Brit. J. Pharmacol. (1964), **22**, 193–203.

SECRETORY RESPONSES OF EXTRAMEDULLARY CHROMAFFIN TISSUE

BY

E. MUSCHOLL* and MARTHE VOGT

From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

(*Received October* 21, 1963)

When the isolated inferior mesenteric ganglion of the dog was perfused with Locke solution, the effluent fluid contained noradrenaline and adrenaline. At rest the output of noradrenaline ranged from 0.7 to 40 ng/min, and that of adrenaline from 0.7 to 25 ng/min. Electrical stimulation of the inferior splanchnic nerves did not alter the catechol amine content of the perfusate, and stimulation of the ascending mesenteric nerves caused an increase to little more than twice the resting value. When acetylcholine or dimethylphenylpiperazinium iodide was infused into the preparation, the catechol amine content of the effluent fluid was 6- to 100-times the resting level. Two drugs with muscarinic action (pilocarpine and 3-acetoxy-1-benzyl-1-methylpyrrolidinium bromide) produced only very small increases in catechol amine release. Angiotensin, in doses ranging from 20 ng to 2 μg, inhibited release of catechol amines, and bradykinin had a weak stimulatory effect. It is suggested that the catechol amines released into the effluent fluid were derived from chromaffin tissue in the ganglion ; evidence for innervation of this tissue was unobtainable, but its catechol amines were readily released by the injection of nicotinic drugs.

Kohn (1903) made a detailed study of mammalian extramedullary chromaffin tissue (paraganglia) and demonstrated its ubiquity in sympathetic ganglia and plexuses, but was not convinced that it was innervated. Zuckerkandl (1901) did not find any evidence of innervation of the large paraganglion which bears his name. Yet Pines (1924) claimed that by staining with methylene blue he could demonstrate the innervation of the paraganglia by sympathetic fibres. Iwanow (1932) reinvestigated the question and stated that any nerve supply present was sparse, inconstant and quite different from the rich innervation of adrenal medullary cells. Physiologists, on the other hand, have recently inclined to the view that preganglionic fibres to sympathetic ganglia innervate ganglion cells and chromaffin cells (see Eccles & Libet, 1961), and it seemed of interest to try to obtain physiological evidence that this was so.

The inferior mesenteric ganglion of the dog was chosen for this purpose. Like all prevertebral sympathetic ganglia it contains a large number of paraganglia, and it should therefore be possible to perfuse the ganglion and to determine the release of substantial amounts of catechol amines on stimulation of innervating nerves. The superior cervical ganglia, as indeed all paravertebral ganglia, are not suited for this purpose since they contain only a few scattered chromaffin cells.

* Present address : Pharmakologisches Institut der Universität Mainz, Germany.

METHODS

Operative techniques. After a single experiment on an adult dog, puppies were exclusively used for the perfusion because their tissues were not so tough and required less handling and dissection to obtain a preparation which was suitable for perfusion. Puppies of either sex and weighing usually between 6 and 8 kg were anaesthetized with ether followed by chloralose (75 mg/kg). Artificial ventilation was often required towards the end of the dissection. The dog was eviscerated and the stump of the inferior mesenteric artery was left long. The ganglion and its nerves get their arterial supply from this artery and from the aorta ; the venous drainage runs into the inferior vena cava. On both sides a piece of ureter, about 6 cm long, was carefully excised, all small vessels in the opened peritoneal fold being tied with fine ligatures. The ureters cover the region of the inferior mesenteric ganglion ; an attempt to include a length of the ureters in the perfused tissue, though it saved time, yielded a preparation both with a poor rate of flow and which became extremely oedematous. A piece of aorta and a length of vena cava were then isolated from the rest of the body by cutting all those vascular branches which did not run in the direction of the ganglion ; the isolated segments extended distally about 1 cm beyond the iliac bifurcations, and orally to a line which crossed the ascending mesenteric nerves about 1.5 cm above the apex of the ganglion. Depending on the purpose of the experiment, the piece of the organ of Zuckerkandl enclosed in the mesh of the ascending mesenteric nerves (see Fig. 1) was either included in the perfused tissue or dissected out. It was important to interfere as little as possible with the tissue between aorta and vena cava so as not to impair the venous drainage.

