Br. J. Pharmac. Chemother. (1967), **30**, 655–667.

THE RELEASE OF CATECHOLAMINES FROM THE ADRENAL MEDULLA BY PEPTIDES

BY

JANINA STASZEWSKA-BARCZAK* AND J. R. VANE

From the Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London

(*Received May* 1, 1967)

In the cat, angiotensin and bradykinin release catecholamines from the adrenal medulla (Feldberg & Lewis, 1964, 1965), but eledoisin does not (Staszewska-Barczak & Vane, 1965; Lewis & Reit, 1966). The present experiments extend the observations on the releasing actions of these peptides and compare their effects in cat and dog. Some of the results were communicated to the Physiological Society (Staszewska-Barczak & Vane, 1964).

METHODS

Cats of either sex weighing 2–5 kg were anaesthetized with ethylchloride and ether; anaesthesia was then maintained with chloralose (80 mg/kg intravenously). Dogs of either sex weighing 6–15 kg were anaesthetized with ether and anaesthesia was then maintained with chloralose (100 mg/kg intravenously).

A carotid or brachial artery was cannulated with polyethylene tubing to supply blood to an extra-corporeal circulation for the blood-bathed organ technique (Vane, 1964) and to record the blood pressure on a mercury manometer. To detect the release of catecholamines a continuous stream of arterial blood was superfused over the rat isolated stomach strip (Vane, 1957) and the chick isolated rectum (Mann & West, 1950); the blood was then returned to the animal through a cannula in a jugular vein. With these organs superfused in series, it was possible to distinguish between the release of adrenaline which relaxed both rat stomach and chick rectum and noradrenaline which only relaxed the rat stomach (Armitage & Vane, 1964; Staszewska-Barczak & Vane, 1965). In some experiments the series also included a rat colon which contracted to angiotensin (Regoli & Vane, 1964). Heparin ("Pularin," Evans; 1,000 i.u./kg intravenously) was injected into the animal before the external circulation of blood was started. Artificial ventilation was maintained with a pump. To make intra-arterial injections to the adrenal glands a fine polyethylene catheter was introduced into the aorta through a femoral artery, so that the tip of the catheter lay upstream to the origins of the adrenal arteries. Thus, an "intra-arterial" injection mixes with that portion of the cardiac output which flows down the aorta below the diaphragm but only a small aliquot of the injected substance reaches the adrenal glands. Intra-arterial injections made in this way have the advantages, first, that they by-pass the lungs and, secondly, that the blood taken for assay from a carotid artery contains only that portion of the injected substance which recirculates. The position of the catheter was checked at the end of the experiment.

The amounts of catecholamines released by intra-arterial or intravenous infusions or injections of peptides were determined by comparing the effects of the released catecholamines on the blood-bathed organs with the effects of intravenous injections or infusions of adrenaline and noradrenaline. The

* Present address: Experimental Pathology Research Centre, Polish Academy of Sciences, Dworkowa 3, Warsaw, Poland.

release of catecholamines by histamine was also studied in many of the experiments. In cats, all the substances tested were compared with angiotensin, and in dogs with bradykinin. Whenever possible, all the substances were compared for activity in the same animal. When the actions of blocking agents were tested, the effects of particular doses of the peptides were determined immediately before and then several times during the 60 min after giving the blocking agent.

In some cats and dogs, after the chloralose had been given, the muscles of the neck were dissected to expose the atlanto-occipital membrane. Later in the experiment, in order to interrupt spinal cord transmission, either the spinal cord was cut (in cats) or 10 ml. 1% lignocaine was injected through the membrane into the cord (in dogs).

Drugs

The following drugs were used, doses of salts are expressed as base: (−) adrenaline bitartrate (British Drug Houses), angiotensin (Hypertensin, Ciba), bradykinin (Parke Davis), eledoisin (Sandoz), hexamethonium bromide (May & Baker), histamine hydrogen phosphate (Burroughs Wellcome); kallidin (Sandoz), mecamylamine hydrochloride (Merck, Sharp and Dohme), (—) noradrenaline bitartrate (Bayer Products), oxytocin ("Pitocin" Parke Davis), pentolinium tartrate (May & Baker), vasopressin ("Pitressin," Parke Davis).

RESULTS

Intra-arterial injections

In both cats and dogs, injections of angiotensin, bradykinin or kallidin intra-arterially regularly released catecholamines. The release was dose dependent, as is illustrated in Fig. 1. When the adrenal glands were excluded from the circulation, there was no detectable output of catecholamine into the circulation, showing that the release was from the adrenal medulla.

To compare the activities of the peptides, doses were chosen which liberated 0.5–2 μg adrenaline; from these the amount of peptide required to liberate 1 μg adrenaline was calculated. In Fig. 1, angiotensin (1 μg intra-arterially) produced a release of catecholamines equivalent to more than 1 μg adrenaline. Angiotensin (2 μg intra-arterially) released between 1 and 2.5 μg adrenaline and angiotensin (4 μg intra-arterially) released just less than 4 μg adrenaline. Angiotensin sometimes contracted the rat stomach strip and this prevented the full relaxation induced by the released catecholamine; such an effect is seen in Fig. 1; after the injection of angiotensin (1 μg intra-arterially) the adrenaline release was greater than 1 μg as shown by the relaxation of the chick rectum, which is insensitive to angiotensin, but the rat stomach strip relaxed much less. When greater amounts of catecholamines were released the contractor action of angiotensin was masked. With the other peptides, in the doses used, bradykinin, kallidin and eledoisin neither contracted nor relaxed the rat stomach strip or the chick rectum. At the end of the experiments, when the animal had been adrenalectomized, it was also possible to test the reactions of the assay tissues to adrenaline injected together with a peptide. Apart from angiotensin, which reduced the effects of adrenaline on the rat stomach strip, there was no interference with the registration of the effects of adrenaline on the assay organs when the peptides (1–10 μg) were injected intravenously at the same time as adrenaline (1–2 μg). Histamine had no direct effect on the rat stomach strip but, in large doses, induced a contraction of the chick rectum (Staszewska-Barczak & Vane, 1965).

