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THE ACTION OF ANGIOTENSIN AND BRADYKININ ON THE SUPERIOR CERVICAL GANGLION OF THE CAT

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It was recently shown that angiotensin and bradykinin are potent releasers of catecholamines from the suprarenal medulla (Feldberg & Lewis, 1964). The present experiments were designed to determine whether these peptides also act on the sympathetic ganglia. Direct evidence is provided showing that both angiotensin and bradykinin stimulate the cells of the superior cervical ganglion of the cat.

METHODS

The experiments were performed on cats of either sex weighing 2·5–4·0 kg. After inducing anaesthesia with ethyl chloride and ether, spinal preparations were made as described by Burn (1952).

With the cat lying on its back, contractions of both nictitating membranes were recorded. The cat's head was slightly elevated and rigidly held in position by tying its jaws tightly around a transverse rod clamped to uprights at the sides of the operating table. A fine silk thread sewn through the middle of the border of each nictitating membrane cartilage was passed around a small pulley and tied to a frontal writing isotonic lever for recording movements of the membrane on a smoked drum. The resting load on each nictitating membrane was 5 g, and the contractions were magnified 14×. In most experiments arterial blood pressure was recorded with a mercury manometer through a glass cannula in the right femoral artery.

Close intra-arterial injections either to the superior cervical ganglion or to the nictitating membrane were made through the central end of the lingual artery in the manner described by Trendelenburg (1959). In most experiments the right and left lingual arteries were cannulated so that injections could be made to both sides. An 8 in. (20 cm) length of fine polyethylene tubing (0·61 mm external diameter) was tied into the lingual artery with the tip resting near the external carotid artery. The dead space in the tubing was about 0·04 ml. To achieve a net injection volume of 0·1 ml., the total volume for each intra-arterial injection was 0·14 ml. To reach the ganglion from the lingual artery the injected drugs have to pass back in the external carotid to the arteries supplying the ganglion, that is, they have to be injected retrogradely. For such retrograde injections the external carotid was first clamped just distal to the origin of the lingual artery and the injection was then made rapidly within 2 sec. The clamp was removed 60 sec later. Injections to the membrane were

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made more slowly within 6–7 sec, with the external carotid artery left unclamped so that the uninterrupted flow of blood effectively prevented any material injected in this manner from passing backward to the ganglion. After each intra-arterial injection, the fluid in the dead space was aspirated and the tubing was flushed twice with 0·2 ml. 0·9 % NaCl solution.

For intravenous injections, a polyethylene cannula was tied into the right femoral vein. In all experiments heparin (0·5 mg/kg) was administered intravenously and both vago-sympathetic trunks were cut low in the neck.

In two cats the right superior cervical ganglion was denervated in an aseptic operation under pentobarbitone sodium anaesthesia, 9 and 13 days before the actual experiments, by removing a 1 cm segment of the right vago-sympathetic trunk low in the neck.

For electrical stimulation of the cervical sympathetic nerve it was separated from the vagus nerve, placed on bipolar platinum electrodes and covered with warm paraffin oil to prevent drying. Square wave shocks of various parameters were applied to the nerve with a 'Physiological Electronic Stimulator' (Cinetronics Ltd).

Substances used: Bradykinin, synthesized at Parke, Davis & Co., Ann Arbor, was kindly supplied by Dr E. D. Nicolaides; angiotensin was the synthetic hypertensin—CIBA (val^5-hypertensin II-asp-β-amide); acetylcholine chloride, hexamethonium bromide, atropine sulphate, histamine dihydrochloride, mepyramine maleate, l-adrenaline-D-bitartrate. All doses given in the text refer to the base.

RESULTS

The rapid retrograde arterial injections of angiotensin or bradykinin toward the superior cervical ganglion produced contractions of the ipsilateral nictitating membrane. The contractions occurred after bilateral adrenalectomy and resulted from stimulation of the ganglion, because they did not occur when the injections were made after removal of the ganglion or after its post-ganglionic trunk was cut. Neither peptide had a stimulating effect on the smooth muscle of the nictitating membrane since the injections towards the membrane did not produce contractions. The ganglion-stimulating effects of angiotensin and of bradykinin are illustrated in Fig. 1 and 2.

In Fig. 1 at *a*, *b* and *c*, are seen the graded contractions of the right nictitating membrane in response to rapid retrograde injections to the right ganglion of 0·1, 0·3 and 1 μg angiotensin. An injection of 0·3 μg directly towards the membrane (at *d*) did not elicit a contraction. After extirpation of the right superior cervical ganglion (between *d* and *e*) the rapid retrograde injection of 1 μg angiotensin (at *e*) no longer contracted the nictitating membrane. None of the injections caused the contralateral nictitating membrane to contract. Similarly, when rapid retrograde injections of 0·3 and 1 μg angiotensin were made to the left superior cervical ganglion (at *f* and *g*) only the left nictitating membrane contracted. The contractions of the nictitating membrane began within 10–20 sec of the injections. In Fig. 2 at *a*, *b* and *d*, are seen the contractions produced by rapid retrograde injections of 5, 0·5 and 1 μg bradykinin to the ganglion. Only the ipsilateral membrane contracted. When injected directly towards

the membrane (at *c*) bradykinin even in a dose of 10 μg did not produce a contraction.