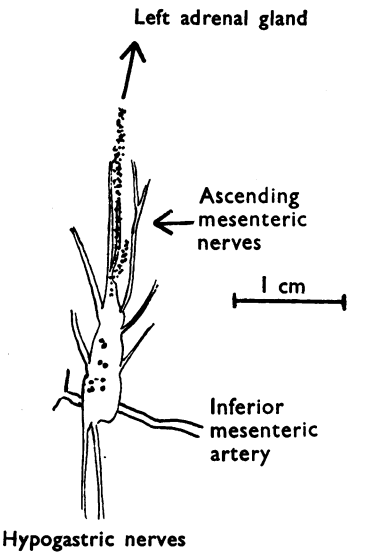

Fig. 1. Schematic drawing on the inferior mesenteric ganglion of the dog. Chromaffin tissue is stippled. The inferior splanchnic nerves are represented by two pairs of bundles entering the lateral borders of the ganglion.

Perfusion was carried out by gravity. Modified Locke solution (concentrations in g/l. : NaCl 8.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.3, glucose 1.0 and ascorbic acid 0.01) was carefully filtered into a Mariotte bottle. From there the solution was fed into a vertical Liebig condenser of 100 ml. capacity, through the jacket of which hot water circulated, keeping the Locke solution at a temperature of 40 to 42° C. A mixture of 95% oxygen with 5% carbon dioxide was bubbled through the whole length of the fluid in the condenser. The effective perfusion pressure ranged from 70 to 75 cm of water. Drugs were injected from a 0.25 ml. syringe connected to a length of fine-bore polyethylene tubing threaded through the Locke solution in the condenser and ending in the perfusion cannula. Immedi-

iately before infusion, the Locke solution in the polyethylene tubing (dead space, 0.18 ml.) was replaced by the drug solution, and the infusion of the required volume, usually 0.2 ml., was carried out over a period of 8 or 10 min.

To start the perfusion, the aorta was cut between ligatures just below the bifurcation, 1,500 U of heparin were injected into the dog and 500 U of heparin were dissolved in the warm perfusion fluid in the condenser. A polyethylene cannula connected to the perfusion apparatus by 75 cm of narrow-bore silicone-rubber tubing was filled with the heparinized Locke solution, and tied into the inferior mesenteric artery. The upper end of the aorta was then cut between ligatures, the isolated segment of the vena cava cut out, and the preparation, consisting of a piece of aorta and vena cava, the inferior mesenteric artery and the ganglionic tissue with all its branches, was transferred to a small polyethylene platform wedged into a funnel. The distance between the condenser in which the perfusion fluid was warmed and the arterial cannula was shortened to about 10 cm by removing the tubing between cannula and condenser ; as soon as the warmer fluid reached the preparation, perfusion rate increased and blood and Locke solution were seen to escape from small veins entering the vena cava which had been slit open. The funnel was covered with a sheet of parafilm, a warming lamp directed towards it, and a period of about 10 min allowed for washing out some of the blood and for the temperature in the system to stabilize. The temperature of the effluent fluid from the funnel varied between 25 and 31° C in different preparations. At the inflow side the temperature must have been higher. The temperature was considered adequate since Dolivo & Larrabee (1958) have reported that oxygen consumption elicited by activity in the rat superior cervical ganglion is little affected by temperature changes between 23 and 36° C.

Electrical stimulation. Up to three pairs of platinum electrodes were used to stimulate the inferior splanchnic and ascending mesenteric nerves. They were left in place during the whole perfusion. Rectangular pulses of 1 msec duration at a frequency of 15 shocks/sec were used at voltages ranging from 6 to 18 V. The current strength flowing through the electrodes was checked in every experiment with the help of an oscilloscope ; it ranged from 3 mA for weak to 8 mA for strong stimuli. The duration of stimulation was from 5 to 12 min.

Estimation of catechol amines. The effluent fluid was collected in ice-cooled measuring cylinders ; it always contained some blood. N-hydrochloric acid (0.1 ml.) was added to each 10 ml. collected.

In order to denature the proteins, each sample of effluent fluid (usually 6 to 15 ml.) was washed into a round-bottom flask with two volumes of absolute ethanol and the mixture was evaporated to dryness *in vacuo* (50° C bath temperature). Further treatment was as described previously for blood (Vogt, 1952) ; it included paper-chromatographic separation of the amines in a phenol-hydrochloric acid mixture, elution and bioassay on the pithed rat. The bioassay of both amines was done on this preparation, which can be rendered sensitive to as little as 0.5 ng of adrenaline by injection of 1.5 mg/kg of pronethalol (Vanov & Vogt, 1963). The extracts were always applied to paper for chromatography on the day of the experiment, and the assays were usually carried out on the following day. The dried eluates can, however, be kept at −17° C for a few days without loss of activity.

