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The cardiovascular actions of WR-149,024 (1,18-diamino-7,13-diaza-9,10-dithiaoctadecane tetrahydrochloride)

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Summary

1. The general cardiovascular properties of WR-149,024 (a straight chain sulphur-containing aliphatic amine) in dogs and cats are reported.

2. Intravenous administration of this compound produced an immediate hypotension and bradycardia in intact anaesthetized dogs. These effects were independent of the parasympathetic nervous system since they were also present in atropinized and bilaterally vagotomized dogs.

3. Ascending aortic blood flow increased after administration of WR-149,024 despite a reduction in blood pressure, contractile force and heart rate. It appears that the initial hypotension is due to a decrease in total peripheral vascular resistance since WR-149,024 produced relatively little change in force of contraction or heart rate in the isolated, blood-perfused heart preparation.

4. WR-149,024 reversed the pressor effects of adrenaline within 10 min of injection while at the same time the vasopressor response to angiotensin or the vasodepressor response to isoprenaline was not altered. α-Adrenoceptor blockade was still evident up to five days after dosing.

5. WR-149,024 did not block phenylephrine inhibition of intestinal motility. These findings suggest that WR-149,024 initiates a relatively specific and prolonged α-adrenoceptor blockade.

Introduction

Pharmacological blockade of the vasopressor response of sympathomimetic amines by the ergot alkaloids was first reported by Dale (1906) and subsequently many diverse classes of chemicals have been shown to possess similar activity. Recently, WR-149,024, a sulphur-containing aliphatic amine was observed to possess selective α-adrenoceptor blocking activity (Demaree, Brockenton, Heiffer & Rothe, 1971a ; Herman, Heiffer, Demaree & Vick, 1971).

$$\begin{array}{l} H_2N\text{-}(CH_2)_5\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}S \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad |.4HCl \\ H_2N\text{-}(CH_2)_5\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}S \end{array}$$

WR-149,024

WR-149,024 is efficacious in the prevention of anaphylactic shock in mice (Demaree, Frost, Heiffer & Rothe, 1971b) and the treatment of haemorrhagic shock

in dogs (Caldwell & Demaree, 1971). Although the efficacy of this agent has been demonstrated in these two conditions, the basic systemic effects have not been previously reported. This study describes the general cardiovascular effects of WR-149,024 in the dog and cat.

Methods

Animals

Adult beagle dogs of either sex (9–12 kg) were anaesthetized with 30 mg/kg sodium pentobarbitone (intravenously). A cuffed endotracheal tube was inserted and connected to a pneumotachograph screen to record respiratory (tidal) air movement. A catheter, connected to an electronic pressure transducer, was placed in the abdominal aorta through a femoral artery to record arterial blood pressure (1 mmHg≡1·333 mbar). A femoral vein was cannulated for drug administration. Heart rate was determined using the beat to beat mode of a cardiotachometer.

Two cats were also anaesthetized and prepared as described above. Unless specified all animals were bilaterally vagotomized and given atropine sulphate, 1 mg/kg, intravenously during surgical preparation.

Initial cardiovascular actions of WR-149,024 (9 dogs)

WR-149,024 (6·25, 12·5 or 25·0 mg/kg) was dissolved in 10 ml normal saline and administered intravenously over a 2 min period. The effects of this compound on blood pressure and heart rate were determined over a 60-min period. All recordings were made on a Hewlett-Packard polygraph.

Ascending aortic blood flow studies (4 dogs)

The chest was opened and a Biotronex flow probe, connected to a sine wave electromagnetic flow meter, was placed around the ascending aorta. Force of contraction was obtained from a Walton–Brodie strain gauge arch sutured to the right ventricle of the heart. The dogs were artificially respired by means of a positive pressure respirator. Dose-response curves were then obtained for the cardiovascular effects of bolus intravenous injections of adrenaline and noradrenaline (0·25 to 2·0 μg/kg).

