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HORMONES OF THE SYMPATHETIC NERVOUS SYSTEM AND THE ADRENAL MEDULLA*

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The substances which determine the action of adrenergic nerves and of the internal secretion of the adrenal medulla are of equal interest from the physiological and the pharmacological point of view. In the elucidation of this problem as a whole, pharmacological methods of analysis have largely been used, and with considerable success, demonstrating the value of pharmacological tools in solving physiological and even chemical problems.

The concept of a neurohumoral transmission of nerve impulses takes us back to Elliott (1905) in England, who, on account of the similarity of action of sympathetic nerve stimulation and adrenaline, propounded the hypothesis that such nerve impulses became effective by liberating adrenaline at the nerve endings. To prove this was not so easy, however, and in spite of several attempts it was not until 1921 that Loewi, in Graz, and Cannon and Uridil, in Boston, demonstrated the release of an active adrenaline-like substance as a result of sympathetic nerve stimulation. It was, as in Elliott's day, considered almost self-evident that this substance was adrenaline. At a time, however, when everybody else seemed to be satisfied with the identity of the actions of adrenaline and sympathetic nerve stimulation, Barger and Dale (1910-11), in their classical study on sympathomimetic amines, noted significant differences in this respect, and also pointed out that better agreement was found with the non-methylated ethanol-amino base, *noradrenaline*. This most important recognition was, however, overlooked for a long time, and as late as 1939 Cannon and his co-workers believed that adrenaline was the mediator of the adrenergic nerves.

Adrenaline
(methylaminoethanolcatechol)

Noradrenaline

On the other hand, Cannon and Rosenblueth (1933) discovered that stimulation of sympathetic nerves produced actions which necessitated the assumption of two different active substances, which were called sympathin I and E owing to their inhibitory and excitatory actions. These substances were believed to be formed by the

interaction of the mediator and constituents of the reacting cells. With increasing knowledge of the action of sympathomimetic amines the idea gradually developed, however, that *noradrenaline* might be responsible for the excitatory reactions and adrenaline for the inhibitory ones (Bacq, 1934; Melville, 1937; Stehle and Ellsworth, 1937; Greer *et al.*, 1938), although these concepts did not seem to become generally accepted.

A closer analysis of the sympathomimetic action of suitably purified extracts of organs and nerves, undertaken in our laboratory in 1944-6, revealed, however, that the responsible substance or substances differed distinctly from adrenaline in several respects (Euler, 1946).

The suspicion that the activity might be due to *noradrenaline* proved fruitful, and it could be shown by various biological and chemical tests that there was a close agreement between the actions of the extracts and *noradrenaline*. Holtz *et al.* (1947) demonstrated independently the probable presence of *noradrenaline* in urine and in suprarenal extracts. In the same year Gaddum and Goodwin (1947) showed that the substance liberated on electrical stimulation of sympathetic nerves could be *noradrenaline*, and shortly afterwards Folkow and Uvnäs (1948) obtained evidence for the liberation of *noradrenaline* in vascular reflexes in the cat. Tullar (1948) succeeded in resolving racemic synthetic *noradrenaline*, or "arterenol," into its optical isomers, and L-*noradrenaline* became available. By biological and chemical methods the active sympathomimetic principle in nerves and L-*noradrenaline* were shown to have a close agreement, strongly supporting their identity.

The pharmacology of *noradrenaline* then naturally aroused great interest, and some of its more important actions are mentioned below, especially in comparison with its more time-honoured and well-known homologue, adrenaline.

Action on Smooth-muscle Organs

As already noted by Barger and Dale, *noradrenaline* has a rather weaker inhibitory effect than adrenaline on smooth-muscle organs, such as the uterus and intestine of the cat. Large variations in the effects occur, however, on different organs. Thus the relative effects on the intestine do not differ much, adrenaline being only about twice as active at most. On the rat's uterus, on the other hand, the inhibitory action of adrenaline may be

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as much as 300 times stronger than that of *noradrenaline*. Other organs, such as the iris, the cat's uterus, bronchial muscles, and the fowl's rectal caecum, are intermediate in their responses.

