RESEARCH PAPERS

THE NATURE OF AVIAN AND AMPHIBIAN SYMPATHIN

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IT is now well established that noradrenaline is present with adrenaline in most mammalian suprarenal glands, though its relative amount varies widely. In the rabbit, for example, only traces are found[1], whilst in the cat up to half the amount of total amine may be present as noradrenaline. It seemed of interest, therefore, to investigate the glands of birds and amphibia, since noradrenaline is less potent than adrenaline in many excitor actions in these animals (e.g., on the frog heart and blood vessels), whereas in excitor actions in most mammals it is more potent than adrenaline. Besides, birds and reptiles are in general herbivorous animals compared with carnivora such as the cat, dog, rat and man. Birds of prey are carnivorous, but we have not yet succeeded in securing adrenal glands from these animals for purposes of comparison.

To study the possible precursors of adrenaline, many workers have used injections of insulin to exhaust the suprarenal medulla. The discharge of amines from the medulla helps to restore the normal blood sugar, with the result that the gland is overworked and contains more of the precursors, though less of the normal total content. Poll[2], for example, found that the chromaffin reaction of adrenal tissues was much diminished by the injection of insulin in rats, mice, pigeons, and frogs. Burn, Hutcheon and Parker[3] estimated the total amount of active material, and the percentage of adrenaline in this amount, present in the suprarenal medulla of the rat following injections of insulin. They obtained evidence that noradrenaline is a precursor of adrenaline in the rat and that, after several hours' depletion of the medulla, the supply of noradrenaline is restored more rapidly than it can be methylated to form adrenaline. In dogs and cats, Bülbring[4] had previously shown that minced suprarenal tissue, when incubated with adenosine triphosphate, converts noradrenaline to adrenaline; conversion is most vigorous in tissue prepared from glands stimulated through the splanchnic nerve beforehand. Bülbring and Burn[5] have also obtained evidence of the conversion of noradrenaline to adrenaline in the perfused suprarenal gland of the dog, by observing a disappearance of noradrenaline and a corresponding increase in the amount of adrenaline. On the other hand, it appears that in the rabbit noradrenaline may not be a precursor of adrenaline, since on only very few occasions has it been detected in the suprarenal medulla[6]. We have, therefore, tried the effect of injections of insulin on the suprarenal medullæ of chicken and pigeon in an attempt to ascertain possible precursors of adrenaline in these animals. A preliminary note concerning noradrenaline and the adrenal glands of the domestic fowl has already been published[7].

METHODS

45 adult pigeons were used for this study. 16 were kept without food overnight and injections were given in the morning after taking blood samples from the wing vein for sugar estimations (Hagedorn and Jensen method). The doses of insulin injected intramuscularly were 800 and 1,600 I.U./kg. At given times after the injection, the pigeons were killed after taking further blood samples for sugar estimations. The suprarenals were removed, weighed, and ground in a mortar with 10 ml. of 0·1 N hydrochloric acid per g. of fresh gland and a little sand. The contents of the mortar were then transferred to a centrifuge tube and centrifuged. The clear supernatant fluid was adjusted to *p*H 4 and kept in the cold until the test for total activity and noradrenaline content was carried out. Extracts of the glands from the non-injected pigeons were prepared at the same time. 8 pigeons were injected intramuscularly with adrenaline hydrochloride solution (4 mg. of base per kg.) and killed at given times after the injection. Similar gland extracts were prepared from these birds.

To obtain control gland extracts of fowls, both adult birds and day-old chicks were used. Further adult birds received a dose of insulin intramuscularly (80 I.U./kg.) and these were killed at given times after the injection. Suprarenal gland extracts were prepared as described above.

For the frog experiments, groups of 5 frogs were killed, and their suprarenals and kidney attachments were extracted with 0·1 ml. of 0·1 N hydrochloric acid per frog. These experiments were carried out between October and February.