Figure 3 illustrates the effect of cutting the post-ganglionic trunk on responses to bradykinin. The strong contraction of the nictitating membrane produced on rapid retrograde injection of 50 μg bradykinin (at *a*) no longer occurred after the trunk was cut (at *b*).

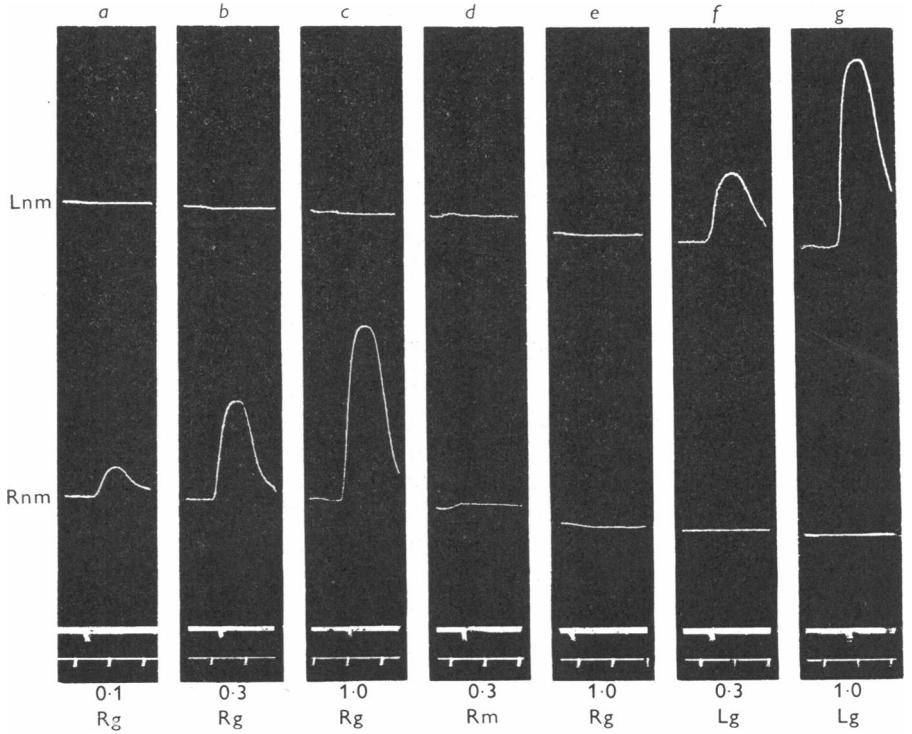

Fig. 1. Records of the left (top; Lnm) and right (bottom; Rnm) nictitating membranes of a spinal cat 2·9 kg. At the signals rapid retrograde injections of angiotensin 0·1 μg (at *a*), 0·3 μg (at *b*) and 1·0 μg (at *c*) to the right (Rg), and 0·3 μg (at *f*) and 1·0 μg (at *g*) to the left (Lg) superior cervical ganglion. At *d*, slow injection of 0·3 μg angiotensin towards the right nictitating membrane (Rm). At *e*, rapid retrograde injection of 1 μg angiotensin to the right side after removal of the right ganglion between *d* and *e*. Time marker 30 sec.

The ganglion was more sensitive to angiotensin than to bradykinin and the sensitivity to both peptides varied greatly in different experiments. With angiotensin strong stimulation of the ganglion was usually obtained with 1 μg, but occasionally a ganglion was insensitive to 10 μg. In a number of experiments the ganglion responded to 0·1 μg. With bradykinin on the other hand, 10 μg were usually required to stimulate the ganglion,

but in a few experiments 5μg were sufficient, and in one experiment the ganglion responded to as little as 0·5μg (Fig. 2). In addition to this difference in sensitivity, there was a difference in latency. The time between injection and onset of contraction was shorter after bradykinin (5–10 sec) than after angiotensin (10–20 sec).

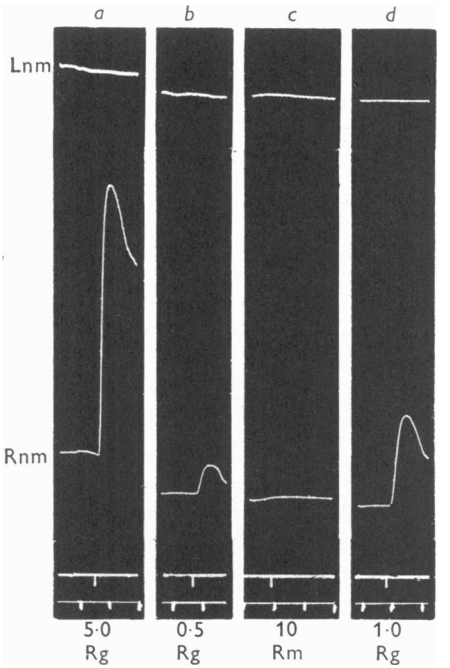

Fig. 2. Records of the left (top; Lnm) and right (bottom; Rnm) nictitating membranes of spinal cat 4·0 kg. At the signals, responses to rapid retrograde injections of bradykinin 5μg (at *a*), 0·5μg (at *b*) and 1μg (at *d*) to the right superior cervical ganglion (Rg). At *c*, injection of 10μg bradykinin directly towards the right nictitating membrane (Rm). Time interval 30 sec.

