J. Physiol. (1965), **178**, *pp.* 239–251
With 8 text-figures
Printed in Great Britain

FURTHER STUDIES ON THE EFFECTS OF PEPTIDES ON THE SUPRARENAL MEDULLA OF CATS

BY W. FELDBERG AND G. P. LEWIS

From the National Institute for Medical Research, Mill Hill, London, N.W.7, and Ciba Laboratories Ltd., Horsham, Sussex

(*Received* 11 *September* 1964)

Recently we have found that bradykinin and angiotensin are potent releasers of the medullary hormones of the suprarenal glands. This was shown in cats in which the released catecholamines were detected by their action on the denervated nictitating membrane (Feldberg & Lewis, 1964). The present experiments are a continuation of this work and demonstrate that the peptides do not act on the cholinergic nerve endings of the adrenal glands when releasing the hormones, but on receptors in the medullary cell different from those activated by acetylcholine. Renin, which forms angiotensin, and several analogues of angiotensin, were also found to release the medullary hormones. Finally, experiments are described which show that under certain conditions angiotensin produces a delayed contraction of the denervated nictitating membrane in adrenalectomized cats.

METHODS

The experiments were performed on 2·3–3·3 kg cats anaesthetized with ethylchloride and ether followed by intravenous chloralose (70 mg/kg), and eviscerated by removal of the stomach, intestines and spleen. Both splanchnic nerves were cut. In order to inject the peptides and other substances into the abdominal aorta above the suprarenal glands a metal cannula was tied into the central stump of the coeliac artery. The method of cannulation and the procedure of injection were the same as described in the previous paper.

Contractions of the denervated right nictitating membrane (and sometimes also of the left innervated one) and changes in arterial blood pressure were recorded on a smoked drum or with an ink writing lever. To denervate the nictitating membrane the right superior cervical ganglion was removed in an aseptic operation under pentobarbitone sodium anaesthesia 12–15 days before the actual experiment. The arterial blood pressure was recorded with a mercury manometer through a cannula inserted into the right femoral artery.

In several cats both kidneys were removed under chloralose anaesthesia by opening the abdomen, and the cats were maintained in deep anaesthesia until the actual experiment was performed. In one cat the two greater splanchnic nerves were cut below the diaphragm by the retroperitoneal approach in an aseptic operation under pentobarbitone sodium anaesthesia; the cat was allowed to recover and was used for the actual experiment 17 days later.

Substances used. Bradykinin was synthesized at Parke Davis and Co, Ann Arbor, and kindly supplied by Dr E. D. Nicolaides. Angiotensin was the synthetic hypertensin-CIBA (val^5-hypertensin II-asp-β-amide). The analogues of angiotensin were synthesized at CIBA

Laboratories, Basle, and kindly supplied by Dr B. Riniker. Renin was lyophilized renin (control no. 6705) prepared by Nutritional Biochemicals Corp., Cleveland, Ohio. Acetylcholine was used as the chloride; all values given refer to the salt. Adrenaline was used as the bitartrate; all values given refer to the base.

RESULTS

Analogues of angiotensin

The ability of angiotensin to release the medullary hormones from the suprarenal glands is shared by its analogues, but to a different degree. Of the thirteen analogues examined, two were more active than angiotensin, one was as active and the others were less active when compared on a weight for weight basis. In the last column of Table 1 are shown the potencies of these analogues relative to angiotensin. Each analogue was assayed on three eviscerated cats by injection into the coeliac artery and recording the contractions of the denervated nictitating membrane. As the contractions were no longer obtained after removal of the suprarenal glands they were due to release of the medullary hormones. The table includes for comparison the values obtained by other workers on the relative potencies of these analogues in raising the arterial blood pressure in nephrectomized rats. There is a correlation in relative potencies in these two tests.

Renin

Fig. 1 illustrates, in an eviscerated cat, the rise in blood pressure and the contraction of the nictitating membrane after an intravenous injection of 0·5 mg renin. The rise in blood pressure begins earlier, within a few seconds of the injection, than the contraction of the membrane which begins after a latency of about 1 min. In the experiment shown in Fig. 1*c*, the main contraction was preceded by a small transient contraction. Since such contractions occur spontaneously, and are not regularly seen following an injection of renin, it is probably not an effect of the renin. The responses of the blood pressure and nictitating membrane last for about 10 min; then both effects decline and disappear at about the same time. Angiotensin has been found to be more potent in contracting the denervated nictitating membrane on injection into the coeliac artery than on intravenous injection (Feldberg & Lewis, 1964); by contrast, the responses to renin are practically the same with either method of injection. This is evident from a comparison of Figs. 1 and 2; in the experiment shown in Fig. 1 the renin was injected intravenously, in that of Fig. 2 arterially, the dose in both experiments being 0·5 mg.

