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PHARMACOLOGICAL OBSERVATIONS ON THE HYPOTENSIVE ACTION OF EXTRACTS OF TELEOST FISH UROPHYSES (UROTENSIN I) IN THE RAT

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1 Intravenous injections of urotensin I regularly caused a long-lasting, dose-related, lowering of blood pressure and an increase in heart rate in conscious rats, or a reduction in perfusion pressure in the isolated hind limb of the rat.

2 After subcutaneous administration, the hypotensive effect of urotensin I was greater in extent and in duration (> 24 hours).

3 Anaesthesia with ether, chloralose, pentobarbitone and thiobarbitone caused a decrease in blood pressure and only slightly diminished the hypotensive effect of urotensin.

4 Mecamylamine, hexamethonium, atropine, phenoxybenzamine, propranolol and diphenhydramine did not alter the effect of urotensin in conscious rats or in the isolated hind limb, although the effects of the respective agonists, i.e. nicotine, acetylcholine, noradrenaline, isoprenaline and histamine were inhibited.

5 In conscious rats, pressor effects of adrenaline, noradrenaline, nicotine and angiotensin II, and depressor effects of acetylcholine and bradykinin, were decreased or inhibited, whereas the hypotensive effect of phenoxybenzamine was potentiated by previous administration of urotensin I. Carotid occlusion reflex was partially inhibited by lower doses of urotensin and abolished by higher doses in rats lightly anaesthetized with chloralose. Urotensin elicited postural hypotension in rats anaesthetized with pentobarbitone.

6 The increase in heart rate produced by urotensin was not affected by phenoxybenzamine, but was abolished by propranolol or ganglion blocking agents (mecamylamine or hexamethonium).

7 It is concluded that urotensin elicits hypotension in the rat by a direct dilatory action on the resistance vessels causing a simultaneous reflex tachycardia.

Introduction

Diverse biological activities have been found in extracts of bony fish urophyses (see Lederis, 1970). One of these extracts increases systemic blood pressure in the eel (Bern, Nishioka, Chester-Jones, Chan, Rankin & Ponniah, 1967; Chan, Chester-Jones & Ponniah, 1969) while lowering of blood pressure by urophysis extracts in rats anaesthetized with pentobarbitone has been observed by Kobayashi, Matsui, Hirano, Iwata & Ishii (1968). This effect was not caused by a 'cholinergic substance', which was previously found in fish urophyses (Kobayashi, Uemura, Oota & Ishii, 1963), nor by contamination with histamine or substance P. Another hypotensive component, in addition to that described by Kobayashi *et al.* (1968), causing a sudden and short-lasting lowering of rat blood pressure was 'unmasked' when urophysial extracts were treated with trypsin and/or chymotrypsin (Lederis, 1970). The long-lasting hypotensive effect of urophysial extracts was not altered by treatment of urethane-anaesthetized rats with phenoxybenzamine or with propranolol (Lederis & Osmond-Jones, unpublished observation).

Chromatography on Sephadex G-25 and in Bio Gels P_2, P_{10}, P_{20}, and P_{60} showed the existence of three separate principles with which the above activities are associated (Zelnik & Lederis, 1971; 1973).

In the present experiments an attempt was made to obtain some information concerning the site and mode of action of the long-acting

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rat-hypotensive principle urotensin I (Bern & Lederis, 1969; Lederis, 1972). Attempts were also made to find a sensitive and reliable assay preparation that would make possible quantitative estimations of the rat hypotensive activity.

This paper describes the results of pharmacological observations on conscious rats, on animals anaesthetized with ether, thiopentone, pentobarbitone and chloralose and on the isolated hind limb of the rat. Some of the findings have been presented in a preliminary form (Medaković, 1972; Medaković & Lederis, 1972).

Methods

Conscious and anaesthetized rats

Rats of either sex, weighing 200-500 g, were anaesthetized with ether. A common carotid artery and the ipsilateral external jugular vein were cannulated. Free ends of both cannulae were passed under the skin and allowed to protrude 3-4 cm from the skin behind the ears of the rat. After surgery and before the start of experiments rats were kept overnight in individual cages and had free access to food and water.

During the experiment the rats were kept in a box with an open top. The carotid cannula was connected via a Statham transducer (P23Gb) to a recorder (Beckman RM Dynograph). Pulse pressure and heart rate were recorded in addition to blood pressure by selecting the appropriate sensitivity of the recording system and the speed of the paper. Injections (in 0.9% w/v NaCl solution) were given into the jugular vein in volumes not exceeding 0.2 ml. The animals were usually used for several experiments, on consecutive days. When the influence of urotensin on the effect of other substances (e.g. adrenaline, noradrenaline, acetylcholine, angiotensin II, bradykinin) was studied, they were administered during the maximal effect of urotensin (3-7 min after it was applied) and the injections were repeated until the recovery of the blood pressure from hypotension caused by urotensin.