An interesting feature in the response to the two catechol derivatives has been observed by Burn and Hutcheon (1949): after denervation of the nictitating membrane in the cat the sensitivity is increased only moderately for adrenaline but up to nine times for *noradrenaline*. I shall not enter into a discussion of this remarkable change in action. It has been suggested that it is due to the possible removal by denervation of normally occurring inactivating enzymes.

In contracting the uterus of the pregnant cat *noradrenaline* is more active than adrenaline.

Action on Heart and Circulation

Some of the most conspicuous actions of *noradrenaline* and adrenaline are their circulatory effects. Whereas adrenaline produces the well-known rise in systolic blood pressure, it has only little action on the diastolic pressure; in fact, this may be lowered, leaving the mean pressure almost unchanged. The minute-volume of the heart is increased. Again, the unaltered level of mean blood pressure in conjunction with the increased minute-volume signifies that the sum total of the vascular effects of adrenaline is a vasodilatation. This is in sharp contrast to the effect of *noradrenaline*, which produces an almost parallel rise in systolic and diastolic blood pressure and also bradycardia (Barcroft and Konzett, 1949; Swan, 1949; Kappert *et al.*, 1950). *Noradrenaline* in moderate doses produces no change in the minute-volume of the heart, and since the mean blood pressure rises one must infer a general vasoconstriction (Goldenberg *et al.*, 1948).

The only vascular areas which so far have been found to respond to *noradrenaline* with vasodilatation are the coronary arteries and possibly the intestinal vessels when small doses are employed. *Noradrenaline* also has a smaller constrictor action on the renal blood flow than adrenaline (Burn and Hutcheon, 1949).

It is not surprising that *noradrenaline* has been linked with hypertension, since many features in hypertension seem to be consistent with an action of *noradrenaline*. Goldenberg *et al.* (1948) have measured the blood-pressure-raising effect of a constant infusion of *noradrenaline* in various doses on normotensive and hypertensive patients and observed a rather stronger response in hypertensives. The reason for this difference, which has also been noted by Moeller and Koppermann (1950), has not yet been elucidated, however. If the reason were a relative insufficiency in the inactivation of *noradrenaline* one might expect to find an increased output of the catechol amines in the urine; but this does not seem to be the case, in essential hypertension at least.

Action on the Metabolism

The calorogenic action of adrenaline has been known for a long time, and has been demonstrated not only on the total oxygen consumption in animals and man but also on isolated organs and minced tissues. In man a dose of 0.01 mg. per kg. given subcutaneously will increase the oxygen consumption by some 25%. *Noradrenaline* is much less active in this respect, as borne out by a series of recent investigations in animals and man. Goldenberg and his associates found hardly any

action at all on infusion of 0.15 μ g. of *noradrenaline* per kg. per minute in man, in contrast to adrenaline, which in the same dose increased oxygen consumption by some 25%. Bigger doses of *noradrenaline* had a moderately stimulating effect on the metabolism.

The glycaemic effect of *noradrenaline* is likewise much less marked than that of adrenaline, and the relative activity has been estimated at about 1:10. Also, *noradrenaline* is less active in the formation of lactic acid.

The recent increase in interest in the pituitary-adrenal system, in connexion with the use of cortical hormones and A.C.T.H. in the treatment of chronic arthritis and other conditions, has directed attention to the mechanism of the release of these hormones. It has been shown that adrenaline is effective in liberating cortical hormones and also that this effect is mediated by the hypophysis. The fall in blood eosinophils caused by adrenocortical hormones has been used as a quantitative test of the release of A.C.T.H.; and the effects of adrenaline and *noradrenaline* in this respect have been compared. Humphreys and Raab (1950) found *noradrenaline* to be only one-sixth as active as adrenaline. Also adrenaline is more active in decreasing the ascorbic acid content of the suprarenals (Nasmyth, 1949).

