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THE SECRETION OF THE DENERVATED ADRENAL MEDULLA OF THE CAT

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In 1917 Stewart and Rogoff tried to determine whether the chronically denervated adrenal medulla shows any secretory activity. They came to the conclusion that " no epinephrine is normally released except through nerves." In view of the recent development of sensitive methods for the detection of adrenaline and noradrenaline, and in view of the possible role played by noradrenaline in the maintenance of vascular tone, the problem was reinvestigated.

METHODS

Operations.—Ten cats were used. In a preliminary aseptic operation under ether, the larger and the lesser splanchnic nerves were cut bilaterally where they emerge from the diaphragm. In addition, both lumbar sympathetic chains were excised, from the first ganglion down to the third or fourth. The removal of the sympathetic chains ensures the section of any fibres which may enter the adrenal without first joining the splanchnic nerves (Elliott, 1913 ; McFarland and Davenport, 1941). Recovery from the operation was uneventful, except for an occasional transient diarrhoea. Between 17 and 56 days after the operation, the cats were anaesthetized with chloralose and adrenal blood collected after injection of heparin. In most instances, the blood was obtained from a cannula introduced into the lower end of the inferior vena cava after preliminary evisceration, bilateral nephrectomy, ligation of the aorta at the level of the renal arteries, and of the adreno-lumbar veins at the lateral border of the adrenals. The vena cava was occluded above the entry of these veins by a ligature which had frequently to be placed inside the liver tissue so as not to obstruct the flow from the right adrenal. For the purpose of arterial injections of KCl, a cannula was tied into the stump of the coeliac artery. The cannula was connected by a short piece of rubber tubing to the butt of a syringe needle. Slow infusions were made by a syringe, the contents of which could be delivered gradually by pushing its plunger with the help of a screw turning in a metal frame which was attached to the barrel. In a few animals the left adreno-lumbar vein entered the renal vein. In these cats, provided infusion into the coeliac artery was not intended, evisceration, right nephrectomy, and ligation of aorta and vena cava could be dispensed with. After removal of the left kidney, blood was obtained from the left adrenal by tying a cannula into the distal end of the left renal vein and occluding its central end.

Estimations of sympathomimetic amines.—All blood samples were collected in ice-cooled tubes, centrifuged without delay, and small portions of the native plasma tested for adrenaline on the rat's uterus on the same day. The remaining plasma was extracted, and the amines in the extracts separated by paper chromatography and eluted from the paper. The eluates were evaporated to dryness and the residue taken up in a

volume of water equal to one-tenth of the original volume of plasma. These solutions were assayed on the rat's uterus or on the rat's blood pressure (see below) as soon as possible; they were neutralized with solid $NaHCO_3$ immediately before use. Most assays were carried out on the day after the operation. If some of the tests had to be postponed to the following day, the evaporated eluates were stored in the refrigerator in the dry state and not dissolved till shortly before their assay.

Preparation of the extracts.—Plasma (5 ml.) was added to ethanol (30 ml.) containing 0.1 per cent (v/v) conc. HCl. After chilling for not less than half an hour, the mixture was centrifuged, the precipitate washed with more acid ethanol, centrifuged, and the combined supernatants evaporated to dryness *in vacuo* (bath temperature 45–50° C.). By means of small quantities of NaCl-saturated ethanol (total volume 6 ml.) the residue was transferred into a 15-ml. centrifuge tube, chilled, spun, the deposit washed with 1 ml. NaCl-ethanol, spun, and the combined supernatants evaporated to dryness as before. With the help of four 0.5 ml. portions of a mixture of equal parts of acetone and ethanol, the residue was transferred into a 15-ml. centrifuge tube, the solvent in the tube concentrated *in vacuo* to less than 1 ml., the tube chilled, centrifuged, and the supernatant applied to filter-paper for chromatography. The residue was washed with 0.25 ml. of the acetone-ethanol mixture, centrifuged, and the supernatant also applied to the paper.