WR-149,024 (19 mg/kg) was administered in 10 ml of saline over a two min period and the responses to 1 μg/kg of adrenaline were recorded every 10 min until two consecutive, identical responses were obtained. The initial injections of noradrenaline and adrenaline were then repeated.

Cardiovascular responses to catecholamines and angiotensin (15 dogs, 2 cats)

Dose-response relationships of the blood pressure changes to i.v. bolus injections of noradrenaline (0·125 to 2·0 μg/kg), adrenaline (0·125 to 2·0 μg/kg), isoprenaline (0·0625 to 1·0 μg/kg) and angiotensin (0·2 to 1·6 μg/kg) were obtained. WR-149,024 was administered in doses of 5, 10, and 20 mg/kg using a 3 min injection time and the responses to the agonist were repeated one hour later. At the end of 60 min, propranolol (0·5 mg/kg) was administered and the agonist challenges were repeated.

In four additional dogs, the responses to adrenaline, noradrenaline, angiotensin and isoprenaline were obtained one, two or five days after the intravenous injection of WR-149,024 (10 mg/kg).

Femoral vascular bed studies (2 dogs)

These dogs were anaesthetized as described above but did not receive atropine and their vagi were left intact. The hind limb was acutely denervated and circulation to the paw was occluded by a tourniquet at the level of the lower tibia. Blood flow in the left femoral artery was measured continuously by means of an electromagnetic flow meter. Carotid artery pressure was recorded simultaneously with blood flow.

The femoral artery was continuously perfused with saline (0·7 ml/min) via a small needle introduced into the lumen. Noradrenaline ($4{\cdot}6 \times 10^{-10}$M to $5{\cdot}8 \times 10^{-8}$M) or angiotensin ($5{\cdot}0 \times 10^{-11}$M to $1{\cdot}73 \times 10^{-9}$M) was given into this flow as a bolus of 0·2 ml. Dose-response curves were constructed for the maximum changes in blood flow. WR-149,024 (10 mg/kg) was injected intravenously over a one min period. The sequence of the doses and agonists were randomized.

In vivo *intestinal motility*

Two adult beagles were given 10 mg/kg of morphine (subcutaneously) followed in 30 min by 30 mg/kg of Na pentobarbitone (intravenously) and atropine 1 mg/kg (intraperitoneally).

A femoral artery and vein were cannulated for the measurement of arterial blood pressure and the introduction of drugs, respectively. A small loop of ileum was exposed through a short midline incision. One of two small water filled balloons, about 5 ml capacity, connected by flexible catheters to a Sanborn differential pressure transducer (Model 268) was placed in the lumen of the ileum and one in the abdominal cavity and the incision closed. The connexions to the transducer were such that the resultant signal displayed the intraluminal intestinal pressure with effects of respiratory movements subtracted mechanically (Ahlquist & Levy, 1959).

Responses of the gut and blood pressure to phenylephrine (5 and 10 μg/kg) and adrenaline (1 μg/kg) were observed before and 1 h after the i.v. injection of WR-149,024 (10 mg/kg). Phentolamine (1 mg/kg) was then given followed 15 min later by the administration of phenylephrine.

Isolated heart preparation

Three adult mongrel dogs (10–12 kg) were anaesthetized with Na pentobarbitone (30 mg/kg, i.v.) and the heart removed and perfused with autologous blood according to a previously described procedure (Vick & Herman, 1971). Coronary perfusion pressure was obtained by means of a needle-tipped catheter inserted into the perfusion circuit and attached to a pressure transducer. The electrocardiogram and heart rate were determined by means of needle-tipped electrodes inserted into the left and right ventricles. The force of contraction was measured with a Walton–Brodie strain gauge sutured to the left ventricle.