Responses like those seen in Figs. 1 and 2 have always been obtained with a first injection of 0·5 mg renin injected either intravenously or arterially. With a second injection, even when given as long as 1 hr after

the first, the responses of the arterial blood pressure, as well as of the nictitating membrane, are either greatly diminished or absent. Thus the first injection produced a condition of tachyphylaxis, which is also known to occur after renin in rats, rabbits and dogs (Goldblatt, Lamfrom & Haas, 1953; Page, McCubbin, Schwarz & Bumpus, 1957; Bock & Gross, 1961).

TABLE 1

	Relative activity	
	Pressor activity in nephrectomized rats	Release of catecholamines from cat suprarenal glands
α-L-Asp.Arg.Val.Tyr.Val.His.Pro.Phe. (NH_2) (Angiotensin)	1	1
α-L-Asp.Arg.Val.Tyr.Val.His.Pro.Phe. (Natural angiotensin)	1(*a*)	1·0, 1·0, 1·0 } 1
β-L-Asp.Arg.Val.Tyr.Val.His.Pro.Phe.	2(*c*)	1·5, 2·5, 2·0 } 2
α-D-Asp.Arg.Val.Tyr.Val.His.Pro.Phe.	2(*c*)	2·0, 3·0, 1·0 } 2
Suc*.Arg.Val.Tyr.Val.His.Pro.Phe.	0·5(*d*)	0·4, 0·25, 0·6 } 0·4
α-L-Asp.Val.Tyr.Val.His.Pro.Phe. (NH_2)	0·2(*b*)	0·1, 0·15, 0·1 } 0·12
α-L-Asp.Arg.Val.Phe.Val.His.Pro.Phe.	0·1(*b*)	0·03, 0·1, 0·03 } 0·05
α-L-Asp.Arg.Val.Phe.Val.His.Pro.Phe. (NH_2)	0·1(*b*)	0·1, 0·03, 0·03 } 0·05
Val.Tyr.Val.His.Pro.Phe.	< 0·001(*a*)	0·001, 0·002, 0·003 } 0·002
α-L-Asp.Arg.Val.Tyr.Val.His.Pro.Phe.Pro.Phe. (NH_2)	0·002(*b*)	0·001, 0·001, 0·003 } 0·002
α-L-Asp.Arg.Val.D-Tyr.Val.His.Pro.Phe.	0(*d*)	0·001, 0·005, 0·001 } 0·002
α-L-Asp.Arg.Val.D-Tyr.Val.His.Pro.Phe. (NH_2)	0(*d*)	0·001, 0·001, 0·001 } 0·001
α-L-Asp.Arg.Val.Tyr.Tyr.Val.His.Pro.Phe.	0·01(*c*)	0·001, 0·001, 0·001 } < 0·001
α-L-Asp.Arg.Val.Tyr.Tyr.Val.His.Pro.Phe. (NH_2)	0·01(*c*)	0·001, 0·001, 0·001 } < 0·001

* Succinic acid = desamino aspartic acid.

(*a*) Gross & Turrian (1960); (*b*) Schwyzer (1961); (*c*) Regoli, Riniker & Brunner (1963); (*d*) Schwyzer (1963).

On account of this tachyphylaxis it is not possible to assess the contribution made by the medullary hormones to the effects on blood pressure and nictitating membrane by comparing on the same cat the responses to renin before and after removal of the suprarenal glands. It is necessary to use different cats, comparing the effects of the first injection of renin only.