Bioassay was essential for some of the experiments, in which the basal secretion was so low that fluorimetry would not have been applicable. It required, however, a number of controls when drugs were used to stimulate secretion. Tests for possible interference were made on the assumption that the drug would have travelled in the chromatogram to the region of either of the amines. The effects of pilocarpine, 3-acetoxy-1-benzyl-1-methylpyrrolidinium bromide (AHR 602) and acetylcholine were abolished by atropine (10 mg/kg), which was given subcutaneously to all rats before pithing ; hexamethonium bromide was injected intravenously when samples were assayed which might contain dimethylphenylpiperazinium. The interference of tyramine was mainly excluded by its R_F value. However, any effect of a small contamination of the adrenaline region of the chromatogram was ruled out by carrying out the assay on a rat rendered insensitive to tyramine by injections of reserpine (2 mg/kg subcutaneously on 2 days before the assay). The polypeptides travelled near the front of the chromatogram. A small contamination of the adrenaline region by a "tail" of angiotensin was conceivable and might have led to an overestimate of the adrenaline in the effluent fluid in the experiment with the highest dose (2μg). Since, however, the amount of pressor substance assayed in the

adrenaline region during infusion of angiotensin was much less, and not more, than that in the preceding control sample, any contamination by angiotensin would not have affected the conclusion about an inhibitory action of this compound on release of catechol amines.

Drugs. These were acetylcholine hydrochloride, dimethylphenylpiperazinium iodide, valine$_5$-hypertensin II aspartic-β-amide (Ciba), pilocarpine hydrochloride, histamine hydrogen phosphate, tyramine hydrochloride, 3-acetoxy-1-benzyl-1-methylpyrrolidinium bromide (AHR 602, A. H. Robins, Richmond, Virginia, U.S.A.) and synthetic bradykinin (Parke Davis). The drugs were dissolved in Locke solution from which the sodium bicarbonate, the glucose and the ascorbic acid had been omitted. The doses are expressed in weights of the salts, except for histamine which is expressed as weight of the base. The amounts of noradrenaline and adrenaline estimated in eluates are expressed in terms of the bases.

RESULTS

Resting secretion. The effluent fluid of the perfused inferior mesenteric ganglion always contained some noradrenaline and adrenaline, but the amounts varied greatly in different preparations. The highest secretion, 40 ng/min of noradrenaline and 25 ng/min of adrenaline, was in an experiment in which a large piece of the organ of Zuckerkandl was included in the tissue excised for perfusion. In the remaining experiments there was no relation between the resting secretion and the amount of chromaffin tissue found by dissection after the experiment. The lowest secretion was 0.67 ng/min of each amine by a preparation which produced large increases on perfusion with drugs (see Fig. 2).

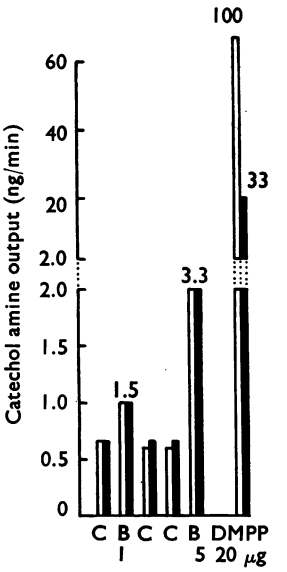

Fig. 2. Catechol amine output into effluent fluid of a perfused inferior mesenteric ganglion. Samples were collected for 10 min and one-half of each effluent sample was extracted and assayed. There were intervals of 2 min between collection of samples 1 and 2 and of samples 4 and 5. Empty columns: adrenaline; filled columns: noradrenaline. Ordinate in ng/min; in the upper half of the figure the scale is contracted 33-fold. C=control; B=bradykinin; DMPP=dimethyl-phenylpiperazinium iodide. The figures above the columns represent the factors by which release has risen above control period; those below the drug abbreviations represent doses in μg.

