J. Physiol. (1953) 120, 15–19

HYDROXYTYRAMINE* AND THE ADRENAL MEDULLA

By D. M. SHEPHERD and G. B. WEST

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

(*Received* 27 *May* 1952)

The adrenal medulla of most animals contains both adrenaline and noradrenaline. Although it is fairly certain that adrenaline is formed from noradrenaline by methylation, the exact way in which noradrenaline is produced in the body is still an open question. Recently, on the basis of paper chromatographic studies, Goodall (1951) claimed that hydroxytyramine (β-(3:4-dihydroxyphenyl)-ethylamine), in addition to adrenaline and noradrenaline, is present in sheep adrenal glands, and that 3:4-dihydroxyphenylalanine (DOPA) is found in the glands of thyroidectomized sheep. These two substances may therefore be precursors of noradrenaline (Blaschko, 1942). It was decided to study this problem in greater detail, and extend it to other animals besides the sheep.

METHODS

Adrenal glands of the sheep, cow, ox, calf and pig were removed immediately after death at the slaughterhouse. The human material was secured from the post-mortem room as soon after death as possible and in many cases this was not more than 3 hr. It was possible in nearly half the specimens to separate the cortex from the medulla. Rabbits, cats and dogs were killed by a blow on the head or by a lethal dose of pentobarbitone, and their adrenal glands were removed as soon as possible. In these animals, six glands were needed each time for preparing medullary extracts.

Cortical, medullary and whole gland extracts of the glands were prepared with 0·5–2 ml. 0·01 N-HCl/g. After centrifuging, known volumes (usually 0·25–0·75 ml.) of the supernatants were precipitated with 4 vol. of ethanol. After further centrifuging, aliquots of the supernatants were evaporated to dryness at 45–50° C and the residues taken up in a small known volume (usually 0·25 ml.) of 0·01 N-HCl. After centrifuging, the clear supernatants were assayed for their adrenaline and noradrenaline contents by paper chromatography and biological assay (Shepherd & West, 1951). To estimate hydroxytyramine, a series of spots was closely applied to chromatography paper to form a continuous straight line. After chromatographing, the position of hydroxytyramine was located by development of a narrow vertical strip cut from the paper. The horizontal strip of paper containing the undeveloped hydroxytyramine was then cut out and the base eluted

* Recently, Sir Henry Dale has suggested that hydroxytyramine be referred to as 'dopamine' in order to stress its chemical relationship to DOPA (cf. tyramine and tyrosine).

with the minimum volume (usually 0·5 ml.) of 0·001 N-HCl. The eluate was applied to a fresh paper and re-chromatographed alongside standard solutions of hydroxytyramine. It was thus possible to estimate the hydroxytyramine content by comparing the intensity of the resulting spot with those of the standard solutions. In all cases, the first chromatograms were developed with potassium ferricyanide and the second with potassium iodate. Butanol–acetic acid–water (4:1:5) and butanol–N-HCl were used as solvents to prove conclusively the presence of hydroxytyramine (see Table 1 for R_F values).

Solutions of (−)-adrenaline, (−)-noradrenaline bitartrate, hydroxytyramine hydrochloride and (±)-dihydroxyphenylalanine in 0·01 N-HCl were used as controls.

TABLE 1. R_F values of some possible precursors of adrenaline in two solvents

	Butanol–acetic acid–water	Butanol–N-HCl
DOPA	0·19	0·22
Noradrenaline	0·28	0·16
Adrenaline	0·36	0·21
Hydroxytyramine	0·39	0·30

TABLE 2. Concentrations (μg/g) of adrenaline (Adren.), noradrenaline (Noradren.) and hydroxytyramine (OH-Tyr.) in the adrenal glands of various animals

		Cortex		Medulla			Whole gland		
Animal	No. of glands	Adren.	Noradren.	Adren.	Noradren.	OH-Tyr.	Adren.	Noradren.	% noradren. in total
Sheep	38	100	40	2000	1600	350	500	250	33
Ox	24	400	150	4000	1500	35	1200	420	26
Cow	4	400	200	4000	1250	17	1250	500	29
Man	48	109	3	1260	314	0	224	34	14
Pig	12	125	75	4000	4000	0	1090	1056	49
Man (phaeochromocytoma)	2	—	—	—	—	—	110	3280	96

RESULTS

In medullary extracts prepared from nineteen freshly killed sheep, hydroxytyramine was identified and estimated by running a second chromatogram of the eluted spot (Figs. 1 and 2). It represented about 9% of the total catechol amine content of the medulla (Table 2). A similar result was obtained on a whole gland extract of a freshly killed newborn lamb. Hydroxytyramine, however, was not detected either in cortical or whole gland extracts of adult animals. It was also absent from further whole gland extracts secured from the Rowett Research Institute, Aberdeen.

In medullary extracts prepared from freshly killed cows and oxen (four and twenty-four glands respectively), hydroxytyramine was identified although it represented only 0·3–0·6% of the total catechol amine content. It was not detected in the corresponding cortical and whole gland extracts. In addition, it was not found in medullary, cortical, or whole gland extracts prepared from pigs, dogs, cats, rabbits and adult human material.

Hydroxytyramine was absent from two extracts of phaeochromocytoma (although over 95% of the total catechol amines in each case was noradrena-

line) and from extracts of Organs of Zuckerkandl containing noradrenaline but no adrenaline (Hunter, Shepherd & West, 1952). It was not found in extracts of glands of calves, kittens, young rabbits and babies all of whose adrenals contain more noradrenaline than adrenaline (Shepherd & West, 1951).

In no case was DOPA detected in the above extracts.