Isolated hind limb of the rat

The isolated hind limb was perfused with Krebs physiological solution, containing 2% dextran and 38.46 mg/l ascorbic acid (as suggested by Fogelman & Grundy, 1970), at 22°C, bubbled with 95% O_2 and 5% CO_2 and perfused at 1.3-2.5 ml/minute. Noradrenaline hydrochloride (0.5-1.5 μg/ml) was added in order to maintain vascular tone. The perfusion pressure, monitored through a Statham P23Db transducer, and a Beckman RM Dynograph, was maintained at or near to 120 mmHg. Test substances were injected into the perfusion cannula in volumes not exceeding 0.02 ml.

The following substances were used: urotensin (Urophysial Reference Preparation; Lederis, 1969; Bern & Lederis, 1969 and partially purified urotensin I, kindly provided by Dr Anita Letter), acetylcholine chloride (Sigma); histamine diphosphate (Sigma); adrenaline hydrochloride (Parke-Davis); noradrenaline bitartrate (Levorphed, Winthrop); isoprenaline hydrochloride (Isuprel, Winthrop); nicotine sulphate (Sigma); tetramethylammonium hydroxide pentahydrate (Sigma); atropine sulphate (Nutritional Biochemicals); mecamylamine hydrochloride (Sigma); hexamethonium bromide (Sigma); propranolol hydrochloride (Inderal, Ayerst Labs); phenoxybenzamine (Benadryl, Parke-Davis); bradykinin triacetate (Sigma); angiotensin II (Hypertensin, Ciba); α-chloralose (Fisher Scientific Co.); thiopentone (Pentothal sodium, Abbot); sodium pentobarbitone (B.D.H. Chemicals); ethyl-ether (Malinkrodt Chem. Works); and heparin (Sodium Heparin Inj., M.T.C. Pharmaceuticals Ltd.). All doses refer to the corresponding salts if not otherwise stated. Prior to injection, the urotensin preparations were adjusted to pH 7 with sodium bicarbonate. The activity of the preparation of urotensin I is expressed in units, a unit being the activity present in an extract of 1 mg of acetone dried urophysis powder of *Gillichthys mirabilis* extracted in 0.25% acetic acid and heated for 3 min in a boiling water bath (Bern & Lederis, 1969).

The nomenclature for urophysial active principles proposed by Bern & Lederis (1969) with the modification of Lederis (1972) is used throughout this paper: urotensin I = mammalian (rat) hypotensive principle, urotensin II = piscine smooth muscle stimulating- and eel pressor principle.

Results

General observations

Conscious rats appeared to be mildly sedated by intravenous injections of urotensin and did not show any signs of discomfort.

No apparent changes occurred in the function of the alimentary tract. Breathing appeared to be stimulated during the phase of lowered blood pressure. Immediately after the injection, flushing of the paws and ears was noted. No change in salivation or in nasal and lacrimal secretion was observed, and there was no chromodacryorrhoea.

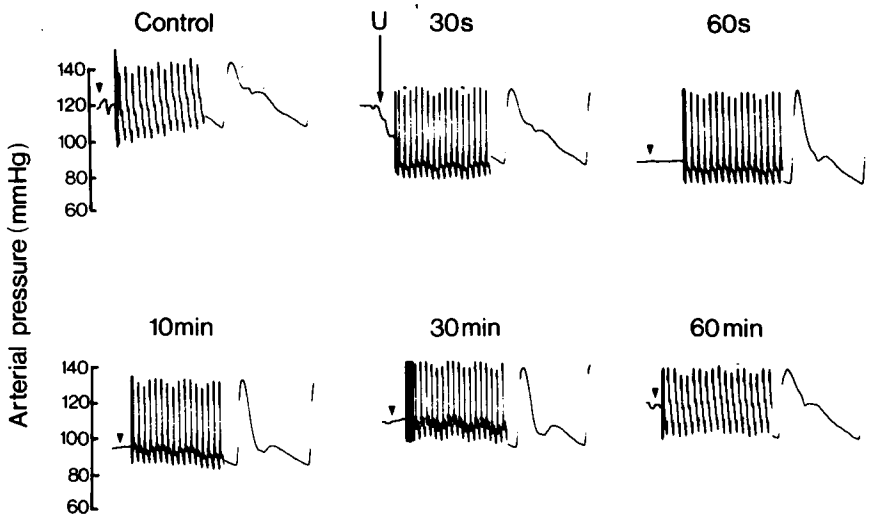

Fig. 1 Blood pressure recording from the cannulated carotid artery of a conscious rat (mean blood pressure (▾), heart rate, pulse pressure and pulse pressure contour). Typical hypotensive effect of urotensin I (U at arrow; 20 mu/100 g, i.v.). Note that the diastolic pressure fell more than the systolic and that the fall in pressure was accompanied by an increase in heart rate.