In a number of cats, particularly late in the course of the experiment, opening the clamp within 60 sec after a rapid retrograde injection of 10–50μg of bradykinin regularly produced a slow renewed contraction of the ipsilateral membrane. Such a secondary contraction is illustrated in Fig. 4. In two experiments, opening the clamp produced a contraction also of the contralateral membrane which had not initially contracted. When removal of the clamp was delayed for >60 sec, the secondary response was smaller or did not occur. The cause of the secondary response has not been investigated. It was not seen with angiotensin.

Chronic denervation of the ganglion

Degeneration of the preganglionic sympathetic fibres did not abolish the ganglion stimulating action of angiotensin or bradykinin. In fact, the chronically denervated ganglion became more sensitive to these peptides as it does to other substances. This is illustrated in the experiment of Fig. 5. Both nictitating membranes were equally sensitive to intravenous adrenaline (at *a*), but the chronically denervated ganglion responded to

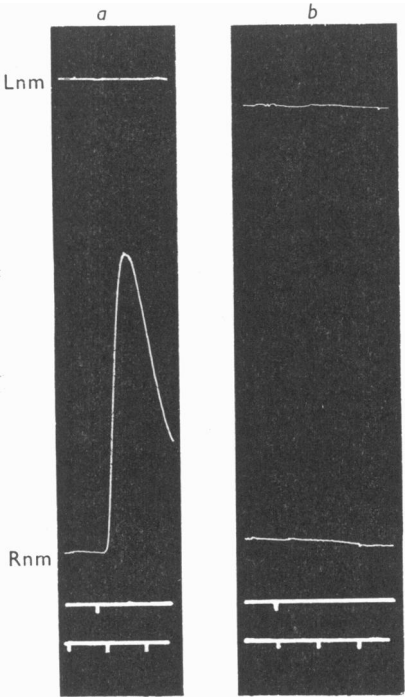

Fig. 3. Records of the left (top; Lnm) and right (bottom; Rnm) nictitating membrane of spinal cat 3·0 kg. At the signals, rapid retrograde injections of 50 μg bradykinin to the right superior cervical ganglion. Between *a* and *b* the right post-ganglionic sympathetic trunk was cut. Time marker 30 sec.

a rapid retrograde injection of 0·1 μg angiotensin (at *b*), whereas the ganglion of the other side did not respond even to 1 μg (at *c*). With bradykinin the difference in sensitivity was not so pronounced; nevertheless, the contraction to a rapid retrograde injection of 30 μg was stronger on the side where the ganglion had been chronically denervated (at *d* and *e*). There was also an increased sensitivity of the chronically denervated ganglion to retrograde injections of acetylcholine, which was of the same order as that seen with angiotensin.

Tachyphylaxis

A characteristic feature of the actions of angiotensin and bradykinin at the superior cervical ganglion was the occurrence of tachyphylaxis. This tachyphylaxis was specific in that angiotensin rendered the ganglion insensitive only to itself but not to bradykinin, and vice versa. With angiotensin the ganglion usually regained its full sensitivity after 30 min, with bradykinin after 20 min. These findings are illustrated in Figs. 6–8.

Figure 6 shows the effect of four consecutive rapid retrograde injections of 1 μg angiotensin given at different intervals. There was good recovery in sensitivity of the ganglion 30 min after the first injection, but when the interval was reduced to 10 min, as between the second and third injection, the ganglion no longer responded to the angiotensin (at *c*). Some recovery was again attained during the following 30 min as shown at *d*.

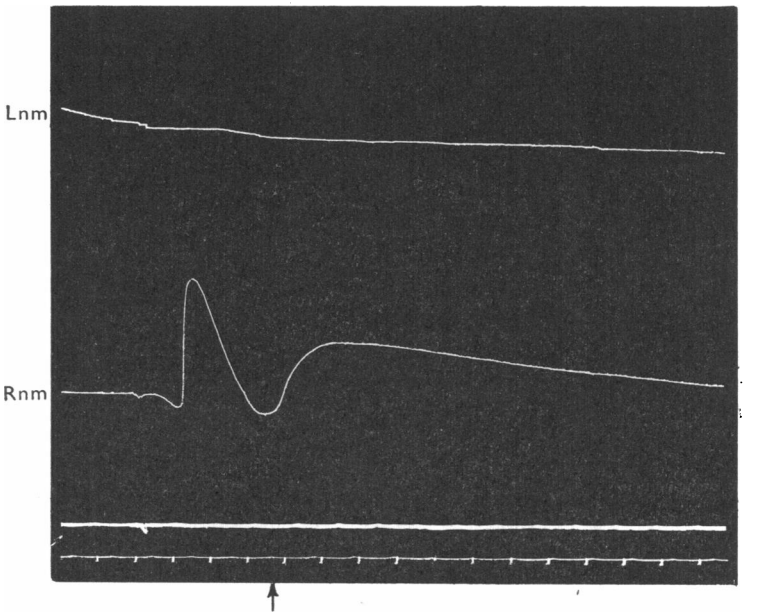

Fig. 4. Records of the left (top; Lnm) and right (bottom; Rnm) nictitating membranes of spinal cat 3·2 kg. At the signal, response to a rapid retrograde injection of bradykinin 10 μg to the right superior cervical ganglion. At arrow (↑) right external carotid artery unclamped. Time marker 30 sec.