The percentage of adrenaline (percentage methylated) in the mixture of noradrenaline and adrenaline amounted to about 50% in most preparations, though it was occasionally as low as 12 and as high as 63%. Previous analyses of the ganglia themselves had shown a very variable proportion of adrenaline (Vanov & Vogt, 1963), and this result is readily explained by the variability in the amount of chromaffin tissue to be found on histological examination of the ganglia. When there is little chromaffin tissue, noradrenaline contributed by the ganglion cells and their axons is mainly responsible for the catechol amines of the ganglion ; when chromaffin tissue is abundant, its catechol amines will mask the contribution by the neurones. This effect is illustrated by an experiment in which a piece of the organ of Zuckerkandl was excised from a dog after its inferior mesenteric ganglion had been set up for perfusion. The concentration of amine in the excised tissue was extremely high (noradrenaline, 1.4 mg/g ; adrenaline, 2.7 mg/g ; 65% methylated) ; since the concentration in ganglionic tissue proper, as judged from superior cervical ganglia in which chromaffin cells are scarce, is only of the order of 10 μg/g (28% methylated) it is clear that the catechol amine content of inferior mesenteric ganglia is largely determined by the amount of chromaffin tissue present.

It is likely that in these experiments the amines in the effluent fluid were mainly derived from chromaffin cells. Some direct evidence was obtained in the experiment referred to earlier in which a piece of chromaffin tissue was found to contain a mixture of amines of which 65% were methylated ; in the corresponding effluent fluid the value was 63%. The question remains whether in those experiments in which the percentage methylation of the effluent fluid was low there was also less adrenaline in the chromaffin tissue, or whether some of the noradrenaline in the effluent fluid was ganglionic in origin.

In newborn infants extramedullary and medullary chromaffin tissues exhibit the same percentage methylation (West, Shepherd, Hunter & Macgregor, 1953). There are no values available of the percentage methylation in the adrenal medulla of puppies ; for adult anaesthetized dogs it lies between 62 and 80% (mean 74%, Lund, 1951). It may well be lower in puppies and cover the range observed in the effluent fluid from the inferior mesenteric ganglion.

Rate of flow through the perfused tissue varied from 0.5 to 3.5 ml./min ; the rate of the resting secretion bore no relation to the rate of flow, which must, to a large extent, have depended on the amount of extraneous tissue included in the preparation. There was, however, sometimes a tendency for the flow to slow down, and for resting amine release to diminish, in the course of an experiment, and oedema was always very obvious at the end. Perfusion time was not extended for longer than 90 min ; this limitation was, however, not imposed by the viability of the preparation, but by the number of samples which could be analysed on one day.

Electrical stimulation. The arrangement of the nerves entering and leaving the inferior mesenteric ganglion is very variable, but follows the pattern illustrated in Fig. 1. The nomenclature is that proposed by Langley & Anderson (1896). A number of preganglionic nerve strands ("inferior splanchnic nerves") which stem from the lumbar sympathetic chains enter the ganglion from both sides. From the upper pole of the ganglion there originates a plexus, the "ascending mesenteric nerves" ; it

TABLE 1

OUTPUT OF CATECHOL AMINES FROM THE PERFUSED INFERIOR MESENTERIC GANGLION OF THE DOG IN FIVE EXPERIMENTS

The figures represent (left, noradrenaline ; right, adrenaline) catechol amine release (ng/min) into samples of effluent fluid collected for periods of 10 min. Intervals of 2 to 10 min were allowed between some of the collections. All experiments were terminated by an injection of dimethylphenylpiperazinium iodide (DMPP) in order to test the viability of the preparation. Results are given in the sequence in which they were performed for each experiment. Values in parentheses refer to parameters of stimulation and doses of drugs. Ach = acetycholine ; AHR 602 = 3-acetoxy-1-benzyl-1-methylpyrrolidinium bromide

Expt.	Controls		Nerves stimulated: Inferior splanchnic	Ascending mesenteric	All		Control	Ach or AHR 602		Controls		DMPP
1	3·0, 3·5	3·0, 3·5			3·0, 3·5 (10V, 4mA)	3·0, 3·5 (18V, 8mA)					2·3, 2·7	17·5, 13·8 (20 μg)
2			4·5, 3·5 (15V, 7mA)	9·0, 7·6 (15V, 7mA)	8·0, 7·0 (15V, 7mA)						4·0, 3·0	27·0, 17·0 (20 μg)
3	1·5, 0·4			2·5, 0·5 (18V, 7mA)							5·0, 0·7	69·0, 8·3 (20 μg)
4	6·0, 4·0		6·0, 4·0 (14V, 8mA)	9.0, 6·0 (14V, 6mA)			5·0, 3·5	30·0, 14·0 (Ach, 24 μg)	Lost, 6·0 (Ach, 100 μg)		2·8, 2·0	17·5, 5·0 (20 μg)
5							7·0, 6·0	14·0, 8·0 (AHR 602, 2 mg)		6·5, 8·0	3·7, 2·8	60·0, 45·0 (20 μg)

