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LIBERATION OF ADRENALINE FROM THE SUPRARENAL GLAND OF THE RABBIT

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Much work has been devoted to *nor*adrenaline in recent years. There is evidence that this substance is present in adrenergic nerves, in the adrenal medulla of cattle and other animals, and in human medullary tumours (for references see West, 1950). It is released into the blood stream when the splanchnic nerves (Bülbring and Burn, 1949; Gaddum and Lembeck, 1949) and nerves to the liver and spleen of the cat (Mann and West, 1950) are stimulated. Holtz and Schümann (1950), however, showed that only adrenaline could be detected in extracts of adrenal glands of rabbits. Their test organs were the blood pressure of a cat after cocaine, the isolated fresh rabbit ileum, and the isolated stored rabbit ileum.

We had been investigating the nature of extracts of adrenal glands of various mammals and birds and had also found that there was little evidence to suggest the presence of *nor*adrenaline in rabbit glands. This conclusion was confirmed when the work was extended to include analysis of the plasma in the adrenal vein before and after stimulation of the splanchnic nerve. Some *nor*adrenaline is produced by the gland and is found in samples of plasma collected soon after stimulation commences, but continued stimulation results in its disappearance, only adrenaline being detected at this stage. This finding is in direct contrast to that already reported in the cat, despite the fact that in this animal methylation is much more active in glands which have been stimulated through the splanchnic nerve just before they were minced than in non-stimulated glands removed as quickly as possible under the most favourable conditions (Bülbring, 1949).

METHODS

Preparation of the adrenal extracts.—All glands whether stimulated or unstimulated were removed from the rabbits as soon after death as possible. After removal of the capsule by careful dissection, the glands were weighed and ground with sand and 10 ml. 0.1 N-HCl/g. (Holton, 1949). The acid extracts were filtered and the filtrates (1 ml. = 0.1 g. gland) were assayed on the pharmacological preparations described below.

Collection of blood.—Rabbits were anaesthetized with intravenous urethane (1 g. per kg.) and eviscerated. In a few experiments, chloralose (110 mg. per kg.) was used as the anaesthetic. Blood pressure was recorded with a mercury manometer and usually remained high if the evisceration was completed rapidly. The left renal vessels were then tied. The left adrenolumbar vein was tied just peripherally to the left adrenal vein. All other branches of the inferior vena cava were tied below the right renal vein. Both the aorta and the inferior vena cava were tied below the left renal vessels, and a small L-shaped cannula (of volume 0.1 ml.) inserted in the vena cava just above the ligature. The inferior vena

cava was then closed centrally by a thread between the left adrenal vein and the right renal vein. Adrenal blood flowed out through the cannula and was collected in tapered centrifuge tubes cooled in ice-water. The blood was rapidly centrifuged and pharmacological tests applied to the plasma, which was kept in ice-water until tested. In general, the collection of each sample took three minutes and provided 2–5 ml. blood. When the blood was not required, it was returned to the animal via the external jugular vein. Chlorazol fast pink (0.1 g. per kg.) was used throughout as the anticoagulant. In some experiments the vessels to both kidneys and to the right adrenal gland were tied.

Stimulation.—The left splanchnic nerve was prepared for stimulation with shielded platinum electrodes. Continuous stimulation was applied for a given time from an induction coil (usually Faradic stimulation at 12.5 cm. on 5 V.), and then the glands were removed for assay.

Assay methods.—Samples of the gland extracts and of the plasma were used in the following pharmacological tests: (1) The fresh isolated ileum of a rabbit, sensitive to 10^{-8} adrenaline or 2×10^{-8} *nor*adrenaline in a bath of Tyrode solution at 34° C. (2) The stored isolated ileum of a rabbit in a similar bath (volume 10 ml.). In later experiments it was found advantageous to use a larger bath (volume 50 ml.) since frothing with the plasma samples was sometimes extensive. Pieces of ileum stored for 4 days in Tyrode solution at 4° C. were used, since at this stage *nor*adrenaline is two to three times as active as adrenaline in inhibiting pendular movement (Table I and Fig. 1). (3) The blood pressure of a rabbit under urethane anaesthesia (1.5 g. per kg.), after cocaine (8 mg. per kg.