The effects on the two assay organs showed that the catecholamine released was mainly if not all adrenaline. Had noradrenaline also been released, the rat stomach would

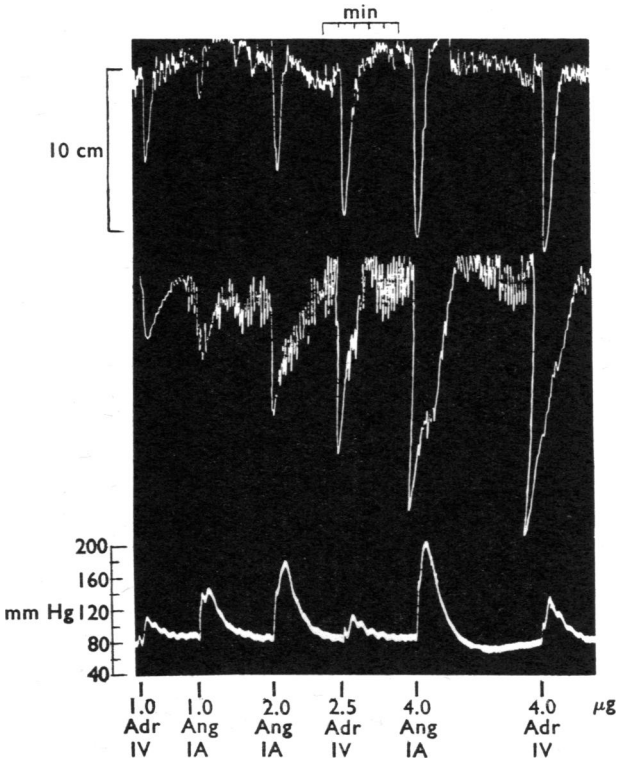

Fig. 1. Cat (4.2 kg) anaesthetized with chloralose. A rat stomach strip (top record) and a chick rectum (middle record) were superfused with carotid arterial blood ; the bottom record is blood pressure. The tracing shows the effects of intravenous injections of adrenaline (1, 2.5 and 4 μg) compared with intra-arterial injections of angiotensin (1, 2 and 4 μg). Angiotensin has no effect on the chick rectum, but contracts the rat stomach strip. This contraction sometimes interferes with the registration of the adrenaline release, as can be seen after angiotensin (1 μg i.a.). With the other injections the exactness of correspondence of the relaxations of the two assay organs after adrenaline and angiotensin showed that angiotensin was releasing mainly if not all adrenaline. Time scale in min ; vertical scales in 10 cm and mm Hg in this and in all subsequent figures.

have shown a greater relaxation than the chick rectum, in comparison to the effects of intravenous adrenaline.

The comparison of adrenaline-releasing activities was made both in the cat and the dog (Table 1). In the cat, angiotensin (mol. wt. 1,038) was the most powerful substance tested: on a molar basis it was 10 times more potent than bradykinin (mol. wt. 1,131) and 60 times more potent than histamine (mol. wt. 111). From the mean values and also in all of the individual experiments, kallidin was more active in releasing adrenaline than bradykinin. This is illustrated in Fig. 2 which shows that angiotensin (0.5 μg intra-arterially) released about 2 μg adrenaline. Kallidin (3 μg) and bradykinin (10 μg) both released about 1 μg adrenaline. Eledoisin was tested in amounts up to 20 μg, but no adrenaline release was detected, even though there was a pronounced hypotensive effect.

TABLE 1

AVERAGE DOSE IN MICROGRAMS±S.E. (NO. OF EXPERIMENTS) CALCULATED TO RELEASE 1 μg ADRENALINE, WHEN THE SUBSTANCE IS INJECTED INTRA-ARTERIALLY

Vasopressin (up to 2 i.u.) and oxytocin (up to 2 i.u.) were ineffective

	Angiotensin	Bradykinin	Kallidin	Eledoisin	Histamine*
Cat	0·8±0·13 (13)	8·1±1·7 (13)	5·8±0·95 (7)	— (5)	4·7±0·89 (11)
Dog	7·4±2·5 (17)	1·6±0·29 (21)	1·4±0·39 (7)	1·4±0·36 (8)	12·0±2·9 (20)

* These figures incorporate some from experiments by Staszewska-Barczak & Vane (1965).