The determination of the proportion of adrenaline and noradrenaline in the extracts was made on the cat's blood pressure and nictitating membrane by the method of Burn, Hutcheon and Parker[3]. In some experiments, atropine (1 mg./kg.) and mepyramine (1 mg./kg.) were given[8]. The total activity in each extract was estimated by comparing its pressor action in the spinal cat, and sometimes its relaxor action in the isolated rabbit ileum, with that of adrenaline. In most experiments, confirmation of the biological values was achieved by paper chromatography[9]. An ascending column was used with butanol-acetic acid-water as the solvent.

RESULTS

Insulin and Adrenaline Injections in the Pigeon. The mean control value of total activity in the extracts of the suprarenal glands of 20 pigeons was 1·35 ± 0·11 mg. of adrenaline, and 1·65 ± 0·15 mg. of noradrenaline, per g. of fresh tissue. Hence, noradrenaline predominates to the extent of 55 per cent. of the total activity (see Fig. 1). After massive doses of insulin (since pigeons are very insensitive to this drug), exhaustion of the suprarenal gland occurred as shown by a drop in total activity to about one-third of that originally present (Table I). This

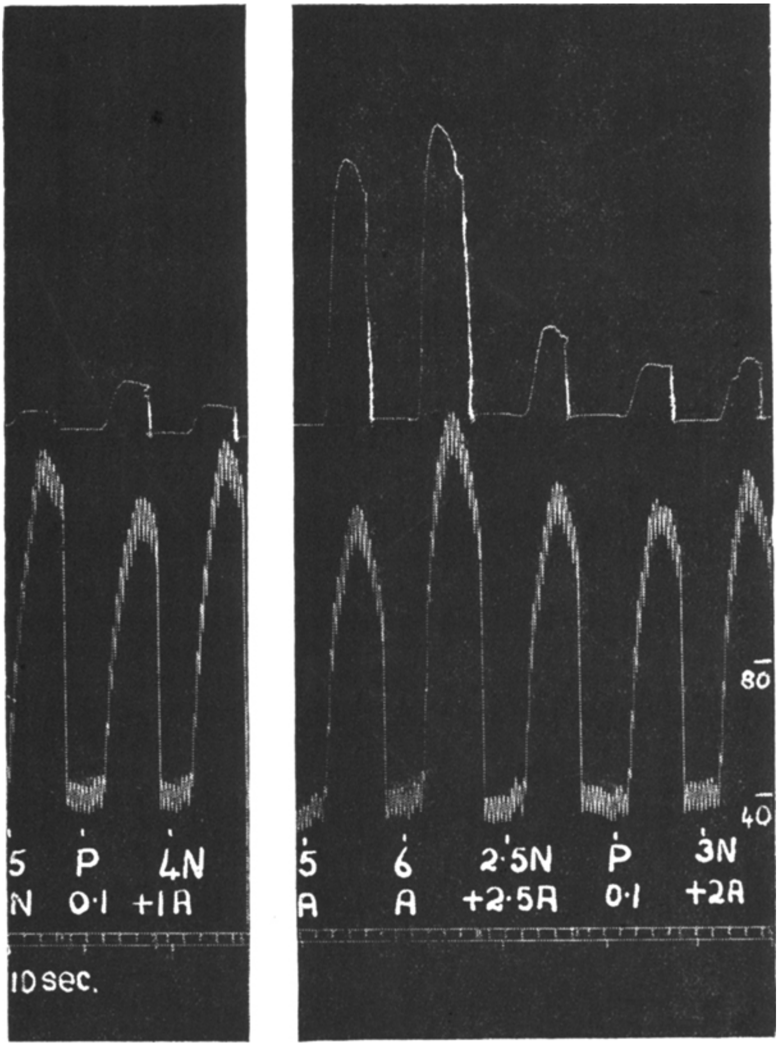

FIG. 1. Spinal cat 2 kg. Upper curve, nictitating membrane: lower curve, blood pressure. Time in 10 sec. Doses in ml. or μg. 0·1 ml. of pigeon extract (P) produces effects which are equated by those produced by a mixture of 2 μg. of adrenaline (A) and 3 μg. of noradrenaline (N).

fall reached its peak at 4 hours after injection at a time when the fall in blood sugar was most marked (see Fig. 2 and Table II). Indeed, the change in total activity was roughly parallel to the change in blood sugar.