anastomoses with fibres from the solar plexus, and in its meshes is embedded a single or double chromaffin strand, the organ of Zuckerkandl. The lower pole of this organ ends a little above the ganglion, and its upper end often stretches to the left adrenal medulla. It is usually visible in the living dog because of its pinkish colour, which distinguishes it from the surrounding whitish nerve fibres.

The inferior splanchnic nerves were stimulated on their own six times, and in no instance was there convincing evidence of release of catechol amines. When, however, the ascending mesenteric nerves were stimulated, a rise in catechol amine content of the effluent fluid occurred in three of five experiments (examples Table 1, Nos. 2, 3 and 4) ; the increase ranged from 50 to just over 100% of the basal concentration. The same increase happened in four of five experiments in which all the nerves were stimulated simultaneously. Usually the two amines increased to the same extent, but the adrenaline content twice remained stationary when the noradrenaline content rose. It appears that a small rise in catechol amine content of the effluent fluid may occur whenever the ascending mesenteric nerves are stimulated ; since these nerves form a network around the distal end of the organ of Zuckerkandl, stimulation of the plexus amounts to placing a strand of chromaffin tissue directly on the electrodes. Vasomotor effects of nerve stimulation were negligible.

Action of Drugs. Of the drugs tried two had nicotinic and two muscarinic actions, and two were polypeptides. In addition, tyramine and histamine were tested once. All drugs were given by slow infusion into the cannula during the whole or the greater part of a single collection of effluent fluid.

A large release of catechol amines was obtained with the nicotine-like drugs acetylcholine and dimethylphenylpiperazinium (Table 1). Acetylcholine was used in doses of 12, 24 and 100 μg ; the largest effect was obtained with 24 μg, whereas 100 μg caused some vasoconstriction and a smaller release. Dimethylphenylpiperazinium (10 or 20 μg) was at least as potent a stimulus to secretion of catechol amines as was acetylcholine ; it was henceforth used to test whether the perfusion was satisfactory and the preparation alive by injecting it during the collection of a last sample at the end of each experiment. Both acetylcholine and dimethylphenylpiperazinium usually caused a fall in the percentage of amine which was methylated, but after a dose of dimethylphenylpiperazinium both amines twice rose by the same factor.

The next compounds tested, pilocarpine and AHR 602, belong to a group of drugs which stimulate muscarinic receptors in sympathetic ganglia. Their action is not abolished by hexamethonium but is by low doses of atropine. In contrast to the nicotinic drugs, they have very little stimulating action on the adrenal medulla (Root, 1951 ; Trendelenburg, 1955 ; Jones, Gomez Alonso de la Sierra & Trendelenburg, 1963). They are pressor in the spinal cat, and 30 μg of pilocarpine are about equipressor with 80 μg of AHR 602. The two drugs should have little activity in this preparation if the amines in the perfusate are exclusively produced by the chromaffin cells.

The injection of 10 μg of pilocarpine caused a very small increase in the catechol amine content of the perfusate, but 50 μg reduced the release to one-third of the

basal secretion without in any way diminishing the flow. The change was irreversible, and subsequent infusions of acetylcholine (24 μg) or histamine (2.5 and 10 μg) failed to cause any release of catechol amines.

AHR 602, the pharmacology of which was first described by Franko, Ward & Alphin (1963), was chosen because it is an even weaker stimulant of the adrenal medulla than is pilocarpine (Jones *et al.*, 1963). It was infused in doses of 0.1, 1.0 and 2.0 mg. There was a small increase, at most a doubling, of the catechol amine content of the effluent fluid, and this effect was not dependent on dose. There was not, as with pilocarpine, damage to the preparation, which remained fully responsive to dimethylphenylpiperazinium (Table 1, expt. 5).