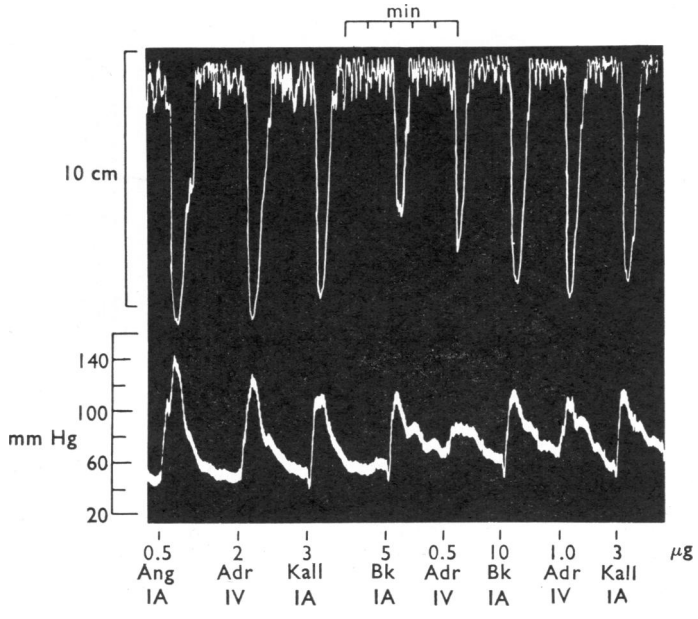

Fig. 2. Cat (2.9 kg) anaesthetized with chloralose. A rat stomach strip (top record) was superfused with carotid arterial blood; the lower record is blood pressure. This tracing compares the release of adrenaline after intra-arterial injections of angiotensin (0.5 μg), kallidin (3 μg) and bradykinin (5 μg and 10 μg). Angiotensin was more than 6 times more potent than kallidin which was about 3 times more potent than bradykinin.

In contrast to the cat, kallidin and bradykinin were both much more potent than angiotensin in the dog. Eledoisin also released adrenaline in this species and was more potent than angiotensin. Figure 3 shows a record from an experiment in which kallidin (1.5 μg) and bradykinin (3 μg) released about 3 μg noradrenaline, but angiotensin (20 μg) released less than 2 μg adrenaline. Eledoisin (3 μg) released almost 10 μg adrenaline and even 0.5 μg eledoisin released more than 2 μg adrenaline. Thus, in this dog eledoisin was more potent even than kallidin, but Table 1 shows that this was not generally so. Neither vasopressin (up to 2 i.u.) nor oxytocin (up to 2 i.u.) released catecholamine in the cat or dog.

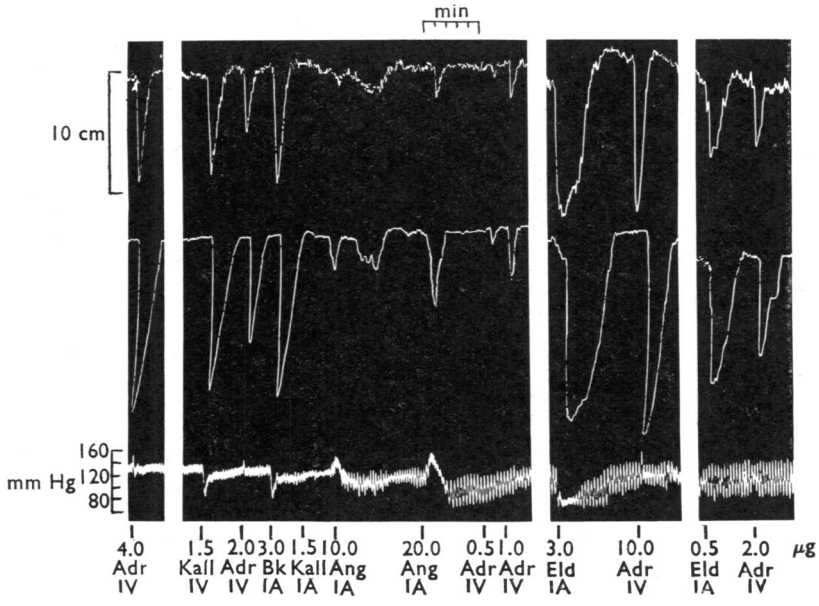

Fig. 3. Dog (9.7 kg) anaesthetized with chloralose. A rat stomach strip (top record) and a chick rectum (middle record) were superfused with carotid arterial blood; the bottom record is blood pressure. The tracing compares the peptides for potency in releasing catecholamine. Kallidin (1.5 μg i.a.) released about 3 μg adrenaline, as did bradykinin (3 μg i.a.). Intravenous kallidin (1.5 μg) was ineffective. To release 1.5 μg adrenaline, 20 μg angiotensin i.a. had to be injected. After eledoisin (3 μg i.a.) the release of adrenaline corresponded in its peak effect to an injection of 10 μg: the release was very prolonged after eledoisin, even with the smaller dose (0.5 μg) which released more than 2 μg adrenaline. Note that there was no adrenaline release associated with the prolonged fall in blood pressure after angiotensin and that there was little or no fall in blood pressure after the smaller dose of eledoisin.

Intravenous injections

When angiotensin, bradykinin and kallidin were injected intravenously, the release of adrenaline was less than when the same dose was given intra-arterially. In order to achieve the same release, up to 10 times the intra-arterial dose of the kinins and 2–4 times the dose of angiotensin had to be injected intravenously (Fig. 4). The first part of the tracing shows that reduction of blood pressure to the carotid sinuses by carotid occlusion did not lead to a detectable release of catecholamine. Bradykinin (1 μg intra-arterially) released about 1.5 μg adrenaline. When given intravenously, although the fall in blood pressure was greater and more prolonged, bradykinin (10 μg) gave a smaller release of adrenaline. With angiotensin, the injection of 5 μg intra-arterially gave a much greater release than 5 μg intravenously. In this experiment the spinal cord of the dog was then blocked by injecting lignocaine through the exposed atlanto-occipital membrane. Carotid occlusion had no further effect but angiotensin (5 μg intra-arterially) still released about 1 μg adrenaline and bradykinin (1 μg intra-arterially), released about 2 μg adrenaline. This experiment shows that in the dog, as in the cat, the release of adrenaline by bradykinin and angiotensin is not dependent on central or reflex effects: similar results were obtained with kallidin.