Differences in action between the two catechol derivatives have also been noticed with regard to the effect on the central nervous system. It is a common observation that, in man, adrenaline produces a peculiar feeling of anxiety, discomfort, and irritation. *Noradrenaline*, in doses which cause a much larger rise in blood pressure, has no such effect, or a rather weak one.

Toxicity

An interesting difference between adrenaline and *noradrenaline* from the pharmacological point of view has been observed as regards toxicity. Already in 1906 Biberfeld had noted that *noradrenaline* was considerably less toxic than adrenaline for rabbits and rats. This has been confirmed by Tainter *et al.* (1948), who found that *L-noradrenaline* was no less than eight times less toxic on mice in single cages than *L-adrenaline*.

Out of these differences in action one may draw certain conclusions not only about the nature of the physiologically active catechols but also about the indications when these substances are to be used as therapeutic agents. Adrenaline has been used, sometimes rather indiscriminately, in circulatory collapse, a use which is largely based on an erroneous conception of its mode of action. For the restoration of blood pressure *noradrenaline* is much better suited than adrenaline and has already proved valuable in combating low blood pressure in operational shock. It may be administered with advantage as an infusion. *Noradrenaline* would also cause less disturbing side-actions when used as a vasoconstrictor together with local analgesics. The satisfactory actions of adrenaline in counteracting circulatory collapse, sometimes noted, may well be due to the fact that the so-called "natural adrenaline" on the market may contain up to 20% *noradrenaline*.

Some Chemical Properties

Adrenaline and *noradrenaline* may be chemically separated from each other, either by pure chemical methods, using the difference in solubility of certain salts, or by partition in different solvents. Craig's counter-current distribution technique allows a good separation when phenol and 0.02/N HCl are used as solvents.

Also partition chromatography on starch columns, and paper chromatography, may be used. *N*-butanol-hydrochloric acid or phenol-water mixtures have proved useful.

Noradrenaline shows a greater resistance to oxidation than adrenaline. Thus a solution of *noradrenaline* at pH 3.5-4 is very slowly oxidized by iodine to the coloured product *iodonoradrenochrome*, whereas adrenaline is rapidly oxidized. An interesting difference is also found in the fluorescence in ultra-violet light of oxidation products of the two substances. The fluorescent substance formed by *noradrenaline* (*noradrenolutin*) emits a less intense light than does *adrenolutin*.

Estimation

Noradrenaline may be assayed biologically or colorimetrically against a chemically pure standard. Usually the problem is complicated by the fact that *noradrenaline* almost always occurs together with adrenaline, which necessitates a more complicated procedure. However, the variations in the activity ratio of adrenaline and *noradrenaline* on various test preparations make it possible to estimate the two catechols in a mixture. A useful pair of test preparations is the cat's blood pressure, where *noradrenaline* is some two to four times more active than adrenaline, and the fowl's rectal caecum or the rat's uterus, where adrenaline is much more active than *noradrenaline*.

The extract is evaluated in terms of either one of the two catechols against a standard and the relative activity of the two catechols determined in the two tests. By a simple formula (Euler, 1949), the amount of each catechol can be computed. This procedure, of course, necessitates extracts which are essentially free from any contaminating active substance. Adsorption on alumina gives satisfactory extracts. If the amounts of catechol derivatives are large enough, as for instance in suprarenal extracts, the assay can be made colorimetrically, which is simpler. In this case use is made of the different oxidation velocities at pH 4 and pH 6. At pH 4 all of the adrenaline is oxidized to the coloured compound in one and a half minutes, whereas only some 10% of the *noradrenaline* is oxidized. At pH 6 all of the adrenaline and *noradrenaline* is oxidized to the coloured compound in three minutes. A simple calculation gives the actual amounts of the two substances (Euler and Hamberg, 1949).

