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FURTHER STUDIES ON THE ACTIONS OF PEPTIDES ON THE SUPERIOR CERVICAL GANGLION AND SUPRARENAL MEDULLA

BY

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The finding that angiotensin and bradykinin are potent releasers of catechol amines from the suprarenal medulla (Feldberg & Lewis, 1964, 1965) led to investigation of their actions on the superior cervical ganglion, and it was found that minute amounts of both peptides stimulated the ganglion (Lewis & Reit, 1964, 1965). The present experiments are a continuation of this work. They deal with the mode of action of angiotensin and bradykinin on the ganglion, and a comparison is made with their actions on the suprarenal medulla.

The relative activities of several analogues of angiotensin and bradykinin were determined and the actions of angiotensin and bradykinin were compared with those of histamine and 5-hydroxytryptamine. Further, the sensitivity of the ganglion and the suprarenal medulla to the peptides was examined after morphine and that of the ganglion after nicotine and preganglionic stimulation as well. On the ganglion a few experiments were also carried out with other biologically active peptides, such as oxytocin, vasopressin, substance P and eledoisin.

METHODS

Most experiments were performed on cats of either sex, weighing 2.4 to 4 kg. Anaesthesia was induced with ethyl chloride and ether, following which some cats were given chloralose (75 mg/kg, intravenously) and others were rendered spinal as described by Burn (1952). A few experiments were carried out on dogs anaesthetized with pentobarbitone sodium (40 mg/kg) or chloralose (80 mg/kg) intravenously after induction with ethyl chloride and ether, and on rabbits anaesthetized with 25% urethane (6 ml./kg, intravenously).

With the animal lying on its back, its head was held rigidly in position by tying its jaws around a transverse rod clamped to uprights at the sides of the operating table. Contractions of both nictitating membranes were recorded isotonically on a smoked drum or with an ink-writing lever. The resting load on each nictitating membrane was 5 g for cat and dog and 2 g for rabbit, and the contractions were magnified twentyfold. In some experiments, arterial blood pressure was recorded from the right femoral artery, either with a mercury manometer or with a Statham pressure transducer coupled to a potentiometric pen recorder (Leeds-Northrup).

For intravenous injections, a polyethylene cannula was tied into the right femoral vein. In all experiments, both vagosympathetic trunks were cut low in the neck. For electrical stimulation of the cervical sympathetic nerve, it was usually separated from the vagus nerve, placed on bipolar

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platinum electrodes and covered with warm paraffin oil to prevent drying. Sometimes the combined vagosympathetic trunk was stimulated. Rectangular wave stimuli of 0.5 msec duration were applied at various frequencies and intensities with a Physiological Electronic Stimulator (Cinetronics Ltd.).

Close intra-arterial injections either to the superior cervical ganglion or to the nictitating membrane were made into the external carotid artery by means of a cannula tied into the central end of the lingual artery as described by Trendelenburg (1959). In most experiments, the right and left lingual arteries were cannulated so that injections could be made to both sides. Drugs injected whilst the external carotid artery was clamped just distal to the origin of the lingual artery passed retrogradely down the external carotid to the arteries supplying the ganglion. Drugs injected whilst the external carotid artery was left unclamped were borne up the external carotid by the uninterrupted flow of blood to the arteries supplying the nictitating membrane. For details of cannulation and injection see previous paper (Lewis & Reit, 1965).

In cats perfusion of the superior cervical ganglion on the right side was carried out, using chloralose anaesthesia, essentially by the method of Kibjakow (1933) as modified by Feldberg & Gaddum (1934). The perfusion fluid was Locke solution of the following composition (g/l.): NaCl, 9.2 ; KCl, 0.42 ; $CaCl_2$, 0.24 ; $NaHCO_3$, 1.8 ; and glucose, 1.0. The solution was gassed with 95% oxygen and 5% carbon dioxide and its *p*H after equilibration was 7.4. It was warmed to body temperature by the method of Perry (1953), being passed through a polyethylene tube inserted into the oesophagus at its junction with the stomach and out through an incision at the level of the larynx.

Drugs dissolved in Locke solution were injected intra-arterially to the perfused ganglion through a sleeve of rubber tubing into the perfusion fluid just as it entered the common carotid artery. In all experiments on perfused ganglia, a polyethylene cannula was tied into the central end of the contralateral lingual artery in order to make close intra-arterial injections also to the ganglion with its circulation intact.

Experiments on the suprarenal medulla were performed on cats in which the right nictitating membrane had been denervated 6 to 12 days earlier by removing the right superior cervical ganglion in an aseptic operation, using pentobarbitone sodium anaesthesia. For the experiments, the cats were anaesthetized with chloralose, and eviscerated by removal of stomach, intestines and spleen. If not otherwise stated the greater splanchnic nerves were left intact. Close intra-arterial injections to the suprarenal glands were made into the abdominal aorta just proximal to their arterial supply by means of a cannula tied into the central end of the coeliac artery, as described by Feldberg & Minz (1931).

Polypeptides. Bradykinin, lys-bradykinin (kallidin) and the octapeptide arg.pro.pro.gly.phe.ser.phe.arg were synthesized at Parke Davis & Co., Ann Arbor, U.S.A., and kindly supplied by Dr E. D. Nicholaides. Met-lys-bradykinin was synthesized at Schering A.C., Berlin, W. Germany, and was kindly supplied by Dr E. Schröder. Angiotensin was the synthetic Hypertensin-CIBA (val^5-Hypertensin II-asp-β-amide). The analogues of angiotensin were synthesized at Ciba Laboratories, Basel, Switzerland, and were kindly supplied by Dr B. Riniker.

Oxytocin was synthetic oxytocin of two types: Syntocinon brand of injection of oxytocin, B.P., Sandoz Ltd., 10 U/ml. (450 U=1 mg); and pure oxytocin synthesized by Dr J. Rudinger of the Czechoslovak Academy of Science, Prague, and kindly supplied by Dr D. Smythe of the National Institute for Medical Research, Mill Hill (300 U = 1 mg). Vasopressin was vasopressin B.P. (Pitressin, 20 pressor U/ml., Parke Davis & Co.). Substance P: preparation RO 1-9256/3, prepared by Hoffmann La Roche, Basel, and adopted as a standard by Sir John Gaddum in 1959 was kindly supplied by Sir John Gaddum. Each sealed tube contained 1 mg of extract mixed with 2 mg of lactose as stabilizer (Substance P activity = 75 U/mg). Eledoisin was synthetized at Sandoz Ltd. (ELD 950, 0.07 mg/ml.) and, with ampoules of the eledoison-free vehicle, was kindly supplied by Dr D. S. Freestone.

Other substances used were (−)-adrenaline-D-bitartrate, histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate, nicotine hydrogen tartrate, phentolamine methane sulphonate (Rogitine, Ciba), morphine sulphate and cocaine hydrochloride. All doses in the text refer to the base except those for morphine sulphate and cocaine hydrochloride, which refer to the salts.

