to 2·0 μg.), Ad (10·0 ng. to 5·0 μg.), NA (10·0 ng. to 5·0 μg), 5-HT (40 ng. to 3·0 μg.), Hm (0·1 to 5·0 μg.), $BaCl_2$ (0·1 to 0·5 mg.) and KCl (3·0 to 5·0 mg.), all caused contractions of the artery strips. Large, reproducible contractions were obtained with small doses of drugs. There appeared to be a linear relation between the logarithm of the dose and the magnitude of the response.

Contractions due to ACh were antagonised by atropine (0·001 to 0·015 ng.), those due to Hm by mepyramine (0·001 to 0·01 ng.), 5-HT by LSD (0·02 to 1 ng.), Ad and NA by phentolamine (0·01 to 0·015 ng.). Some of these antagonisms are shown in Figure 2. Drug antagonisms were specific, atropine did not antagonise Hm induced contractions and mepyramine did not antagonise contractions due to ACh. Reserpine (1·0 to 25·0 μg.) and hydrallazine (25·0 to 500·0 μg). antagonised the contractile responses to all of the spasmogens tested. No evidence of specificity or selectivity was obtained. Recovery with hydrallazine was usually rapid and complete (Fig. 3) but in a few experiments it was incomplete. After reserpine it was rare for the tissue to recover, although occasionally recovery was seen after 6 to 12 hours (Fig. 4). This points to the strong affinity of this drug for arterial smooth muscle. In some experiments using smaller doses of hydrallazine (5·0 to 10·0 μg.) there was a slight potentiation of the contractions due to ACh and Hm.

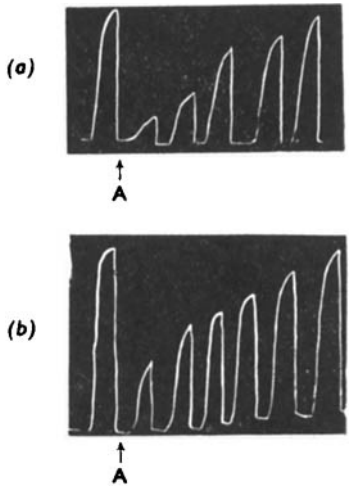

FIG. 3. (*a*) Effect of hydrallazine upon noradrenaline-induced contractions of horse carotid artery strips. All contractions due to 0·66 μg. of NA for 5 minutes. At A, 26·5 μg. hydrallazine 20 minutes before NA. (*b*) Effect of hydrallazine upon acetylcholine-induced contractions of horse carotid artery strips. All contractions due to 0·13 μg. of ACh for 5 minutes. At A, 100 μg. of hydrallazine 20 minutes before ACh.

Furchgott[4], in experiments with strips of rabbit aorta has shown that the presence of a high concentration of a stimulating drug during exposure to a specific antagonist, can protect against the effects of the antagonist. Such "self protection" implies that the antagonist is blocking the same receptors with which the stimulant drug combines, since these have been saturated by the high concentration of the stimulant drug and are, therefore, not available to the antagonist. For example, atropine will specifically antagonise contractions caused by ACh but if the initial concentration of ACh is high, then atropine is less effective. This has been taken

to imply that atropine is acting on the same receptors and that ACh is protecting itself. If it can be assumed that when there is no "self protection," there is no specificity, then this method can be used to differentiate between drugs which act on specific receptors and those which do not. A few experiments have been carried out on this assumption.

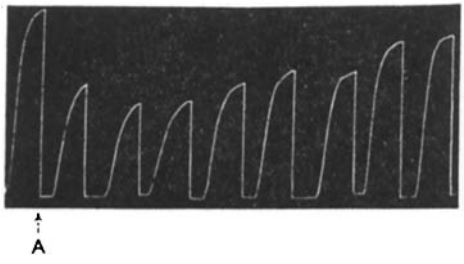

FIG. 4. Effect of reserpine upon acetylcholine-induced contractions of horse carotid artery strips. All contractions due to 0·05 μg. of ACh for 5 minutes. At A, 12·5 μg. reserpine 20 minutes before ACh.

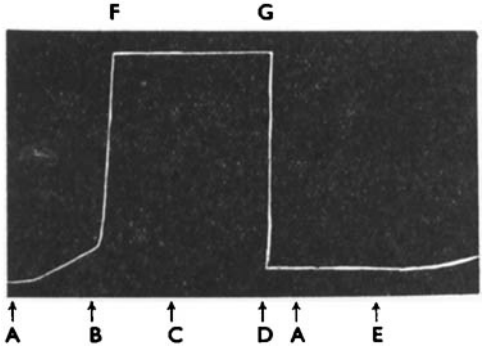

FIG. 5. Failure of a high dose of histamine to protect against hydrallazine. At A, 1 μg./ml. Hm for 5 minutes. At B, 10 μg./ml. Hm. for 15 minutes. At C, 25 μg./ml. hydrallazine for 20 minutes. At D, wash out. At E, small contraction due to 1·2 μg./ml. Hm for 5 minutes. Due to the magnitude of the contraction following 10 μg./ml. Hm a "stop" had to be placed on the lever to prevent this leaving the drum. This accounts for the plateau FG.

Artery strips were stimulated using small concentrations of ACh, Hm, Ad or NA (1·0 to 5·0 μg.). The low concentration of the stimulant was allowed to act for 5 minutes and then a second, higher dose of 0·5 mg. to 1·0 mg. of the same drug was added. The second dose was left in the bath for 15 minutes and reserpine (5·0 to 25·0 μg.) or hydrallazine (25·0 to 500·0 μg.) added and left in contact with the tissue for a further 20 minutes. After washing out, the addition of the smaller dose was repeated. The second contraction was usually much reduced (Fig. 5). This was very clear with ACh and Hm but the effect of a second dose of Ad or NA was not reduced to the same extent (Fig. 6), thereby showing some affinity of hydrallazine for adrenergic receptors.

DISCUSSION

Strips of horse carotid artery have been shown to be suitable for demonstrating the activity of drugs which cause contraction of smooth muscle. We have observed that horse arterial muscle reacts in a typical fashion towards ACh, Ad, NA, Hm, 5-HT, $BaCl_2$ and KCl and the specific antagonists of the first five drugs. Reserpine and hydrallazine relaxed arterial smooth muscle irrespective of the nature of the stimulant drug used and were capable also of causing direct relaxation of artery

strips. The experiments described point to non-specific drug effects rather than to an action upon specific receptors, although there is some evidence for affinity towards adrenergic receptors. Reserpine may act by virtue of an interference with the metabolic processes which underly the contraction of intestinal smooth muscle[5–7]. It seems not unlikely that reserpine and hydrallazine are acting in a similar manner on arterial smooth muscle.

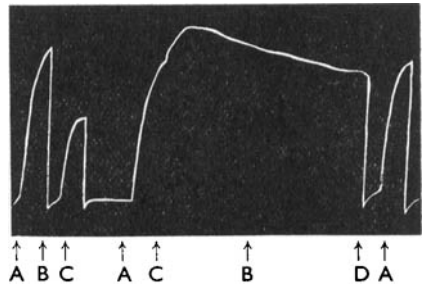

FIG. 6. Self protection by a high dose of adrenaline against hydrallazine At A, 1 μg./ml. Ad. for 5 minutes. At B, 20 μg./ml. hydrallazine for 20 minutes before Ad. At C, 10 μg./ml. Ad for 15 minutes. At D, wash out.

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