The object of using NaCl-saturated alcohol for the second extraction was to reduce the amount of potassium salts in the solution (Barsoum and Gaddum, 1935), and that of using acetone-alcohol in the final extraction to lessen the amounts of inorganic constituents and of acetone-insoluble organic compounds applied to the paper.

Extracts of adrenal gland were prepared by grinding up one gland in 10 ml. 0.15 N-HCl, centrifuging, precipitating 1 ml. of the supernatant with 7 ml. ethanol, discarding any precipitate, and treating this alcoholic extract like the supernatant of a plasma extract in NaCl-ethanol. Assays were done only after chromatographic separation of the amines.

Chromatography.—In principle, the methods described by Crawford and Outschoorn (1950) were used, but three points of difference in the present procedure call for mention.

(1) The solvent employed for developing the chromatograms was a mixture of phenol with 0.1 N-HCl as used by Crawford (unpublished). Of different concentrations tried, 15 per cent (v/w) 0.1 N-HCl in phenol gave optimal separation without causing the occurrence of patches of waterlogging of the paper, as larger amounts of acid frequently did. The jars were filled with CO_2 or N_2 instead of SO_2.

(2) In the absence of SO_2 losses occurred unless the filter-paper was washed before use with 0.01 N-HCl, a procedure found by Crawford (unpublished) to be helpful in the fluorimetric estimation of noradrenaline.

(3) Chromatography was carried out at a temperature of 25–26° C., in order so to accelerate the flow that the process of separation was completed in 14–16 hours (overnight).

Assays.—The adrenaline, unless present in large amounts, was assayed on the rat's uterus stimulated by carbachol (Gaddum and Lembeck, 1949). Since this preparation is, on the average, 150 times more sensitive to adrenaline than to noradrenaline, interference with the estimation of adrenaline by the simultaneous presence of noradrenaline is usually negligible (Gaddum, Peart, and Vogt, 1949). Thus it was possible to assay both native plasma, which contained the two substances, and eluates of paper strips containing adrenaline only. When plasma was tested, difficulties were frequently encountered owing to the fact that certain samples, even when lacking any stimulating effect of their own on the uterus, were found to "mask" an appreciable quantity of added

adrenaline. Matching had therefore to be done with solutions of adrenaline in adrenaline-free plasma (i.e. arterial plasma, since all animals used had denervated adrenals) and not with adrenaline in saline. Even so, the results from a plasma which "masks" must be considered as somewhat unreliable, since the arterial blood need not contain the same amount of interfering substances as the adrenal effluent. Precautions were also necessary when eluates were being tested. The phosphates and traces of other chemicals which are present in the eluates may affect the isolated uterus and mimic or mask the presence of traces of adrenaline. Thus the volume of eluate added to the uterus bath has to be kept below the threshold of such interfering substances. The threshold is established by preparing an eluate from a strip of paper not containing any adrenaline. It was usually well above the quantity of eluate required for an assay. In cases of doubt, the neutralized solution was heated for 5 min. in a boiling waterbath, so that any adrenaline was destroyed, and the responses to the heated solution were examined without and with added adrenaline.

The assay of samples of adrenal blood collected during stimulation of the gland by KCl offered no difficulties. The adrenaline was estimated in the native plasma on the rat's uterus and in the eluate on the rat's blood pressure. The use of native plasma caused no errors, since the concentration of adrenaline was so high that the plasma could be diluted sufficiently to eliminate the action of masking substances. There was good agreement between the two methods.

Noradrenaline was only assayed after chromatographic separation from adrenaline, the assay of mixtures by parallel tests on different organs having been shown by Gaddum and Lembeck (1949) to involve very large errors. Noradrenaline was estimated by the rise in blood pressure produced in the rat under urethane anaesthesia, as described by Crawford and Outschoorn (1950). One modification of their procedure was the intravenous administration to the rat (Outschoorn, unpublished) of hexamethonium hydrobromide (5–10 mg./kg.). The threshold for noradrenaline is greatly lowered by hexamethonium, 1 mμg. noradrenaline being usually detectable at the beginning of the experiment. The sensitivity, however, gradually declines, and can only be partially restored by giving more hexamethonium.