RESULTS

Analogues of angiotensin and bradykinin

The stimulating action of angiotensin and bradykinin on the superior cervical ganglion is shared to different degrees by several of their analogues. A comparison has previously been made of the effects of these analogues on the arterial blood pressure and on the suprarenal medulla (Feldberg & Lewis, 1964, 1965). The relative activities for three analogues of each peptide on the ganglion, the suprarenal medulla and the arterial blood pressure are shown in Table 1. One analogue of angiotensin, β-angiotensin, was more and two were less active than angiotensin, whereas all three analogues of bradykinin were less active than bradykinin. The octapeptide was relatively more active on the ganglion than on the suprarenal medulla or the arterial blood pressure, but the relative activities of the other two analogues of bradykinin on the three preparations were about the same.

TABLE 1

RELATIVE ACTIVITIES OF ANALOGUES OF ANGIOTENSIN AND BRADYKININ IN CATS
Each value is the mean of three experiments. *For analogues of angiotensin. †For analogues of bradykinin. §From Feldberg & Lewis (1965) and unpublished experiments

Compound	Amino-acid sequence	Stimulation of superior cervical ganglion	Release of catechol amines from suprarenal glands§	Pressor* or depressor† activity
α-Angiotensin	α-Asp.arg.val.tyr.val.his.pro.phe. (NH_2)	1	1	1
β-Angiotensin	β-Asp.arg.val.tyr.val.his.pro.phe.	1·3	2	1·6
	α-Asp.arg.val.phe.val.his.pro.phe.	0·02	0·05	0·1
	α-Asp.arg.val.D-tyr.val.his.pro.phe.	0·0001	0·002	0·001
Bradykinin	Arg.pro.pro.gly.ser.phe.pro.phe.arg	1	1	1
Lys-bradykinin (Kallidin)	Lys.arg.pro.pro.gly.ser.phe.pro.phe.arg	0·5	0·6	0·8
Met. lys-bradykinin	Met.lys.arg.pro.pro.gly.ser.phe.pro.phe.arg.	0·5	0·3	0·8
	Arg.pro.pro.gly.ser.phe.phe.arg.	0·05	0·002	0·002

Comparison with other substances at the ganglion and suprarenal gland

In previous investigations the actions of angiotensin and bradykinin were compared with that of acetylcholine in causing the release of catechol amines from the suprarenal medulla (Feldberg & Lewis, 1965) and with those of acetylcholine and histamine in stimulating the superior cervical ganglion (Lewis & Reit, 1965). In the present experiments further comparisons of the two peptides have been made with histamine at the suprarenal glands and with 5-hydroxytryptamine at both sites. In addition the effect of morphine on the responses to all these substances was investigated at both sites.

In order to compare in the same cat the actions of the peptides and other substances on the superior cervical ganglion with their actions on the suprarenal medulla, the right nictitating membrane, sensitized by chronic denervation, served to detect the catechol amines released from the suprarenal medulla, the left membrane to detect stimulation of the ganglion.

Histamine. Previously it was shown that, on a molar basis, angiotensin was about 100 times and bradykinin about five times more active than histamine at the ganglion (Lewis & Reit, 1965). We have now observed that, at the suprarenal glands, a similar

order of relative molar potency prevailed. For example, in one experiment, on injection into the central stump of the coeliac artery, histamine (1 μg), bradykinin (1 μg) and angiotensin (0.1 μg) released the equivalent of 1 μg of adrenaline and in another experiment the same amount of adrenaline was released by histamine (0.1 μg), bradykinin (0.1 μg) and angiotensin (0.01 μg). On a molar basis, therefore, angiotensin (molecular weight, 1,038) was about seventy times and bradykinin (molecular weight, 1,131) about ten times more active than histamine (molecular weight, 111) in releasing medullary catechol amines.

5-Hydroxytryptamine. At the superior cervical ganglion 5-hydroxytryptamine, though more active than histamine, was still considerably less active than angiotensin. 5-Hydroxytryptamine was usually less active than bradykinin although in one experiment

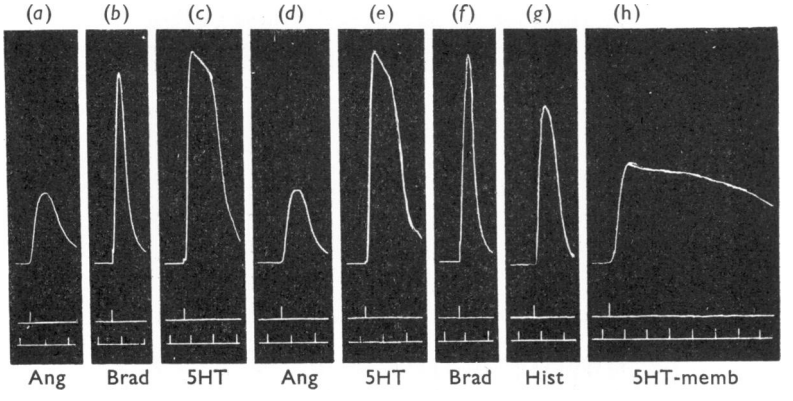

Fig. 1. Record of contractions of the left nictitating membrane of a spinal cat, 2.9 kg. At the signals, there were rapid retrograde intra-arterial injections: (*a*) and (*d*) 1 μg of angiotensin (Ang), (*b*) and (*f*) 30 μg of bradykinin (Brad), (*c*) and (*e*) 3 μg of 5-hydroxytrpytamine (5HT), and (*g*) 20 μg of histamine (Hist), all to the left superior cervical ganglion. In (*h*), intra-arterial injection of 10 μg of 5-hydroxytryptamine towards the left nictitating membrane (5HT-memb). Intervals between injections: *a* and *b*, 8 min; *b* and *c*, 21 min; *c* and *d*, 9 min; *d* and *e*, 7 min; *e* and *f*, 5 min; *f* and *g*, 16 min; and *g* and *h*, 7 min. Time marks, 30 sec.

it was somewhat more active. In this experiment, illustrated in Fig. 1, retrograde injections to the ganglion of 3 μg of 5-hydroxytryptamine (*c* and *e*) produced responses larger than those produced by 1 μg of angiotensin (*a* and *d*) or 20 μg histamine (*g*) and almost equivalent to those produced by 30 μg of bradykinin (*b* and *f*). Thus on a molar basis, 5-hydroxytryptamine (molecular weight, 176) was about fifteen times less active than angiotensin, about 1.5 times more active than bradykinin and about ten times more active than histamine. However, the action of 5-hydroxytryptamine on the ganglion was variable and, in the experiment of Fig. 2, it was about sixty times less active than angiotensin and six times less active than bradykinin on a molar basis. In this experiment the retrograde injection to the ganglion of 10 μg of 5-hydroxytryptamine (*c*) produced a response somewhat less than that produced by 1 μg of angiotensin (*a*) and similar to that of 10 μg of bradykinin (*b*).