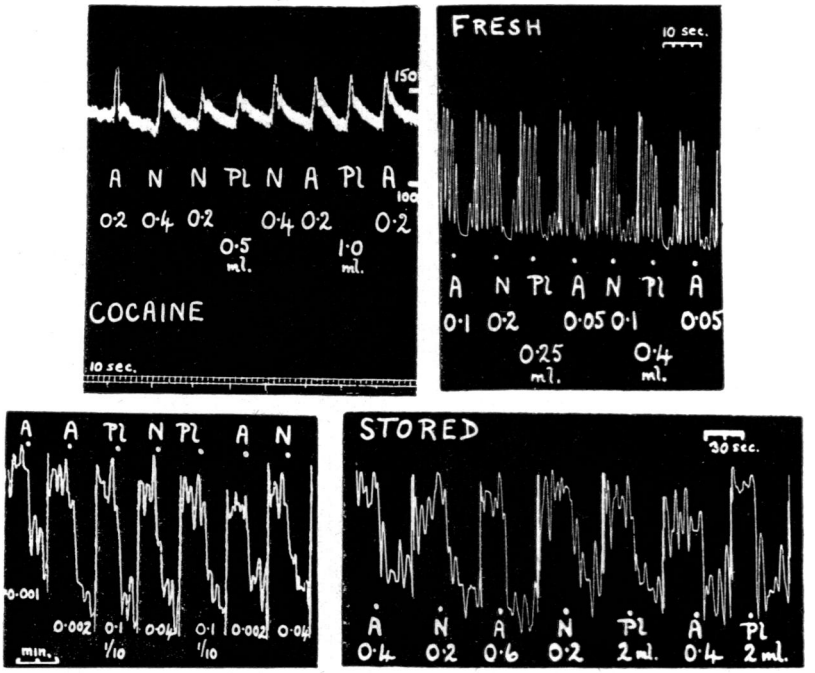

FIG. 1.—Parallel assays (Rabbit No. 3). Plasma (Pl) from the adrenal vein during stimulation of the splanchnic nerve. A, adrenaline; N, *nor*adrenaline. Doses in μg. and ml. Records: top left, blood pressure of a rabbit; top right, fresh rabbit ileum; bottom left, chick rectum; bottom right, stored rabbit ileum. Adrenaline equivalent is 0.2 μg. per ml. plasma.

TABLE I

THE INFLUENCE OF STORAGE ON THE RESPONSE OF THE RABBIT ILEUM TO *nor*ADRENALINE AND ADRENALINE

Bath volume = 10 ml. Doses shown produce a just submaximal inhibition of pendular movement

Amine	Equiactive doses (μg.) after storage for 1 to 7 days							
	0	1	2	3	4	5	6	7 days
Adrenaline (A) ..	0.05	0.1	0.2	0.3	0.6	1.0	1.0	2.0
*Nor*adrenaline (N) ..	0.1	0.1	0.2	0.2	0.2	0.4	0.4	0.4
Ratio N/A ..	2	1	1	0.7	0.3	0.4	0.4	0.2

intravenously) and double vagotomy. (4) An isolated rectum of a week-old chick in a bath of Tyrode at 37° C., sensitive to 2×10^{-10} adrenaline or 4×10^{-9} *nor*adrenaline. (5) An isolated uterus from a non-pregnant rat in dioestrus, sensitive to 10^{-10} adrenaline or 5×10^{-9} *nor*adrenaline. Usually, tests (1) and (2) and two of the other three tests were carried out on all samples. Solutions of *l*-adrenaline and *l-nor*adrenaline were prepared in 0.01 N-HCl.

RESULTS

Adrenaline content of normal and stimulated glands.—Parallel quantitative assays by different methods were carried out on the gland extracts obtained in each experiment (Fig. 1). The estimates were taken in pairs and values for adrenaline and *nor*adrenaline calculated using the formula developed by Bülbring (1949). The values shown in Table II indicate that there is considerable variation in the amine content of the glands, but in nearly every case only adrenaline was detected. The concentration of adrenaline in the stimulated glands was higher than in the unstimulated ones. Two unstimulated glands contained some *nor*adrenaline, results which were confirmed by the method of estimation devised by Burn, Hutcheon, and Parker (1950).