Each preparation was allowed to stabilize for approximately 15 min after which control responses to 0·5 or 1·0 μg adrenaline were obtained. WR-149,024, dissolved in 5 ml saline, was injected into the perfusion circuit over a 2 to 3 min period at doses of 12·5 mg (1 heart) and 25 mg (2 hearts). Each heart was rechallenged with adrenaline at 15, 30 and 60 min after WR-149,024 injection.

Chemicals and drugs

1,18-Diamino-7,13-diaza-9,10-dithiaoctadecane tetrahydrochloride is a white, crystalline powder. All doses of this compound are expressed as the free base.

The following drugs were commercially obtained: (−)-adrenaline hydrochloride, (−)-noradrenaline bitartrate, (−)-isoprenaline hydrochloride, (−)-phenylephrine hydrochloride, sodium heparin, atropine sulphate, angiotensin II amide, phentolamine methanesulphonate and (±)-propranolol hydrochloride. With the exceptions of atropine sulphate and phentolamine methanesulphonate the doses are expressed as the free base.

Results

Initial pharmacological actions of WR-149,024

The injection of 6·25, 12·5 or 25·0 mg/kg of WR-149,024 caused immediate hypotension and bradycardia (Fig. 1). These effects were not dose-dependent since 6·25 mg/kg depressed blood pressure 58 ± 22% and heart rate 82 ± 5%, whereas 25 mg/kg caused a 52 ± 9% decrease in blood pressure and a 70 ± 9% decrease in heart rate. Although recovery of both parameters began within five min, blood pressure ultimately stabilized slightly below control levels. Heart rate returned to control levels by 15 min and stabilized above control levels for the remainder of the 60 min observation period.

WR-149,024 also induced an immediate 10–15 s period of apnoea followed by a rapidly shallow breathing pattern for the next 10 minutes.

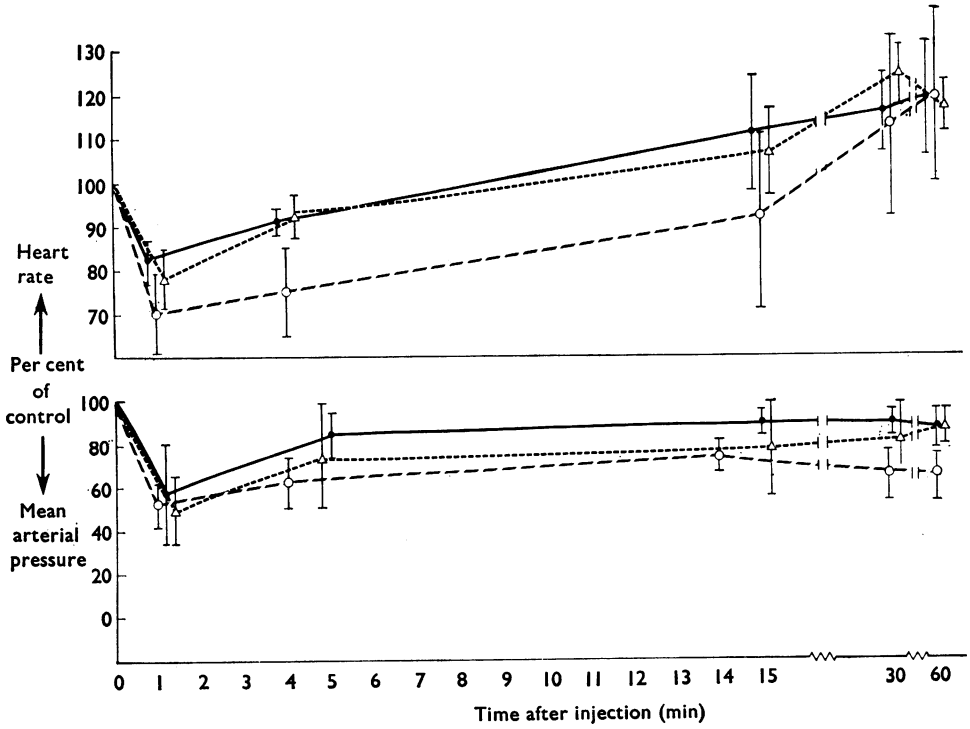

FIG. 1. Effects of various doses of WR-149,024 on heart rate and mean arterial pressure at various times after intravenous administration. Each point is the average of three observations with the vertical bar indicating the standard error. ●——● 6·25 mg/kg, △ - - - △ 12·5 mg/kg, ○ - - - ○ 25 mg/kg.