The experiment of Fig. 1 illustrates in addition three features of the action of 5-hydroxytryptamine. Firstly the contractions of the nictitating membrane were more prolonged following ganglion-stimulation by 5-hydroxytryptamine than by either

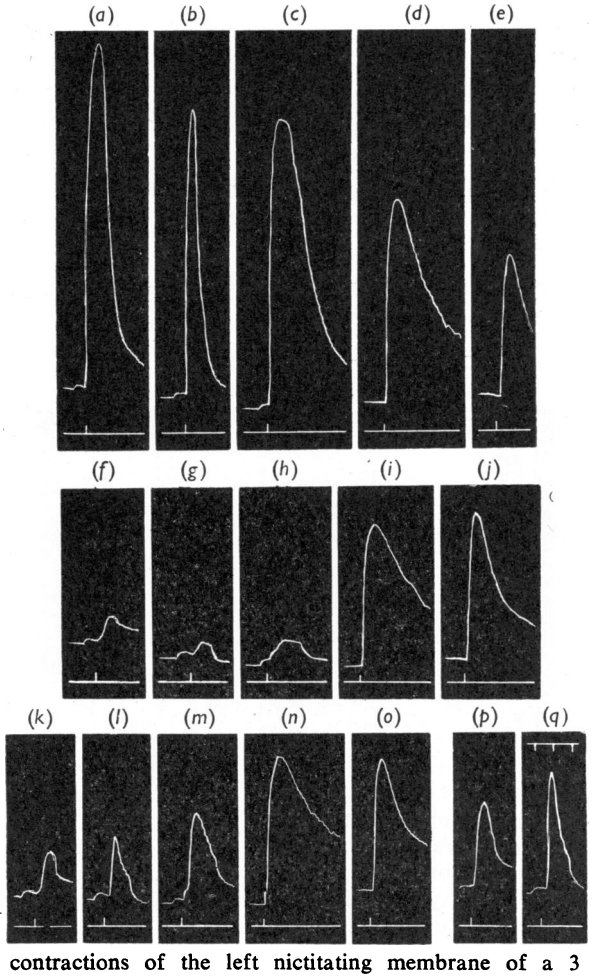

Fig. 2. Record of contractions of the left nictitating membrane of a 3 kg cat anaesthetized with chloralose. At the signals rapid retrograde intra-arterial injections of 1 μg of angiotensin (in *a*, *f*, *k* and *p*), 10 μg of bradykinin (in *b*, *g*, *l* and *q*), and 10 μg of 5-hydroxytryptamine (in *c*, *h* and *m*) to the left superior cervical ganglion; intra-arterial injection of 10 μg of 5-hydroxytryptamine towards the left nictitating membrane (in *d*, *i* and *n*); and intravenous injection of 10 μg of adrenaline (in *e*, *j* and *o*). Between (*e*) and (*f*), intravenous injection of morphine sulphate, 10 μg/kg. Intervals from morphine injection to (*f*), 8 min; (*k*), 67 min; and (*p*), 115 min. Time marks, 30 sec.

bradykinin or histamine; they thus resembled more the responses to angiotensin. Secondly, there was no interference between the action of 5-hydroxytryptamine and the peptides. A previous injection of 5-hydroxytryptamine to the ganglion did not affect the ganglion-stimulating action of either angiotensin (compare *a* with *d*) or bradykinin (compare *b* with *f*), and previous injections of the peptides did not interfere with the ganglion-stimulating action of 5-hydroxytryptamine. Thirdly, 5-hydroxytryptamine stimulated the nictitating membrane directly (*h*). This direct stimulating affect of 5-hydroxytryptamine was observed by Thompson (1955) on the isolated nictitating membrane.

The direct action of 5-hydroxytryptamine on the nictitating membrane interfered to some extent with the assessment of its activity in releasing catechol amines from the suprarenal medulla, especially since the chronically denervated membrane exhibited supersensitivity to 5-hydroxytryptamine as well as to the catechol amines. Thus in some experiments 5-hydroxytryptamine still produced a contraction of the supersensitive nictitating membrane on injection into the coeliac artery after removal of the suprarenal glands. None the less, it was regularly observed that 5-hydroxytryptamine was less active at the suprarenal glands than either histamine or the peptides, and in terms of relative molar potency it was estimated to be at least five times less active than histamine, fifty times less active than bradykinin and 500 times less active than angiotensin.

Effect of morphine. Injected retrogradely to the ganglion morphine inhibited the ganglion-stimulating action of angiotensin and bradykinin in doses of 0.1 to 1 μg which, however, did not inhibit the response of the nictitating membrane to preganglionic nerve stimulation. In higher doses morphine slightly reduced the responses to preganglionic stimulation, probably by inhibiting the release of the adrenergic transmitter from the postganglionic fibres as described by Trendelenburg (1957a).

When injected intravenously, morphine abolished the action of the peptides at the ganglion but did not reduce or only slightly reduced their action at the suprarenal medulla,

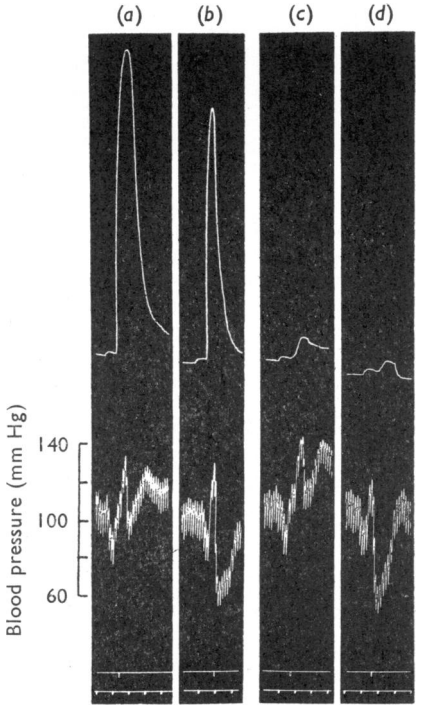

Fig. 3. Records of contractions of the left nictitating membrane (top) and arterial blood pressure (bottom) of the same cat as for Fig. 2. At the signals rapid retrograde intra-arterial injections of 1 μg of angiotensin (in *a* and *c*) and 10 μg of bradykinin (in *b* and *d*) to the left superior cervical ganglion. Between (*b*) and (*c*), intravenous injection of morphine sulphate, 10 μg/kg. Time marks, 30 sec.

and did not affect their actions on arterial blood pressure. These findings are illustrated in Figs. 2, 3 and 4. Fig. 2 shows the inhibiting effect of intravenous morphine (10 μg/kg) on ganglionic responses to the peptides and to 5-hydroxytryptamine, and the subsequent partial recovery. The responses to angiotensin (1 μg), bradykinin (10 μg) and 5-hydroxytryptamine (10 μg) (*a*, *b* and *c*) injected retrogradely to the ganglion were nearly abolished (*f*, *g* and *h*) by the morphine injection. Some recovery occurred after about 1 hr (*k*, *l* and *m*), and further recovery after another hour (*p* and *q*). The direct response of the membrane to 5-hydroxytryptamine (10 μg) was also reduced by the morphine but to a much smaller extent (compare *d* with *i*). In contrast the response to intravenous adrenaline (10 μg) was unaffected (compare *e* with *j*). Neither the pressor response to angiotensin nor the depressor response to bradykinin was affected by morphine injected intravenously in doses which nearly abolished the ganglionic responses of the peptides. This is illustrated in Fig. 3, which is from the same experiment as Fig. 2.