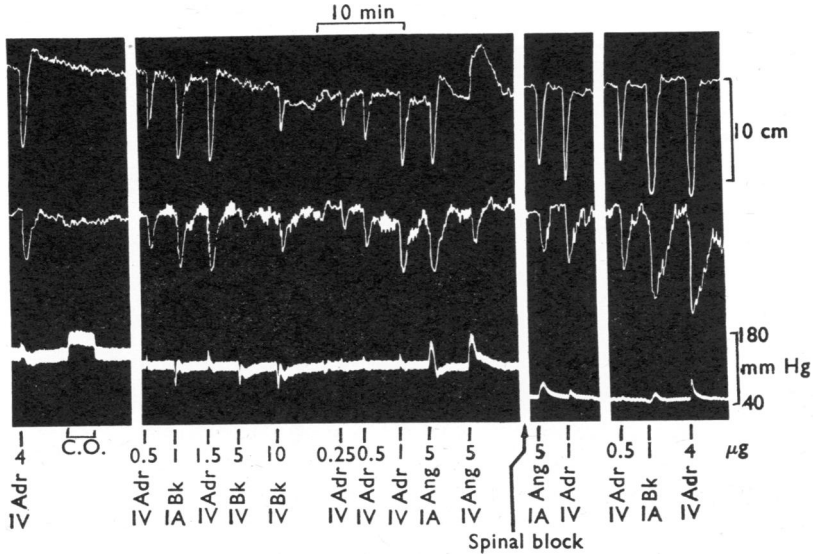

Fig. 4. Dog (6.6 kg) anaesthetized with chloralose. A rat stomach strip (top record) and a chick rectum (middle record) were superfused with blood from a brachial artery; the lower record is of blood pressure. The first panel shows that during carotid occlusion, there was no detectable release of catecholamine into the circulation. The second panel shows that bradykinin (1 μg i.a.) released more than 1 μg adrenaline, whereas 10 μg bradykinin i.v. released less than 1 μg adrenaline, even though the fall in blood pressure was greater. With angiotensin, 5 μg i.a. released about 1 μg adrenaline whereas 5 μg i.v. released less. Note the contraction of the rat stomach when the angiotensin given intravenously reached the assay organs via the brachial arterial blood. After spinal block with lignocaine (8 ml. of a 10 mg/ml. solution injected into the spinal cord through the atlanto-occipital membrane) carotid occlusion no longer raised the blood pressure (not shown), proving the block to be effective. Both angiotensin and bradykinin still released adrenaline when injected intra-arterially.

When eledoisin was given intravenously to the dog, the release of adrenaline was always greater than when given intra-arterially. In the four experiments in which the comparison was made, the intra-arterial doses of eledoisin calculated to release 1 μg adrenaline were 3.0, 0.5, 1.2 and 2.3 μg. In the same dogs, the intravenous doses were 0.4, 0.3, 1.0 and 1.5 μg.

Effects of ganglion-blocking agents

In four experiments in cats, after determining the relative potencies of the peptides, a ganglion-blocking agent (pentolinium or hexamethonium) was given and the potencies once more tested. Such an experiment is illustrated in Fig. 5. Before pentolinium (3 μg intra-arterially) kallidin released 0.8 μg adrenaline, bradykinin (10 μg intra-arterially) released 2 μg and angiotensin (0.5 μg intra-arterially) released about 0.4 μg adrenaline. After pentolinium (2 mg/kg intravenously) the peptides released much more adrenaline into the blood stream; bradykinin (5 μg), kallidin (3 μg) and angiotensin (0.5 μg) all released about 2 μg adrenaline. Similar results with these three peptides were obtained after ganglion block in 5 dogs. However, in 6 experiments, one of which is illustrated in Fig. 6, the release of adrenaline induced by either intravenous or intra-arterial eledoisin