The effect of urotensin I on the blood pressure of conscious rats

Intravenous injection. Urotensin was injected in doses ranging from 10-15 mu/100 g body weight. After a short latent period (ranging from 10-30 s) mean pressure fell gradually during the following 1-2 min, by 7-56 mmHg and remained low for 30-120 minutes. The extent of the fall and the duration of effect were dose-dependent. During hypotension caused by urotensin, subsequent injections of urotensin produced further lowering of blood pressure.

The extent of fall in pressure was greater with high doses and was influenced by the initial level of the pressure. In rats with initial pressure of 110-120 mmHg, urotensin doses of 10-20 mu/100 g body weight decreased the mean blood pressure by at least 10%. Many rats, however, were more sensitive to urotensin. In one experiment the mean blood pressure was lowered by 37 mmHg after 20 mu/100 g and by 20 mmHg after only 5 mu/100 g body weight. In another similar experiment pressure was lowered by 45 mmHg by a dose of 12.5 mu/100 g, whilst the dose of 4 mu/100 g elicited a drop of 18 mmHg.

Pulse pressure was increased during the hypotension caused by urotensin. This was due to a more extensive drop in diastolic pressure than in systolic pressure. The former dropped by 40% more than the latter. The contour of the pulse pressure was changed in that its diastolic portion was considerably lowered, indicating dilatation of the resistance vessels (Best & Taylor, 1966). When urotensin at 1 mu/100 g was injected, no apparent drop in blood pressure occurred. However, an increase in the pulse pressure, lowering of its diastolic phase and the increase in the heart rate were distinct, suggesting that some vasodilatation occurred even with this low dose of urotensin.

A typical effect of urotensin on blood pressure parameters and on heart rate is shown in Figure 1.

Intramuscular and subcutaneous injection. Urotensin was injected subcutaneously in five and intramuscularly in four conscious rats. When injected subcutaneously in a dose of 250 mu/100 g, which is approximately 100 times higher than the usual threshold dose after intravenous administration, urotensin elicited a gradual and long-lasting decrease in blood pressure in four out of five animals. The decrease was apparent at 6 to 13 min after injection and hypotension gradually developed. In one rat, hypotension was still present 24 h after urotensin was injected. In all the animals, mean blood pressure fell to extremely low levels (35-50 mmHg).

After an intramuscular injection (100 mu/100 g) into conscious rats, blood pressure fell over 5 to 15 min from 140 to 78 mmHg in one rat. The recovery took from 4 to 5 hours.

A lower subcutaneous dose of urotensin (50 mu/100 g) elicited in one experiment a drop in

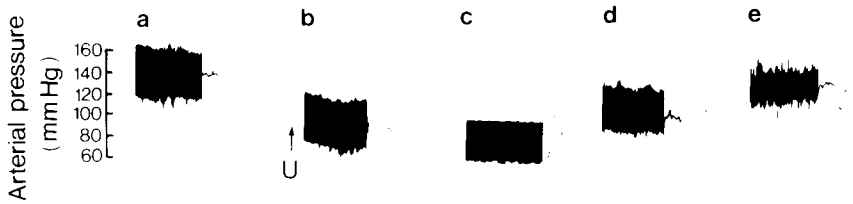

Fig. 2 Conscious rat. Blood pressure recording (mean blood pressure (▾) and pulse pressure contour) before (a) and 15 min (b), 30 min (c), 2.5 h (d) and 5 h (e) after the subcutaneous injection of 100 mu/100 g of partially purified urotensin I (U).

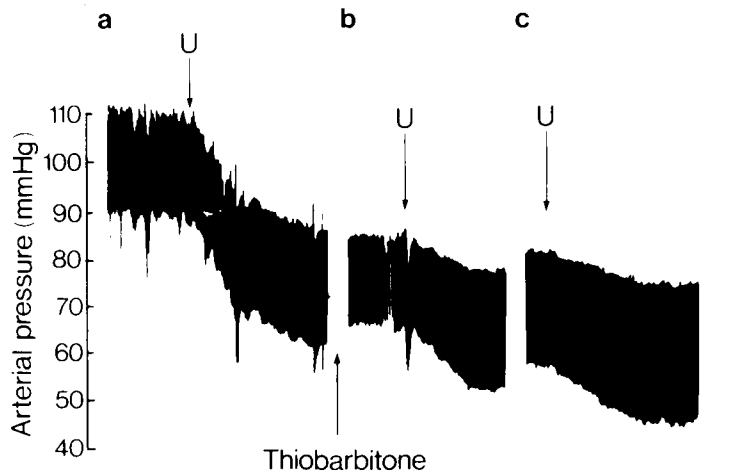

Fig. 3 The hypotensive effect of urotensin I (U at arrow, 15 mu/100 g i.v.) in (a) was recorded while the animal was conscious and in (b) and (c) under thiopentone anaesthesia (10 mg/kg i.v.). Note that thiopentone lowered blood pressure (from 100 to 80 mmHg) and that the subsequent effect of urotensin was decreased.