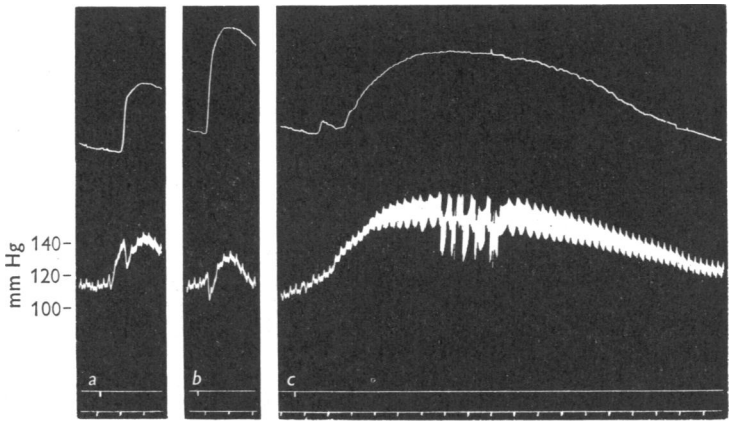

Fig. 1. Contractions of the denervated nictitating membrane (upper record) and arterial blood pressure (lower record) of a 2·5 kg eviscerated cat in chloralose anaesthesia. Both splanchnic nerves cut. At the signals intravenous injection of 0·2 μg angiotensin (at *a*), 0·5 μg adrenaline (at *b*) and 0·5 mg renin (at *c*). Time signals: 30 sec.

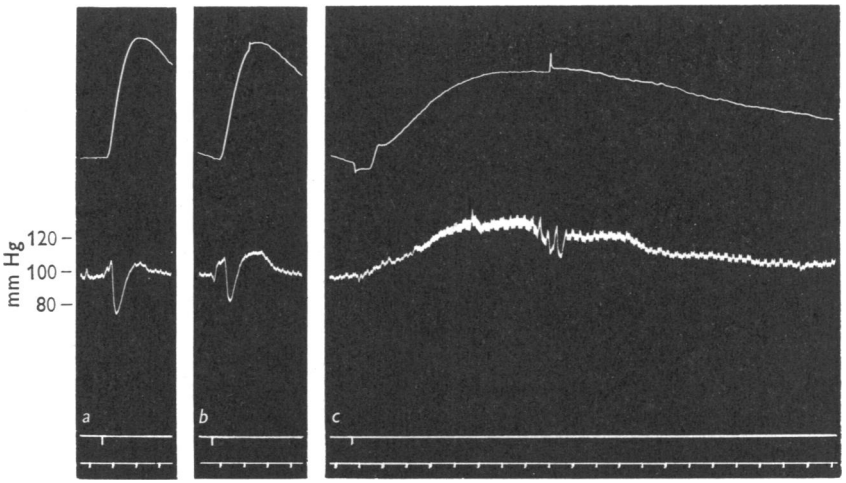

Fig. 2. Contractions of the denervated nictitating membrane (upper record) and arterial blood pressure (lower record) of a 2·5 kg eviscerated cat in chloralose anaesthesia. Both splanchnic nerves cut. At the signals intravenous injection of 0·5 μg adrenaline (at *a*) and injection into the coeliac artery of 50 ng angiotensin (at *b*) and of 0·5 mg renin (at *c*). Time signals: 30 sec.

Therefore in three cats the suprarenal glands were removed before any renin was injected but after the sensitivity of the nictitating membrane to adrenaline and of the suprarenal medulla to angiotensin had been established. In these experiments the first injection of 0·5 mg of renin, given intravenously in two, and arterially in one, no longer contracted the nictitating membrane but still caused a rise in blood pressure. The result obtained in the cat with arterial injection is shown in Fig. 3. The pressor response was of the same magnitude as that obtained in the experiments of Figs. 1 and 2 with intact suprarenal glands, but the nictitating membrane did not contract (at *e*), although its sensitivity to adrenaline (*a* and *d*) was greater than that in the experiments of Figs. 1 and 2. The sensitivity of the glands to angiotensin, tested before their removal (at *b*), was of the

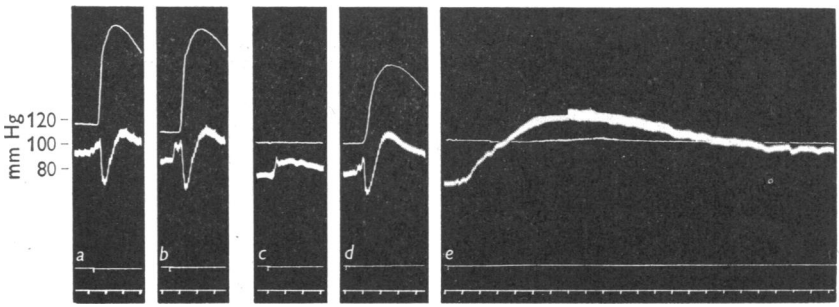

Fig. 3. Contractions of the denervated nictitating membrane (upper record) and arterial blood pressure (lower record) of a 2·35 kg eviscerated cat in chloralose anaesthesia. Both splanchnic nerves cut. At the signals intravenous injections of 0·2 μg adrenaline (at *a* and *d*) and injection into the coeliac artery of 20 ng angiotensin (at *b* and *c*), and 0·5 mg renin (at *e*). Between *b* and *c* the adrenal glands were removed. Time signals: 30 sec.

same order as in the experiment of Fig. 2. In both experiments angiotensin released the equivalent of 10 times its own weight of adrenaline: 20 ng released 0·2 μg in the experiment shown in Fig. 3, and 50 ng released 0·5 μg in the experiment illustrated in Fig. 2.