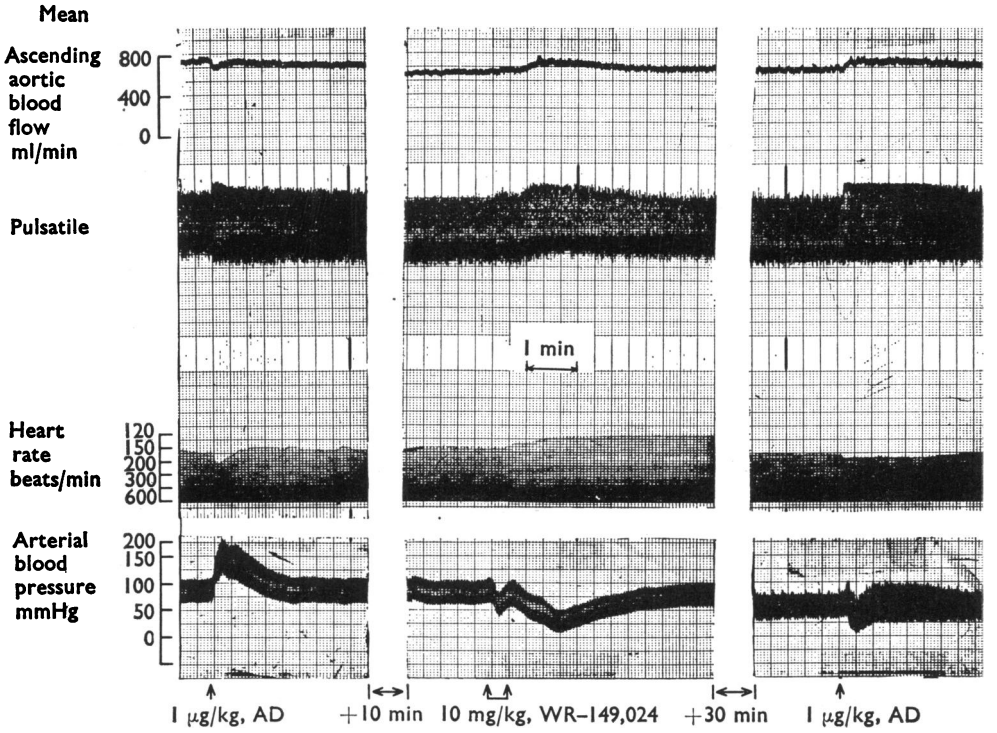

FIG. 2. Effect of WR-149,024 and adrenaline on mean and pulsatile ascending aortic blood flow, heart rate and arterial blood pressure in an open-chest dog.