Distribution

Noradrenaline is widely distributed in the body and has in fact been found in all nerves containing adrenergic fibres, and consequently in almost all organs and tissues, including the blood, and also in aqueous humour, urine, and glandular secretions. Only the placenta and perhaps the bone marrow seem to lack these ergones. In the placenta there are no nerves, and in the bone marrow one could imagine that there would be little need of vasomotor nerves, since there is no margin for expansion or contraction. The amounts found in typical adrenergic nerves, such as the splenic nerves, are quite large (10-20 μ g. per g.). In the spleen the content is considerable (3-4 μ g. per g.), indicating that the peripheral parts of the nerves contain comparatively large amounts. The content in the heart is also relatively large. In the central nervous system no more than 0.2 μ g. per g. was found (rabbit)—that is, something like 1% of the content of the splenic nerves. The same is the case for histamine.

The amount of adrenaline occurring together with *noradrenaline* in adrenergic nerves and organs is usually only a few per cent. of the total catechol amines, showing that *noradrenaline* is the dominating adrenergic neurohormone. The Table shows the absolute catechol amine content (in μ g. per g.) of some organs and nerves, and also the relative amount of adrenaline. After stimulation, the two substances apparently are released in

Organ	Cow		Sheep	
	Total Catechol μ g. per g.	% Adrenaline	Total Catechol μ g. per g.	% Adrenaline
Spleen	1.1-2.6	4-11	1.6 2.6 3	5 3 20
Heart	0.2-0.4	20-26	0.9 0.08	20 2.5
Lung				
Brain	0.02	13		
Kidney	0.1	4		
Striated muscle ..	0.04		0.03	5
Lymph gland ..	0.8		0.3	2.5
Submaxillary gland ..			0.6-1.1	15-37
Splenic nerves ..	7.6-9.6	1.1-3.5	5.1-8.3 1.1-1.3	2-7 2-8
Mesenteric nerves ..	3		3.5	2
Vagus nerves ..	0.1		0.5-1.5	4.5-7.5
Sympathetic trunk ..	2.5-5		1.1-3.8	1.6-7.5
Sciatic trunk ..			0.18	2

about the same proportion as they occur in the nerve (Peart, 1949). After denervation the content in the nerve and the corresponding organs diminishes greatly so as to leave even less than 0.5% of the original amount (Cannon and Lissák, 1939; Goodall, 1950b).

Occurrence in Blood and in Urine.—*Noradrenaline* seems to be normally present in small amounts in the venous blood of man and animals. In purified extracts it has been demonstrated biologically in amounts of the order of 1-2 μ g. of *noradrenaline* per 100 ml. of blood (Euler and Schmitterlów, 1947). It is likely that some adrenaline is also present in the blood. The presence of the catechol amines in the blood is also compatible with the fact that they occur normally in urine, as shown originally by Holtz *et al.* (1947) and confirmed by us. The larger part is present in a free form, but apparently some is conjugated and may be liberated by acid hydrolysis. The amount in the urine of normal man is of the order of 30 μ g. of *L-noradrenaline* a day and some 10 μ g. of *L-adrenaline*.

Suprarenals and Chromaffin-cell Tumours.—The first intimation of the presence of *noradrenaline* in the suprarenals was made in 1947 by Holtz and his associates. Their findings were soon confirmed, and extended, by several groups in various countries. It was isolated from suprarenal extracts in 1949 (Bergström, Euler, and Hamberg, 1949). By using paper chromatography the separation of *noradrenaline* and adrenaline was first demonstrated by James (1948), and this method also indicated that these two catechol derivatives were the only ones to be found normally in most suprarenal extracts. The relative amounts of adrenaline and *noradrenaline* vary greatly in different animals and also in the same animal during different conditions. In the rabbit almost all of the catechol amines are adrenaline, but in the cat the *noradrenaline* may constitute more than 50% of the total. In chromaffin-cell tumours the *noradrenaline* content may be very high, as originally shown by Holton (1949) and confirmed by several other authors. In other cases, however, adrenaline and *noradrenaline* may occur in about equal amounts, depending on the site and nature of the tumour.