In view of the demonstration by Feldberg & Lewis (1963) that angiotensin and bradykinin are very active in releasing amines from the adrenal medulla, these polypeptides were infused into the inferior mesenteric ganglion. Angiotensin (2.0 and 0.1 μg, tested on two different preparations) reduced the flow through the tissue and the catechol amine content of the effluent fluid. Even after 0.1 μg of angiotensin, dimethylphenylpiperazinium had nearly lost its stimulating effect. In another experiment, the dose was further lowered to 20 ng ; flow was reduced by 20%, catechol amine release was diminished, but a subsequent dose of dimethylphenylpiperazinium was fully active. The preparation was obviously not damaged, but angiotensin failed to release any catechol amines. Bradykinin was then tried. Though it is less potent as a releasing agent of medullary amines than is angiotensin, its vasodilator properties ensure that lack of stimulating action cannot be caused by damage through vasoconstriction. Fig. 2 illustrates the results : 1 μg of bradykinin caused a just perceptible rise, and 5 μg a trebling of the catechol amine output into the perfusate ; these are high doses, 50 ng producing a pressor effect when injected into the cat adrenal medulla (Feldberg & Lewis, 1963). How weak an effect the action of even 5 μg of bradykinin represents is obvious from the response to a subsequent dose of dimethylphenylpiperazinium which increased the noradrenaline release 100-fold, and the adrenaline release 33-fold.

Tyramine releases catechol amines from sympathetic nerve endings in the perfused heart (Lindmar & Muscholl, 1961) ; though it is less effective than dimethylphenylpiperazinium, 10 μg releases about a quarter of the quantities of noradrenaline liberated by 10 μg of dimethylphenylpiperazinium. Tyramine (80 μg) was therefore infused into the inferior mesenteric ganglion, but no catechol amines were liberated, though this preparation has previously responded to 12 μg of acetylcholine and, at the end of the experiment, 10 μg of dimethylphenylpiperazinium increased by 650% the noradrenaline released into the effluent fluid.

DISCUSSION

Stimulation of the inferior splanchnic nerves did not release catechol amines from chromaffin tissue inside the inferior mesenteric ganglion. Yet the tissue was viable, as shown by its response to dimethylphenylpiperazinium, and it is difficult to escape the conclusion that the preganglionic fibres do not innervate the chromaffin cells. A small release of amines did, however, occur when the lower tip of the organ of

Zuckerkandl, enclosed in the meshes of the ascending mesenteric nerves, was placed on the electrodes and stimulated. On the assumption that chromaffin cells are not excitable electrically (Rosenblueth & Cannon, 1934), there are two likely explanations for this observation : either fibres innervating the organ of Zuckerkandl run in the ascending mesenteric nerves, but, if this is so, it is surprising that the effect was so small ; or else some cholinergic fibres coursing in the plexus but supplying structures other than the paraganglion released acetylcholine, some of which diffused to the chromaffin cells and liberated small amounts of catechol amines. The results suggest that the innervation of extramedullary chromaffin tissue is either nonexistent or sparse, thus confirming Iwanow's (1932) views.

Bülbring (1944) and Reinert (1963) have found small quantities of catechol amines in the perfusate of the superior cervical ganglion of the cat. In Bülbring's experiments, stimulation of the preganglionic fibres increased the concentration of what was then, before noradrenaline had been identified as the main adrenergic transmitter, considered to be adrenaline. Reinert, who estimated noradrenaline in the effluent fluid, found concentrations of the same order as those reported by Bülbring, but only occasionally observed an increase on orthodromic or antidromic stimulation. Four sources of the catechol amines in the perfusate have to be considered : vasomotor fibres, the sympathetic ganglion cells and their axons, cells of the carotid body and chromaffin cells. Vasomotor fibres may be excluded as a major source since Reinert found no difference in noradrenaline production between normal and chronically denervated superior cervical ganglia. Reinert considered the ganglion cells and their axons to be the source of the noradrenaline, but did not exclude another possible source, the carotid body. Chungcharoen, Daly & Schweitzer (1952) have shown that the preparation of the superior cervical ganglion unavoidably includes the carotid body in the irrigated area. The glomus cells contain noradrenaline (Muscholl, Rahn & Watzka, 1960) ; in the glomus of most species there are also chromaffin cells which presumably contain adrenaline as well as noradrenaline. Thus the carotid body is undoubtedly a potential source of catechol amines in the perfused superior cervical ganglion preparation of the cat. Chromaffin cells are very infrequent in the cat superior cervical ganglion. This is also obvious from its very low content of adrenaline (Muscholl & Vogt, 1958). In spite of that they are a possible source for the noradrenaline in the perfusate of a superior cervical ganglion.