the mean blood pressure from 124 mmHg to 105 mmHg (30 min after the injection). The changes in the contour of the pulse pressure, produced by subcutaneous and intramuscular injections of urotensin, were similar to the changes observed after intravenous injection: the diastolic phase of the pulse pressure was considerably lowered. In the experiment shown in Fig. 2, the recovery of the pulse pressure contour began 2 h after the injection and it regained its original shape only 5.5 h after the injection.

The influence of different anaesthetic agents on the hypotensive action of urotensin

Two successive intravenous injections of a low dose of urotensin were first tested on the conscious rat. Then, the animal was anaesthetized and the same dose of urotensin was given, usually twice. The two effects obtained in the anaesthetized rat were compared with each other, as well as with the control effects.

A fall of blood pressure resulted on administration of one of the four anaesthetic agents used (ether, thiopentone, pentobarbitone and chloralose) although the anaesthesia was shallow in all cases (reaction to pain, positive corneal reflex). From four to six animals were used in each case. The extent of the fall varied from experiment to experiment. The action of thiopentone (10 mg/kg i.v.) on the effect of urotensin in a representative experiment is shown in Figure 3. After the induction of anaesthesia, the blood pressure fell from 98 to 82 mmHg. The effect of urotensin (10 mu/100 g) was then somewhat decreased as compared with the control effect.

In rats anaesthetized with ether, pentobarbitone (40 mg/kg i.p.) or chloralose (40 mg/kg as 2% solution), blood pressure fell in most experiments

and the hypotensive effect of urotensin was then less pronounced than in conscious animals, as shown for thiopentone anaesthesia (Figure 2).

Action of mecamylamine, hexamethonium, atropine, propranolol and diphenhydramine on the blood pressure effects of urotensin

The influence of mecamylamine and hexamethonium, of atropine, of dibenzyline and propranolol and of diphenhydramine, on the hypotensive action of urotensin was studied in conscious rats. All observations were repeated in at least four animals. In order to evaluate the degree of blockade of the specific receptors, effects of the respective agonists (i.e. nicotine, actylcholine, noradrenaline, isoprenaline and histamine, respectively) were observed before and after the injection of a given antagonist.

The ganglionic blocking agents, mecamylamine (up to 2 mg/100 g) and hexamethonium (4 mg/100 g) abolished the hypertensive effect of nicotine (30 μg), leaving the effect of urotensin unchanged. These observations indicated that (nicotinic) ganglionic transmission was not involved in the production of hypotension by urotensin. Accordingly, a site of urotensin action located more peripherally than the autonomic ganglia was considered. After injection of atropine (0.1 mg/100 g), which abolished the effect of acetylcholine (1 μg), the effect of urotensin was moderately potentiated.

Propranolol (0.1 mg/100 g) abolished the hypotensive effect of a high dose of isoprenaline (2 μg/100 g) but did not change the hypotensive effect of urotensin. Blockade of histamine receptors by a high dose (0.8 mg/100 g) of diphenhydramine was also without effect on the action of urotensin. According to these observations, the hypotension elicited by urotensin was not mediated by muscarinic receptors, β-adrenoceptors or histamine receptors, nor was it due to a release by urotensin of endogenous transmitter substances which affect cholinoceptors, β-adrenoceptors, muscarinic or histamine receptors.

The action of urotensin on the blood pressure effects of noradrenaline, adrenaline, nicotine, angiotensin II, acetylcholine and bradykinin in conscious rats

In this series of experiments a possible influence of urotensin on the actions of nicotine, noradrenaline, adrenaline, acetylcholine, bradykinin and angiotensin II was studied. All observations were repeated in four or more rats. After control effects of the respective agents had been obtained,

