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FORMATION OF CATECHOL AMINES DURING SPLANCHNIC STIMULATION OF THE ADRENAL GLAND OF THE CAT

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In 1951 Hökfelt found that, following depletion of the catechol stores of the adrenal by insulin, several days were required for complete restoration of the normal amine content. He concluded from his observations that the rate of synthesis of adrenaline must be slow. Recently Udenfriend, Cooper, Clark, and Baer (1953) and Udenfriend and Wyngaarden (1956) observed a very low rate of incorporation of ^{14}C into adrenaline and noradrenaline after the injection of ^{14}C-labelled tyrosine or phenylalanine. However, after administration of ^{14}C-labelled dopa, the incorporation was rapid. The calculated half-lives of both amines were of the order of 3–6 days. This low turnover suggested that at least one step in their formation was slow. Since the turnover rates of noradrenaline and adrenaline were equal, the methylation of noradrenaline could not be a limiting factor.

In the foregoing papers no observations were made on the effect of electrical stimulation of the splanchnic nerves. Elliott (1912) described an extensive investigation of this, though he assumed that only one active amine was concerned. In his first experiments he divided the splanchnic nerves on one side and observed the effect of administering morphine, ether, chloroform and other drugs. He found that the innervated gland which still received impulses from the brain was always depleted of amines as compared with the gland the nerve fibres to which had been cut. In contrast to these experiments there were others in which he divided the splanchnic nerves on both sides, and then applied electrical stimulation for periods up to 7 hours to those of one side. When he removed the glands at the end of this time he found little or no depletion of the amine store of the stimulated side. He said: "So slight is the change in the residual adrenalin caused by faradization of the splanchnic nerves that it would never have sufficed to convince me of the existence of the splanchnic control." Elliott's results suggested that in these experiments there must have been a vigorous formation of noradrenaline and perhaps also of adrenaline during the period of stimulation, for he recorded the blood pressure throughout and observed that it was continuously raised.

In his paper Elliott quoted Tscheboksaroff (1910), who also stimulated the splanchnic nerves and "in a very clear series of experiments . . . observed that, after stimulation, the gland on the stimulated side yielded a more potent extract, that is contained more adrenalin, than that of the other side."

In order to obtain more information on the rate of formation of noradrenaline and adrenaline experiments have been carried out in which the effects of splanchnic stimulation on medullary outflow and amine content of the cat's adrenal were determined. Results have been obtained which are interpreted to mean that splanchnic stimulation increases both the rate of synthesis and the methylation of noradrenaline.

METHODS

Cats under ether were given 80 mg./kg. chloralose intravenously. The stomach, intestines, pancreas, and spleen were removed, and the blood supply to the liver through the hepatic artery and portal vein was arrested. By dissection upwards under the diaphragm, the left splanchnic nerves were exposed and cut; the peripheral ends were prepared for stimulation with shielded platinum electrodes. The right splanchnic nerves were similarly exposed and cut. Stimulation was applied for 5 min. from an induction coil, the primary circuit being interrupted by Lewis's rotary contact breaker at a rate of 20/sec. Both vagi were cut, and the blood pressure was recorded from the carotid artery. The outflow of medullary amines was estimated by matching as closely as possible the blood-pressure response to splanchnic stimulation by infusing a mixture of equal parts of adrenaline and noradrenaline at a constant rate. The mixture was infused into the external jugular vein. The comparison of the effect of stimulation with that of infusion was made both by the height of the rise of blood pressure and also by the area of the rise deter-

mined with a planimeter. The two methods gave approximately the same results.

After 10 periods of stimulation for 5 min. at 5-min. intervals, both glands were removed as rapidly as possible, and ground with sand in 0.025M-HCl. The *p*H was adjusted to 5.5 by the addition of sodium acetate. The suspensions were placed in a boiling water bath for 3 min. and they were filtered and the filtrates kept for assay. The quantity of amines as well as the proportion of adrenaline and noradrenaline in the preparations were determined by the method of Burn, Hutcheon, and Parker (1950), using the blood pressure and nictitating membrane of the spinal cat. In a number of experiments the total amine content was determined by the chemical method of Euler and Hamberg (1949).