From the results obtained on the adrenalectomized cats it is thus evident that the response of the nictitating membrane to renin is fully accounted for by the release of the medullary hormones, whereas their contribution to the rise in arterial blood pressure is insignificant.

Nephrectomy. In 1938, Tigerstedt & Bergman found that in rabbits the pressor response to renin is prolonged 12 hr after removal of the kidney. According to experiments on rats by Blacquier, Hoobler, Schroeder, Gomez & Kreulin (1962), such prolongation develops gradually several hours after nephrectomy. The present experiments show that the effect also occurs in cats. Some prolongation of pressor response is seen 8 hr after neph-

rectomy, but the effect is more pronounced after 12 hr and is then apparently maximal because the prolongation is still the same after 17 hr. A prolongation after 12 hr is illustrated in Fig. 4. In this experiment evisceration was carried out shortly before the intravenous injection of 0·5 mg of renin so as to allow comparison with the response shown in Fig. 1. In contrast to the rise in blood pressure the response of the denervated nictitating membrane is not prolonged by nephrectomy. This can be explained by the fact that the tachyphylaxis of the suprarenal medulla to angiotensin, produced by renin, develops whilst the blood pressure is still high (see p. 246, and Fig. 6).

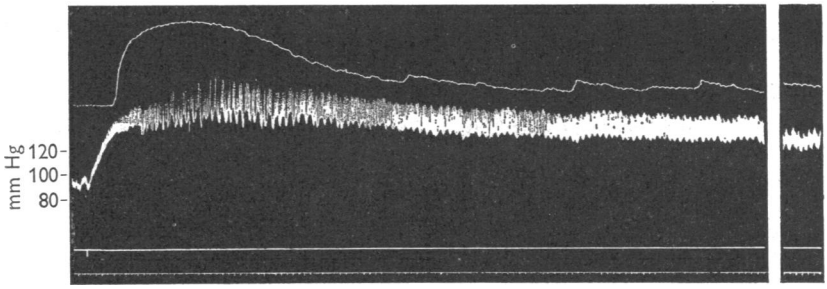

Fig. 4. Contraction of the denervated nictitating membrane (upper record) and rise in arterial blood pressure (lower record) following an intravenous injection (at the signal) of 0·5 mg renin in a 3·3 kg cat. Nephrectomy 12 hr, evisceration and cutting of splanchnic nerves 1 hr, before the renin injection. Time signal: 10 sec; interval of 20 min between the two sections of the record.

Responses of the suprarenal medulla under different conditions

Chronic bilateral splanchnicotomy. Siehe (1934) has shown that the chronically denervated suprarenal medulla retains its sensitivity to acetylcholine, histamine and peptone. The present experiments show that it also responds to angiotensin and bradykinin. In one experiment in which the right and left splanchnic nerves had been divided 17 days before the actual experiment the injection into the coeliac artery of 0·005 μg angiotensin and 0·05 μg bradykinin was sufficient to produce a detectable release of the medullary hormones as shown by contractions of the denervated nictitating membrane and blood pressure changes. In this experiment 0·2 μg adrenaline injected intravenously produced a contraction of the nictitating membrane greater than that produced by 0·005 μg angiotensin or 0·05 μg bradykinin injected arterially and smaller than that produced by arterial injection of 0·01 μg angiotensin or 0·1 μg bradykinin. If the output of medullary hormones is expressed in terms of adrenaline, this would mean that in this experiment angiotensin released nearly 30 times, and bradykinin about 3 times its own weight of adrenaline.