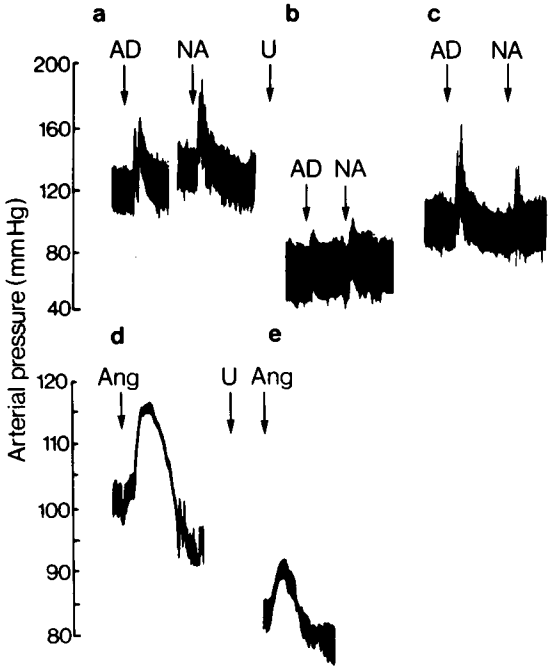

Fig. 4 (a) Control effects of adrenaline (AD) 2 μg/kg and noradrenaline (NA) in the conscious rat. Between (a) and (b) urotensin (U) 50 mu/100 g was injected. Note the drop in blood pressure and the inhibition of the effects of adrenaline and noradrenaline (in b). Full response to adrenaline returned 30 min after urotensin (c).

(d) Control effect of angiotensin II (Ang) 2 μg/kg and (e) partial inhibition of this effect after urotensin (U) 40 mu/100 g.

urotensin was injected and the injections of the same agents were repeated during the hypotension elicited by urotensin. Typical experiments are shown in Figures 4 and 5. Figure 4 (above) shows that the effects of noradrenaline and adrenaline were distinctly inhibited during the hypotension induced by urotensin. The effect of nicotine was decreased to a lesser extent than that of noradrenaline. The inhibitory action of urotensin on the hypertensive effect of angiotensin II is shown in Figure 4e.

Figure 5 (above) shows that the fall in blood pressure produced by acetylcholine (1 μg) decreased or was inhibited during hypotension by urotensin. In some experiments acetylcholine did not produce a response after urotensin even if the dose was increased considerably (5 μg/100 g).

Bradykinin (0.1 μg/100 g) elicited a short-lasting hypotension. When bradykinin was given 10 to 15 min after administration of urotensin, the effect of bradykinin on blood pressure was considerably diminished (Figure 5e).

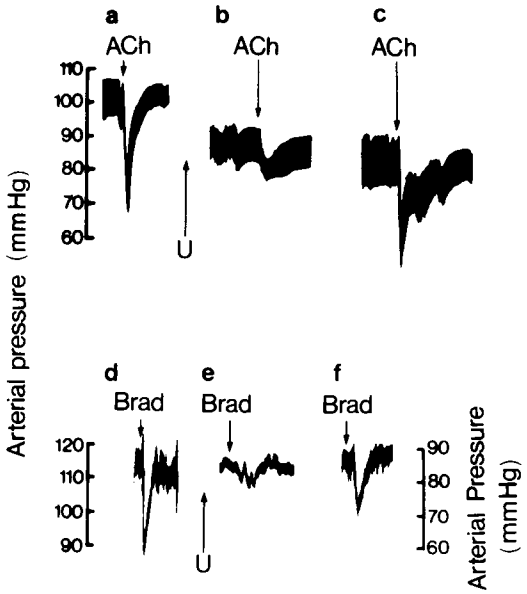

Fig. 5 Blood pressure recording in the conscious rat. (a) control effect of acetylcholine (ACh) 1 μg and the effect of urotensin (U) 50 mu/100 g; (b) partial inhibition of the effect of acetylcholine by urotensin; (c) reappearance of the response to acetylcholine (30 min after urotensin).

(d) control effect of bradykinin (Brad) 0.1 μg/100 g. Between d and e urotensin (U) 50 mu/100 g was injected. Partial inhibition of the effect of bradykinin 5 min (e) and 15 min (f) after urotensin.

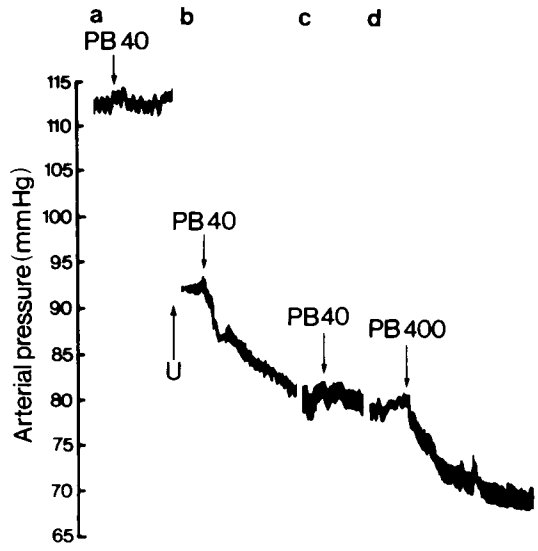

Fig. 6 Blood pressure recording in a conscious rat. (a) Subthreshold dose of phenoxybenzamine (PB at arrow, 40 μg/100 g) elicited a strong effect when injected after 20 mu/100 g urotensin (U) in (b). Potentiation of the effect of phenoxybenzamine was not present when it was injected 30 min after urotensin (c). Note that a high dose of phenoxybenzamine (400 μg/100 g) lowered the blood pressure further (d).