Figure 7 illustrates a corresponding experiment with five consecutive rapid retrograde injections of 50 μg bradykinin. A comparison of the effect produced by the first with that of the second injection given 10 min later, shows that the ganglion was still nearly insensitive to this dose of

bradykinin. However, when the injections were given 15 min apart good recovery was obtained (at *c* and *d*), and when the interval was 20 min there was full recovery (at *e*).

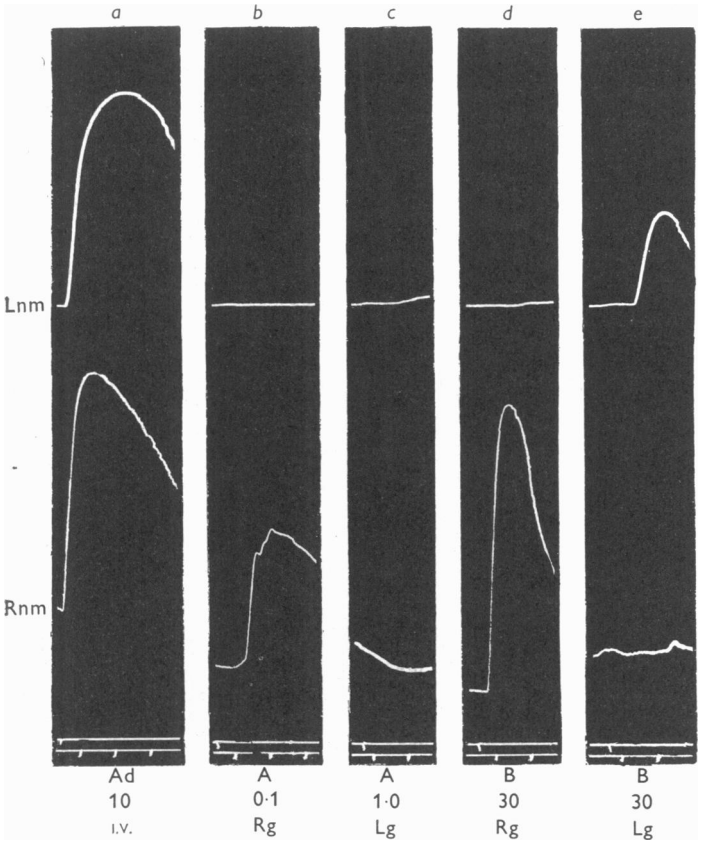

Fig. 5. Records of the normal left (top; Lnm) and the decentralized right (bottom; Rnm) nictitating membranes of a spinal cat 2·6 kg; after preganglionic denervation of the right superior cervical ganglion 13 days previously. At the signals, responses to intravenous adrenaline (Ad) and to rapid retrograde injections to the respective superior cervical ganglion (Rg, Lg) of angiotensin (A) and bradykinin (B): 10 μg adrenaline at *a*, 0·1 μg angiotensin and 30 μg bradykinin to the right ganglion at *b* and *d*, 1·0 μg angiotensin and 10 μg bradykinin at *c* and *e* to the left ganglion. Time marker 30 sec.

The experiment of Fig. 8 illustrates the absence of cross-tachyphylaxis between angiotensin and bradykinin. The first two injections (at *a* and *b*) are of 1 μg angiotensin given 30 min apart, a sufficient interval for the ganglion to have regained its sensitivity to this peptide. Forty minutes later, 50 μg bradykinin was given (at *c*). This injection did not render the

ganglion insensitive to the subsequent injection of 1 μg angiotensin given 10 min later (at *d*) and this injection in turn did not render the ganglion insensitive to 50 μg bradykinin given again (at *e*) after an interval of 10 min.

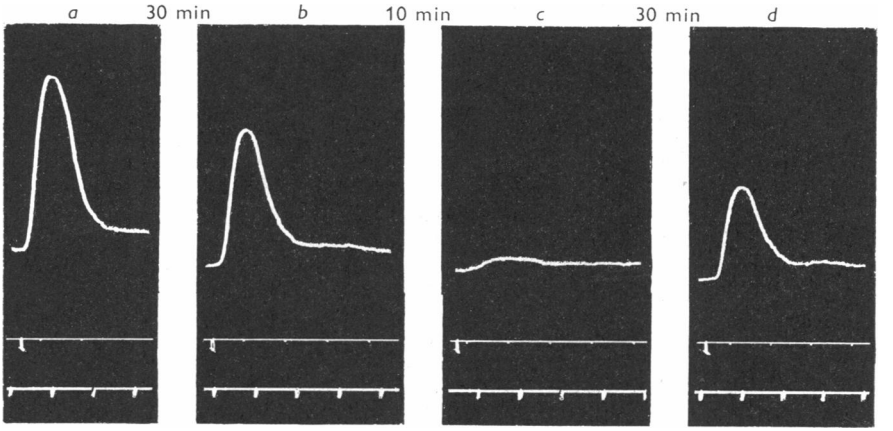

Fig. 6. Record of the right nictitating membrane of a spinal cat 2·8 kg. At the signals, responses to rapid retrograde injections of angiotensin 1 μg to the right superior cervical ganglion. Interval between injections, *a–b*, 30 min; *b–c*, 10 min; and *c–d*, 30 min. Time marker 30 sec.