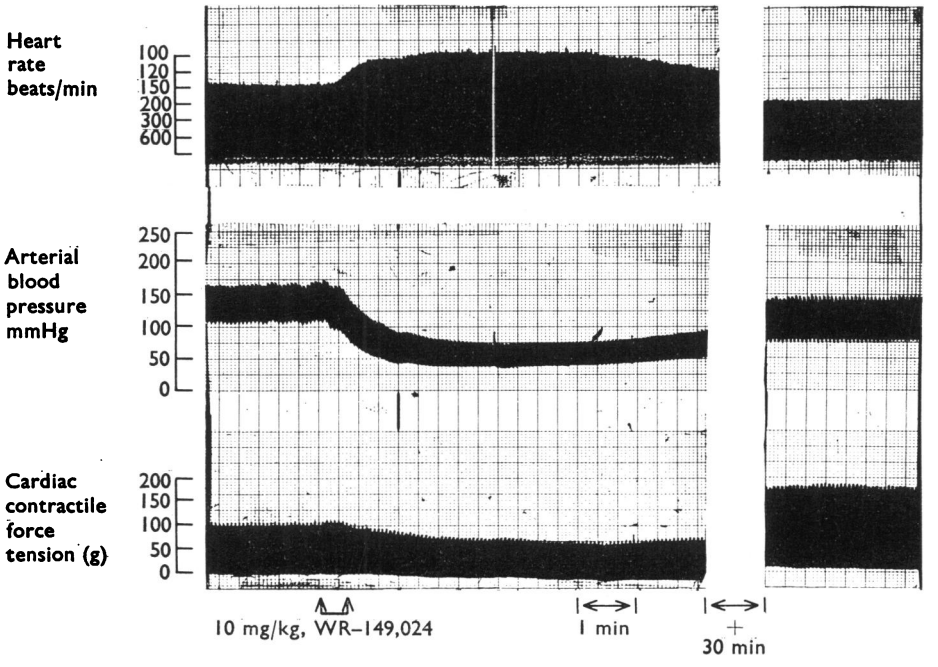

FIG. 3. Effect of WR-149,024 on heart rate, arterial blood pressure and cardiac contractile force in an open-chest dog.

Ascending aortic blood flow series

WR-149,024, in a dose of 10 mg/kg, caused a slight increase in cardiac output during the injection period and a simultaneous decrease in blood pressure and heart rate (Fig. 2). The ability of WR-149,024 to reverse the vasopressor response to adrenaline was actually apparent within 10 min following administration and was well established by 30 minutes. Adrenaline reversal occurred without a decrease in cardiac output.

Figure 3 demonstrates that in addition to hypotension and bradycardia, WR-149,024 produced a depression in the cardiac contractile force but this too was transient. Cardiac contractile force had returned to well above control values 35 min after WR-149,024 administration. The heart rate and arterial pulse pressure were also increased over control levels at this time.

Responses to catecholamines and angiotensin

The vasopressor responses to adrenaline and noradrenaline were reversed or attenuated following WR-149,024. However, the vasopressor responses to angiotensin and the vasodepressor responses to isoprenaline were not affected by