TABLE II

CONCENTRATION OF ADRENALINE (μG/G.) IN EXTRACTS OF SUPRARENAL GLANDS OF RABBITS

Rabbit No. :	13	14	15	16	17	18	19	20	21	Mean
Right unstimulated	474	370*	625	600	714	200	420	500	580	498
Left unstimulated	418	399	625	555	714	132	380	410	620	472

Rabbit No. :	1	12	2	3	4	11	5	8	9	10	Mean
Right unstimulated	281	225	670	756	556	375	566†	410	476	419	418
Left stimulated ..	400	475	558	890	556	500	620	555	593	520	568
Stimulation time (min.)	15	15	16	30	50	59	62	90	91	120	—

* This sample also contained 50 μg. *l-nor*adrenaline/g. † This sample also contained 55 μg. *l-nor*adrenaline/g.

Adrenaline content of plasma from suprarenal vein.—Before stimulation occurred control samples of plasma were collected over 3–5 minutes in each experiment. When these were assayed by four of the pharmacological tests, the mean value for adrenaline for eleven rabbits was 0.084 μg./ml. (Table III). *Nor*adrenaline was

TABLE III

Amine	Concentrations of the two amines (μg./ml.) in control plasma taken from the suprarenal vein of rabbits, Nos. :											
	1	2	3	4	5	7	8	9	10	11	12	Mean
l-adrenaline	0.09	0.14	0.10	0.18	0.05	0.03	0.05	0.06	0.10	0.02	0.10	0.084
*l-nor*adrenaline ..	0	0.02	0	0	0	0	0.01	0	0	0.03	0	0.005

detected in three of the specimens. Several samples had to be discarded because they contained stimulating substances in large quantities. It is of interest to note that this value agrees with that found by West (1947b) for dialysed blood (0.098 μg. adrenaline per ml.), and that *nor*adrenaline was not detected then, since quantitative agreement between the different fluorimetric, physiological, and chemical tests was shown when the results were calculated as adrenaline.

Adrenaline content of suprarenal vein plasma following stimulation.—During continuous stimulation of the splanchnic nerve, plasma samples were collected and subjected to parallel assays on the same day. An example of adrenal sympathin is shown in Table IV, where the calculated value for *nor*adrenaline is zero—i.e.,

TABLE IV

PARALLEL ASSAYS (RABBIT NO. 3). CONCENTRATION OF ADRENAL SYMPATHIN IN THE FIRST POST-STIMULUS SAMPLE OF PLASMA IN TERMS OF ADRENALINE AND *nor*ADRENALINE (μG./ML.)

Sympathin estimated as	Rabbit ileum		Rabbit blood pressure	Chick rectum
	Fresh	Stored		
l-adrenaline (A)	0.3	0.3	0.3	0.35
*l-nor*adrenaline (N)	0.6	0.1	0.6	7.00
Ratio A/N	0.5	3	0.5	0.05

Calculated value (Bülbring, 1949) gives mean of 0.31 μg. *l*-adrenaline per ml. *Nor*adrenaline value is zero.

all the sympathin was present as adrenaline. This was the first post-stimulus sample (collected 0–3 minutes), and other samples from this rabbit indicated that some *nor*adrenaline was released during the early stages of stimulation, but it was not present in later samples (Table V). With continued stimulation in many rabbits, it was possible to show that the percentage of adrenaline in the sympathin present in the suprarenal blood decreased to 68 per cent during the first nine minutes of continuous stimulation, but after a further six minutes recovery had taken place

TABLE V

RABBIT NO. 3. CONCENTRATION OF ADRENAL SYMPATHIN IN PRE- AND POST-STIMULUS SAMPLES OF PLASMA

Amine	Control value	μg./ml. after continuous stimulation for				
		0–3 min.	3–6 min.	6–9 min.	9–12 min.	27–30 min.
l-adrenaline	0.10	0.31	0.20	0.15	0.21	0.30
*l-nor*adrenaline	0	0	0.09	0.11	0.01	0
% adrenaline ..	100	100	70	58	95	100

and little or no *nor*adrenaline could be detected (Table VI). In one rabbit (No. 6), it was impossible to obtain a control value, although the stimulated samples were easily estimated.