Hexamethonium. When the suprarenal medulla is rendered insensitive to acetylcholine by hexamethonium, the responses to angiotensin and bradykinin are unaffected. A typical experiment is illustrated in Fig. 5. Before the administration of hexamethonium the contractions of the denervated nictitating membrane to injections of 0·1 μg angiotensin and 10 μg acetylcholine into the coeliac artery (at *a* and *c*) were practically indistinguishable and the contraction to 1 μg bradykinin (at *b*) was somewhat less. After the intravenous injection of 50 mg hexamethonium the responses to angiotensin and bradykinin (at *d* and *e*) remained the same, whereas acetylcholine (at *f*) no longer caused a detectable release of the medullary hormones.

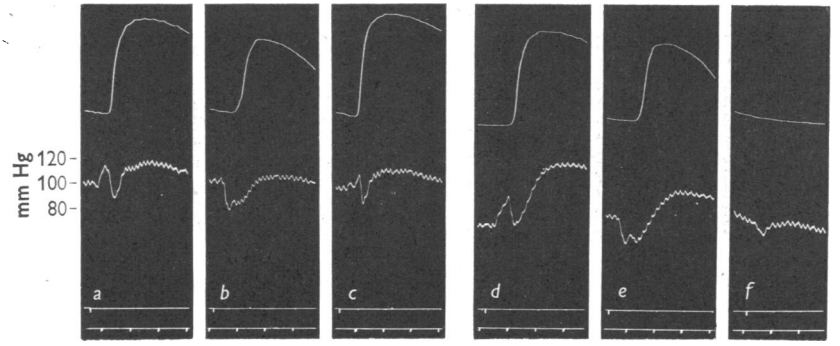

Fig. 5. Contractions of the denervated nictitating membrane (upper record) and arterial blood pressure (lower record) of a 2·8 kg eviscerated cat in chloralose anaesthesia. Both splanchnic nerves cut. At the signals injections into the coeliac artery of 100 ng angiotensin (at *a* and *d*), 1 μg bradykinin (at *b* and *e*), and 10 μg acetylcholine (at *c* and *f*). Between *c* and *d* intravenous injection of 50 mg hexamethonium. Time signals: 30 sec.

Renin. During the tachyphylaxis produced by renin, the nictitating membrane retains its sensitivity to adrenaline, but the medullary cells of the suprarenal glands no longer respond to previously effective doses of angiotensin. In nephrectomized cats this tachyphylaxis of the glands was found to develop whilst the blood pressure was still high. This is illustrated in Fig. 6, which is from the same experiment as that shown in Fig. 4, and shows the effect of adrenaline and angiotensin before (at *a*, *b* and *c*), and about 30 min after the renin injection (at *d*, *e* and *f*) whilst the blood pressure is still elevated. The tachyphylaxis has rendered the medullary cells insensitive to 0·4 μg angiotensin (at *d*) and the response to 1 μg (at *f*) has become similar to that of 0·2 μg (at *b*) given before the renin injection. On the other hand, the sensitivity of the nictitating membrane to 0·5 μg adrenaline is little affected.

Tachyphylaxis following the injection of renin produces an alteration of the sensitivity of the suprarenal medulla which is opposite to that brought about by hexamethonium. The sensitivity of the medullary cell to angiotensin as well as to bradykinin is greatly reduced, but the sensitivity to acetylcholine is increased or remains unchanged. This is illustrated in Fig. 7 which shows responses of the denervated nictitating membrane and of the arterial blood pressure to intravenous adrenaline and arterial

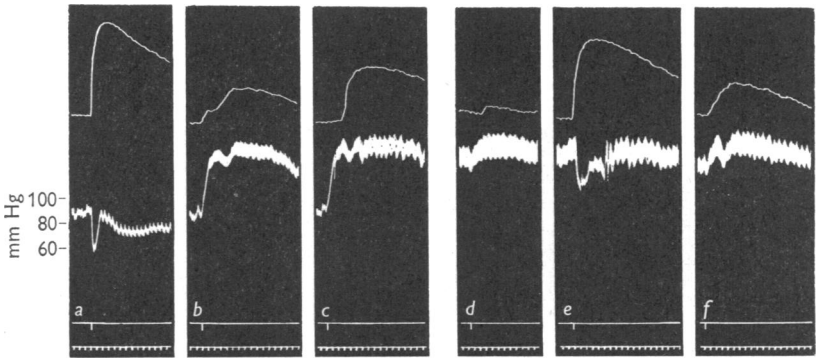

Fig. 6. The same cat as in Fig. 4. Responses of the denervated nictitating membrane (upper record) and arterial blood pressure (lower record) to intravenous injection of 0·5 μg adrenaline (at *a* and *e*), injection into the coeliac artery of angiotensin 200 ng (at *b*), 400 ng (at *c* and *d*) and 1000 ng (at *f*). Responses *a*, *b* and *c* were obtained shortly before, and responses *d*, *e* and *f*, 30 min after the injection of renin. Time signal: 10 sec.