TABLE I

TOTAL ACTIVITY IN GLAND EXTRACTS AFTER INJECTION OF INSULIN (AVERAGE VALUES AFTER 800 AND 1600 I.U./KG.)

Pigeon	Times after insulin			
	0 hr.	2 hr.	4 hr.	8 hr.
Total activity (mg./g.)	3·00	2·05	1·09	1·55
Adrenaline (mg./g.)	1·35	0·89	0·47	0·70
Noradrenaline (mg./g.)	1·65	1·16	0·62	0·85
Percentage of noradrenaline	55	56	57	54

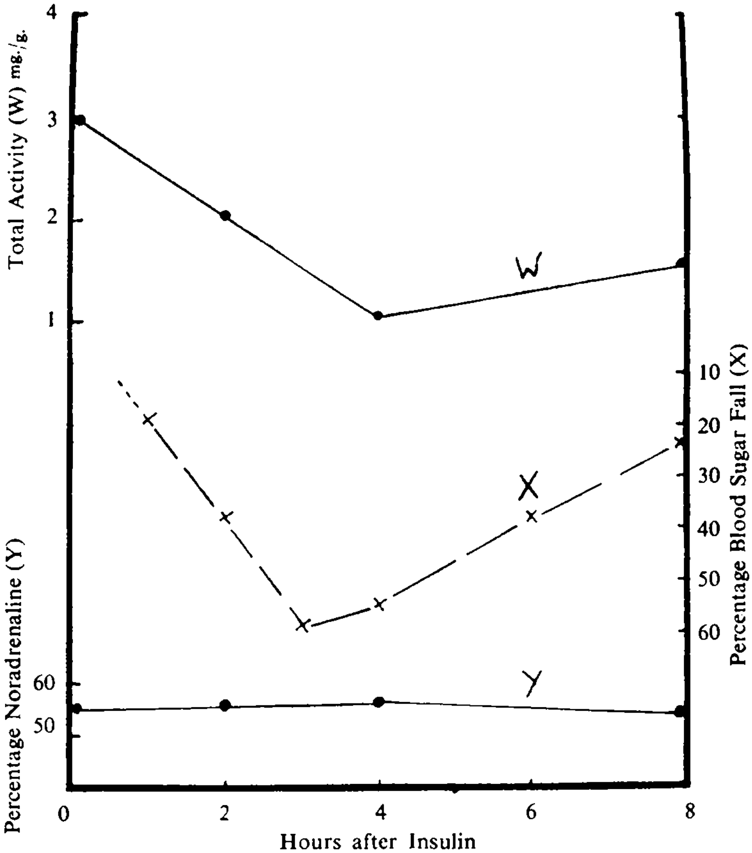

FIG. 2. The effect of insulin on the total activity (W) and relative noradrenaline content (Y) in the suprarenal gland of pigeons. Note that the percentage fall in blood sugar (X) closely follows the change in total activity and that there is no change in the relative noradrenaline content.

TABLE II

PERCENTAGE BLOOD SUGAR FALLS IN PIGEONS AFTER INJECTION OF INSULIN

Dose of insulin (I.U./kg.)	Times after insulin					
	1 hr.	2 hr.	3 hr.	4 hr.	6 hr.	8 hr.
800	19	33	60	49	23	18
1600	20	44	58	61	53	30
Mean	19	38	59	55	38	24

Mean fasting blood sugar -- 131 mg. per cent.