The action of phenoxybenzamine on the hypotensive effect of urotensin

Since urotensin was found to inhibit the effects of noradrenaline and adrenaline on the rat blood pressure, a possible participation of blockade of α-adrenoceptors in the effect of urotensin on blood pressure was examined in more detail. Phenoxybenzamine was injected in doses sufficiently high to ensure a complete blockade of α-adrenoceptors (1-2 mg/100 g). A fall of blood pressure (from 106 mmHg to 89 mmHg in one experiment) followed the injection of phenoxybenzamine and the effect of subsequently administered noradrenaline was abolished. However, injection of urotensin still elicited an additional fall in pressure. There was no inhibition by phenoxybenzamine of the hypotensive effect of urotensin in five experiments. In two out of those five experiments the hypotension induced by urotensin was more extensive after phenoxybenzamine than before, although the initial blood pressure had already been lowered by phenoxybenzamine, suggesting that synergism between urotensin and phenoxybenzamine might exist. In another experiment, a low, near threshold dose of phenoxybenzamine (40 μg/100 g) which did not lower the blood pressure by itself, elicited a marked effect when injected 3 min after urotensin (Figure 6).

The effect of urotensin I on the carotid occlusion relfex and on postural blood pressure regulation

The inhibitory action of urotensin on vascular effects of noradrenaline, adrenaline, angiotensin II, acetylcholine and bradykinin suggested that the hypotension elicited by urotensin might be due to a lowered sensitivity of the resistance blood vessels to physiological-vascular regulatory mechanisms. This was examined by studying the action of urotensin on the carotid occlusion reflex. In rats under ether anaesthesia, one common carotid artery was clamped for 15 s and the increase in blood pressure was recorded before and after

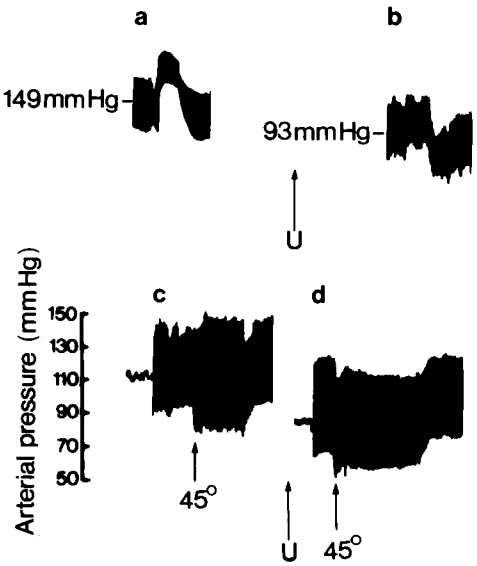

Fig. 7 Rat lightly anaesthetized with ether. Carotid occlusion relfex (•) before (a) and after (b) urotensin 40mu/100 g.
Rat anaesthetized with sodium pentobarbitone (4 mg/100 g i.p.). Inclination of the animal 45° (at arrow). (c) before and (d) after urotensin (U) 50 mu/100 g.

urotensin. The control-clamping of the carotid artery elicited an immediate rise in blood pressure (Figure 7a). Since there was no simultaneous increase in heart rate and in pulse pressure, the hypertensive response to the carotid occlusion can be considered as having been due mainly to reflex peripheral vasoconstriction. This reflex was partially inhibited by urotensin (20 mu/100 g) in three out of five rats (not affected in one and completely abolished in one). When urotensin (40 mu/100 g) was injected it was abolished in three out of four rats (only partially inhibited in one).

For postural experiments, rats anaesthetized with pentobarbitone (4 mg/100 g) were placed in a plastic single rat cage. After control recordings of blood pressure were taken, the cage with the rat was inclined, lowering the hind part of the animal by 45°. Usually only a short-lasting fall of blood pressure was recorded in this control test, which was followed by immediate recovery in spite of the inclined posture of the animal.

The same procedure was repeated after intravenous injection of urotensin I (10-50 mu/100 g). As the lower part of Fig. 7 shows, blood pressure fell (from 80 to 72 mmHg) after the posture was changed, and in contrast to the control test, the blood pressure did not return to that recorded before the inclination.