angiotensin, acetylcholine and bradykinin, before and after an intravenous injection of 0·5 mg renin. There is a slight increase in the response to 0·5 μg adrenaline (*f* compared with *a*) but a much greater increase in the response to 5 μg acetylcholine (*i* and *e*) after the renin injection. On the other hand, the strong contractions produced by 0·1 μg angiotensin and 2 μg bradykinin (at *b* and *c*) were abolished (at *g* and *h*) after the renin injection. Nevertheless, the 2 μg bradykinin still produced some release of catecholamines as evidenced by the pressor response which was similar to that obtained with 0·4 μg (at *d*) before the renin injection.

Delayed contractions of the nictitating membrane to angiotensin after adrenalectomy

Angiotensin is not completely without effect on the denervated nictitating membrane when the suprarenal glands have been removed. It then produces a delayed contraction, but usually only when given in a relatively high dosage, i.e. after intravenous injection of 10–20 μg. The contraction

begins after a latency which is as long as 2–5 min, during which time there may be some relaxation of the membrane. When the contraction begins, the pressor response is already on the decline. A typical result is shown in Fig. 8.

In cats in which the intravenous injection of 20 μg angiotensin produced such a delayed contraction, the same dose injected into the ipsilateral carotid through the lingual artery was without effect on the nictitating membrane. Thus the delayed contraction is not a direct action of angiotensin on the nictitating membrane. It appears to be a response to

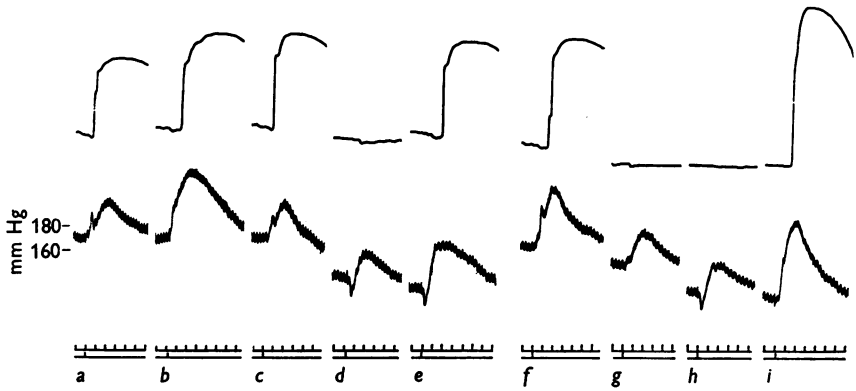

Fig. 7. Contractions of the denervated nictitating membrane (upper record) and blood-pressure changes (lower record) of a 2·7 kg eviscerated cat in chloralose anaesthesia. Both splanchnic nerves cut. At the signals intravenous injection of 0·5 μg adrenaline (at *a* and *f*) and injections into the coeliac artery of 100 ng angiotensin (at *b* and *g*) 5 μg acetylcholine (at *e* and *i*) and of 0·4 μg (at *d*) and 2 μg (at *e* and *h*) bradykinin. Between *e* and *f* intravenous injection of 0·5 mg renin. Time signals: 30 sec.

catecholamines released from sites other than the adrenal medulla, a conclusion favoured by the following two observations. In two adrenalectomized cats in which the denervated nictitating membrane was exceptionally sensitive to adrenaline, responding with a strong contraction to 0·01 μg, a delayed contraction was obtained with as little as 1 μg intravenous angiotensin. In two other cats in which the innervated nictitating membrane was nearly as sensitive to adrenaline as the denervated one the delayed contraction following an intravenous injection of 20 μg angiotensin caused contractions of both membranes.

If the delayed contraction of the nictitating membrane to angiotensin in adrenalectomized cats results from release of catecholamines then the sites of release appear not to be, or not solely to be, within the lower part of the body because the delayed contractions to intravenous angiotensin

were always greater than those following injections of the same dose into the central stump of the coeliac artery.