For the calculation of the results obtained a modification of the method of Gaddum and Lembeck (1949) was employed. The total vasoconstrictor amine content—T.A.—was calculated from the relation

$$\text{T.A.} = \frac{\text{Ae}}{\alpha \times \text{Nf} + \text{Af}} \times 100$$

where Ae is the total activity on the blood pressure expressed as μg. adrenaline; Nf and Af are the percentages of noradrenaline and of adrenaline in the sample as determined from the responses of the nictitating membrane and of the blood pressure, and α is the ratio of noradrenaline activity to adrenaline activity on the blood pressure. Values obtained were expressed as μg. amine/g. tissue wet weight. The right unstimulated gland was assumed to represent a valid control in these experiments. Evidence in favour of the assumption was given by Elliott (1912) and also by Elmes and Jefferson (1942).

Results

Fig. 1 illustrates a typical experiment, as well as the general procedure employed in these studies. At A the left splanchnic nerve was stimulated at a rate of 20/sec. for 5 min. At these high frequencies a progressive deterioration of the medullary discharge was observed. Five minutes after cessation of excitation 25 μg. of a 50–50 mixture of adrenaline and noradrenaline was infused during a 5-min. period (B). At C the stimulation was repeated. This procedure was continued until 10 stimulations and 10 infusions were recorded. In a number of experiments 10 successive stimulations were recorded, with infusions at the beginning and at the end of the experiment. In the particular experiment illustrated, the quantity of amines released from the left gland by splanchnic stimulation amounted to approximately 25 μg./5 min. or 2 μg./kg. cat/min. The figures for all experiments are given in Table I.

Fig. 2 shows a typical experiment illustrating the method for determining both the total amine content and also the proportion of adrenaline in the sample. The details of one experiment were as follows.

Expt. 9 (Table I). Right adrenal 190 mg.; left adrenal 223 mg. The total amine content of the right unstimulated gland was 871 μg./g. by the biological method, and 758 μg./g. by the colorimetric method. The figure for the stimulated left gland was lower, being 515 μg./g. biologically and 466 μg. colorimetrically. Thus, taking the biological figures the stimulated gland had lost 356 μg./g. (Table II). Now the total amine discharged into the blood was estimated as 160 μg., or 713 μg./g. weight of left adrenal gland. Thus, reckoning per g. of left adrenal gland, 357 μg./g. of total amine was newly synthesized, this being

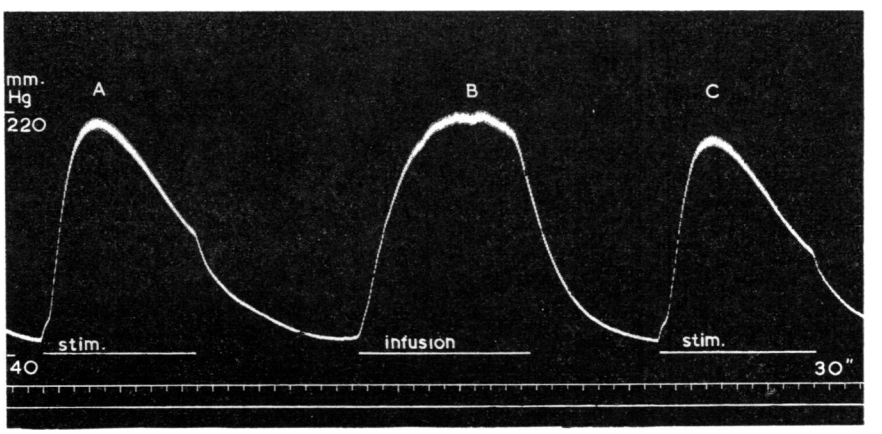

FIG. 1.—Cat under chloralose. Eviscerated. Record of blood pressure (A) during stimulation of left splanchnic nerve for 5 min., (B) during infusion of a mixture of 12.5 μg. noradrenaline and 12.5 μg. adrenaline, (C) during stimulation as before.