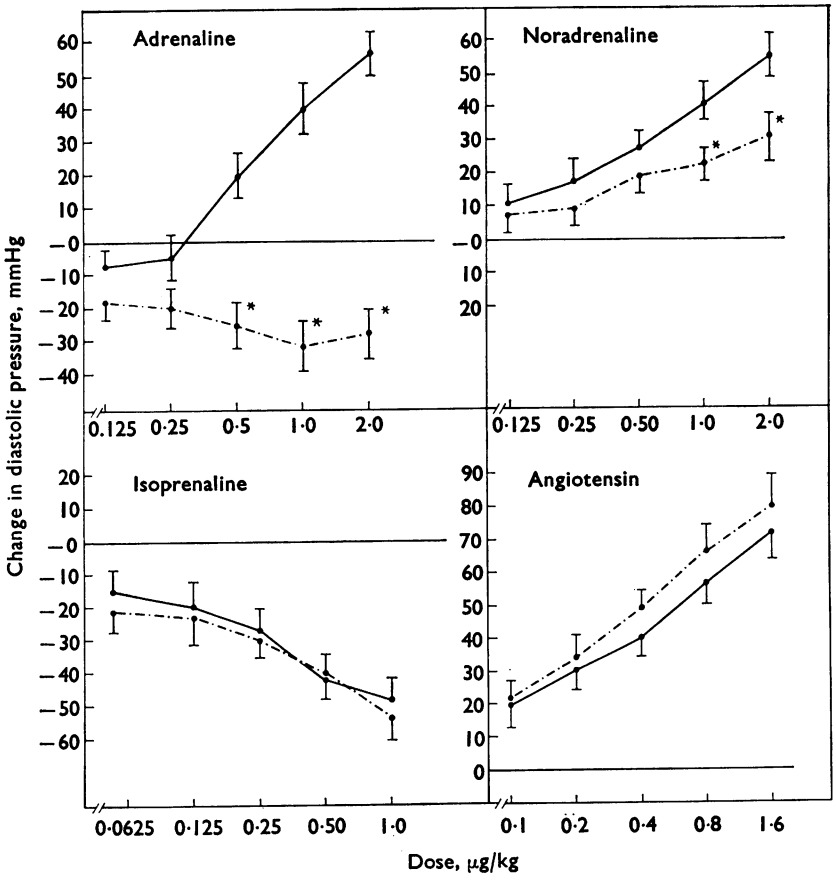

FIG. 4. Changes in diastolic pressure to intravenous doses of various catecholamines and angiotensin before and one hour after administration of WR-149,024. Pressure measurements made at peak responses after administration of agonists. Control ●-·-● ; after WR-149,024 ●——●. *$P<0{\cdot}05$.

WR-149,024. The dose-response relationships among these agonists before and after the administration of WR-149,024 are given in Figure 4.

Once adrenaline reversal was present a pressor response could be obtained only by injection of a large dose of adrenaline (32 μg/kg). Propranolol, given after adrenaline reversal was established, partially restored the pressor effect of adrenaline. Similar results were obtained in 2 cats.

In the 3 dogs observed 24 and 48 h after WR-149,024 administration (10 mg/kg), the blood pressure responses to adrenaline and noradrenaline, in doses up to 32 μg/kg, were reversed or attenuated, respectively. Responses to angiotensin and isoprenaline were unchanged from control. Adrenaline reversal was still apparent in two dogs five days after administration of 10 mg/kg of WR-149,024.

Femoral vascular bed

In two experiments angiotensin and noradrenaline caused a dose-related decrease in femoral artery blood flow. The vascular responses to angiotensin in this preparation were not altered by administration of WR-149,024. In contrast, the response to noradrenaline was less at each dose. The results of one of these experiments are summarized in Figure 5.

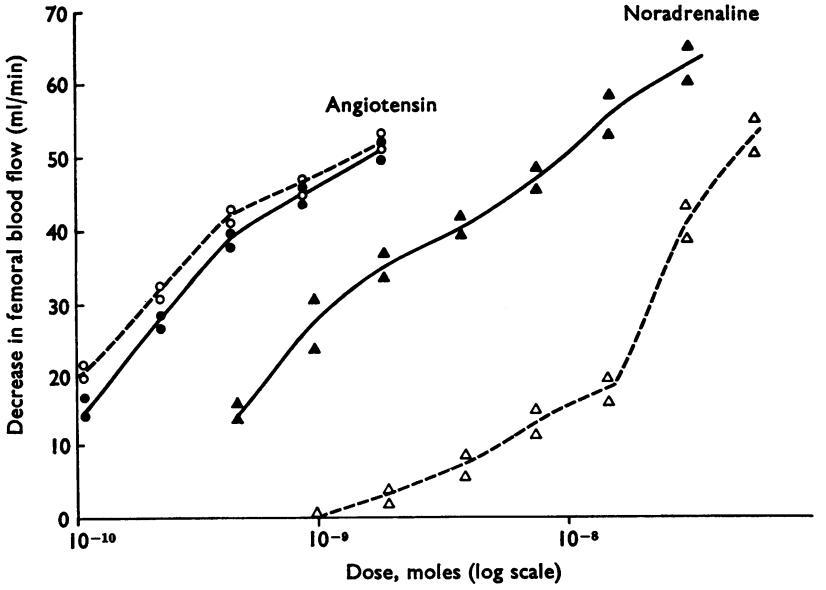

FIG. 5. Decrease in femoral blood flow in one dog after intravenous injections of angiotensin and noradrenaline before (——) and one hour after (– – –) an intravenous injection of 10 mg/kg of WR-149,024. Lines go through midpoint between two determinations.