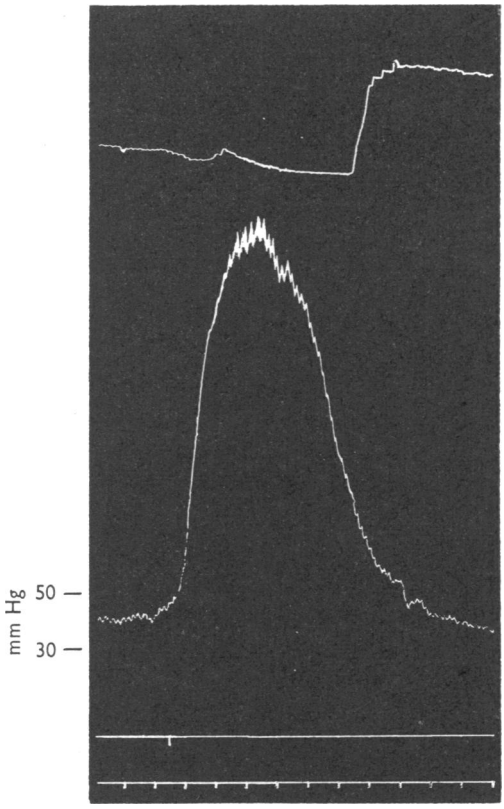

Fig. 8. Contraction of the denervated nictitating membrane (upper record) and rise of arterial blood pressure (lower record) following an intravenous injection of 20 μg angiotensin in a 3·2 kg cat, adrenalectomized, eviscerated and with cut splanchnic nerves. Time signals: 30 sec.

DISCUSSION

Previously we found (Feldberg & Lewis, 1964) that bradykinin and two of its analogues cause a release of medullary hormones from the suprarenal glands with a relative potency similar to that found on smooth-muscle preparations. In the present experiments several analogues of angiotensin were found to cause release of the medullary hormones; when compared with their pressor effect in rats again the same relative potency was encountered. It is known that peptone, too, causes a release of adrenaline from the suprarenal glands (Siehe, 1934), whereas the peptides oxytocin,

vasopressin and substance P lack this property (Feldberg & Lewis, 1964). The question therefore arises whether the action of peptone on the suprarenal medulla depends on a sequence of amino acids resembling that present in either the bradykinin or angiotensin molecules.

The property of angiotensin and bradykinin to release the medullary hormones from the suprarenal glands cannot be explained by an action of the peptides on cholinergic nerve endings in the suprarenal medulla, because chronic denervation of the greater splanchnic nerves did not diminish their effect on the release of catecholamines. True, this procedure may not have resulted in complete denervation of the glands, but it would have eliminated the greater part of the nerve supply. Further evidence that the peptides do not act through a release of acetylcholine from cholinergic nerve endings, but act directly on the medullary cells, is provided by the failure of hexamethonium to reduce the sensitivity of the medullary cells to the peptides whilst abolishing the effects of acetylcholine. This finding necessitates the assumption that different receptors exist for acetylcholine and for the two peptides. It is also possible to block the peptide receptors without diminishing the sensitivity of the medullary cells to acetylcholine; this condition is produced by renin. The blocking actions of hexamethonium and renin thus differentiate between the two receptors. In this connexion it is interesting to recall the observation that large doses of nicotine reduce the sensitivity of the medullary cells to acetylcholine to a much greater extent than to histamine (Szczygielski, 1932); or 'paralysing' doses of choline render the medullary cells insensitive to choline itself but reduce their sensitivity to acetylcholine or nicotine only slightly (Gutmann, 1932).

The release of the medullary hormones by renin is unlikely to be due to an action of renin itself on the suprarenal glands, but rather to the angiotensin formed by the renin. If renin would itself have such an action, it should be more effective in releasing these hormones on injection into the coeliac artery than when given intravenously; but such a difference was not observed.

Previously we discussed the possibility that the vasoconstrictor effect of angiotensin which persists after removal of the suprarenal glands may result from a local release of noradrenaline from the adrenergic nerve endings in blood vessels. The fact that angiotensin does not act on the nerve endings in the suprarenal medulla does not preclude this possibility since these are cholinergic. The finding, after removal of the suprarenal glands, of a delayed contraction of the denervated nictitating membrane when relatively large doses of angiotensin were injected intravenously, may in fact provide the first indication for a local release of noradrenaline in the walls of blood vessels. The delayed contraction is not a direct action

of angiotensin, since it did not occur on injection into the carotid artery through the lingual artery, and the fact that the size of the contraction depended on the sensitivity of the nictitating membrane to adrenaline suggested that it resulted from circulating catecholamines. Whether the amines originate from extra-medullary chromaffin tissue or adrenergic nerve endings is, however, an open question.