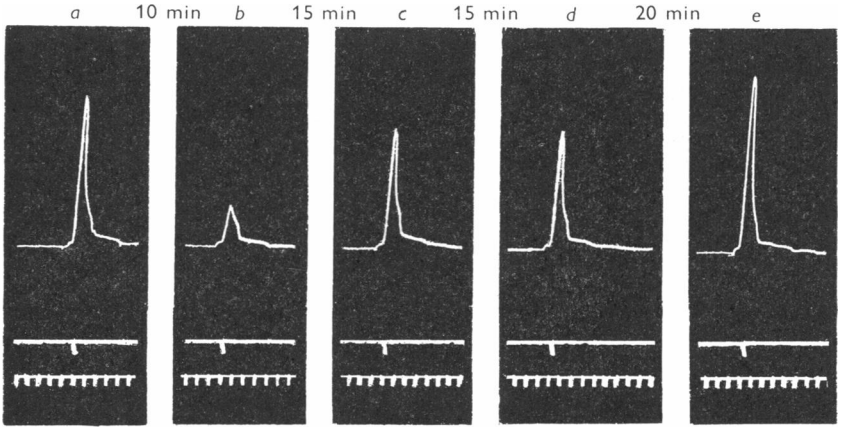

Fig. 7. Record of the right nictitating membrane of a spinal cat 3·8 kg. At the signals, responses to rapid retrograde injections of 50 μg bradykinin to the right superior cervical ganglion. Intervals between the injections: *a–b*, 10 min; *b–c*, 15 min; *c–d*, 15 min; and *d–e*, 20 min. Time marker 30 sec.

Comparison with acetylcholine and histamine

The ganglion-stimulating action of angiotensin and bradykinin was compared with that of acetylcholine and of histamine, selected as repre-

sentatives of the nicotine-like and the non-nicotinic stimulants of autonomic ganglia. On a weight-for-weight basis acetylcholine was found to be approximately as active as bradykinin, and histamine was usually somewhat more active. Rapid retrograde injections of 20–30 μg of acetylcholine could be made every 5 min without producing diminution of the response of the ganglion cells, whereas with histamine the interval had to

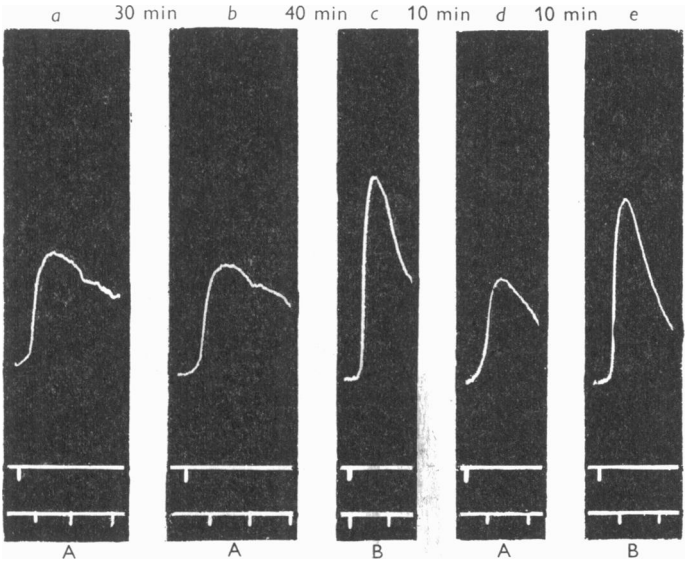

Fig. 8. Record of the right nictitating membrane of a spinal cat 3·0 kg. At the signals, responses to rapid retrograde injections of angiotensin (A) 1 μg (at *a*, *b* and *d*) and of bradykinin (B) 50 μg (at *c* and *e*) to the right superior cervical ganglion. Intervals: *a*–*b*, 30 min; *b*–*c*, 40 min; *c*–*d* and *d*–*e*, 10 min. Time marker 30 sec.

be about 20 min to avoid loss of sensitivity since tachyphylaxis occurs, as found by Robertson (1954) and later by Trendelenburg (1954). Thus, in this respect, histamine resembled the peptides. No cross-desensitization occurred, with one exception. Histamine rendered the ganglion insensitive to angiotensin, but angiotensin did not desensitize the ganglion to histamine. This is illustrated in Fig. 9. At *a* and *b* are shown the control contractions produced by rapid retrograde injections of 1 μg angiotensin and of 20 μg histamine given 20 min apart. The histamine rendered the ganglion insensitive to histamine, as seen at *c* when the same dose was injected again after an interval of 10 min. After these two injections of histamine the sensitivity of the ganglion to 1 μg angiotensin (at *d*) given 10 min after the second histamine injection was also greatly reduced. But within 30 min the ganglion had regained its sensitivity to angiotensin and the injection of 1 μg (at *e*) produced again the same strong response as at the

beginning of the experiment. This injection of angiotensin, however, did not reduce the sensitivity of the ganglion to a subsequent injection of histamine given 10 min later (at *f*).