The effect of urotensin I on heart rate in conscious rats

Urotensin increased heart rate in conscious rats. This increase was observed in 76 out of 79 experiments after doses of urotensin varying from 1 mu-50 mu/100 g had been injected. The increase in heart rate became evident, as a rule, together with the fall of blood pressure and recovered gradually as the pressure recovered. However, in some experiments heart rate returned to normal even though the blood pressure was still low. In one experiment after urotensin (1 mu/100 g) the mean blood pressure did not decrease. However, a lowering of diastolic phase of the pulse pressure contour occurred and this was accompanied by an increase in heart rate (11%).

The extent of the increase in heart rate varied from experiment to experiment, and seemed to be related to the dose injected, to the degree of the fall in blood pressure and to the initial heart rate, recorded before the injection of urotensin. After higher doses of urotensin, which elicited a more marked fall in blood pressure, the increase in heart rate tended to be more pronounced, in particular when the initial heart rate was low.

Phenoxybenzamine (1 mg/100 g) did not alter the cardiac effects of urotensin. However, the increase in heart rate did not occur in experiments in which propranolol (300 μg/100 g) was administered. Propranolol alone decreased the heart rate considerably, yet it did not inhibit the lowering of blood pressure by urotensin. The ganglionic blocking agents, mecamylamine (1 mg/100 g) and hexamethonium (2 mg/100 g) also completely prevented changes in heart rate during urotensin hypotension.

The perfusion pressure in the isolated hind limb was lowered by doses of urotensin I (0.1-1 mu). The reduction in pressure was dose-related (Medaković & Lederis, 1972). Atropine (200 μg) abolished the fall in blood pressure produced by acetylcholine (2 ng) but did not affect that of urotensin. Similarly, the effect of urotensin was not inhibited by mecamylamine (800 μg), which abolished the pressor action of nicotine (30 μg) and tetramethylammonium (50 μg). Propranolol (30 μg) and diphenhydramine (300 μg) did not affect the responses to urotensin, while abolishing the effects of isoprenaline (1 μg) and histamine (3 μg) respectively.

Discussion

Urotensin was previously found to decrease mean blood pressure in rats anaesthetized with pentobarbitone (Kobayashi *et al.*, 1968) or urethane (Lederis, 1970).

In the present experiments urotensin lowered blood pressure in conscious rats to a greater extent than that which was observed in animals anaesthetized with urethane. All three parameters, i.e. systolic, diastolic and mean pressure, were lowered by urotensin. However, diastolic pressure fell more than systolic, the diastolic phase of the pulse pressure was lowered, and there was an accompanying increase in heart rate. Fall in diastolic pressure can be partially offset by a simultaneous increase in heart rate. Thus the fall in diastolic pressure caused by urotensin would be more extensive than recorded if there was no increase in heart rate. This combination of changes in blood pressure parameters suggests that the hypotension caused by urotensin was due predominantly or entirely to a decrease in the peripheral vascular resistance (drop of the diastolic pressure), which reflexly increased heart rate. This is supported by the absence of increase in heart rate not only in rats treated with propranolol, but also in those in which autonomic ganglia had been blocked by mecamylamine or hexamethonium.

The duration of the hypotensive effect of urotensin (i.v.) was found to be shorter in the conscious rat than that observed in rats anaesthetized with urethane (Lederis, Bern, Nishioka & Geshwind, 1971), and the threshold dose was lower. The latter finding suggested that the action of urotensin might be diminished by anaesthetic agents. This could be shown to be the case in rats anaesthetized with ether, chloralose, thiobarbitone or pentobarbitone. However, all four anaesthetic agents lowered the initial blood pressure, so that urotensin was injected at a lower blood pressure than in unanaesthetized rats. In contrast to urethane, the duration of the hypotensive action of urotensin was not prolonged by the anaesthetic agents used in the present experiments. Systolic, diastolic and mean pressure were changed by urotensin in the same manner in anaesthetized and in conscious rats. Also, in contrast to anaesthesia with urethane, where an extensive loss of sensitivity to the second injection of urotensin has been observed (Zelnik, 1971), several consecutive responses to urotensin could be obtained in conscious rats and in those anaesthetized with ether, chloralose, pentobarbitone or thiobarbitone, respectively. Since the responses to urotensin were less pronounced in rats with low initial blood pressure, the loss of sensitivity observed in urethane anaesthesia (Zelnik, 1971) might have been due to very low pre-injection blood pressure levels, which are usually caused by this anaesthetic. Wilder (1957) showed that the lower the initial value, the smaller is the response to a substance which lowers blood pressure. The possibility cannot be excluded, therefore, that the effects of urotensin were less in rats anaesthetized with ether, chloralose, pentobarbitone, and thiobarbitone because of reduced 'initial' blood pressure.