In vivo *motility*

The increase in arterial blood pressure and the decrease in gut motility produced by phenylephrine are depicted in Figure 6. Adrenaline also produced a similar response. After the introduction of WR-149,024 (10 mg/kg), the adrenaline pressor response was reversed and the pressor responses to phenylephrine were attenuated. However, the inhibitory actions of phenylephrine and adrenaline on gut motility were not affected.

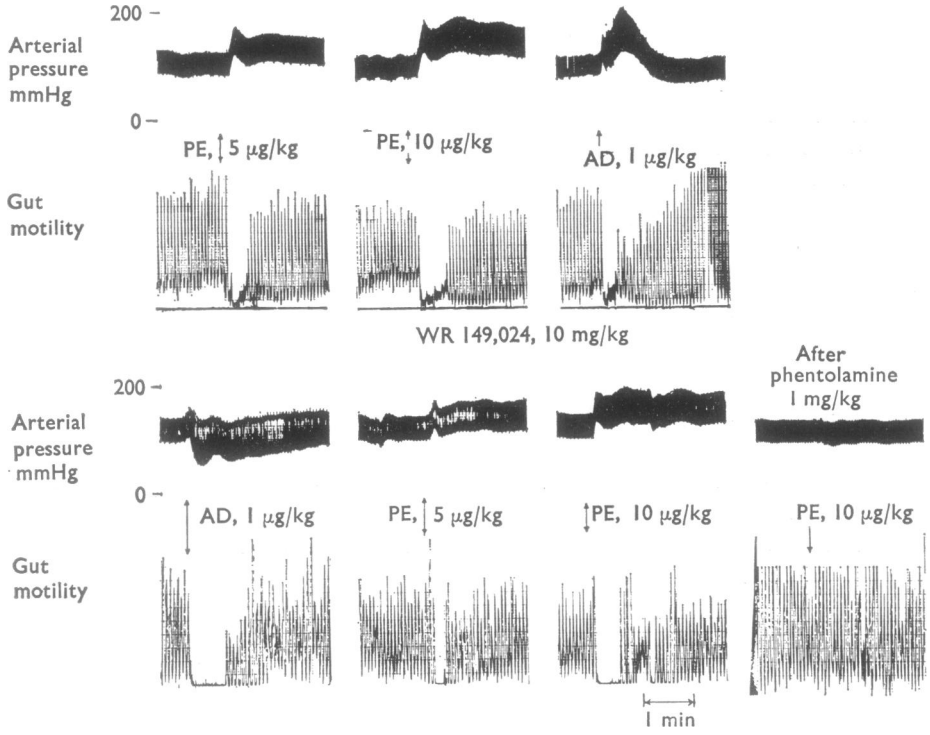

FIG. 6. Effects of phenylephrine (PE) and adrenaline (AD) on arterial blood pressure and gut motility before and one hour after WR-149,024 administration and 15 min after administration of phentolamine.

Phentolamine (1 mg/kg) further attenuated the vasopressor effect of phenylephrine and completely blocked its inhibitory effect on gut motility.

Isolated dog heart

The injection of either 12·5 or 25·0 mg of WR-149,024 into the isolated heart preparation caused a 5 to 11% decrease in force of contraction and a 0 to 6% decrease in heart rate. Both parameters were at or near control levels five min after injection. No significant changes were noted in coronary perfusion pressure during these experiments.

Adrenaline (1 μg) produced a brief 140 to 152% increase in force of contraction and a 124 to 134% increase in heart rate over control levels. These responses were essentially unchanged after either 12·5 or 25·0 mg of WR-149,024.

