THE SUPRARENAL GLANDS OF THE HARE AND HORSE

By G. B. West

From the Department of Pharmacology and Therapeutics, University of St. Andrews Medical School, Dundee

Received May 6, 1953

It is now well known that extracts of many mammalian suprarenal glands contain noradrenaline in addition to adrenaline, though the relative amount of each amine varies widely[1]. Whereas in the rabbit and guinea-pig methylation of noradrenaline is almost complete, the predominating amine in the pigeon, fowl and frog is noradrenaline[2]. Another catechol amine, hydroxytyramine, has been identified in the adrenal medulla of the sheep, ox and cow[3], but its presence bears no relationship to the total or relative catechol amine content of the tissue.

With the aid of paper chromatography, Goodall[4] found that the percentage of adrenaline and noradrenaline in the suprarenals of some African mammals varied according to the animal family. For example, the percentage of noradrenaline was very low in the rodents and differed markedly from that found in the feline family. From their habits, Goodall suggested that the hunted animals (primates, rodents and ruminants) have predominantly adrenaline in their suprarenals, whilst the hunters or aggressive animals (lion and cat) have chiefly noradrenaline.

We have now examined the suprarenals of the wild hare (for comparison with the tame rabbit) and the horse (for comparison with the slower-moving farm animals). Particular attention was paid to the identification and estimation of possible precursors of adrenaline and noradrenaline.

Methods

Hares were shot on a local farm and their suprarenal glands were removed as soon as possible (sometimes in the field) into bottles containing 2 ml. of 0·01N hydrochloric acid. The glands were taken to the laboratory for weighing and grinding up with sand and the surrounding acid. In a similar manner, the glands of horse were collected as soon as possible after death at the slaughter-house. After centrifuging the extracts, the clear supernatant liquids were assayed for their adrenaline and noradrenaline contents by paper chromatography and biological assay[1]. Further concentration was effected by the addition of 4 volumes of ethanol, centrifugation, evaporation to dryness and elution into a small volume of water. Careful examination of the paper chromatograms of these concentrated extracts was carried out in every case to detect possible precursors. Solutions of the following substances were prepared in 0·01N hydrochloric acid—*l*-adrenaline, *l*-noradrenaline bitartrate, hydroxytyramine hydrochloride, *dl*-dihydroxyphenylalanine, *dl*-dihydroxyphenylserine, lactyl-adrenaline and lactyl-noradrenaline[5], *dl-p*-hydroxyphenylethanolamine hydrochloride (*p*-norsynephrine), *dl-m*-hydroxyphenylethanolamine hydrochloride, tyramine hydrochloride, tyrosine and phenylalanine. Most of these drugs were obtained through the courtesy

of Sterling-Winthrop Research Institute, New York; Hoffmann-la-Roche, Basle; and Bayer Products Ltd., London.

RESULTS

Suprarenals of the hare. 10 experiments were carried out using extracts of the suprarenal glands of hares, and the mean results are 309 μg. of adrenaline and 41 μg. of noradrenaline per g. of fresh tissue (noradrenaline 12 per cent.). The glands are about the same size as those of the rabbit which contain 470 μg. of adrenaline and 10 μg. of noradrenaline/g.[1], and this means that the hare has a greater reserve of noradrenaline and less adrenaline than the rabbit.

Suprarenals of the horse. 29 experiments were completed in this study. It was possible in many cases to separate the cortex from the medulla, thereby enabling extracts of each part of the gland to be made without contamination by the other part. The means of all results are: whole gland, 666 μg. of adrenaline and 166 μg. of noradrenaline/g. (noradrenaline 20 per cent.); medulla only, 3200 μg. of adrenaline and 800 μg. of noradrenaline/g.; cortex only, 160 μg. of adrenaline and 42 μg. of noradrenaline/g. These values are recorded in Table I and contrasted with those already found for other farm animals[3]. It will be seen that the horse in this group contains the lowest relative amount of noradrenaline, whilst the pig (which is certainly not an aggressive animal like the lion) contains about equal quantities of both pressor amines.

TABLE I

CONCENTRATIONS (μg./g.) OF ADRENALINE AND NORADRENALINE IN THE ADRENAL GLANDS OF SOME FARM ANIMALS

Animal	Cortex		Medulla		Whole gland		Noradrenaline in total per cent.
	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	
Horse ..	160	42	3200	800	666	166	20
Ox ..	400	150	4000	1500	1200	420	26
Cow ..	400	200	4000	1250	1250	500	29
Sheep ..	100	40	2000	1600	500	250	33
Pig ..	125	75	4000	4000	1090	1056	49

Possible precursors of noradrenaline. Extracts of the suprarenal glands of the horse after concentration were chromatographed in the usual manner using solutions of the amines for controls. The developers used included aqueous potassium iodate[1], aqueous potassium ferricyanide[6], a mixture of potassium dichromate and formaldehyde (to produce the fluorescence reaction)[7], ninhydrin in butanol, *p*-nitraniline[8], Folin and Ciocalteu reagent, and acid and alkaline Pauly reagent. Table II illustrates how each of the possible precursors can be detected. In all the horse and hare gland extracts, only adrenaline and noradrenaline were identified. Traces of tyrosine and phenylalanine were present in most extracts, and an unknown spot (R_F value, 0·32) was found in some. This latter spot may be an ascorbic acid derivative, though there is no complete proof of this.