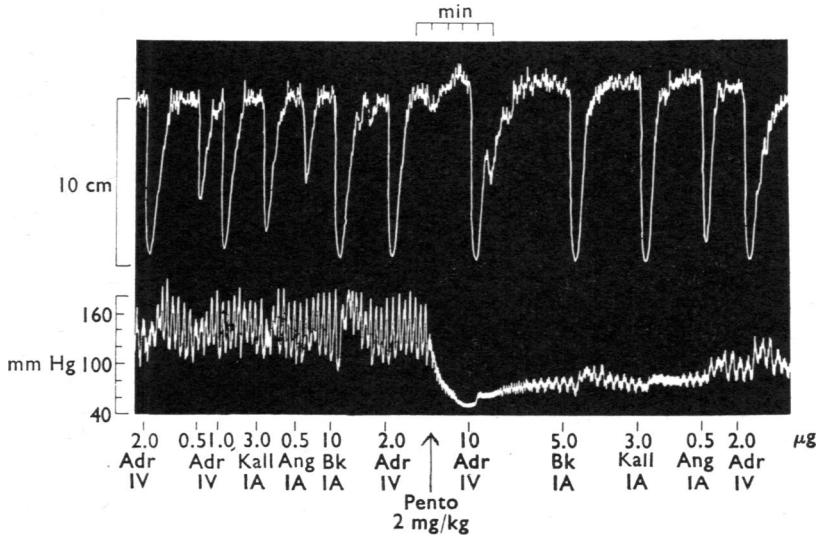

Fig. 5. Cat (2.9 kg anaesthetized with chloralose). A rat stomach strip (top record) was superfused with carotid arterial blood. The lower record is blood pressure. Kallidin (3 μg i.a.) released about 0.8 μg adrenaline, bradykinin (10 μg i.a.) released about 2 μg and angiotensin (0.5 μg i.a.) about 0.4 μg adrenaline. After pentolinium (2 mg/kg i.v.), the adrenal medulla was more sensitive to the peptides and bradykinin (5 μg i.a.), kallidin (3 μg i.a.) and angiotensin (5 μg i.a.) all released around 2 μg adrenaline).

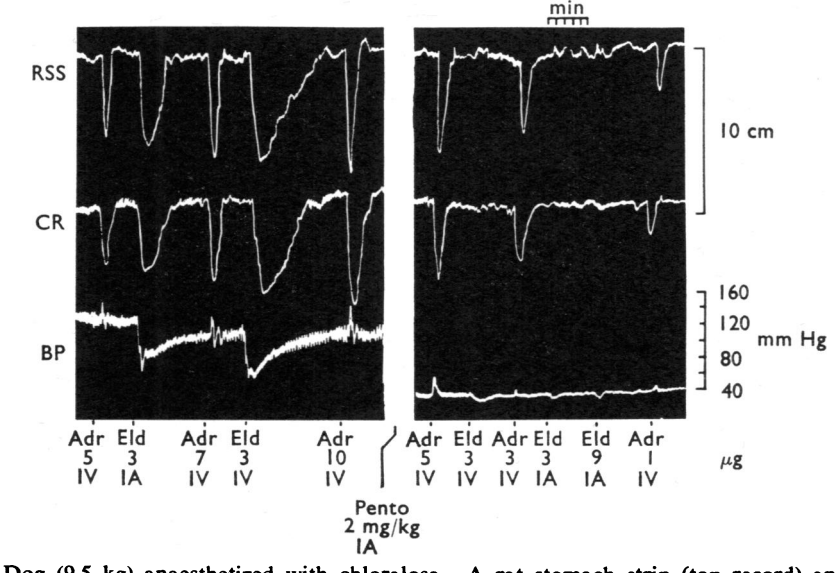

Fig. 6. Dog (9.5 kg) anaesthetized with chloralose. A rat stomach strip (top record) and a chick rectum (middle record) were superfused with carotid arterial blood. The bottom record is blood pressure. Eledoisin (3 μg) intra-arterially released 6 μg adrenaline whereas the same dose intravenously released about 9 μg adrenaline. Note that the release was prolonged so that the relaxation of the assay organs lasted much longer than after the injection of adrenaline. After pentolinium (2 mg/kg i.a.) eledoisin was no longer effective as a stimulant of adrenaline release and even three times the dose released no adrenaline.

was abolished by ganglion block. In this experiment, before pentolinium, the peak release of adrenaline induced by eledoisin (3 μg intra-arterially) corresponded to 6 μg adrenaline, whereas the same dose of eledoisin intravenously released about 8 μg adrenaline. The release of adrenaline induced by eledoisin tended to be prolonged, making it difficult to quantitate in terms of injections of adrenaline. After pentolinium (2 mg/kg intra-arterially) neither intravenous nor intra-arterial eledoisin induced release of catecholamine, even when the dose was increased to 9 μg.

Peptide infusions

The effects of intra-arterial infusions of bradykinin and angiotensin were determined in both the cat and the dog. In the cat, infusion of bradykinin of less than 2 μg/min had no effect. When the rate was increased to 5–10 μg/min there was an initial output of adrenaline, but this rapidly declined. With higher rates of bradykinin infusion (10–50 μg/min), the initial output of adrenaline was larger, but was still not maintained. However, with these higher infusion rates, another phenomenon was observed in which the output of adrenaline became sporadic (Fig. 7). In this experiment, an infusion rate of

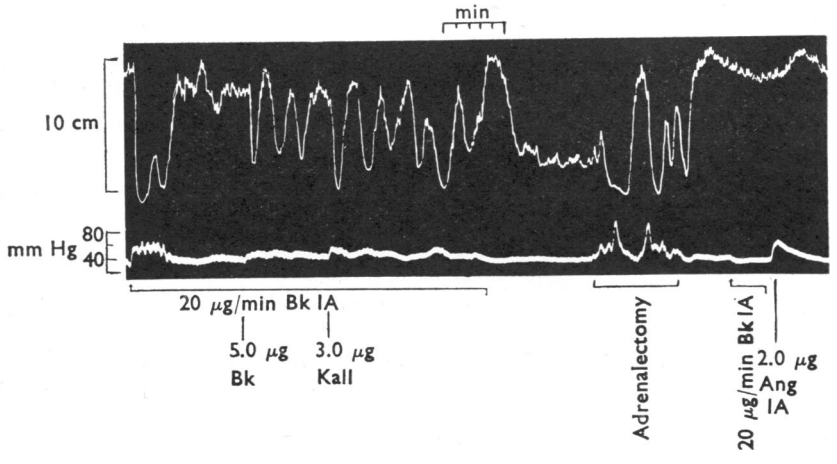

Fig. 7. Cat (2.9 kg) anaesthetized with chloralose. A rat stomach strip (top record) was superfused with carotid arterial blood. The lower record is of blood pressure. When an intra-arterial infusion of bradykinin (20 μg/min) was started, there was a good output of adrenaline but this passed off. Injections of bradykinin (5 μg i.a.) and kallidin (3 μg i.a.) during the infusion appeared to stimulate further adrenaline release but interpretation was difficult because there were spasmodic bursts of secretion. When the infusion stopped, the adrenaline secretion stopped for a minute or so but rapidly returned. Adrenalectomy abolished the secretion and then an infusion of bradykinin had no effect. An injection of angiotensin (2 μg i.a.) also failed to release adrenaline into the circulation, but did cause a small direct effect on the rat stomach.

