Brit. J. Pharmacol. (1965), **24**, 561–565.

RELEASE OF MEDULLARY AMINES FROM THE ISOLATED PERFUSED ADRENAL GLAND OF THE DOG

BY

MARTHE VOGT

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

(*Received September* 18, 1964)

In a previous paper (Muscholl & Vogt, 1964) the release of catechol amines from extramedullary chromaffin tissue had been examined, and certain differences found between the responses of extramedullary chromaffin cells perfused with Locke solution and the well-known reactions of the adrenal medulla *in situ*. Thus electrical stimulation of preganglionic fibres to the ganglion containing the chromaffin tissue did not cause a release of adrenaline or noradrenaline, as does stimulation of the nerve supply to the adrenal medulla. Furthermore, injection into the perfusion fluid of bradykinin or angiotensin had little or no stimulant action on extramedullary, but was known to have powerful action on medullary chromaffin tissue.

The present work was undertaken in order to find out whether these differences were due to the one tissue having been studied while the circulation was intact and the other while perfused with Locke solution, or whether the differences were inherent in the nature of the two tissues. The effect was therefore examined of stimulation of the splanchnic nerves and of the injection of a series of drugs including angiotensin and bradykinin on release of adrenaline and noradrenaline by the dog Locke-perfused isolated adrenal gland.

METHODS

Conditions for the perfused adrenal medulla were made as similar as possible to those used for the perfusion of the extramedullary chromaffin tissue (Muscholl & Vogt, 1964). Puppies (bodyweight 2.2 to 7.5 kg) were used as donors as before, and the composition and temperature of the Locke solution, perfusion apparatus, pressure head, and gassing mixture were the same. The dissection was that used for perfusion of dog adrenals with blood (Vogt, 1951), but the adrenal gland, attached to a segment of aorta and a length of vena cava, both of which had been completely separated from the rest of the body, was cut out and placed on a small platform sunk into a polyethylene funnel from which the effluent fluid escaping from the opened vena cava was collected. Perfusion was through the superior mesenteric artery, and was started while the adrenal gland was still *in situ*, just as described previously for the tissue supplied by the inferior mesenteric artery. The flow through the adrenal gland was faster than through the extramedullary chromaffin tissue and ranged from 3 to 9 ml./min. Collection periods usually lasted 2 min; either the whole or one-half of such a 2-min sample was extracted, and the catechol amines were separated and assayed as in the earlier work. Recoveries of the amines averaged 70%.

The following drugs were used: angiotensin (Hypertensin II aspartic-β-amide, Ciba), bradykinin (Parke Davis), dimethylphenylpiperazinium iodide (Parke Davis), histamine bis-(dihydrogen phosphate) (B.D.H.), 2,4,5-trihydroxyphenylethylamine (6-hydroxydopamine) hydrobromide (Merck, Sharp & Dohme), methylreserpate methyl ether hydrochloride (Su 8842, Ciba), reserpine (Serpasil, Ciba) and tyramine hydrochloride.

Doses are expressed as weights of the salts except for histamine, adrenaline and noradrenaline, which are given in terms of the base.

Electrical stimulation of the splanchnic nerves was carried out with shielded platinum electrodes using rectangular pulses of 1 msec duration at a frequency of 15 shocks/sec and supramaximal voltage.

When drugs had been infused, their possible interference with the bioassay of noradrenaline and adrenaline was carefully checked. The polypeptides travel to the front of the chromatogram, and so does dimethylphenylpiperazinium, so that this alone excluded gross contamination of the regions eluted for assay purposes. However, if small " tails " of the compounds had extended into the adrenaline region, there was so much adrenaline in the perfusates that the amount of eluate injected into the rat was only a small fraction of the total and would not have contained enough of the infused drug to produce effects of its own.

6-Hydroxydopamine travels to the noradrenaline region of the chromatogram but, its pressor activity being about 3,000-times less than that of noradrenaline, only subthreshold quantities were present in the amount of eluate used for injection. Tyramine travels faster than adrenaline in the chromatogram, and the amount which might have contaminated the adrenaline region was therefore also found to be too small to produce a pressor effect.

The other drugs were inactive in the doses in which they might have been present in the portion of eluate injected for assay, even if they had travelled to the same region of the chromatogram as noradrenaline or adrenaline.

RESULTS

The Locke-perfused adrenal gland continuously releases catechol amines into the effluent fluid but this spontaneous release declines to a low level. The first control sample was therefore usually collected after a waiting period of between 10 and 24 min from the time of transfer of the gland to the perfusion apparatus.