SUMMARY

1. The experiments are a continuation of an earlier investigation (Feldberg & Lewis, 1964) in which it was shown that angiotensin and bradykinin are potent releasers of the medullary hormones of the suprarenal glands in cats.

2. In cats anaesthetized with chloralose the effects on the suprarenal medulla of angiotensin, several of its analogues, renin and bradykinin, were examined. The released medullary hormones were detected by their contraction of the denervated nictitating membrane.

3. The property of angiotensin to release the medullary hormones is shared by many of its analogues. Their relative potency on the medullary cells is about the same as that in raising the blood pressure in nephrectomized rats.

4. Renin, the enzyme which forms angiotensin in the blood, also causes a release of the medullary hormones. The effect is not an action of renin itself, but an action of the formed angiotensin.

5. Nephrectomy prolongs the pressor effect of renin. This prolongation does not extend to the effect on the suprarenal medulla, because tachyphylaxis of the suprarenal glands develops whilst the blood pressure is still raised.

6. Chronic bilateral splanchnicotomy does not lessen the sensitivity of the suprarenal medulla to angiotensin or to bradykinin. Their action is therefore not on the cholinergic nerve endings in the gland, but on the medullary cells.

7. Different receptors appear to exist for angiotensin and bradykinin on the one hand, and for acetylcholine on the other. This conclusion is based on the changes in sensitivity of the medullary cells produced by hexamethonium and by renin. Hexamethonium renders the cells insensitive to acetylcholine but does not reduce their sensitivity to the two peptides. In contrast, renin greatly reduces the sensitivity of the cells to angiotensin and bradykinin, but enhances that to acetylcholine.

8. After removal of the suprarenal glands relatively large doses of angiotensin given intravenously produce a delayed contraction of the denervated nictitating membrane. This contraction is not due to a direct action of angiotensin on the membrane since it does not occur on injection

into the carotid artery. The contraction appears to result from an action of catecholamines released either from adrenergic nerve endings or from extra-medullary chromaffine tissue.

REFERENCES

BLACQUIER, P., HOOBLER, S., SCHROEDER, J., GOMEZ, A. & KREULIN, T. (1962). Effect of bilateral nephrectomy on pressor response to renin. *Amer. J. Physiol.* **203**, 339–343.

BOCK, K. D. & GROSS, F. (1961). Renin and angiotensin tachyphylaxis. *Circulation Res.* **9**, 1044–1050.

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

GOLDBLATT, H., LAMFROM, H. & HAAS, E. (1953). Physiological properties of renin and hypertensin. *Amer. J. Physiol.* **175**, 75–83.

GROSS, F. & TURRIAN, H. (1960). Pharmacology of hypertensin and synthetic analogues. In *Polypeptides which Affect Smooth Muscle*, pp. 137–151. London: Pergamon Press.

GUTMANN, P. (1932). Die Empfindlichkeit der Nebennieren von Katzen auf Cholin und Acetylcholin. *Arch. exp. Path. Pharmak.* **166**, 612–623.

PAGE, I. M., MCCUBBIN, J. W., SCHWARZ, H. & BUMPUS, F. M. (1957). Pharmacologic aspects of synthetic angiotensin. *Circulation Res.* **5**, 552.

REGOLI, D., RINIKER, B. & BRUNNER, H. (1963). The enzymic degradation of various angiotensin II derivatives by serum, plasma or kidney homogenate. *Biochem. Pharmacol.* **12**, 637–645.

SCHWYZER, R. (1961). Synthetische Analoge des Hypertensins. I Einleitung. *Helv. chim. Acta*, **44**, 667–674.

SCHWYZER, R. (1963). Chemical structure and biological activity in the field of polypeptide hormones. *Pure and Applied Chemistry.* **6**, 265–295.

SIEHE, H. J. (1934). Die Reaktion des denervierten Nebennierenmarkes auf humorale Sekretionsreize. *Pflüg. Arch. ges. Physiol.* **234**, 204–209.

SZCZYGIELSKI, J. (1932). Die adrenalinabsondernde Wirkung des Histamins und ihre Beeinflussung durch Nikotin. *Arch. exp. Path. Pharmak.* **166**, 319–332.

TIGERSTEDT, R. & BERGMAN, P. G. (1938). Niere und Kreislauf. *Skand. Arch. Physiol.* **8**, 223.