Discussion

WR-149,024, a straight chain aliphatic amine, shown to be effective in the treatment of several conditions of cardiovascular shock, was found to exert both immediate and prolonged actions on the cardiovascular system. The intravenous administration of WR-149,024 produced an immediate hypotension and bradycardia in the intact, anaesthetized dog and cat. Since these effects were also observed in atropinized and bilaterally vagotomized dogs, the reduced heart rate and depressor response were independent of the parasympathetic nervous system. Herman *et al.* (1971) also observed the initial hypotension and bradycardia following WR-149,024

administration in rats, indicating that these phenomena are not species specific. In the present experiments, ascending aortic blood flow did not decrease with administration of WR-149,024 despite a reduction in contractile force and heart rate. These latter effects do not appear to be due to a direct myocardial action since WR-149,024 had little effect on force of contraction and heart rate in the isolated dog heart. It therefore seems likely that the initial hypotension is due to a decrease in total peripheral vascular resistance.

WR-149,024 produced adrenaline reversal and antagonized the pressor response to noradrenaline. Since WR-149,024 did not potentiate the isoprenaline depressor responses, it is unlikely that this agent exerts its action by enhancing β-adrenoceptor responses. Likewise, WR-149,024 did not antagonize the pressor effects of angiotensin. This would tend to rule out nonspecific interference with vasoconstriction. Propranolol partially restored the pressor effects of adrenaline after they had been abolished by WR-149,024. Propranolol has also been reported to partially restore the adrenaline pressor response after blockade with phenoxybenzamine (Olivares, Smith & Aronow, 1967). These properties indicate that WR-149,024 is an α-adrenoceptor blocking compound, as previously reported (Herman *et al.*, 1971 ; Demaree *et al.*, 1971a).

The onset of stable α-adrenoceptor blockade occurred within 10 min and was much more rapid than that observed with WR-2823, the phosphorylated monomer of WR-149,024, which required 45 to 60 min for maximum effect (Heiffer, Herman, Vick, Demaree, Mundy & Reynolds, 1969). This difference in onset of action is probably due to the time required for the dimerization of WR-2823 to WR-149,024 which is the α-adrenoceptor blocking form. Demaree *et al.* (1971a) have also demonstrated that WR-149,024 is more potent and has a more rapid onset of action than WR-2823 in antagonizing responses to noradrenaline on the isolated rabbit aorta.

The α-adrenoceptor blockade produced by WR-149,024 could be surmounted, since larger doses of adrenaline (32 μg/kg) partially restored the pressor response. These properties of reversibility and rapid onset of action are possessed by other α-adrenoceptor antagonists, such as tolazoline and phentolamine (Herman, Jourdan & Bonnet, 1941). WR-149,024 has a duration of action similar to that of dibenamine or phenoxybenzamine (Nickerson & Goodman, 1947) since altered adrenaline responses have been observed at least five days following administration.

Another interesting observation, in view of the ability of WR-149,024 to create α-adrenoceptor blockade in the vascular system, is the secondary tachycardia, increase in cardiac contractile force and pulse pressure seen 15 to 30 min after administration of WR-149,024. Kirpekar & Cervoni (1963) have previously demonstrated that two other α-adrenoceptor blocking agents, phenoxybenzamine and phentolamine, are capable of elevating circulating catecholamine levels. Possibly these later effects of WR-149,024 are due to an increase in circulating catecholamines.

The inability of WR-149,024 to block α-adrenoceptor inhibition of intestinal motility in doses which produce α-adrenoceptor blockade in the vasculature is especially noteworthy. This finding suggests that there are differences in the receptors mediating these two responses. Moreover, this property, along with the demonstrated inability of this substance to interfere with the cardiovascular actions of acetylcholine, histamine or 5-hydroxytryptamine (Herman *et al.*, 1971) reveals WR-149,024 to be quite specific and selective as a pharmacological antagonist.

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