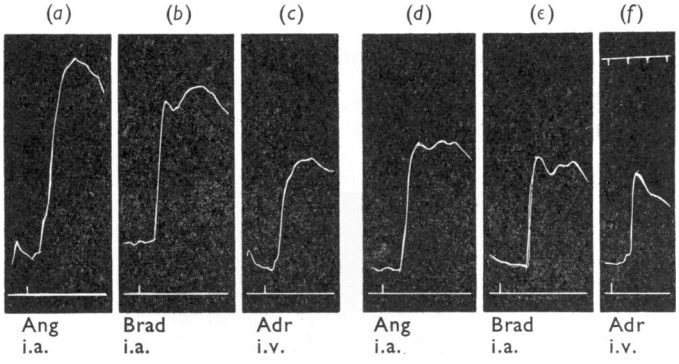

Fig. 4. Record of contractions of the denervated right nictitating membrane of a 2.7 kg eviscerated cat anaesthetized with chloralose. The right superior cervical ganglion had been removed 6 days previously. At the signals, injections into the central end of the coeliac artery (i.a.) of 0.1 μg of angiotensin (Ang) and 1 μg of bradykinin (Brad) and intravenous injections of 0.5 μg of adrenaline (Adr). Between (*c*) and (*d*), intravenous injection of morphine sulphate, 100 μg/kg. Time marks, 30 sec.

The finding that morphine had a much weaker blocking action at the suprarenal gland than at the ganglion is illustrated in the experiment of Fig. 4, in which close arterial injections of the peptides were made to the suprarenal glands before and after an intravenous injection of morphine (100 μg/kg), tenfold greater than the effective blocking dose at the ganglion. Before the morphine injection the amounts of catechol amines released by the close arterial injections of angiotensin (0.1 μg) and bradykinin (1 μg) were estimated by comparison with the response of the denervated nictitating membrane to intravenous adrenaline to be equivalent to about 1 μg of adrenaline. Afterwards the release of catechol amines by the peptides was only slightly reduced; it was still equivalent to somewhat more than 0.5 μg of adrenaline.

Effects of nicotine on the ganglion-stimulating action of the peptides

Paton & Perry (1953) and Trendelenburg (1957b) have shown that nicotine blocks ganglionic transmission in two phases: first by depolarization block, which is nonspecific

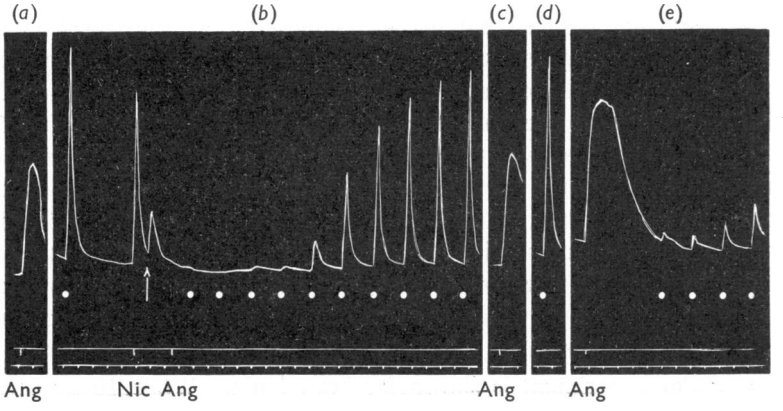

Fig. 5. Record of contractions of the right nictitating membrane of a 3.1 kg spinal cat. At the signals, rapid retrograde intra-arterial injections of 1 μg of angiotensin (Ang) and 200 μg of nicotine (Nic) to the right superior cervical ganglion. At the arrow, release of the clamp on the external carotid artery resulting in a small additional nicotine-induced contraction. At the white dots, supramaximal stimulation of the right cervical sympathetic nerve for 5 sec (20 shocks/sec, 10 V). Between (*d*) and (*e*), five rapid retrograde intra-arterial injections at 2 min intervals of 200 μg of nicotine to the right superior cervical ganglion. The injection of angiotensin in (*e*) was 2 min after last nicotine injection. Time marks, 30 sec.

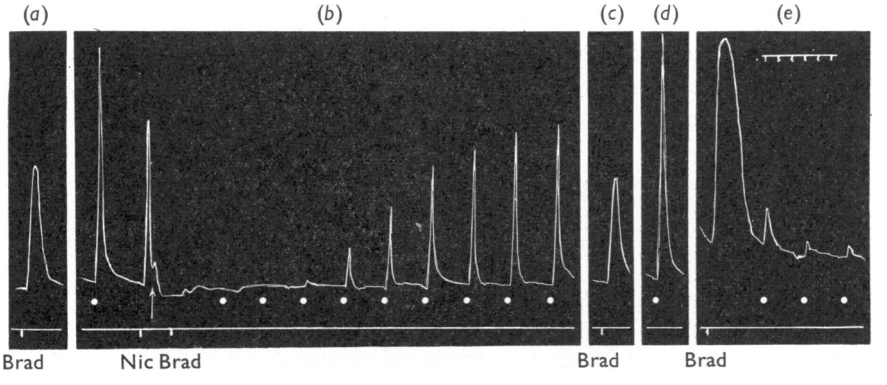

Fig. 6. Record of contractions of the left nictitating membrane of a 2.9 kg cat anaesthetized with chloralose. At the signals, rapid retrograde intra-arterial injections of 10 μg of bradykinin (Brad) and 200 μg of nicotine (Nic) to the left superior cervical ganglion. At the arrow, release of the clamp on the external carotid artery resulting in a very small additional nicotine-induced contraction. At the white dots, supramaximal stimulation of the left cervical sympathetic nerve for 5 sec (20 shocks/sec, 10 V). Between (*d*) and (*e*), five rapid retrograde intra-arterial injections at 1.5-min intervals of 200 μg of nicotine to the left superior cervical ganglion. The injection of bradykinin in (*e*) was 2 min after last nicotine injection. Time marks, 30 sec.

in that it renders ineffectual all substances previously studied which stimulate ganglion cells, and second by competitive block, which succeeds the first type and is specific for those substances which act upon the nicotinic receptors for acetylcholine. In the present experiments, the ganglion-stimulation by angiotensin and bradykinin was prevented during the nicotine depolarization block while, during the competitive block, the actions

of the peptides were not reduced but in fact enhanced. As illustrated in Fig. 5, the responses to angiotensin (1 μg) injected retrogradely to the ganglion and to preganglionic nerve stimulation were abolished shortly after an injection of nicotine 200 μg (*b*). Recovery of the responses to preganglionic electrical stimulation began after 6 min, and was complete after 12 min, as was the recovery of the response to angiotensin when it was next injected after 31 min (*c*). Between (*d*) and (*e*), a larger amount of nicotine (1 mg) was injected to the ganglion in five doses of 200 μg over a period of 8 min to produce the more prolonged competitive block. During this block responses to electrical stimulation, previously fully effective (*d*), were considerably reduced (*e*) and recovery was less rapid than after the previous injection of the smaller dose of nicotine. Yet the response to 1 μg of angiotensin (*e*) rather than being inhibited was greatly enhanced.