Matching.—In the biological assays, matching was done by "bracketing" the unknown sample with two doses of standard differing by a factor of 2; thus if a result is listed as 7.5 mμg., it was found to be smaller than 10 and greater than 5 mμg. Usually the discrimination of the biological preparation was not sufficient to narrow down the range of the result any further.

Recovery.—Recovery experiments, in which 1 μg. adrenaline and 1 μg. noradrenaline were added to 5 ml. arterial plasma (taken from cats with denervated adrenals), showed the recovery to be rarely less than 75 per cent and sometimes more. The recovery of the two amines in a particular experiment did not differ significantly, so that the estimated percentage methylation should have been unaffected by occasional losses. When losses occurred they were always in the extraction and not in the chromatographic procedure, which allowed, within the limits of the error of the assay, full recovery of amounts as small as 5 mμg. adrenaline.

RESULTS

Resting secretion per minute.—Small quantities of sympathomimetic amines were found in all twelve samples of adrenal plasma tested. The mean secretion of both glands per min. was 21 mμg. adrenaline and 34 mμg. noradrenaline with a range shown in Table I. These outputs corresponded to an average concentration in the plasma of 1.4×10^{-8} adrenaline and 2.7×10^{-8} noradrenaline.

TABLE I

ADRENALINE AND NORADRENALINE IN PLASMA FROM DENERVATED ADRENALS

No. of cat	No. of sample	Post-operative interval (days)	Adrenaline (mμg./min.) assayed on		Noradrenaline (mμg./min.)	Blood flow (ml./min.)	Body weight (kg.)
			Uterus	Blood pressure			
1	•	52	5[1]		<48		5.0
2	1	56	46[1]	46	73	1.83	4.0
	2		46[1]	42	91	0.91	
3*	1	42	13[1]	15	43	0.86	3.6
	2		32[1]	40	80	0.32	
4	1	19	5[1]		6	1.0	3.5
	2		*1,500*[1]	*2,000*	*2,000*	*0.5*	
5	1	18	15[1]		20	1.25	3.3
	2		*3,500*[1]	*1,750*	*660*	*0.87*	
6	1	17	7[1]		19	0.57	2.5
	2		*2,500*[1]	*3,300*	*1,600*	*0.41*	
7*	1	23	10[2]		8	1.3	3.0
	2			*220*	*290*	*0.58*	
8*	1	26	48[2]		24?	4.8	2.8
	2		*2,600*[1]	*2,000*	*1,440*	*6.4*	
9*	1	28	28[2]		6	2.2	2.6
	2		*2,200*[1]	*2,600*	*630*	*0.7*	
10*	1	30	25[2]		8	1.18	1.8
	2		*46*†[1]	*110*	*70*	*0.92*	

* Indicates that blood was obtained from one gland only. In order to make the results directly comparable, the figures in columns 4–7 are doubled. [1] Native plasma tested. [2] Eluate tested. † Severe masking. *Italics:* during infusion of KCl.

Note.—In assays giving figures with more than one digit, the second digit is only approximate. Figures like 91 and 73 mμg./min. result from the conversion of observed concentrations into secretion per min.

In any one experiment the secretion per minute was steady, the concentration rising as the blood flow diminished. The only exception was Exp. 3, in which the blood pressure and the blood flow through the gland were intentionally reduced by severe haemorrhage between collection of the two samples of adrenal blood. Under the conditions of anoxia prevailing when the second sample was obtained the production of amines per minute by the glands had risen to twice its initial value.

Effect of KCl.—Arterial injection of KCl greatly accelerated the rate of secretion and the total amount of amines released per minute was usually of the order of 4,000 mμg.

Percentage methylation.—On the average, the percentage methylation (Table II) in the plasma collected from the resting glands was lower than the mean figure of 59 per cent reported by Bülbring and Burn (1949) for nine normal cats during a first electrical stimulation of the splanchnic nerves. The two sets of figures, however, have almost the same range, and in two denervated glands (cats 8 and 9) the percentage methylation was high even by normal standards.