Our results suggest that the ganglion cells and their axons are not the source of catechol amines in the perfusate of a sympathetic ganglion. This view is based on the following findings :

1. The proportion of adrenaline in the catechol amine mixture was usually high and in the experiment in which this percentage was also estimated in the chromaffin tissue, this proved to be identical with that of the perfusate.

2. Drugs (pilocarpine and AHR 602) which stimulate "muscarine receptors" in the ganglion but have little action on adrenal medullary tissue only slightly increased the amine content of the effluent fluid. If the nonstimulated perfused ganglion cells gave off noradrenaline into the vessels draining it, one would expect this process to be enhanced during stimulation, yet electrical stimulation of the preganglionic fibres

did not increase the noradrenaline released into the effluent fluid of the inferior mesenteric ganglion, and the small effect produced by the two muscarinic drugs appears to have been exerted on the chromaffin tissue since adrenaline was released with the noradrenaline.

It is perhaps a general rule that the stores of catechol amines are more labile in chromaffin tissue than in adrenergic neurones : granules prepared from the adrenal medulla release catechol amines on incubation with calcium ions, whereas no such release occurs in granules prepared from splenic nerves or stellate ganglia (Schümann & Philippu, 1963).

If we accept the view that the catechol amines in the effluent fluid originated from the chromaffin tissue, it would appear that within this tissue chemical stimuli frequently release proportionately more noradrenaline than adrenaline.

It would be of interest to know whether the differences found in the responses to drugs of extramedullary and medullary chromaffin tissues are due to genuine differences in the reactivity of the cells or are a consequence of the artificial conditions of the perfusion. In the brisk responses to acetylcholine and dimethylphenylpiperazinium, and in the poor responses to the specific ganglion stimulants pilocarpine and AHR 602, the perfused tissue behaved exactly like an adrenal gland. There was, however, surprising lack of reactivity to angiotensin and bradykinin ; the difference cannot be connected with the fact that the adrenal medulla is innervated and the paraganglia seem to lack a nerve supply : the chronically denervated adrenal gland responds normally not only to acetylcholine (Siehe, 1934) but also to the two polypeptides (Feldberg & Lewis, personal communication), which had little or no releasing action in the inferior mesenteric ganglion. Further work will have to establish whether this is a genuine physiological difference.

There are a few observations which suggest that the paraganglia may respond to stimuli by releasing adrenaline *in vivo*. Thus in adrenalectomized patients (Birke, Euler & Ström, 1959) some adrenaline is excreted in the urine and this excretion rises after muscular exercise. This adrenaline may come from paraganglia (though these are said to be atrophic in adult man) or from sympathetic nerve endings, if Peacock, Shaldon, Tyler & Badrick (1962) are right in their assumption that in man the adrenergic transmitter contains a high proportion of adrenaline. If the paraganglia are not innervated and yet play a physiological role, their activity should be controlled by some humoral stimulus, but the present experiments give no guidance as to the nature of the stimulus.

We wish to thank Mr J. E. McEwen for his expert technical assistance, Dr B. V. Franko (A. H. Robins Co.) for a gift of AHR 602, Dr. C. D. Falconer (Ciba Laboratories, Horsham) for angiotensin, Dr A. Spinks (I.C.I.) for pronethalol and Parke Davis for dimethylphenylpiperazinium iodide. This work was supported in part by the Air Force Office of Scientific Research, OAR, through the European Office, Aerospace Research, U.S.A.F. Our thanks are also due to the Deutsche Forschungsgemeinschaft for a grant (to E.M.) towards travel expenses.

REFERENCES

BIRKE, G., EULER, U. S. VON & STRÖM, G. (1959). Observations on some dimensions and functions of the circulatory system after adrenalectomy in breast cancer and diabetes. *Acta med. scand.*, **164**, 219–229.

BÜLBRING, E. (1944). The action of adrenaline on transmission in the superior cervical ganglion. *J. Physiol. (Lond.)*, **103**, 55–67.

CHUNGCHAROEN, D., DALY, M. DE BURGH & SCHWEITZER, A. (1952). The blood supply of the superior cervical sympathetic and the nodose ganglia in cats, dogs and rabbits. *J. Physiol. (Lond.)*, **118**, 528–536.