Formation and Fate in the Body

The discovery of Holtz and associates that animal organs contain an enzyme capable of decarboxylating dihydroxyphenylalanine (D.O.P.A.) into hydroxytyramine (L-dopa-decarboxylase) indicated a possible way of formation of *noradrenaline*, as already pointed out by Blaschko in 1942. Langemann (1950) found dopa-decarboxylase in considerable quantities in suprarenals. An important contribution has been made by Goodall (1950a), who found certain amounts of hydroxytyramine in normal sheep's hearts. He also demonstrated D.O.P.A. in sheep suprarenals after thyroidectomy. Hydroxytyramine seems to occur regularly in urine. One may therefore depict the formation of *noradrenaline* along the following lines.

The process by which a hydroxy group is introduced in the side chain is still unknown, although it has been observed to occur *in vitro*.

Noradrenaline is partly inactivated by means of an amine oxidase and a catechol oxidase in the body, and partly eliminated in the urine, where it appears chiefly in free form.

On certain occasions the amounts excreted are rather higher than normally, as, for instance, in muscular work (Euler and Hellner, 1951) and in cases of chromaffin-cell tumours. In the latter cases excretions amounting to more than 1,000 μg . a day have been observed (Engel and Euler, 1950), a circumstance which has been utilized as a way of diagnosing such tumours. In such cases the urine may be assayed directly on the cat's blood pressure.

Highly interesting are the findings of Blaschko and Burn (1950) concerning the inactivation of a mixture of *noradrenaline* and *adrenaline* by means of an amine-oxidase preparation from rabbit liver. They found that, as incubation proceeded, the relative amount of *noradrenaline* steadily fell from 50 to 10% and the percentage of *adrenaline* rose from 50 to 90%. Independently Udden in our laboratory found that *noradrenaline* is much more readily inactivated on incubation with splenic nerve tissue of cattle than is *adrenaline*.

The form in which *noradrenaline* and *adrenaline* are present in the nervous tissue is still unknown. However, both catechol amines form ether-soluble compounds with lecithin. In equilibrium with a water phase the amounts taken up by the lecithin-ether are greatly dependent on the pH. Thus at pH 7 some 25–30% of the catechol amines may occur in the ether-lecithin phase, whereas at pH 5 only some 5% is bound to the lecithin in ether solution (Norlander, 1950).

One may imagine that the catechol amines could be bound to phosphatids in the nerve and liberated by a shift in acidity.

Significance of *Noradrenaline* in the Body.—The significance of the occurrence of *noradrenaline* in the organism has been questioned in some quarters, where it is thought that *noradrenaline* is merely a precursor of *adrenaline* and serves no independent function. This is, however, in obvious opposition to the findings that the

normal reflex vascular effects in reality are effectuated chiefly by *noradrenaline*. It appears that *noradrenaline* is the sympathetic neuro-ergone for general service, the *adrenaline* acting as an adjuvant for more special purposes, especially as regards metabolic actions.

Alterations in Distribution of *Noradrenaline* and *Adrenaline*.—A disturbance in the distribution of the two catechol amines is encountered in connexion with various endocrine disturbances, such as after hypophysectomy, in thyrotoxicosis, after thyroidectomy, and in other conditions. This applies to the suprarenal medulla, and other organs as well. In the rat's spleen, after hypophysectomy, the *adrenaline* percentage is considerably increased (Euler and Hökfelt, 1950), and after thyroidectomy it is markedly decreased (Goodall, 1950b). A large field is opening up here which may be of interest for the interpretation of certain vegetative disorders in connexion with hormonal imbalance.

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Miss Edna Jackson has been appointed a Deputy Chief Nursing Officer of the Ministry of Health in succession to Mrs. E. Harman, who has resigned following her marriage. Miss Jackson's duties cover a wide field of public health nursing, particularly in relation to mothers and young children, midwifery, home nursing, and the preventive health services of the local health authorities. She was previously Public Health Nursing Officer in the North-west Region of the Ministry.