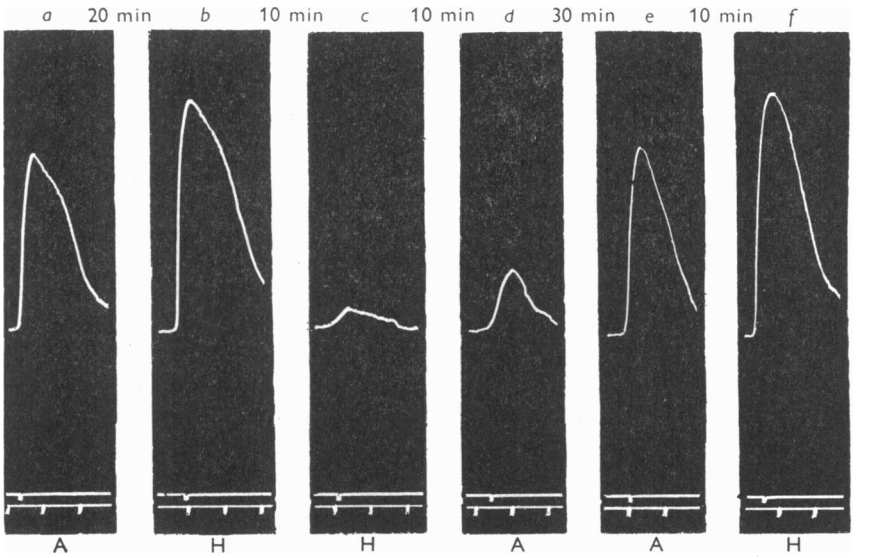

Fig. 9. Record of the right nictitating membrane of a spinal cat 3·0 kg. At the signals, responses to rapid retrograde injections of angiotensin (A) 1 μg (at *a*, *d* and *e*) and of histamine (H) 20 μg (at *b*, *c* and *f*) to the right superior cervical ganglion. Intervals: *a*–*b*, 20 min; *b*–*c*, 10 min; *c*–*d*, 10 min; *d*–*e*, 30 min; and *e*–*f*, 10 min. Time marker 30 sec.

The fact that histamine reduced the response to angiotensin but not to bradykinin suggested an interaction of histamine and angiotensin at receptors in the ganglion. This view is supported by the finding that the anti-histamine drug, mepyramine, not only inhibited the ganglion stimulating action of histamine, but also reduced the effectiveness of angiotensin, but not of bradykinin. A typical experiment is shown in Fig. 10 in which rapid retrograde injections of angiotensin 0·1 μg, bradykinin 30 μg, and histamine 10 μg, were made before and after intravenous administration of mepyramine 0·5 mg/kg. After mepyramine, the response to histamine (compare *c* and *d*) was abolished, that to angiotensin (compare *a* and *f*) was considerably reduced, but that to bradykinin was, if anything, somewhat enhanced (compare *b* and *c*). The reduction of the angiotensin response cannot be attributed to the previous injection of histamine, since it was made 50 min earlier.

Ganglionic transmission

Angiotensin and bradykinin did not interfere with responses of the nictitating membrane to preganglionic electrical stimulation of the cervical sympathetic nerve. When the nerve was stimulated with submaximal shocks (0·5 msec square wave pulses at frequencies of 2 and 16 c/s for 5 sec) every 1–1·5 min, and the peptides were injected between stimulations, the responses of the subsequent two or three periods of stimulation were enhanced; hence there was summation of the responses to nerve stimulation and to the peptides.

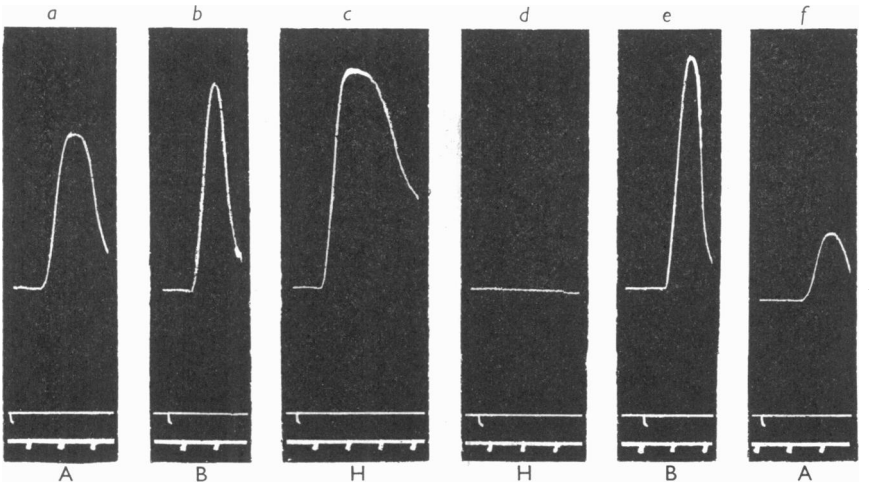

Fig. 10. Record of the right nictitating membrane of a spinal cat 2·5 kg. At the signals, responses to rapid retrograde injections of angiotensin (A) 0·1 μg (at *a* and *f*), bradykinin (B) 30 μg (at *b* and *e*) and histamine (H) 10 μg (at *c* and *d*) to the right superior cervical ganglion. Between *c* and *d*, intravenous injection of mepyramine (0·5 mg/kg). Time interval between *d* and *f* 50 min. Time marker 30 sec.

When the effects of preganglionic stimulation were blocked by hexamethonium, the responses to both peptides remained unaltered. In the experiment of Fig. 11, a rapid retrograde injection of 5 mg hexamethonium was sufficient to block completely the effect of preganglionic stimulation as shown in *d*. This dose of hexamethonium, when given shortly before a rapid retrograde injection of angiotensin 1 μg (at *e*) or bradykinin 30 μg (at *f*) did not alter the responses of the ganglion to the peptides.