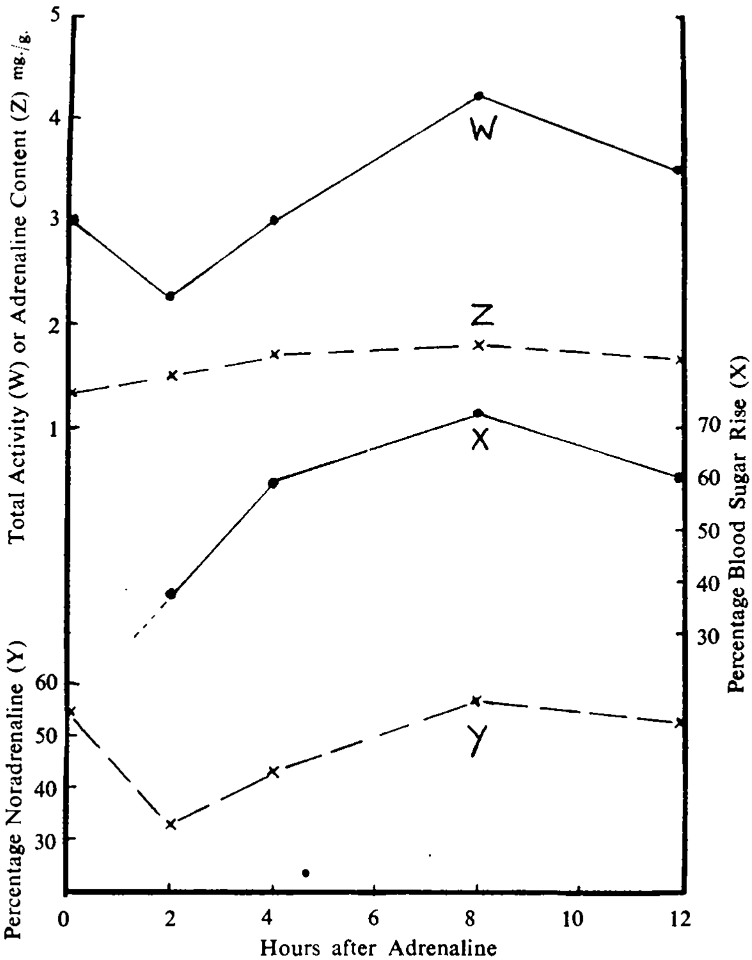

FIG. 3. The effect of adrenaline (4 mg./kg.) on the blood sugar (X), and on the total activity (W), relative noradrenaline (Y), and absolute adrenaline (Z) contents in the suprarenal gland of pigeons.

On the other hand, the percentage of noradrenaline in the gland extracts did not change despite the large drop in total activity.

When a large dose of adrenaline (4 mg. of base per kg.) was given, total activity fell slightly during the first 2 hours after injection and then rose to a peak at 8 hours (Fig. 3) before returning to normal levels.

TABLE III

TOTAL ACTIVITY IN GLAND EXTRACTS AFTER INJECTION OF ADRENALINE (4 MG./KG. INTRAMUSCULARLY)

Pigeon	Times after adrenaline				
	0 hr.	2 hr.	4 hr.	8 hr.	12 hr.
Total activity (mg./g.)	3·00	2·25	3·00	4·20	3·50
Adrenaline (mg./g.)	1·35	1·50	1·71	1·80	1·65
Noradrenaline (mg./g.)	1·65	0·75	1·29	2·40	1·85
Percentage of noradrenaline...	55	33	43	57	53
Percentage blood sugar rise...	—	37	59	73	60

The rise in blood sugar was also maximal at 8 hours after injection (Table III). Unlike the effect following the injection of insulin, the percentage of noradrenaline in the mixture in this case fell during the first 2 hours, after which there was a return to normal values.