Comparison of the figures obtained from the same cat under different conditions shows a striking similarity between percentage methylation in the blood collected at rest, in that obtained during stimulation of the medulla by KCl, and in the gland itself (cats 7–9). In cats 5, 6, and 10, on the other hand, methylation was found to be higher during stimulation by KCl than when the gland was at rest; as

TABLE II

Cat No.	Sample No.	Percentage methylation of sympathomimetic amines		
		Adrenal plasma		Right adrenal gland
		At rest	During KCl infusion	
2	1	39		
	2	34		
3	1	25		
	2	31		
4		45	47	
5		43	80	
6		29	64	
7		56	43	38
8		66	62	69
9		82	79	83
10		23	53	
Mean of Nos. 4–10		49	61	
Mean of all determinations ..		43		

a result of this the mean percentage methylation in the secretion produced by chemical stimulation of the denervated gland does not differ from the value found for nervous stimulation of the normal gland.

Within the experimental period covered (up to 56 days), there was no correlation between the quantity or composition of medullary secretion and the interval between denervation and experiment.

Discussion

The experiments by Meier and Bein (1948), in which normal vasomotor responses were restored in the acutely adrenalectomized animal by infusing small doses of noradrenaline intravenously, suggest that a small quantity of this substance is continuously secreted by the adrenal medulla. The present experiments, carried out on denervated adrenals in which the nerves had been allowed time to degenerate, show that, in complete absence of nervous stimuli, blood leaving the adrenal medulla invariably contains noradrenaline, but also some adrenaline. The percentage methylation in this secretion tends to be low, but figures above 50 per cent occur, and the range covered is the same as that reported in the literature for normal cats.

Injection of KCl into such glands did not affect the percentage methylation except for three cats, in which it appeared to raise it. Since, however, the estimation of the percentage methylation in the resting samples, in which the concentrations encountered are often below 10^{-8}, is subject to large errors, these few observations can no more than suggest that injection of KCl may be able to increase the low percentage methylation of a denervated gland. The good agreement in percentage methylation between a gland excised at the beginning of an experiment and the blood leaving the other gland rather points to the degree of methylation present in the medullary tissue, and not the type of stimulus employed, determining the composition of the secretion. Further support for this view can be seen in the fact that

the average percentage methylation in the effluent of denervated glands stimulated by KCl is the same as that reported in the literature for normal glands secreting as a result of brief splanchnic stimulation.

Anoxia increases the secretion of the denervated adrenal medulla, though it is unable to accelerate it to an extent comparable with that occurring in the innervated gland. This observation is in good agreement with the observation by Bülbring, Burn, and Elío (1948) on the isolated adrenal of the dog.

The difference between the conclusions to be drawn from this work and the views held by Stewart and Rogoff (1917) are probably due to the recent improvement in experimental methods for the identification of minute quantities of sympathomimetic amines. The alternative explanation is that a few fibres escaped destruction in the operations carried out in the present experiments and that they might be the cause of the slight " resting " secretion ; this is not a very likely explanation, because there is no anatomical difficulty in tracing the splanchnic nerves and the sympathetic chains in a cat.

SUMMARY

1. In ten cats, the nerves to the adrenal glands were cut and allowed to degenerate. Adrenal blood collected from these animals contained both adrenaline and noradrenaline, the mean secretion per min. amounting to 21 mμg. adrenaline and 34 mμg. noradrenaline. Though the percentage methylation tended to be low, its range covered that described for adrenal blood of normal cats in which the splanchnic nerves were being stimulated.

2. Arterial injection of KCl released sympathomimetic amines in the same proportion as stimulation of the splanchnic nerves does in the normal cat.

3. In spite of great individual variations, in any one animal the percentage methylation of the secretion at rest, of the secretion during injection of KCl, and of the stores in the medulla is usually about the same.

4. The observations under (2) and (3) suggest that it is the composition of the stores and not the type of stimulus at work which determines the proportion in which the adrenal medulla releases its two amines.

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