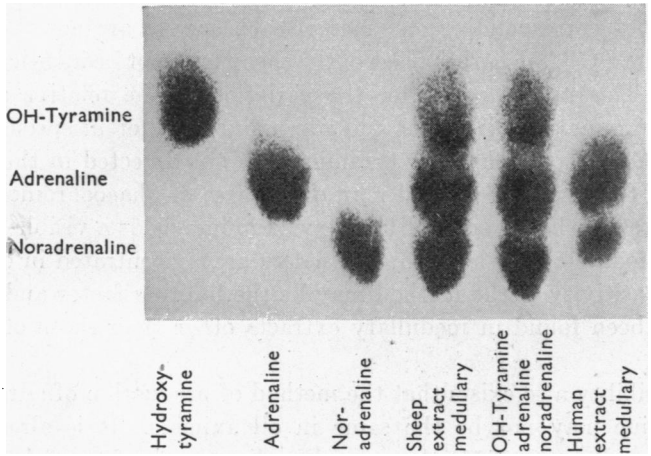

Fig. 1. Paper chromatogram. Solvent: butanol–acetic acid–water (ascending method). 48 hr at room temperature. Adrenaline, noradrenaline and hydroxytyramine are present in adrenal medullary extracts of the sheep, but hydroxytyramine is absent from human medullary extracts.

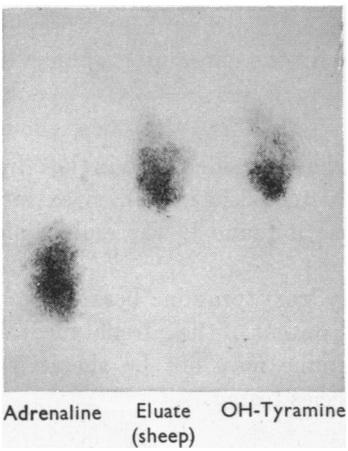

Fig. 2. Paper chromatogram. Solvent: butanol–acetic acid–water (ascending method). 48 hr at room temperature. Eluate of hydroxytyramine area from undeveloped chromatogram of sheep medullary extract re-chromatographed between standard adrenaline and hydroxytyramine solutions.

DISCUSSION

We have confirmed that hydroxytyramine is a normal constituent of adrenal medullary tissue of the sheep and have shown that it is also present in cows and oxen. The evidence supports the suggestion that this substance may be a precursor of noradrenaline in the body. In his scheme for the biosynthesis of adrenaline, Blaschko (1942) postulated three enzymic steps, and it is possible that the corresponding enzymes of each species are not the same; for example, the DOPA-decarboxylase of the sheep may not be identical with that of the ox. This may account for the variation in the relative amounts of hydroxytyramine, noradrenaline and adrenaline in different species (Table 2).

It is of interest that hydroxytyramine was not detected in the medullary extracts of the pig or of normal man or of cases of phaeochromocytoma. It must be stressed, however, that hydroxytyramine yields a visible colour with potassium iodate only when more than 4 μg are concentrated in one spot, so that the sensitivity of the method may be the limiting factor and the reason it has not been found in medullary extracts other than those of sheep and cattle.

The possibility also exists that the method of production of adrenaline and noradrenaline may not be the same in all animals. It is already known (Erspamer & Boretti, 1951) that noradrenaline can be formed by the action of mushroom juice or of ultra-violet light on norsynephrine (β-hydroxy-β-(4-hydroxyphenyl)-ethylamine), an amine occurring naturally in the salivary glands of the octopus. Other possible precursors of noradrenaline are epinine (*N*-methyl-β-(3:4-dihydroxyphenyl)-ethylamine) and dihydroxyphenylserine (α-amino-β-hydroxy-β-(3:4-dihydroxyphenyl)-propionic acid), and we have paid particular attention in the chromatographic work to checking that they are absent.

SUMMARY

1. Hydroxytyramine has been identified in the adrenal medulla of the sheep, ox and cow. It was not detected in medullary extracts of adult pig, dog, cat, rabbit and man, nor was it found in the embryonic tissue of the cow, cat, rabbit and man.

2. The presence of hydroxytyramine bears no relationship to the total or relative catechol amine content of the adrenal medulla. It is suggested, therefore, that hydroxytyramine may not be the immediate precursor of noradrenaline in all the species tested.

We wish to record our thanks to Dr A. T. Phillipson of the Rowett Research Institute, Aberdeen, for sheep glands, to Dr M. Goldenberg of Columbia University for supplying the phaeochromocytoma extracts, and to Prof. A. C. Lendrum and members of the Pathology Department of the University for the human glands. We are also grateful to Mr W. Cameron for photographing the developed chromatograms.

REFERENCES

BLASCHKO, H. (1942). The activity of L(−)-dopa decarboxylase. *J. Physiol.* **101**, 337–349.

ERSPAMER, V. & BORETTI, G. (1951). Identification and characterization, by paper chromatography, of enteramine, octopamine, tyramine, histamine and allied substances in extracts of posterior salivary glands of Octopoda and in other tissue extracts of vertebrates and invertebrates. *Arch. int. Pharmacodyn.* **88**, 296–332.

GOODALL, McC. (1951). Studies on adrenaline and noradrenaline in heart and suprarenals. *Acta physiol. scand.* **24**, Suppl. 85.

HUNTER, R. B., SHEPHERD, D. M. & WEST, G. B. (1952). Organs of Zuckerkandl. *J. Physiol.* **116**, 6*P*.

SHEPHERD, D. M. & WEST, G. B. (1951). Noradrenaline and the suprarenal medulla. *Brit. J. Pharmacol.* **6**, 665–674.