In Fig. 6 a similar experiment is shown for bradykinin. While 10 μg of bradykinin injected to the ganglion was ineffective during the depolarization block produced by 200 μg of nicotine (*b*), it elicited an enhanced response during the competitive block (*e*) following the larger dose of nicotine injected between (*d*) and (*e*).

Effect of preganglionic electrical stimulation on the ganglion-stimulating action of the peptides

Preganglionic supramaximal stimulation at high frequency is another procedure which enhances the ganglion-stimulating action of the peptides. This type of enhancement following a volley of supramaximal preganglionic stimuli has been reported for the ganglionic effects of acetylcholine, carbachol and tetramethylammonium (Volle, 1962), the muscarine-like agent 4-(*m*-chlorophenylcarbamoyloxo)-2-butynyltrimethylammonium (McN-A-343), histamine and nicotine (Jones, 1963; Trendelenburg & Jones, 1965). It

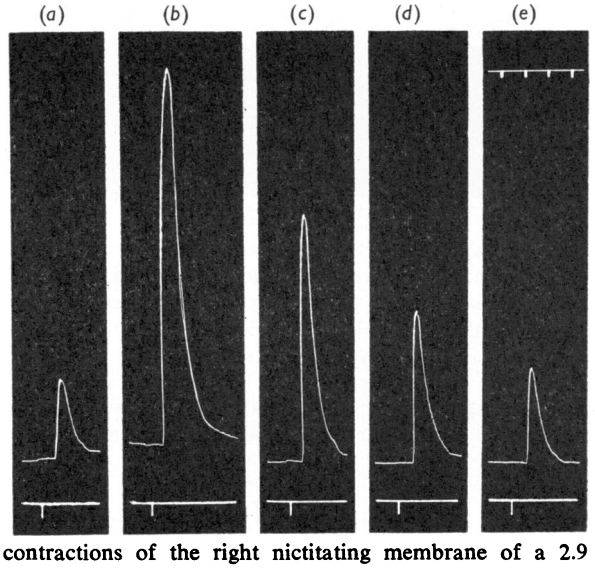

Fig. 7. Record of contractions of the right nictitating membrane of a 2.9 kg cat anaesthetized with chloralose. At the signals, rapid retrograde intra-arterial injections of 10 μg of bradykinin to the right superior cervical ganglion at 20-min intervals. At 5 min before (*b*), supramaximal stimulation of the right cervical sympathetic nerve for 5 sec (32 shocks/sec, 20 V). Time marks, 30 sec.

is illustrated for bradykinin in Fig. 7. Between (*a*) and (*b*), a 5-sec burst of supramaximal shocks was applied to the cervical sympathetic nerve at 32 shocks/sec. At 5 min later the response of the ganglion cells to 10 μg of bradykinin was increased approximately four times. This potentiation gradually disappeared during the next hour. With angiotensin a similar result was obtained.

To produce an enhancement by a short volley of supramaximal preganglionic stimuli the stimulus frequency had to be relatively high. With a frequency of 20 shocks/sec a 5-sec burst of supramaximal stimuli did not alter the response of the ganglion to the peptides, even when such bursts of stimuli were repeated at regular intervals of 1 to 1.5 min. This agrees with the finding of Trendelenburg & Jones (1965) that potentiation of the response to McN-A-343 required at least 125 supramaximal shocks and for optimal efficacy this number of shocks had to be applied at a rate of at least 25 shocks/sec.

Action of the peptides on the perfused ganglion

Trendelenburg (1956a) found that the perfused superior cervical ganglion was less sensitive to histamine and 5-hydroxytryptamine than the ganglion with its circulation intact. A reduction of sensitivity has also been described for the perfused suprarenal medulla of cats to histamine (Feldberg, 1940) and of dogs to angiotensin and bradykinin (Vogt, 1965). In the present experiments, the perfused superior cervical ganglion of the cat usually responded to angiotensin and bradykinin as well as, and in some experiments even better than, the ganglion with its circulation intact. A typical experiment in which the ganglion of one side was perfused for 1 hr is shown in Fig. 8.

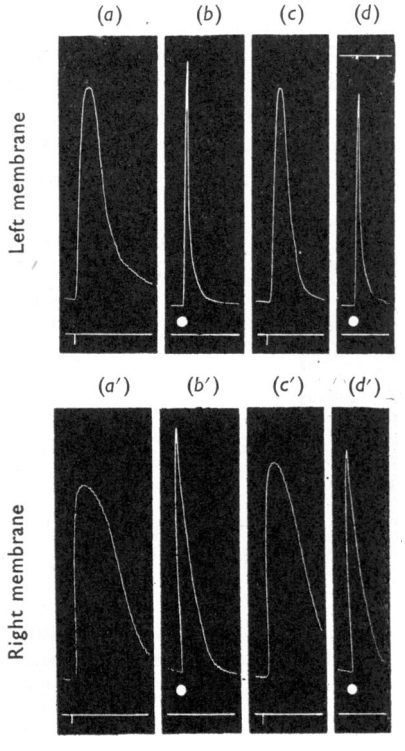

Fig. 8. Records of the left (top) and right (bottom) nictitating membranes of a 2.9 kg cat anaesthetized with chloralose. Top records: at the signals, rapid retrograde intra-arterial injections of 1 μg of angiotensin (*a*) and 10 μg of bradykinin (*c*) to the left superior cervical ganglion (circulation intact). At the white dots, submaximal stimulation of left vagosympathetic trunk for 5 sec (20 shocks/sec, 0.5 V). Bottom records: at the signals, rapid retrograde intra-arterial injections of 1 μg of angiotensin (in *a*′) and 10 μg of bradykinin (in *c*′) to the right superior cervical ganglion after perfusion with Locke solution for 52 and 62 min, respectively. At the white dots, submaximal stimulation of right vagosympathetic trunk for 5 sec (20 shocks/sec, 1 V). Time marks, 30 sec.

The responses of this perfused ganglion (lower record) to angiotensin (1 μg), bradykinin (10 μg) and preganglionic electrical stimulation were more or less of the same magnitude as the responses of the contralateral ganglion (upper record) which had its natural circulation. However, in the perfused preparation the latencies of the responses to the peptides were longer, 15 and 14 sec compared with 6 and 5 sec, and the responses to the peptides as well as to preganglionic stimulation were more prolonged than for the ganglion with its normal blood circulation.