The purpose of the first experiments was to ascertain that, with the technique used for perfusion of extramedullary chromaffin tissue, stimulation of the splanchnic nerves released medullary amines. There was invariably a large release of amines (Table 1). The relative,

TABLE 1

EFFECT ON THE RELEASE OF MEDULLARY HORMONES OF ELECTRICAL STIMULATION OF THE SPLANCHNIC NERVES IN THE ISOLATED PERFUSED LEFT ADRENAL GLAND OF THE DOG

Figures are not corrected for recovery. Weight of adrenal glands, 0·2 to 0·35 g

Experiment No.	Condition	Noradrenaline (ng/min)	Adrenaline (ng/min)	Methylated (%)
1	Before stimulation	62	400	87
	During stimulation	300	1,000	77
2	Before stimulation	450	3,000	87
	During stimulation	2,750	6,000	69
3	Before stimulation	25	125	83
	During stimulation	1,750	2,100	54
4	Before stimulation	62	187	75
	During stimulation	550	1,250	69

but not the absolute, increase in secretion was greater for noradrenaline than for adrenaline, so that there was a fall in the percentage of total amines present in the methylated form (last column, Table 1).

The next problem was that of the efficacy of the polypeptides angiotensin and bradykinin in releasing amines from the perfused adrenal gland. It is clear from Table 2 that release took place, but even the largest doses increased secretion to less than twice the resting value,

TABLE 2

EFFECT ON THE RELEASE OF MEDULLARY HORMONES OF INFUSIONS OF BRADYKININ AND ANGIOTENSIN INTO THE ISOLATED PERFUSED ADRENAL OF THE DOG

Figures are not corrected for recovery

			Noradrenaline		Adrenaline		
Drug	Dose (μg)		Release (ng/min)	Increase (%)	Release (ng/min)	Increase (%)	Methylated (%)
Bradykinin	2·5	Before infusion	20		100		83
		During infusion	30	50	187	87	86
	20·0	Before infusion	93		325		78
		During infusion	150	61	600	85	80
Angiotensin	0·4	Before infusion	15		107		88
		During infusion	17·5	17	114	7	87
	2·0	Before infusion	15		107		88
		During infusion	22·5	50	143	34	87
	8·0	Before infusion	93		325		82
		During infusion	150	68	600	85	88

and the doses required were much higher than those needed in the eviscerated cat (Feldberg & Lewis, 1964). At the end of each perfusion, the viability of the preparation was checked by infusing 20 μg of dimethylphenylpiperazinium iodide. In the experiments of Table 2, this increased secretion of noradrenaline 100-fold and secretion of adrenaline sixfold, in clear contrast to the poor responses of the perfused gland to the polypeptides.

Tyramine hydrochloride (80 μg, single experiment) had not been found capable of releasing catechol amines from extramedullary chromaffin tissue. In four perfusions of the adrenal medulla, tyramine hydrochloride (200 and 400 μg) was used. (These doses can be considered equivalent to that of 80 μg tried in the extramedullary chromaffin tissue through which perfusion fluid was circulating at only about one-fifth of the rate at which it flows through an adrenal gland.) Each time there was release of both amines, in two of the experiments the release of adrenaline being larger than that of noradrenaline. Thus, on one occasion, the noradrenaline output rose by 38% and that of adrenaline by 72%, whereas in another perfusion the corresponding figures were 220 and 620%.

There has been some controversy on the subject whether reserpine and its analogues have a direct action on the adrenal medulla or whether amine depletion *in vivo* occurs entirely through reflex action. In order to avoid artefacts due to precipitation of the drug, reserpine was dissolved in the perfusing Locke solution at the highest concentrations at which opalescence did not occur (8.2×10^{-6} M). There was no release of amines, but some precipitate formed when this solution was kept standing. The next experiment was therefore carried out with half that concentration: no amine was released, and subsequent injections of dimethylphenylpiperazinium proved fully active. A third experiment made use of the fact that methylreserpate methyl ether (Su 8842) forms a water-soluble hydrochloride and is as active as reserpine on the heart (Sharman, Vanov & Vogt, 1962). Perfused in a concentration of 8.6×10^{-5} M it did not release amines from the perfused adrenal gland nor did it interfere with the subsequent action of dimethylphenylpiperazinium.

This was in complete contrast to the action of another drug which severely depletes the heart of its noradrenaline stores—6-hydroxydopamine hydrobromide (Porter, Totaro & Stone, 1963). Infusions of a dose of 0.4 mg caused moderate stimulation of the secretion of both amines, the increase ranging from 70 to 200%.

Feldberg (1940) reported that the perfused adrenal was much less sensitive to histamine than the gland *in situ*, 5 μg of the dihydrochloride producing only a small release of amines. Since no information had been obtained in the previous work on the sensitivity of the extramedullary chromaffin tissue to histamine, three perfusions of the region of the inferior mesenteric ganglion were carried out as before (Muscholl & Vogt, 1964), and different doses of histamine were infused. This tissue, too, was found insensitive: 2 μg produced no release of amines, whereas 8 μg produced once a 25% rise in noradrenaline secretion, another time a doubling of both amines, and the third time a 62% increase in adrenaline secretion. These effects were compared with those of subsequent stimulation of the tissue by potassium chloride (4.4 and 10 mg), which produced much greater effects: the two doses increased noradrenaline secretion respectively by about 300 and 500%, and adrenaline secretion by 100 and 300%.