20 μg/min intra-aterially gave an initially high output of adrenaline from the adrenal medulla, but this declined very quickly, even though the infusion was maintained. To test whether the decline was due to tachyphalaxis bradykinin (5 μg intra-aterially) was rapidly injected, and this still produced a response. However, following this injection the output of catecholamines from the glands became sporadic and for the rest of the

infusion period bursts of adrenaline were secreted, making it impossible to determine whether injections of kallidin or bradykinin in themselves produced a response. When the infusion was stopped the secretion of adrenaline stopped for a minute or so, but very quickly was re-established. After adrenalectomy the secretion stopped once more, showing that it came from the adrenal medulla and thereafter neither bradykinin nor angiotensin released adrenaline. In the dog, similar effects were observed, but the rate of bradykinin infusion needed to produce the sporadic bursts was lower (Fig. 8). An

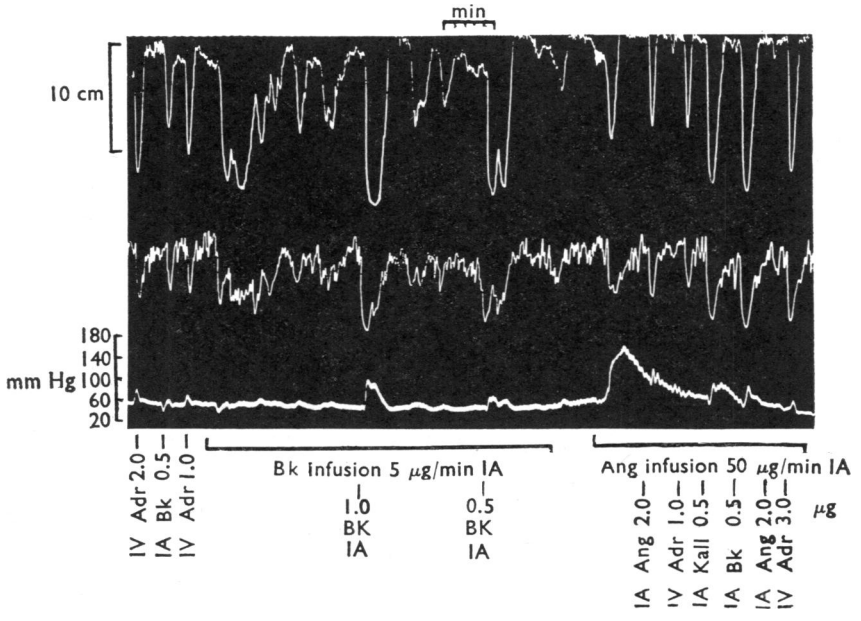

Fig. 8. Dog (7.3 kg) anasthetized with chloralose. A rat stomach strip (top record) and chick rectum (middle record) were superfused with carotid arterial blood. The bottom record is blood pressure. Bradykinin (0.5 μg i.a.) liberated less than 1 μg adrenaline. An infusion of bradykinin (5μg/min i.a.) gave a large initial output but this then declined and became sporadic. During the infusion, injections of bradykinin (1 and 0.5 μg i.a.) now released much more adrenaline. The infusion was stopped and an infusion of angiotensin (50 μg/min i.a.) started. Again there was a short initial output of adrenaline which rapidly waned. The responses to injections of angiotensin (2 μg i.a.) decreased during the infusion whereas those injections of kallidin (0.5 μg i.a.) and bradykinin (0.5 μg i.a.) did not.

infusion of bradykinin (5 μg/min intra-arterially) induced an initially large output of adrenaline which quickly declined and then changed into sporadic bursts. Injections of bradykinin (1 and 0.5 μg intra-arterially) superimposed on the infusion clearly gave increased effects. An infusion of angiotensin (50 μg/min intra-arterially) was then given ; this also induced a release of adrenaline which declined after the first few minutes. Injections of kallidin and bradykinin superimposed on the infusion showed that both peptides were still able to release adrenaline but the effects of injected angiotensin (2 μg) was almost abolished. Thus, when tachyphylaxis to angiotensin had been established, kallidin and bradykinin remained effective, showing that the receptor sites for bradykinin and kallidin differed from those for angiotensin.

Infusions of angiotensin were given to 5 other dogs and 6 cats. Intra-arterial infusions of less than 0.5 μg/min in cats and 5 μg/min in dogs gave little or no catecholamine release. When the infusion rate was increased there was an initial output of adrenaline but this rapidly waned. Further increases in rate of infusion were then ineffective. Even with infusions of 10–20 μg/min in the cat and 20–50 μg/min in the dog, there was still only an initial output of adrenaline, waning rapidly as the infusion was maintained.