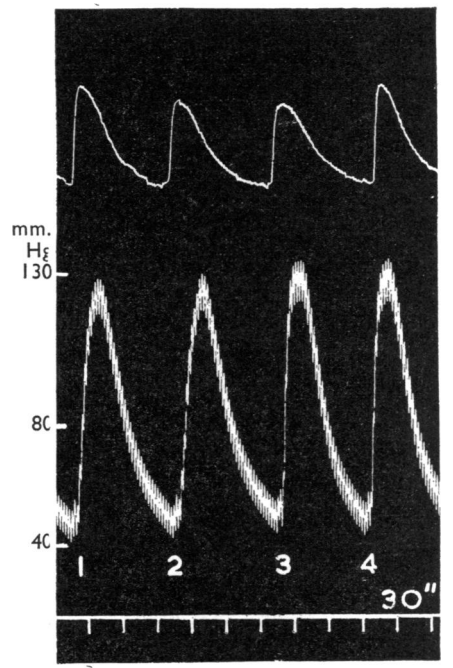

FIG. 2.—Estimation in spinal cat of proportions of noradrenaline and adrenaline in adrenal gland. Upper record, contractions of nictitating membrane; lower record, blood pressure. At 1, 20 μg. adrenaline; 2, 1.7 ml. adrenal extract; 3, 15 μg. adrenaline +5 μg. noradrenaline; 4, 20 μg. adrenaline. The effect of the extract (at 2) on the nictitating membrane was less than that of an equipressor dose of adrenaline (at 1 and 4); hence the extract was less than 100% adrenaline. Similarly the effect of the extract indicated that it contained slightly more than 75% adrenaline injected at 3. Intrapolation showed that it contained 82% adrenaline.

the difference between the amount appearing in the blood and the amount lost from the gland. If the figure for new synthesis is expressed as a percentage of what was present in the unstimulated gland, it is 41% by the biological test and 55% by the colorimetric test.

Table I is a summary of the results obtained with 12 cats, using the procedure just described. Following 10 stimulation periods (total of 50 min.), the mean amine content of the left stimulated gland fell approximately to 75% of that in the right gland. In two experiments (Nos. 6 and 11) there was no appreciable fall, and in one experiment (No. 4) the amine content rose. The results for the percentage of adrenaline in the stimulated gland were of interest because in every experiment this percentage was greater than that in the unstimulated gland. The mean figure for the unstimulated glands was 63%, while that for the percentage of adrenaline in the stimulated glands was 82%.

Formation of Amines.—The amount of pressor amines liberated in the blood by stimulation of the splanchnics is shown in the third column of Table II. The second column gives the loss of amine from the gland, and in every experiment the estimated output was greater than the loss. The excess of the output over the loss is given in the fourth column as the amount of amines synthesized during the experiment. In the final column the amount synthesized is expressed as a percentage of the amine content of the unstimulated gland. The mean figure is 38%.

DISCUSSION

The experiments were designed to obtain an estimate of the amounts of noradrenaline and adrenaline synthesized during splanchnic stimulation. In 12 experiments in which the left adrenal

TABLE I

CHANGES IN AMINE OUTPUT OF STIMULATED ADRENAL GLANDS

Cat	Total Amine Content (μg./g.)		Amine Content of L. Gland as Percentage of R.	Percentage of Adrenaline	
	R.	L.		R.	L.
1	684	284	41·5	50	80
2	638	493	77	75	100
3	1,520	1,320	87	63	75
4	1,080	1,320	122	50	70
5	1,400	1,230	88	50	66
6	438	415	95	70	87
7	1,180	856	72·5	57	65
8	1,250	722	58	55	92
9	871 (758)*	515 (466)	59 (61·5)	62	82
10	1,910	1,110	58	68	86
11	520 (423)	495 (420)	95 (99)	77	82
12	582 (708)	246 (493)	42 (69·5)	87	95
Mean..	1,006	750	74·6	63	82

* Figures in parentheses were determined by the method of Euler and Hamberg (1949).