DISCUSSION

Electrical stimulation of the splanchnic nerves released large quantities of catechol amines from the Locke-perfused adrenal gland of the dog. This result contrasts sharply with the failure to obtain any release from extramedullary chromaffin tissue on stimulation of the inferior splanchnic nerves under precisely the same experimental conditions (Muscholl & Vogt, 1964). It supports the view that this failure was not due to technical factors.

It had not been possible to stimulate the extramedullary chromaffin tissue by angiotensin, and only weak effects had been obtained with bradykinin. The perfused adrenal glands responded, but only poorly, to both polypeptides, and the doses required were very high in comparison with those needed in the eviscerated cat. It seems, therefore, likely that the artificial conditions of the perfusion were responsible for the lack of sensitivity of medullary and extramedullary chromaffin cells. The same is probably true of the lack of sensitivity to histamine. In the present work, large doses were found necessary in order to release amines from extramedullary chromaffin tissue, and Feldberg (1940) found that in the perfused adrenal gland of the cat large doses produced only small effects.

The perfused adrenal gland was found to release amines when perfused with tyramine, and there are not enough results to decide whether in this respect there is a genuine difference from the extramedullary chromaffin tissue which had not been seen to respond.

The failure of reserpine or its soluble ether to release catechol amines agrees well with the finding that denervation protects adrenal glands *in vivo* from the action of moderate doses of reserpine (Holzbauer & Vogt, 1956). Euler, Stjärne & Lishajko (1964) have recently reported a similar lack of response when retrogradely perfusing cattle adrenal glands through the vein with 2.7×10^{-4} M-reserpine.

Locke-perfused extramedullary and medullary chromaffin tissues have thus proved to respond to drugs in a very similar fashion. In both, sensitivities to angiotensin, bradykinin and histamine are low, that to angiotensin even absent in the extramedullary tissue. Both tissues respond well to acetylcholine (Feldberg & Minz, 1931), dimethylphenylpiperazinium, and potassium chloride. The damage due to the perfusion with Locke solution appears to disrupt the receptors for the first group of substances more than those for the second group. In keeping with the fact that the response to the natural transmitter of splanchnic nerve impulses is well preserved, the response to electrical stimulation of these nerves was also maintained.

SUMMARY

1. Electrical stimulation of the splanchnic nerves released quantities of catechol amines ranging from about 0.8 to 5.3 μg/min from the Locke-perfused left adrenal medulla of puppies.

2. Both angiotensin (8.0 μg) and bradykinin (20.0 μg) produced a small release of medullary amines, and the doses required were much higher than *in vivo*.

3. Tyramine hydrochloride (0.4 mg) released larger quantities of medullary amines than the two polypeptides, and so did 6-hydroxydopamine (0.4 mg). Dimethylphenylpiperazinium iodide (20 μg) was more active still. Neither perfusion with reserpine (4.1 and 8.2×10^{-6} M) nor with methylreserpate methyl ether hydrochloride (8.6×10^{-5} M) caused a release of catechol amines from the perfused medulla.

4. Histamine was injected into Locke-perfused extramedullary chromaffin tissue of the puppy; a moderate release of catechol amines was obtained with high doses (8 μg), but the responses were much smaller than those following an injection of potassium chloride (4.4 and 10 mg).

5. The conclusion is that, in Locke-perfused extramedullary or medullary chromaffin tissue, the receptors sensitive to acetylcholine and to potassium chloride survive well, whereas those interacting with angiotensin, bradykinin and histamine are readily disrupted.

I am grateful to J. E. McEwen, F.I.S.T., for his skilled help in the amine extractions. My thanks are due to the following for gifts of chemicals: Ciba Laboratories for Hypertensin and Serpasil, Dr W. E. Barrett (Ciba Pharmaceutical Products Inc.) for methylreserpate methyl ether (Su 8842), Dr C. S. Miller (Merck, Sharp & Dohme Research Laboratories) for 6-hydroxydopamine, and Parke Davis for dimethylphenylpiperazinium iodide.

REFERENCES

EULER, U. S. VON, STJÄRNE, L. & LISHAJKO, F. (1964). Effect of reserpine, segontin and phenoxybenzamine on the catecholamines and ATP of isolated nerve and adrenomedullary storage granules. *Life Sciences*, **3**, 35–40.

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

HOLZBAUER, M. & VOGT, M. (1956). Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.*, **1**, 8–11.

MUSCHOLL, E. & VOGT, M. (1964). Secretory responses of extramedullary chromaffin tissue. *Brit. J. Pharmacol.*, **22**, 193–203.

PORTER, C. C., TOTARO, J. A. & STONE, C. A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *J. Pharmacol. exp. Ther.*, **140**, 308–316.

SHARMAN, D. F., VANOV, S. & VOGT, M. (1962). Noradrenaline content in the heart and spleen of the mouse under normal conditions and after administration of some drugs. *Brit. J. Pharmacol.*, **19**, 527–533.

VOGT, M. (1951). Cortical secretion of the isolated perfused adrenal. *J. Physiol. (Lond.)*, **113**, 129–156.