DISCUSSION

The use of the blood-bathed organ technique for assaying the release of catecholamines from the adrenal medulla into the circulation has been discussed elsewhere (Vane, 1964, 1966 ; Staszewska-Barczak & Vane, 1965). The results of the present paper confirm and extend those of Feldberg & Lewis (1964, 1965) who used a different assay system in the cat. As does histamine (Staszewska-Barczak & Vane, 1965), the peptides release adrenaline rather than noradrenaline from both cat and dog adrenal medullae. Since the medullae of these species may contain up to 90% noradrenaline (West, 1955 ; Butterworth & Mann, 1957), this catecholamine must be sequestered in cells or components of cells not available to extracellular chemical stimulation.

Adrenalectomy abolished the adrenaline release by the peptides. Thus, the sympathetic nerve stimulation which must result from excitation of ganglia or more central structures by angiotensin and the kinins (Lewis & Reit, 1965, 1966) cannot lead to detectable concentrations of catecholamine in the circulating blood. Thus, local uptake of noradrenaline in or around the sympathetic nerve endings may prevent catecholamine from escaping into the circulation in amounts detectable by this method. A mechanism involving uptake of noradrenaline was proposed in the spleen by Brown (1960) and extended to a concept of re-uptake into the catecholamine-storing cell by Paton (1960). In this context, it is interesting that the release of adrenaline by intra-arterial injections of angiotensin and the kinins was increased by ganglion-blocking agents, substances which may prevent the re-uptake of catecholamines (Vane, 1962). It may be that some adrenaline released by the peptides is normally re-absorbed into the medullary cells before ever reaching the blood stream and that the presence of a ganglion blocking agent prevents this re-absorption, thereby increasing the escape into the blood stream.

Calculation from Table 1 of the average molar potencies of the peptides relative to histamine allows our results to be compared with one of the experiments of Lewis & Reit (1966) in a cat. Similar figures are obtained ; angiotensin is 60 times, kallidin 8 times and bradykinin 6 times stronger than histamine. The relative potencies are quite different in the dog ; angiotensin is only 16 times, whereas kallidin is 90 times and bradykinin 70 times more potent than histamine. However, relative potencies of substances in releasing adrenaline after intra-arterial injection may be misleading if used as evidence of their relative importance in physiological or pathological processes. For instance, changes of concentration of endogenous angiotensin in the circulation are likely to be much slower and more prolonged than those following an injection. Thus, in response either to a sudden reduction (Regoli & Vane, 1966) or to a slow reduction (Hodge, Lowe & Vane, 1966) in the blood volume of a dog, the angiotensin generation rate increases

only gradually, sometimes to a level equivalent to an intravenous infusion of 2–3 μg/min. However, even when 5 μg angiotensin/min is infused intra-arterially, there is no detectable adrenaline release in the dog. Even when infusion rates are given which are sufficient to release adrenaline, the output is only transient and passes off during the infusion. This contrasts strikingly with the effects of angiotensin on the adrenal cortex, for infusions of small doses continue to stimulate aldosterone secretion for as long as they are maintained ; this has been demonstrated in man with intravenous infusions for periods of up to 11 days (Laragh, 1967). Thus, whereas the stimulation of aldosterone secretion is likely to be an important physiological function of angiotensin, the stimulation of adrenal medullary secretion is not. It is also unlikely to be important in such pathological states as severe haemorrhage.

The release of adrenaline after bradykinin infusions took a different form from that after angiotensin. Even though the release became intermittent, the gland seemed to maintain sensitivity to injected bradykinin and in some experiments appeared to be more sensitive. The sporadic nature of the release was unexpected; Feldberg (1940) noted a similar sporadic release after injections of snake venom. Our results do not distinguish between a central or direct action of the infusions, but bradykinin injections must have been acting directly on the medulla since their effects resisted ganglion-blocking agents. In some experiments, as previously seen with histamine (Staszewska-Barczak & Vane, 1965) the medulla continued to secrete adrenaline after the bradykinin infusion stopped: this may have been related to local damage to the medulla. It also suggests the possibility that even if circulating bradykinin has little influence on adrenal medullary secretion (because the concentration in arterial blood is too low, Ferreira & Vane, 1967a, b), local generation within the gland itself may stimulate a prolonged secretion of adrenaline.

One further conclusion arises from these results with infusions of peptides. At a time when the adrenal medulla was no longer responding to angiotensin, it still responded to both kallidin and bradykinin, showing that the receptors for angiotensin are different from those for the kinins.

There was an interesting difference in the reactions of the cat and dog to the hypotensive peptide eledoisin. In the cat, eledoisin did not induce catecholamine secretion, even though there was a substantial fall in blood pressure. In the dog, however, there was a strong stimulation of the adrenal medulla, coincident with the fall in blood pressure, which was abolished by ganglion blockade. The possibility that the adrenaline release was induced by the hypotension cannot be eliminated, especially since intravenous injections were more potent than intra-arterial ones. However, stimulation of carotid baroreceptors could not have been responsible for the adrenaline secretion, since carotid occlusion was an ineffective stimulus for the adrenal medulla. Furthermore (*a*) there was often a release of adrenaline with the smaller doses of eledoisin even though the mean blood pressure showed no effect (Fig. 3), and (*b*) other hypotensive effects (Fig. 3) of similar magnitude to that of eledoisin did not induce catecholamine release. It remains to be elucidated whether the adrenaline secretion after eledoisin injection in the dog was induced by stimulation of chemo- or baro-receptors other than those in the carotid sinus, by stimulation of some other afferent nerves, by a central action or by a stimulation of nerve endings susceptible to ganglion block within the medulla.