After more than an hour of perfusion, the responses of the ganglion to electrical stimulation as well as to the peptides gradually became smaller. This parallel decrease in sensitivity reflects the general deterioration of the ganglion which occurs after prolonged perfusion with artificial salt solutions (Birks & MacIntosh, 1961).

In several experiments the outflow from the perfused ganglion was measured by counting the number of drops. Although both peptides are strongly vasoactive, no alteration in the outflow was observed when either peptide was injected.

Other peptides

Oxytocin and vasopressin. When injected retrogradely to the superior cervical ganglion, 1 to 2 μg of oxytocin produced a contraction of the ipsilateral nictitating membrane, but injections of similar doses made directly to the nictitating membrane had no effect. Thus the contraction produced by injection to the ganglion must have resulted from stimulation of the ganglion by oxytocin. The response to oxytocin (Syntocinon) injected retrogradely to the ganglion is illustrated in the experiment of Fig. 9,*c*; for comparison the responses to 10 μg of bradykinin (*a*) and 1 μg of angiotensin (*b*) are shown. The ganglion-stimulating action of oxytocin was also observed on a chronically denervated ganglion, showing that oxytocin was not acting through release of acetylcholine from presynaptic nerve endings. Because of the development of a profound and prolonged tachyphylaxis, in most experiments only one ganglionic stimulation by oxytocin was obtained. During the period of insensitivity to oxytocin

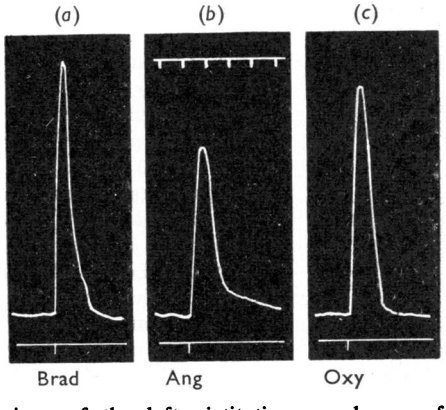

Fig. 9. Record of contractions of the left nictitating membrane of a 3.2 kg cat anaesthetized with chloralose. At the signals, rapid retrograde intra-arterial injections to the left superior cervical ganglion of 10 μg bradykinin (Brad), 1 μg of angiotensin (Ang), and 2 μg of oxytocin (Oxy). Time marks, 30 sec.

the ganglionic responses to angiotensin and bradykinin were unaffected. There was no difference in the action of Syntocinon and pure synthetic oxytocin.

No ganglion-stimulating action could be demonstrated on retrograde injection of up to 2 U (5 μg) of vasopressin. Such injections caused no desensitization of the ganglion to oxytocin, angiotensin or bradykinin nor did they interfere with ganglionic transmission of submaximal preganglionic stimuli.

Substance P and eledoisin. A retrograde injection of Substance P (75 U) did not stimulate the superior cervical ganglion nor did it interfere with the response to submaximal preganglionic stimulation.

Retrograde injections of eledoisin (7 to 28 μg) also did not stimulate the superior cervical ganglion. A small transient inhibition of the response to submaximal preganglionic stimulation sometimes produced by these injections could be attributed to the presence of chlorbutol which is included as a bacteriostatic agent in the vehicle at a concentration of 0.5%.

Eledoisin is also inactive on the suprarenal medulla. An injection of 7 μg of eledoisin into the central stump of the coeliac artery caused no detectable release of catechol amines.

Slow secondary response of the nictitating membrane to bradykinin injected retrogradely to the ganglion

Previously we found that in a small number of cats injection of bradykinin retrogradely to the ganglion produced a slow secondary contraction of the ipsilateral nictitating membrane which began several seconds after the clamp was removed from the external carotid artery (Lewis & Reit, 1965). Two additional features of this secondary response have now been observed. Firstly, it appears to be dependent on the ganglion-stimulating action of bradykinin. This is illustrated by the experiment of Fig. 10, in which a gradual reduction in the ganglionic response to successive injections of 10 μg of bradykinin as a result of tachyphylaxis was associated with a corresponding reduction in the secondary response. Secondly, this response is not affected by the adrenaline antagonist,

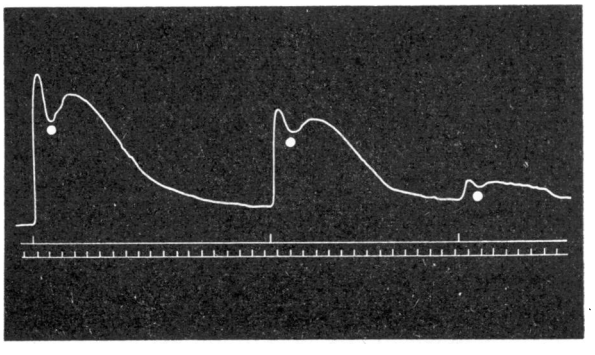

Fig. 10. Record of contractions of the left nictitating membrane of a 2.7 kg cat anaesthetized with chloralose. At the signals rapid retrograde intra-arterial injections of 10 μg of bradykinin to the left superior cervical ganglion. At the white dots, the clamp was removed from the left external carotid artery. Time marks, 30 sec.

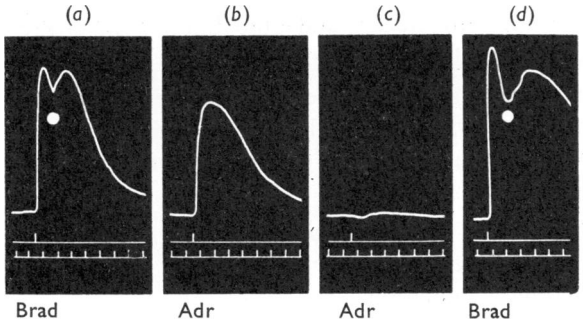

Fig. 11. Record of contractions of the left nictitating membrane. Continuation of the experiment of Fig. 10. At the signals, rapid retrograde intra-arterial injections of 10 μg of bradykinin (Brad) to the left superior cervical ganglion, and intravenous injections of 1 μg of adrenaline (Adr). Between (*b*) and (*c*) intravenous injection of phentolamine, 100 μg/kg. Time marks, 30 sec.

phentolamine. As shown in the experiment of Fig. 11 intravenous injection of 100 μg/kg of phentolamine (between *b* and *c*) abolished the response of the nicitating membrane to 1 μg of adrenaline injected intravenously (compare *c* with *b*), but did not reduce the secondary response to bradykinin (compare *d* with *a*) ; the ganglionic response also remained unaffected.

Effects of the peptides on ganglia of dogs and rabbits

In both species the nictitating membranes were singularly insensitive to injected adrenaline and in the rabbit to stimulation of the cervical sympathetic nerve as well. In the present experiments the sensitivity of the nictitating membranes was enhanced by cocaine and the sensitivity of the ganglia by a conditioning volley of high frequency supramaximal preganglionic stimuli as described in the experiments on cats. Nevertheless only small responses were obtained under these conditions to rapid retrograde intra-arterial injections of 30 to 50 μg of bradykinin in the dog and rabbit and to 10 μg of angiotensin in the rabbit ; in the dog no ganglionic effect of angiotensin could be detected.