TABLE VI

Stimulation time in minutes	Percentage of adrenaline in active material released by the adrenal gland at various times by continuous stimulation of the splanchnic nerve in rabbits, Nos. :										
	1	2	3	4	5	6	7	8	9	10	Mean
0	100	88	100	100	100	—	100	83	100	100	97
3	100	70	100	100	100	47	100	83	100	33	83
6	100	30	70	90	100	30	60	100	100	60	74
9	65	—	58	59	26	94	50	100	—	95	68
12	—	63	95	—	—	—	—	—	—	95	84
15	—	—	—	100	—	—	100	—	—	94	98
Over 15	—	—	100	100	100	100	100	100	100	100	100
Total time in min.	15	16	30	50	62	64	85	90	90	120	

DISCUSSION

The results have confirmed the findings of Holtz and Schümann (1950) that the rabbit suprarenal glands, unlike those of the dog, cat, or man, contain only adrenaline. This is important by itself, but even more interesting is the observation that the gland appears to be capable of adapting itself to stimulation of the splanchnic nerve. Small quantities of *nor*adrenaline were detected in the plasma in the early stages of stimulation, but the glands at no time contained this amine in any appreciable amount, although the adrenaline content was increased by more than 30 per cent in stimulated glands.

Shaw (1938) found that rabbit's blood had an apparent adrenaline content of 0.05–0.06 μg./g., and most of it probably was adrenaline, as shown by the specific colour increase with alkali. West (1947b) obtained a value of 0.098 μg./ml. (as adrenaline) in normal rabbit's blood by using chemical, fluorimetric, and physiological methods. The samples of blood were taken from the ear vein direct into solid sodium

citrate and assayed. The value, however, agrees well with that found in plasma from the suprarenal vein before stimulation (0.084 μg./ml.), and clearly indicates that adrenaline (and not *nor*adrenaline) is adrenal sympathin in the rabbit.

One of the assay processes involved the blood pressure of a rabbit under urethane anaesthesia. It is known that by the jugular route in rabbits adrenaline is a much more active pressor agent than *nor*adrenaline (West, 1948). This is in contrast to the cat or dog where the primary amine is the more potent. However, when cocaine is given, the *nor*adrenaline response is potentiated more than that to adrenaline, and *nor*adrenaline becomes about one-half as active as adrenaline.

Another of the assay processes involved the isolated stored rabbit ileum. Several workers have shown that storage of isolated organs affects the sensitivity and movements of these structures when set up in the ordinary organ bath. West (1947a) reported that cat, rabbit, and rat ileum become less sensitive to adrenaline on storage in Tyrode solution at 4° C., the sensitivity to *dl-nor*adrenaline remaining practically the same. After 5 days' storage, *nor*adrenaline was about four times as active as adrenaline on the rabbit ileum. Schümann (1950) repeated the work with *l-nor*adrenaline and obtained similar results. Our series has been extended to storage for 7 days (Table I). However, after 4 days' storage, pendular movement is still quite prominent, relaxations are easily estimated, and *nor*adrenaline is three times more active than adrenaline, so that this tissue forms a satisfactory pharmacological preparation for assisting in the differentiation of the two amines. It has been suggested that the effect of storage on the sensitivity of tissues to drugs is due to death of the nerves. Such a change would be expected to lead to enhanced responses to adrenaline, sympathin, and certain other sympathomimetic amines, but the only effect observed with the rabbit ileum was a diminished response to adrenaline. The loss of sensitivity to adrenaline measures a general failure of the tissue metabolism. It is known that denervation *in vivo* produces a greater change in the reaction of some tissues to *nor*adrenaline than to adrenaline. Failure of the mechanism of innervation leads to a denervation with an increase (or certainly not a large decrease) in sensitivity to *nor*adrenaline.

Summary

1. When the splanchnic nerve in the rabbit is stimulated and the plasma is obtained from the blood in the suprarenal vein, at first adrenaline only is present. During the first nine minutes of stimulation the adrenaline concentration decreases and *nor*adrenaline appears. At this stage, as much as 32 per cent of the active material in the plasma may be *nor*adrenaline.

2. After 15 minutes' continuous stimulation of the splanchnic nerve, only adrenaline is detected in the plasma in the suprarenal vein.

3. Stimulation of the splanchnic nerve in rabbits for periods varying from 15 to 120 minutes increases the adrenaline content of the suprarenal gland by more than 30 per cent, but no *nor*adrenaline is detected.

4. These findings allow of the conclusion that in the rabbit adrenaline is more important than *nor*adrenaline in the transmission of autonomic nerve impulses.

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