TABLE II

IDENTIFICATION OF THE PRESSOR AMINES AND THEIR POSSIBLE PRECURSORS BY PAPER CHROMATOGRAPHY

Pressor amines and their possible precursors	R_F value (butanol–acetic acid–water)	Colour with developer							
		KIO_3	$K_3Fe(CN)_6$	Fluorescence	Ninhydrin	*p*-Nitraniline	Folin and Ciocalteu reagent	Pauly reagent	
								Acid	Alkaline
Adrenaline	0·36	Pink	Pink	Yellow-green	Purple	Grey-blue	Blue	Brown	Red-brown
Noradrenaline	0·28	Violet	Rose	Turquoise	Brown	Grey-blue	Blue	Brown	Brown
Hydroxytyramine	0·39	Orange-brown	Brown	Yellow	Brown	Grey-blue	Blue	Brown	Red-brown
Dihydroxyphenylalanine	0·21	Greyish-violet	Greyish-violet	Yellow	Purple	Grey-blue	Blue	Brown	Red-brown
Dihydroxyphenylserine	0·15	Pinkish-brown	Pinkish-brown	Turquoise	Purplish-brown	Grey-blue	Blue	Brown	Red-brown
Lactyl-adrenaline	0·57	Brownish-violet	Pink	0	Brown	Green-blue	Blue	Brown	Red-brown
Lactyl-noradrenaline	0·52	Brownish-violet	Rose	0	Brown	Light grey	Blue	Brown	Brown
p-Hydroxyphenylethanolamine	0·43	0	0	0	Purplish-brown	Deep red	Blue	0	Yellow-brown
m-Hydroxyphenylethanolamine	0·46	0	0	Violet	Brown	Deep red	Blue	Delayed yellow	Yellow-brown
Tyramine	0·58	0	0	0	Purple	Grey-blue	Blue	0	Red-brown
Tyrosine	0·30	0	0	0	Purple	Purple	Blue	0	Red-brown
Phenylalanine	0·51	0	0	0	Purplish-blue	Rose	0	0	0

Discussion

We have found that the suprarenal gland of the hare contains a higher proportion of noradrenaline than is usually found in the glands of rabbits. Since the glands in the two animals are about the same size, the hare must have a greater reserve of noradrenaline. This may be related to its exceptional powers of running or to the fact that it is usually a wild animal; its adrenaline reserve is certainly less than that found in the tame rabbit.

The suprarenal gland of the horse contains the lowest percentage of noradrenaline of the 5 farm animals so far tested, yet this is not related to the total amine content. The glands of the pig which contain the highest total amine content also contain the highest percentage of noradrenaline. It appears that there is no easy explanation why the relative noradrenaline content of the suprarenal gland of animals should differ so widely. We still believe that some cortical material may be the controlling factor.

Concerning possible precursors of adrenaline and noradrenaline, hydroxytyramine and dihydroxyphenylalanine were the obvious first choice to search for, since Blaschko[9] presented in 1942 a scheme for the biosynthesis of adrenaline which involved the formation of these two substances. Dihydroxyphenylserine, on the other hand, is the amino-acid corresponding to noradrenaline, decarboxylation producing this pressor amine; the amino-acid, however, has not been found naturally. The lactyl derivatives of adrenaline and noradrenaline are relatively inactive forms of the parent amines, and since lactic acid occurs in fair quantity in the suprarenal medulla they might be the means whereby the gland stores the active material. *p*-Hydroxyphenylethanolamine has been found naturally and can be converted into noradrenaline under the influence of ultra-violet irradiation in the presence of air. The *meta*-compound can likewise be converted to this active amine[10]. Tyramine might be the important step between tyrosine and *p*-hydroxyphenylethanolamine in this scheme of synthesis. However, all of these substances were not detected and the method by which the adrenaline and noradrenaline synthesis in the body occurs is still an open one.

Summary

1. The suprarenal gland of the hare contains adrenaline and noradrenaline; its relative noradrenaline content is greater than that found in the rabbit. The suprarenal gland of the horse contains adrenaline and noradrenaline; its relative noradrenaline content is lower than those found in the sheep, cow, ox and pig. No explanation has been found so far to account for these variations.

2. Hydroxytyramine, dihydroxyphenylalanine, dihydroxyphenylserine, *p*-norsynephrine and tyramine are not present in detectable quantities in suprarenal gland extracts of the hare and horse.

References

1. Shepherd and West, *Brit. J. Pharmacol.*, 1951, **6**, 665.
2. West, *J. Pharm. Pharmacol.*, 1951, **3**, 400.
3. Shepherd and West, *J. Physiol.*, 1953, **120**, 15.

4. Goodall, *Acta physiol. scand.*, 1951, **24**, Supp. 85.
5. Crawford, *Biochem. J.*, 1951, **48**, 203.
6. James, *Nature, Lond.*, 1948, **161**, 851.
7. Shepherd and West, *ibid.*, 1953, in the press.
8. Wickström and Salvesen, *J. Pharm. Pharmacol.*, 1952, **4**, 631.
9. Blaschko, *J. Physiol.*, 1942, **101**, 337.
10. Shepherd and West, *J. Pharm. Pharmacol.*, 1952, **4**, 672.

Correction.

THE QUANTITATIVE DETERMINATION OF CINNAMON IN THE FORM OF POWDER

BY R. DEQUEKER.

This Journal, 1952, **4**, 573.

TABLE II, p. 575

The heading of the column reading: Length of fibres in mm. per g. of powder. . . .

Should read: Length of fibres in m. per g. of powder. . . .