Figure 11 also illustrates the fact that this dose of hexamethonium, sufficient to block ganglionic transmission, only partially inhibited the action of exogenous acetylcholine on the ganglion. After a rapid retrograde injection of 5mg hexamethonium, the injection of 30mg acetylcholine

(at *g*) still produced a response, though smaller than that produced by the same amount of acetylcholine, given at *c*, before hexamethonium. This remaining action of the injected acetylcholine was found to be sensitive to atropine, since it was abolished by a rapid retrograde injection of 50 μg, as shown at *h*. Such an effect of atropine has been described by Ambache (1949), Konzett & Rothlin (1949) and Ambache, Perry & Robertson (1956). The atropine however did not affect the ganglion stimulating action of angiotensin or bradykinin. Their responses persisted unchanged after atropine as shown at *i* and *j*.

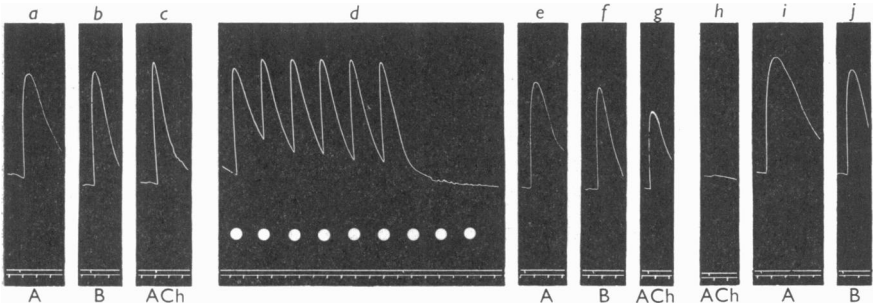

Fig. 11. Record of the right nictitating membrane of a spinal cat 2·7 kg. At the signals, responses to rapid retrograde injections of angiotensin (A) 1 μg (at *a*, *e* and *i*), bradykinin (B) 30 μg (at *b*, *f* and *j*), and acetylcholine (Ach) 30 μg (at *c*, *g* and *h*) to the right superior cervical ganglion. At dots, stimulation of the preganglionic cervical sympathetic nerve for 5 sec (0·5 msec square wave pulses, 20/sec, 3 V). Injections of hexamethonium (C_6) 5 mg to the right superior cervical ganglion at signal in *d* and before *e*, *f* and *g*. Injections of atropine 50 μg to the right superior cervical ganglion before *h*, *i* and *j*. Timer marker 30 sec.

DISCUSSION

Our experiments show that angiotensin and bradykinin are potent stimulators of the superior cervical ganglion. They caused contractions of the nictitating membrane when delivered to the ganglion by rapid retrograde injections into the external carotid artery whilst the blood flow from this artery to the nictitating membrane was interrupted. These contractions were not due to the peptides reaching the general circulation, because they occurred only on the side of the injection. Their ganglionic origin was proved by the fact that they were no longer elicited either when the ganglion had been removed or the post-ganglionic trunk cut. It is unlikely that the peptides owe their ganglion-excitation to some local circulatory effect, since angiotensin is a vasoconstrictor and bradykinin a vasodilator substance.

On a weight-for-weight basis the potency of angiotensin and bradykinin was of the same order as that of other naturally occurring substances, such

as acetylcholine, histamine or 5-hydroxytryptamine, which excite the ganglion (Robertson, 1954; Trendelenburg, 1954, 1956). On a molar basis the peptides are the most potent naturally occurring ganglion-stimulating substances known, since their molecular weights are so large: for angiotensin, 1038; for bradykinin, 1131.

The ganglion-stimulating action of angiotensin and bradykinin does not result from an action on the preganglionic nerve endings but from excitation of the ganglion cells themselves, because after degeneration of the preganglionic fibres the ganglia still responded to the peptides and were, in fact, more sensitive than the innervated one.

The receptors for the peptides in the ganglia are different from those activated by acetylcholine, as evidenced by the following two observations. When the stimulating action of acetylcholine, either injected arterially or released by preganglionic stimulation, was abolished by hexamethonium and atropine the ganglion cells still responded normally to the peptides. Conversely, when as a result of tachyphylaxis the ganglion cells became insensitive to either angiotensin or bradykinin, their sensitivity to exogenous and endogenous acetylcholine was unimpaired.

The finding that no cross-desensitization occurred between angiotensin and bradykinin suggests the presence of specific receptors for each peptide. These receptors are not those activated by histamine, because the tachyphylaxis which developed to each peptide did not extend to this amine. There appears, however, to be some common link between the histamine and angiotensin receptors. Although tachyphylaxis produced by angiotensin did not extend to histamine, the reverse did not hold true: tachyphylaxis produced by histamine did extend to angiotensin. Furthermore, mepyramine not only depressed the stimulating effect of histamine, but also that of angiotensin. Although it is unlikely that angiotensin and histamine act on the same receptor, it might well be that angiotensin through interaction with its specific receptor initiates a sequence of events which subsequently involves the histamine receptor in order to cause stimulation of the ganglion cells. No evidence was found for such a common link between the histamine and bradykinin receptors.