TABLE IV

TOTAL ACTIVITY IN GLAND EXTRACTS AFTER INJECTION OF INSULIN (80 I.U./KG. INTRAMUSCULARLY)

Fowls	Times after insulin			
	0 hr.	2 hr.	4 hr.	8 hr.
Total activity (mg./g.)	10·10	5·63	6·00	8·58
Adrenaline (mg./g.)	2·01	3·13	4·00	4·29
Noradrenaline (mg./g.)	8·09	2·50	2·00	4·29
Percentage of noradrenaline	80	44	33	50

Insulin Injections in the Fowl. The mean control value of total activity in the extracts of the suprarenal glands of birds of all ages was

8·09 ± 0·74 mg. of noradrenaline and 2·01 ± 0·21 mg. of adrenaline per g. of fresh tissue. In the chicken adrenals, therefore, 80 per cent. of the total activity exists as noradrenaline. This is the highest value found in all the species we have so far tested. After a dose of insulin, exhaustion of the gland occurred, due solely to a reduction in the noradrenaline content (Table IV and Fig. 4). In fact, the adrenaline content in the gland steadily increased.

Frog Suprarenal Glands. In 6 series of 5 frogs, the suprarenal glands have been extracted and tested in the usual manner. Results showed a great variation, but the mean values were 3 μg. of adrenaline and 4 μg.

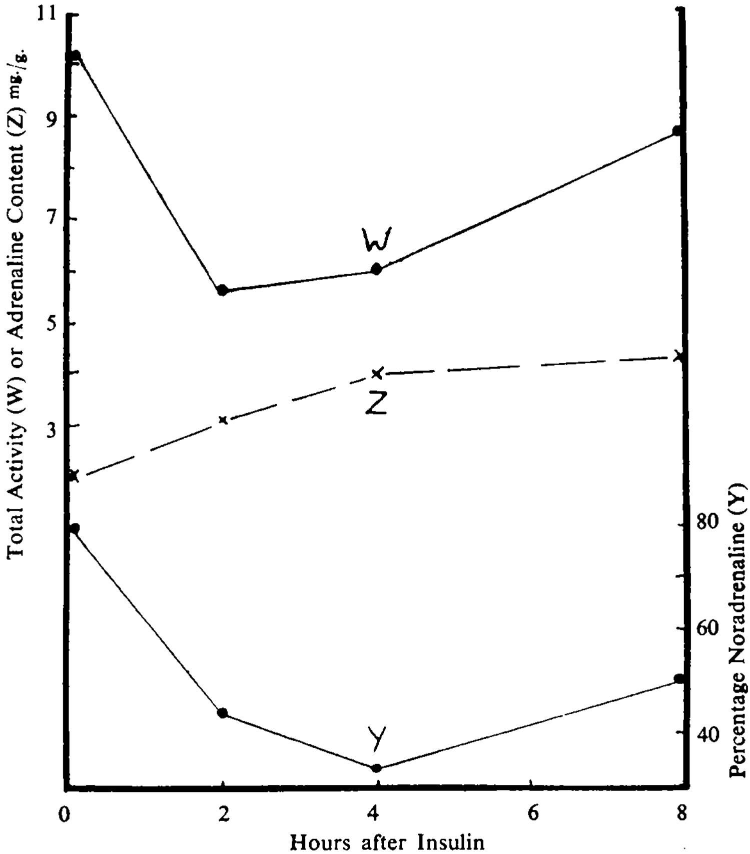

FIG. 4. The effect of insulin (80 I.U./kg.) on the total activity (W), relative noradrenaline (Y) and absolute adrenaline (Z) contents in the suprarenal gland of fowls. Note that the fall in total activity is due solely to a fall in absolute noradrenaline amounts.

of noradrenaline per frog. This means that in the frog, as in the pigeon, noradrenaline predominates in the suprarenal gland to the extent of about 55 per cent. of the total activity. This result is of interest, since workers have found[10,11] that the active substance in extracts of frog hearts is adrenaline and not noradrenaline, whereas noradrenaline is the predominating amine in extracts of mammalian hearts[12].