The present experiments show that the hypotensive effect of urotensin could not be inhibited by ganglionic blocking agents. This indicates that the action was exerted at a site distal to the autonomic ganglia. Since vasodilatation was elicited by low doses of urotensin in the perfused rat hind limb, vascular smooth muscle is likely to be the site of the hypotensive action of urotensin. Further, urotensin does not act by causing a prolonged release of acetylcholine, histamine or an isoprenaline-like metabolite of adrenaline. Such a metabolite of adrenaline has been shown to be present in extracts of mammalian adrenal medulla (Lockett, 1954), in extracts of protein-free plasma of rabbits (Roberts & Lockett, 1961), or released in a cat heart-lung preparation after stimulation of sympathetic chains (Lockett, 1957).

In view of the lack of any influence of atropine on the effect of urotensin it is difficult to propose a feasible interpretation of the inhibition by urotensin of the blood pressure effect of acetylcholine in the conscious rat. The inhibition by urotensin of the hypertensive effect of adrenaline, noradrenaline and nicotine in conscious rats, as well as its lowering of the perfusion pressure kept high by noradrenaline in the isolated hind limb, may imply that urotensin possesses an α-adrenoceptor blocking action. However, the urotensin-induced hyoptension was not altered if conscious rats in the present experiments or if rats under urethane anaesthesia (Lederis & Osmond-Jones, unpublished observations) were treated with phenoxybenzamine either before urotensin, or if this α-adrenoceptor blocker was injected during the effect of urotensin.

It may be of interest to note that the fall in blood pressure produced by phenoxybenzamine was potentiated by the administration of urotensin beforehand. In the present experiments this was the only substance whose effect was potentiated by urotensin. Figure 4 shows that the potentiation was temporary and it ceased before the cessation of hypotension. The potentiation of phenoxybenzamine may be related to the inhibitory action of urotensin on the pressor effects of noradrenaline and adrenaline.

The action of urotensin I on the carotid occlusion reflex as well as the appearance of

postural hypotension, may be related to the inhibition by urotensin of vascular responses to noradrenaline. The peripherally elicited inhibition of those reflexes, which are important in blood pressure regulation, may contribute to the hypotensive effect of urotensin I.

Long-lasting hypotensive effects were recorded after intramuscular or subcutaneous injection of urotensin I. The duration of the effect depended on the dose and in some experiments after subcutaneous injection of urotensin was still present after 24 hours. The fall in blood pressure was not as abrupt as after intravenous injection. The dose that usually sufficed to elicit an effect which lasted for several hours amounted to only 50 mu/100 g (s.c.). The extent of the blood pressure fall was similar to that elicited with urotensin (10-20 mu/100 g) administered intravenously.

Erspamer & Glaeser (1963) described the hypotensive polypeptide, eledoisin, which could be distinguished from other polypeptides, known to possess potent hypotensive action (bradykinin and substance P). Another polypeptide, physalaemin, obtained from the skin of *Physalaemus fuscumaculatus* elicits a potent hypotensive effect in various mammalian species (Bertaccini, Cei & Erspamer, 1965a). The hypotensive effects of these peptides were not appreciably changed by atropine, adrenergic-blocking and ganglion-blocking agents and were described as being chiefly peripheral. Like urotensin I, both eledoisin and physalaemin antagonized the pressor effects of catecholamines, nicotine and angiotensin. The hypotensive effect of urotensin differs from those of eledoisin and physalaemin in that it is uniquely long-lasting. Another dissimilarity is that whereas no activity of urotensin I on smooth muscle (other than vascular) could be detected in the present experiments, eledoisin- and physalaemin-like polypeptides (Erspamer & Erspamer, 1962, and Bertaccini, Cei & Erspamer, 1965b) stimulate other smooth muscle-containing isolated organs (e.g. guinea-pig ileum, rat intestine and colon, rabbit large intestine, dog colon, rat and frog stomach, rabbit, rat, cat and guinea-pig uterus and guinea-pig seminal vesicles).

Finally, it may be mentioned that the long-lasting hypotensive effect of urotensin I in the rat can be clearly distinguished from the effects of substance P and bradykinin, which are of short duration.

Urotensin I has a pronounced and prolonged peripheral vasodilatory and hypotensive action. Its effects do not seem to be limited to the rat, since, in preliminary experiments, it elicited cardiac acceleration, vasodilatation and hypotension in the dog, sheep and the Rhesus monkey (MacCannell, Van Petten, Letter & Lederis, unpublished observations).

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