It has been shown previously that angiotensin is a much more potent releaser than bradykinin of the medullary hormones from the suprarenal glands (Feldberg & Lewis, 1964). The present experiments show a similar difference in potency of the two peptides on the superior cervical ganglion. Independent of these differences, greater amounts of both peptides were required to stimulate this ganglion than the suprarenal medulla although at both sites the method used was that of close arterial injection. It is interesting to note in this connexion that such a difference in potency at the two sites pertains also to histamine and acetylcholine, which excite the

suprarenal medulla in smaller doses than those required to stimulate the superior cervical ganglion (Trendelenburg, 1954; Feldberg & Lewis, 1965). This difference might be the result of a relative inaccessibility of the ganglion cells or their receptors to substances which reach the ganglion via the blood stream and, thus, need not mean that the ganglion cells are actually less sensitive than the medullary cells of the suprarenal gland to these various substances.

The ganglion-stimulating action of angiotensin may be relevant to the observation made by Bickerton & Buckley (1961) as well as by Laverty (1963) that its vasoconstrictor effect is not entirely due to a direct action on the smooth muscle of the blood vessels. It has been suggested by these authors that the vasoconstriction is in part mediated through the sympathetic nervous system. Recently, the finding that angiotensin augments the effects of electrical stimulation of the hypogastric nerve has been put forward in favour of this view, and it has been assumed that angiotensin acts at the peripheral nerve-endings producing a greater output of noradrenaline (Benelli, Della Bella & Gandini, 1964). Stimulation of the ganglion cells as shown in the present experiments may be another, and perhaps even more important, way in which the sympathetic nervous system contributes to the vasoconstriction produced by angiotensin.

The slow and delayed secondary response of the ipsilateral nictitating membrane which sometimes occurred with the rapid retrograde injections of bradykinin, after unclamping the external carotid artery, cannot be due to an action of bradykinin on the membrane, because when the peptide was injected directly towards the membrane no contraction occurred. This failure of bradykinin to stimulate the membrane has also been observed *in vitro* on its isolated smooth muscle (Thompson, 1958). The secondary contraction might be due to the action of some substance liberated locally from the ganglion or its neighbouring structures and carried to the membrane after unclamping the external carotid artery. In two experiments in which there was also a slow response of the contralateral membrane, the substance could have gained access to that membrane through the cranial anastomoses which are known to exist between the carotid arterial circulations of both sides (Chungcharoen, de Burgh Daly, Neil & Schweitzer, 1952). Adrenaline, released from extramedullary chromaffin tissue that is present in and near sympathetic ganglia (Boyd, 1960) might be the stimulating substance reponsible for the secondary membrane contraction. Since such secondary responses were not observed with retrograde injections of angiotensin, the more potent releaser of catecholamines from the suprarenals, one would have to assume that the sensitivity of extramedullary chromaffin tissue differs from that of the suprarenal gland.

SUMMARY

1. In spinal cats the effects of angiotensin and bradykinin on the superior cervical sympathetic ganglion were examined. For this purpose the contractions of the nictitating membrane were recorded in response to rapid retrograde injections of the peptides to the ganglion through the central end of the cannulated lingual artery while the external carotid was clamped distal to the origin of the lingual.

2. Angiotensin and bradykinin injected in this way caused contractions of the ispilateral nictitating membrane. The contractions were not due to a direct action of the peptides on the membrane, since they did not occur when the injections were made slowly and with the external carotid unclamped so as to carry the peptides to the membrane.

3. The contractions resulted from stimulation of the superior cervical ganglion, since they no longer occurred after cutting the post-ganglionic sympathetic trunk or removal of the ganglion.

4. The threshold dose for stimulation of the ganglion varied for angiotensin between 0·1 and 0·3μg and for bradykinin between 0·5 and 10μg. On a molar basis the two peptides are the most potent ganglion-stimulating substances known to occur naturally.

5. The ganglion-stimulating effect of angiotensin and of bradykinin was not due to an action on the preganglionic nerve endings, since it occurred after chronic denervation of the cervical sympathetic ganglion. It was therefore due to a direct action on the ganglion cells.

6. The receptors for the two peptides must be different, since tachyphylaxis of the ganglion cells was readily produced by each peptide but only to itself.

7. The receptors on which angiotensin and bradykinin act are different from those activated by acetylcholine. When as a result of tachyphylaxis the ganglion cells had become insensitive to either peptide, their sensitivity to injected acetylcholine was unimpaired and responses to preganglionic stimulation were not depressed. Conversely, when the ganglion had been rendered insensitive to preganglionic stimulation and to injected acetylcholine by hexamethonium and atropine, they responded normally to the peptides.

8. The receptors on which angiotensin and bradykinin act are different from those activated by histamine, yet there is a common link between the receptors for histamine and angiotensin, but not for histamine and bradykinin, as shown by the following facts. Mepyramine decreased the ganglionic response to histamine and angiotensin, but not to bradykinin. Tachyphylaxis of the ganglion cells produced by histamine extended to angiotensin but not to bradykinin. The receptors for histamine cannot,

however, be the same as those for angiotensin, since tachyphylaxis produced by angiotensin did not extend to histamine.

9. A secondary delayed contraction of the nictitating membrane was sometimes observed with the rapid retrograde injections of bradykinin after unclamping the external carotid artery. It is attributed to the local release in the vicinity of the ganglion of a substance which may be a catecholamine. Such a contraction was not obtained with angiotensin.

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