Discussion

The results show that the suprarenal glands of pigeons, fowls and frogs contain more noradrenaline than adrenaline. This finding is similar to that recorded in glands of whales[13], in tumour tissue from human phaeochromocytomas[14], and the glands of children under 1 year old[15]. The absolute activity values found for the birds are very high, weight for weight, when compared with most mammals (Table V). It appears

TABLE V

THE TOTAL ACTIVITY AND RELATIVE NORADRENALINE CONTENT IN EXTRACTS OF SUPRARENAL GLANDS OF VARIOUS ANIMALS

Animal	Activity (mg./g.)	Percentage noradrenaline in gland	Medullae:Cortices
Fowl	10·10	80	1 : 1
Pigeon	3·00	55	1 : 2
Frog	—	57	– –
Dog	1·70	26	1 : 5
Man	1·25	15	1 : 9
Cat	1·23	33	1 : 18
Rat	1·00	10	1 : 20
Rabbit	0·48	2	1 : 40
Guinea-pig	0·13	3	1 : 63

from Table V that the ratio of medullæ to cortices may be a clue to the relative amount of noradrenaline in the gland. As this ratio decreases, so the relative noradrenaline content decreases. This suggests that the degree of methylation may be related to the relative size of the cortex. It is also possible that the enzyme concerned in methylation (transmethylase) may be a constituent of, or be activated by, cortical tissue.

Burn, Hutcheon and Parker[3] showed that after several hours' depletion of the suprarenal medulla of the rat by insulin the supply of noradrenaline is restored more rapidly than it can be methylated to form adrenaline. This evidence clearly suggests that noradrenaline is a precursor of adrenaline in the rat, as it is in the cat and dog. In the pigeon and fowl, however, insulin produces a different result; there is no change in the relative noradrenaline content in the glands of pigeons, whereas a substantial fall in this component occurs in the fowl, despite a decrease in total activity resulting from the hypoglycæmia. Presumably in the first case, an outpouring of both adrenaline and noradrenaline has taken place, and, in the second case, the fall in total activity has resulted from the liberation of only noradrenaline, since the adrenaline content is unchanged. An alternative explanation of this second result may be that the process of methylation proceeds faster than that of replenishing the store of noradrenaline, though this effect is doubtful. However, we suggest that, since noradrenaline predominates in the resting suprarenal glands of the pigeon, fowl and frog, it may well be a hormone itself and that is not merely a precursor of adrenaline. The result of injecting adrenaline further supports this hypothesis in the pigeon. Here the hyperglycæmia produces changes in the noradrenaline content of the gland which closely follow the changes in total activity.

In the chromatographic work, particular attention was paid to other possible precursors of adrenaline such as dihydroxyphenylalanine or hydroxytyramine, but in all extracts tested no amines other than noradrenaline and adrenaline were identified.

SUMMARY

(1) The suprarenal glands of the pigeon, fowl and frog contain more noradrenaline than adrenaline. In the fowl, 80 per cent. of the total activity may be present as noradrenaline.

(2) When insulin is given to pigeons and fowls, the total activity in the suprarenal medulla declines during the first 4 hours, after which the amount of active material begins to rise.

(3) After the injection of insulin, there is also a fall in the percentage of noradrenaline present in the gland of the fowl. This fall is parallel to the fall in total activity, and suggests that noradrenaline may be a hormone itself and that it is not merely a precursor of adrenaline.

(4) In the pigeon, there is no fall in the percentage of noradrenaline present in the gland, despite the large dose of insulin. Since total activity has been reduced, both noradrenaline and adrenaline must have been released in an effort to counteract the hypoglycæmia.

I wish to record my thanks to Mr. D. M. Shepherd, of this Department, for carrying out the chromatographic studies.

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