DOLIVO, M. & LARRABEE, M. G. (1958). Metabolism of glucose and oxygen in a mammalian sympathetic ganglion at reduced temperature and varied pH. *J. Neurochem.*, **3**, 72–88.

ECCLES, R. M. & LIBET, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. *J. Physiol. (Lond.)*, **157**, 484–503.

FELDBERG, W. & LEWIS, G. P. (1963). Release of adrenaline from cat's suprarenals by bradykinin and angiotensin. *J. Physiol. (Lond.)*, **167**, 46–47*P*.

FRANKO, B. V., WARD, J. W. & ALPHIN, R. S. (1963). Pharmacologic studies of N-benzyl-3-pyrrolidyl acetate methobromide (AHR 602), a ganglion stimulating agent. *J. Pharmacol. exp. Ther.*, **139**, 25–30.

IWANOW, G. (1932). Das chromaffine und interrenale System des Menschen. *Ergebn. Anat. Entwickl.-Gesch.*, **29**, 87–280.

JONES, A., GOMEZ ALONSO DE LA SIERRA, B. & TRENDELENBURG, U. (1963). The pressor response of the spinal cat to different groups of ganglion-stimulating agents. *J. Pharmacol. exp. Ther.*, **139**, 312–320.

KOHN, A. (1903). Die Paraganglien. *Arch. mikr. Anat.*, **62**, 263–365.

LANGLEY, J. N. & ANDERSON, H. K. (1896). The innervation of the pelvic and adjoining viscera. *J. Physiol. (Lond.)*, **20**, 392–406.

LINDMAR, R. & MUSCHOLL, E. (1961). Die Wirkung von Cocain, Guanethidin, Reserpin, Hexamethonium, Tetracain and Psicain auf die Noradrenalin-Freisetzung aus dem Herzen. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **242**, 214–227.

LUND, A. (1951). Release of adrenaline and noradrenaline from the suprarenal gland. *Acta pharmacol. (Kbh.)*, **7**, 309–320.

MUSCHOLL, E., RAHN, K.-H. & WATZKA, M. (1960). Nachweis von Noradrenalin im *Glomus Caroticum*. *Naturwissenschaften*, **47**, 325.

MUSCHOLL, E. & VOGT, M. (1958). The action of reserpine on the peripheral sympathetic system. *J. Physiol. (Lond.)*, **141**, 132–155.

PEACOCK, J. H., SHALDON, C., TYLER, C. & BADRICK, F. E. (1962). The sympathetic postganglionic neurotransmitter substance in man. *Lancet*, ii, 1077–1080.

PINES, I.-L. J. (1924). Über die Innervation des chromaffinen Gewebes des Sympathicus und über das sympathico-chromaffine System im Allgemeinen. *Arch. Psychiat. Nervenkr.*, **70**, 636–647.

REINERT, H. (1963). Role and origin of noradrenaline in the superior cervical ganglion. *J. Physiol. (Lond.)*, **167**, 18–29.

ROOT, M. (1951). Certain aspects of the vasopressor action of pilocarpine. *J. Pharmacol. exp. Ther.*, **101**, 125–131.

ROSENBLUETH, A. & CANNON, W. B. (1934). Direct electrical stimulation of denervated autonomic effectors. *Amer. J. Physiol.*, **108**, 384–396.

SCHÜMANN, H. J. & PHILIPPU, A. (1963). Die Wirkung von Calcium und Magnesium auf die Brenzcatechinaminfreisetzung aus Speichergranula verschiedener Gewebe. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **245**, 97.

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

TRENDELENBURG, U. (1955). The potentiation of ganglionic transmission by histamine and pilocarpine. *J. Physiol. (Lond.)*, **129**, 337–351.

VANOV, S. & VOGT, M. (1963). Catecholamine-containing structures in the hypogastric nerves of the dog. *J. Physiol. (Lond.)*, **168**, 939–944.

VOGT, M. (1952). The secretion of the denervated adrenal medulla of the cat. *Brit. J. Pharmacol.* **7**, 325–330.

WEST, G. B., SHEPHERD, D. M., HUNTER, R. B. & MACGREGOR, A. (1953). The function of the organs of Zuckerkandl. *Clin. Sci.*, **12**, 317–324.

ZUCKERKANDL, E. (1901). Ueber Nebenorgane des Sympathicus im Retroperitonaealraum des Menschen. *Anat. Anz.*, **19**, Erg. H., 95–107.