DISCUSSION

In our previous investigation on the ganglion-stimulating effects of angiotensin and bradykinin, it was shown that each peptide acts upon specific receptors. The present finding that analogues of both peptides had the same relative activities at the ganglion as they were shown to possess at the suprarenal medulla and on vascular smooth muscle (Feldberg & Lewis, 1964, 1965) provides evidence that at all three sites the receptors for angiotensin are different from those for bradykinin but that for each peptide the receptors at the three sites are similar.

The receptors on which angiotensin and bradykinin act at the ganglion were shown to be different from those activated by acetylcholine (Lewis & Reit, 1965) and to resemble those of non-nicotinic ganglion stimulants such as histamine, 5-hydroxytryptamine and muscarine-like compounds. The ganglion-stimulating action of these substances is not

abolished by hexamethonium. This was shown by Trendelenburg (1954, 1956b) for histamine and 5-hydroxytryptamine and for angiotensin and bradykinin in our previous experiments. The present experiments concerning the nicotine-induced ganglion block provide further evidence in favour of this conclusion. It was found that the stimulating effect of the peptides was abolished only during the initial nonspecific phase of the block, whereas during the second or competitive phase of the block, which affects only the nicotinic acetylcholine receptors, the action of the peptides was not reduced but enhanced. Such an enhancement was shown by Trendelenburg (1957b) to occur also for the ganglion-stimulating actions of histamine and 5-hydroxytryptamine. On the other hand, the enhancement of the responses to the peptides following a burst of supramaximal high frequency preganglionic stimuli does not furnish any information concerning the type of receptors activated by the peptides since it is a nonspecific effect which extends to responses produced by nicotinic as well as non-nicotinic ganglion stimulants (Volle, 1962 ; Jones, 1963 ; Trendelenburg & Jones, 1965).

Morphine revealed a resemblance between the peptides and other non-nicotinic ganglion stimulants which, however, did not extend to nicotinic ganglion stimulants. In small doses it blocked the actions of the peptides, histamine and 5-hydroxytryptamine at the ganglion but only reduced the response to nicotine and did not affect that to preganglionic stimulation. The effect of morphine illustrated in addition one of the differences between the actions of the peptides at the ganglion and at the suprarenal medulla since even in large doses morphine only reduced slightly their ability to release medullary catechol amines. The vascular responses to the peptides were unaffected by morphine.

It seems unlikely that morphine acts by blocking specific receptors since it inhibits equally the ganglion-stimulating actions of angiotensin, bradykinin, histamine and 5-hydroxytryptamine, each of which acts on a different receptor. It might act at a common site in their postreceptor pathway. Such a site would be either absent or inaccessible at the cells of the suprarenal medulla and vascular smooth muscle. Another possibility would be that at the ganglion morphine prevents the passage of injected substances from the blood to the nerve cells through connective tissue barriers which are lacking in the other structures.

The sensitivity of the suprarenal medulla to stimulating substances decreases on perfusion with artificial salt solutions, as shown for histamine in the cat (Feldberg, 1940) and for angiotensin and bradykinin in the dog (Vogt, 1965). In contrast, in the present experiments after about 1 hr of perfusion the superior cervical ganglion of the cat responded as well as the ganglion with its blood circulation intact and in some experiments even better. This difference in susceptibility to the devitalizing effect of perfusion may also be accounted for by the connective tissue barrier which surrounds the ganglion cells but not the medullary cells, protecting the former by making them less accessible to substances within the vascular system. It is, however, not certain whether the ganglion retains its sensitivity to other stimulants when perfused with an artificial salt solution because in this condition Trendelenburg (1956a) did not regularly obtain stimulation with histamine.

In dogs there appears to be a great difference in sensitivity to the peptides between the superior cervical ganglion and the suprarenal medulla. Bradykinin had only a weak ganglion-stimulating effect and none at all was obtained with angiotensin. But on

the suprarenal medulla both peptides were shown by Staszewska-Barczak & Vane (1965) to be potent releasers of catechol amines; in fact bradykinin was more potent than in cats. In rabbits, both peptides were weak ganglion stimulants; on the suprarenal glands only bradykinin has so far been examined and found to be active in releasing catechol amines (Lecomte, Troquet & Dresse, 1961; Lewis & Nustad, 1965), but from the results available its potency cannot be ascertained.

Stimulation of ganglion cells is not a property of peptides in general. Although it was obtained with the analogues of angiotensin and bradykinin, of the four other peptides examined, oxytocin, vasopressin, substance P and eledoisin, only oxytocin had such an action and this, like some of its other actions (Bisset, 1963), was followed by pronounced and prolonged tachyphylaxis. The failure to obtain stimulation by substance P agrees with a similar finding by Beleslin, Radmanović & Varagić (1960). They, however, observed changes in sensitivity to preganglionic stimulation, a potentiation after injection of small and a depression after injection of larger doses of substance P. Such changes in sensitivity were not observed in the present experiments. This difference may be due to the use of different preparations of substance P, none of which was pure.

On the suprarenal medulla of cats, all four peptides are inactive, as shown first for oxytocin, vasopressin and substance P (Feldberg & Lewis, 1964) and later for eledoisin (Staszewska-Barczak & Vane, 1965); this has been confirmed in the present experiments. In dogs, however, eledoisin was shown by Staszewska-Barczak & Vane (1965) to release medullary catechol amines. This provides another instance where the actions of peptides in one species need not apply in another.

In our previous paper (Lewis & Reit, 1965), the delayed secondary response of the nicitating membrane which occurs occasionally on unclamping the external carotid artery after retrograde injection of bradykinin to the ganglion was shown to be neither a ganglionic response nor a direct action of bradykinin on the membrane. It was suggested that it " 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," and it was stated that this substance might be adrenaline. The present finding that phentolamine, which abolished the contraction of the nictitating membrane produced by adrenaline, did not affect the delayed secondary response excludes adrenaline as the mediator for this response. The well-known sensitivity of the nictitating membrane to 5-hydroxytryptamine as well as the fact that 5-hydroxytryptamine has been found in the superior cervical ganglion of the rat (Eränko & Härkönen, 1965) raise the question whether local release of this amine may not be responsible for the delayed secondary response.

SUMMARY

1. Further studies of the ganglionic effects of angiotensin and bradykinin have been carried out and a comparison has been made with their actions on the suprarenal medulla.

2. Angiotensin, bradykinin and three analogues of each were tested for potency as stimulants of the superior cervical ganglion of the cat; the peptides in each group had relative activities closely parallel to those on the suprarenal medulla and on vascular

smooth muscle. Angiotensin and bradykinin, which act on different receptors, each has similar receptors at the three sites.