TABLE II

ESTIMATION OF AMINE SYNTHESIS

Cat	Loss of Amine from Left Adrenal (μg./g.)	Estimated Output from Left Adrenal (μg./g.)	Synthesis of Amines (μg./g.)	Synthesis as % of Amine Content of Right Gland
1	400	440	40	6
2	145	360	215	34
3	200	365	165	11
4	240 (gain)	305	545	51
5	170	455	285	20
6	23	335	312	71
7	324	398	74	6
8	528	1,330	802	64
9	356	713	357	41
10	800	1,240	440	23
11	25	238	213	41
12	336	828	492	84
Mean	256	584	328	38

gland was stimulated for a total time of 50 min. the mean output from the gland was 584 μg. Since the mean gland weight was 257 mg. this represented an output of 150 μg./gland in 50 min. or an output of 3 μg./gland/min. If both glands had been stimulated the output would presumably have been 6 μg./cat/min. This figure is somewhat larger but similar to that obtained by Vogt (1952), who stimulated denervated glands of the cat by the intra-arterial injection of KCl and found that the total amount of amines released per min. "was usually of the order" of 4 μg. The figure of 6 μg./cat/min., or 2.4 μg./kg./min., is also in agreement with that of Celander (1954), who, using the same general procedure as in the present series of experiments, gave the figure of 3 μg./kg./min.

The evidence now presented shows that, of the 3 μg./min. liberated from one gland, only 1.3 μg. came from the store in the gland, and 1.7 μg. or 56% was obtained by fresh synthesis. Thus with splanchnic stimulation the two glands of the cat can presumably synthesize 3.4 μg./min., and, taking the average cat weight as 2.5 kg., this is 1.36 μg./kg./min.

Such a result indicates a need to reconsider the conclusion of Hökfelt (1951) and of Udenfriend *et al.* (1953) that the rate of formation of adrenaline is extremely slow. While this may be true under normal conditions when the adrenals are not stimulated, it seems that under the influence of constant impulses from the splanchnic nerves the rate of synthesis may be very much increased, as was suggested by the observations of Tscheboksaroff (1910) and of Elliott (1912), to which reference has already been made. The figure of 1.36 μg./kg./min. is very much larger than the figure of 0.002–0.004 μg./kg./min., mentioned by Udenfriend *et al.* (1953).

The second result of the present experiments was that in every experiment the percentage of adrenaline in the stimulated gland was found to be greater than that in the unstimulated gland, the mean figures being 82% and 63% respectively. This observation is capable of two different explanations. It can be explained by supposing that stimulation released much more noradrenaline than adrenaline from the store in the gland, so that the percentage of adrenaline increased in the store. It can also be explained by supposing that stimulation increased the rate of methylation of noradrenaline. Against the first explanation are the figures of Bülbring and Burn (1949), who found that the proportion of adrenaline to noradrenaline released in the blood stream by splanchnic stimulation varied from a ratio of 98:2 to a ratio of 15:85, with a mean ratio of 59:41. Furthermore Holtz, Engelhardt, Greef, and Schümann (1952) found 80% adrenaline in the amines present in the adrenal vein during splanchnic stimulation. These results give no support to the idea that splanchnic stimulation always releases a greater proportion of noradrenaline than that present in the gland. In favour of the second explanation are the results of Bülbring (1949), who found that the methylation of noradrenaline in adrenal homogenates was much greater if the homogenates were prepared from glands which were stimulated for 30 min. before they were removed from the body. Whereas in homogenates prepared from non-stimulated glands the mean increase in adrenaline was 19%, in homogenates from glands which had been stimulated the mean increase in adrenaline was 60%.

The observations described in this paper thus support the view that the chemical processes in the adrenal gland which underlie the formation of noradrenaline can be greatly accelerated by the stimulation of the splanchnic nerves, and that statements concerning rates of formation in conditions in which there is no stimulation may be inapplicable during splanchnic activity.

Summary

1. The effect of splanchnic stimulation on the synthesis of catechol amines in cat adrenal glands has been investigated.

2. After a stimulation period of 50 min. there was a mean decrease of 256 μg./g. gland and an estimated output of 584 μg. The difference of 328 μg. represents the synthesis. If this value for synthesis is expressed as percentage of what was present in the unstimulated gland it is 38%.

3. The results thus indicate that stimulation liberated 3 μg. of amines from one gland per min., of which 1.3 μg. came from the store in the gland and 1.7 μg. from synthesis. With splanchnic stimulation the two glands of the cat can presumably synthesize 3.4 μg./min. or 1.36 μg./kg./min. This is much higher than figures published for resting glands.

4. It is concluded that splanchnic stimulation increases the rate of synthesis and methylation of noradrenaline.

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