SUMMARY

1. The blood-bathed organ technique was used to measure the release of catecholamines into the blood stream of cats and dogs after intra-arterial and intravenous injections and infusions of angiotensin, kallidin, bradykinin and eledoisin.

2. The catecholamine released was mainly if not all adrenaline. The release of adrenaline was dependent on the dose of the peptide and no release could be detected after adrenalectomy.

3. Angiotensin and the kinins were less potent intravenously than they were intra-arterially.

4. In the cat, angiotensin was the most potent of these peptides followed by kallidin and bradykinin. Eledoisin was not active.

5. In the dog, kallidin and eledoisin were the most potent peptides, followed by bradykinin and angiotensin.

6. Infusions of peptides gave an initial burst of adrenaline secretion. With bradykinin the initial burst was followed by sporadic secretion and sometimes by continued secretion after the infusion had stopped. With angiotensin the secretion rapidly failed after the initial burst.

7. When the medulla had been de-sensitized to angiotensin by an infusion it was still sensitive to kallidin and bradykinin, showing that the receptor sites were different.

8. Ganglion-blocking drugs increased the release of adrenaline by angiotensin and the kinins in both species but abolished the response of the gland to eledoisin in the dog.

9. The results suggest that the release of medullary catecholamines by angiotensin is not of physiological importance, since high concentrations have to be used and even then the secretion is not maintained.

One of us (J. S-B.) would like to thank the Wellcome Trust for a personal grant and Professor G. V. R. Born for hospitality and kindness in his department. We would like to thank Mr. Rodney Ginn for his excellent technical assistance.

REFERENCES

ARMITAGE, A. K. & VANE, J. R. (1964). A sensitive method for the assay of catechol amines. *Br. J. Pharmac. Chemother.*, **22**, 204–210.

BROWN, G. L. (1960). Release of sympathetic transmitter by nerve stimulation. In *Adrenergic Mechanisms*, ed. by Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M. Pp. 116–124. Churchill, London.

BUTTERWORTH, K. R. & MANN, M. (1957). A quantitative comparison of the sympathomimetic amine content of the left and right adrenal glands of the cat. *J. Physiol.*, **136**, 294–299.

FELDBERG, W. (1940). The action of bee venom, cobra venom and lysolecithin on the adrenal medulla. *J. Physiol.*, **99**, 104–118.

FELDBERG, W. & LEWIS, G. L. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. *J. Physiol.*, **171**, 98–108.

FELDBERG, W. & LEWIS, G. L. (1965). Further studies on the effects of peptides on the suprarenal medulla of cats. *J. Physiol.*, **178**, 239–251.

FERREIRA, S. H. & VANE, J. R. (1967a). The detection and estimation of bradykinin in the circulating blood. *Br. J. Pharmac. Chemother.*, **29**, 367–377.

FERREIRA, S. H. & VANE, J. R. (1967b). The disappearance of bradykinin and eledoisin in the circulation and vascular beds of the cat. *Br. J. Pharmac. Chemother.*, **30**, 417–424.

HODGE, R. L., LOWE, R. D. & VANE, J. R. (1966). The effects of alteration of blood volume on the concentration of circulating angiotensin in anaesthetised dogs. *J. Physiol.*, **185**, 613–626.

LARAGH, J. H. (1967). Symposium on renin, angiotensin, aldosterone and hormonal regulation of arterial pressure and salt balance. Introductory remarks. *Fed. Proc.*, **26**, 39–41.

LEWIS, G. L. & REIT, E. (1965). The action of angiotensin and bradykinin on the superior cervical ganglion of the cat. *J. Physiol.*, **179**, 538–553.

LEWIS, G. L. & REIT, E. (1966). Further studies on the actions of peptides on the superior cervical ganglion and suprarenal medulla. *Br. J. Pharmac. Chemother.*, **26**, 444–460.

MANN, M. & WEST, G. B. (1950). The nature of hepatic and splenic sympathin. *Br. J. Pharmac. Chemother.*, **5**, 173–177.

PATON, W. D. M. (1960). In *Adrenergic Mechanisms*, ed. by Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M. Pp. 124–127. Churchill, London.

REGOLI, D. & VANE, J. R. (1964). A sensitive method for the assay of angiotensin. *Br. J. Pharmac. Chemother.*, **23**, 351–359.

REGOLI, D. & VANE, J. R. (1966). The continuous estimation of angiotensin formed in the circulation of the dog. *J. Physiol.*, **183**, 513–531.

STASZEWSKA-BARCZAK, J. & VANE, J. R. (1964). The release of catechol amines from the adrenal medulla by peptides. *J. Physiol.*, **177**, 57–58P.

STASZEWSKA-BARCZAK, J. & VANE, J. R. (1965). The release of catechol amines from the adrenal medulla by histamine. *Br. J. Pharmac. Chemother.*, **25**, 728–742.

VANE, J. R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

VANE, J. R. (1962). In *Recent Advances in Pharmacology*, ed. Robson, J. M. & Stacey, R. S. Pp. 95–121. Churchill, London.

VANE, J. R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmac. Chemother.*, **23**, 360–373.

VANE, J. R. (1966). The estimation of catecholamines by biological assay. *Pharmac. Rev.*, **18**, 317–324.

WEST, G. B. (1955). The comparative pharmacology of the suprarenal medulla. *Quart. Rev. Biol.*, **30**, 116–137.