3. The stimulating action of 5-hydroxytryptamine on the ganglion and the suprarenal medulla did not influence the stimulating action of the peptides on these structures. The stimulating action of histamine on the suprarenal medulla did not interfere with that of the peptides ; it is known that, at the ganglion, tachyphylaxis by histamine extends to angiotensin.

4. On a molar basis, at the ganglion and suprarenal medulla, angiotensin was about 70 to 100 times and bradykinin 5 to 10 times more active than histamine. At the suprarenal glands, angiotensin was about 500 times and bradykinin about 50 times more active than 5-hydroxytryptamine, whilst at the ganglion angiotensin was only about 15 to 60 times and bradykinin at most 6 times more active than this amine.

5. Morphine, 0.1 to 1 μg injected intra-arterially to the ganglion or 10 μg/kg intravenously, blocked the ganglion-stimulating actions of angiotensin and bradykinin. Yet, 100 μg/kg intravenously did not alter the vascular effects of the peptides and caused only a small reduction in their ability to release medullary catechol amines.

6. During the depolarization phase of the nicotine-induced ganglion block the ganglion was insensitive to angiotensin and bradykinin, but during the competitive phase when the nicotinic receptors for acetylcholine were blocked the stimulating action of the peptides was enhanced. In this respect angiotensin and bradykinin resemble other non-nicotinic ganglion stimulants.

7. A burst of supramaximal preganglionic stimuli at high frequency produced a long-lasting enhancement of the ganglion-stimulating effects of angiotensin and bradykinin.

8. Ganglia perfused with Locke solution were as sensitive to angiotensin and bradykinin as ganglia with their circulation intact.

9. The ability of angiotensin and bradykinin and several of their structural analogues to stimulate the superior cervical ganglion and suprarenal medulla of the cat was not shared by vasopressin, substance P and eledoisin. Oxytocin on the other hand stimulated the ganglion, although it does not release catechol amines from the suprarenal medulla.

10. The occasional delayed secondary response of the nictitating membrane on unclamping the external carotid artery after retrograde injections of bradykinin to the ganglion is not due to a local release of adrenaline since it was not affected by phentolamine. It appears to depend upon the ganglion-stimulating action of bradykinin, because when this action was gradually reduced by tachyphylaxis there was a corresponding reduction in the secondary response.

11. Bradykinin had a weak stimulating action on the superior cervical ganglia in the dog and the rabbit. In the rabbit, angiotensin had a similar weak action but, in the few experiments on the dog, it was inactive.

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REFERENCES

BELESLIN, D., RADMANOVIĆ, B. & VARAGIĆ, V. (1960). The effect of Substance P on the superior cervical ganglion of the cat. *Brit. J. Pharmacol.*, **15**, 10–13.

BIRKS, R. I. & MACINTOSH, F. C. (1961). Acetylcholine metabolism of a sympathetic ganglion. *Canad. J. Biochem.*, **39**, 787–827.

BISSET, G. W. 1963. The effect on milk-ejecting activity of modifying two functional groups in oxytocin. In *Oxytocin, Vasopressin and Their Structural Analogues, Proc. 2nd Int. Pharmacol. Meeting*, pp. 21–30. London: Pergamon.

BURN, J. H. (1952). *Practical Pharmacology.* Oxford : Blackwell.

ERÄNKO, O. HÄRKÖNEN, M. (1965). Monoamine-containing small cells in the superior cervical ganglion of the rat and an organ composed of them. *Acta physiol. scand.*, **63**, 511–512.

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

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

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

FELDBERG, W. & GADDUM, J. H. (1934). The chemical transmitter at synapses in a sympathetic ganglion. *J. Physiol. (Lond.)*, **81**, 305–319.

FELDBERG, W. & MINZ, B. (1931). Die Wirkung von Azetylcholine auf die Nebennieren. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **163**, 66–96.

JONES, A. (1963). Ganglionic actions of muscarinic substances. *J. Pharmacol. exp. Ther.*, **141**, 195–205.

KIBJAKOW, A. W. (1933). Uber humorale Ubertragung der Erregung von einem Neuron auf das andere. *Pflügers Arch. ges. Physiol.*, **232**, 432–443.

LECOMTE, J., TROQUET, J. & DRESSE, A. (1961). Stimulation de la medullosurrenalienne par la bradykinine. *Arch. int. Physiol.*, **69**, 89–91.

LEWIS, G. P. & NUSTAD, K. (1965). Bradykinin, release of adrenaline and blood flow. *J. Physiol. (Lond.)*, **179**, 36–37*P*.

LEWIS, G. P. & REIT, E. (1964). Stimulation of the superior cervical ganglion of the cat by angiotensin and bradykinin. *J. Physiol. (Lond.)*, **176**, 28*P*.

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

PATON, W. D. M. & PERRY, W. L. M. (1953). The relationship between depolarization and block in the cat's superior cervical ganglion. *J. Physiol. (Lond.)*, **119**, 439-454.

PERRY, W. L. M. (1953). Acetylcholine release in the cat's superior cervical ganglion. *J. Physiol. (Lond.)*, **119**, 439–454.

STASZEWSKA-BARCZAK, J. & VANE, J. R. (1965.) The release of catecholamines from the adrenal medulla by peptides. *J. Physiol. (Lond.)*, **177**, 57*P*.

THOMPSON, J. W. (1955). The cat nictitating membrane as an isolated preparation. *J. Physiol. (Lond.)*, **129**, 70*P*.

TRENDELENBURG, U. (1954). The action of histamine and pilocarpine on the superior cervical ganglion and the adrenal glands of the cat. *Brit. J. Pharmacol.*, **9**, 481–487.

TRENDELENBURG, U. (1956a). Modification of transmission through the superior cervical ganglion of the cat. *J. Physiol. (Lond.)*, **132**, 529–451.

TRENDELENBURG, U. (1956b). The action of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. *Brit. J. Pharmacol.*, **11**, 74–80.

TRENDELENBURG, U. (1957a). The action of morphine on the superior cervical ganglion and on the nictitating membrane of the cat. *Brit. J. Pharmacol.*, **12**, 79–85.

TRENDELENBURG, U. (1957b). Reaktion sympathischer Ganglien wahrend der Ganglienblockade durch Nicotin. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **230**, 448–456.

TRENDELENBURG, U. (1959). Non-nicotinic ganglion-stimulating substances. *Fed. Proc.*, **18**, 1001–1005.

TRENDELENBURG, U. & JONES, A. (1965). Facilitation of ganglionic responses after a period of preganglionic stimulation. *J. Pharmacol. exp. Ther.*, **147**, 330–335.

VOGT, M. (1965). Release of medullary amines from the isolated perfused adrenal gland of the dog. *Brit. J. Pharmacol.*, **24**, 561–565.

VOLLE, R. L. (1962). Enhancement of postganglionic responses to stimulating agents following repetitive preganglionic stimulation. *J. Pharmacol. exp. Ther.*, **136**, 68–74.