REVIEW ARTICLE

ADRENALINE AND NORADRENALINE

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The discovery that a chemical substance is a natural constituent of living tissues has always led to an increase in experimental work on that subject, resulting in an expansion of knowledge in most branches of medical science. Adrenaline, for example, was regarded for a long time as the specific hormone of the suprarenal medulla and also as the chemical transmitter of the sympathetic nervous system. Its synthesis in 1904 ended, for practical purposes, biochemical research on these structures, it being the first hormone to be isolated and chemically identified. And yet, during the last few years, considerable interest has again been centred on adrenaline, by reason of the fact that noradrenaline occurs with it in nature. The effects of these two substances in man have been extensively studied, and it seems appropriate to take stock of this recent knowledge, particularly because it is possible to define with reasonable certainty the function of adrenaline and noradrenaline in the normal working of the body. Although noradrenaline is now known to be one of the hormones of the suprarenal medulla, adrenaline predominates in man and in most species. Adrenaline, possessing the more general function of stimulating various metabolic processes in conditions of stress, acts as a reserve ready to be liberated in emergency conditions to support the actions of adrenergic nerves which function by liberating chiefly noradrenaline.

The humoral concept of transmission of nerve impulses dates from the time of Elliott[1], who in 1905 made the brilliant suggestion that the sympathetic nerves caused their effects in the body by actually liberating a chemical transmitter substance at their endings which then activated the effector cell. Elliott thought that the transmitter was adrenaline, and indeed for a long time no other substance was seriously considered in its place. This is surprising, for Barger and Dale[2] in 1910 pointed out the difficulties in accepting adrenaline as the transmitter of sympathetic nerve impulses. These authors stated that the action of certain other amines was more like the action of sympathetic nerves than was that of adrenaline. This most important observation was borne in mind by many workers (e.g., Cannon and Uridil[3], Loewi[4], and others) during the next 20 years, and I think Bacq[5] in 1934 was the first to put forward the hypothesis that noradrenaline might be the transmitter substance in adrenergic nerves, a hypothesis which now appears to be true.

Adrenaline is a secondary amine; one atom of hydrogen in the group $-NH_2$ being substituted by CH_3. Noradrenaline differs from adrenaline in the absence of this CH_3 group and is therefore a primary amine (Fig. 1). Like all dihydroxyphenyl derivatives, both adrenaline and noradrenaline

are rapidly oxidised in the presence of oxygen, especially in an alkaline medium, or if heavy metal ions are present. They are, however, more stable in weakly acid solutions and considerably more stable in 5 per cent. dextrose solution or distilled water than in saline solution or whole blood[6]. Both amines were synthesised by Stolz[7] in 1904, but it was not until 1946 that Euler[8] working in Stockholm demonstrated by biological

Noradrenaline (Arterenol) — Adrenaline

FIG. 1. Relationship between the two amines.

methods that a substance closely resembling noradrenaline was present in extracts of fresh ox and cow spleen, the heart of the ox, horse and cat, and also in nerves of the sympathetic chain. This seems to be the turning point in the story of the naturally occurring catechol amines, and with the aid of better techniques the significance of the observations of Barger and Dale has been fully appreciated in the past few years. In 1947, Holtz, Credner and Kroneberg[9] in Austria established that endogenous catechol amines are normally excreted in the urine of man, and that noradrenaline is present with adrenaline in extracts of suprarenal glands. From then on, workers in different parts of the world used very sensitive biological test organs such as the cat's denervated nictitating membrane, the hen's rectal cæcum, and the rat's uterus and colon, to show that noradrenaline is the predominating amine liberated into the blood stream when the hepatic, splenic, abdominal and hypogastric sympathetic nerves in various animals are stimulated (e.g., Mann and West[10,11]). Later work, using in addition various chromatographic and fluorimetric techniques, confirmed and extended these findings. The racemic form of noradrenaline had to be used in the early studies since the more active and naturally occurring lævo-isomer was not available till 1948, as the outcome of the work of Tullar in the United States[12].

In this review, some of the more important actions of noradrenaline will be discussed and contrasted with those of adrenaline. Since adrenaline must be considered to be the chief compound produced by the suprarenal medulla in most species, including man, a functional differentiation between the suprarenal medulla and the sympathetic nervous system must be inferred.

QUANTITATIVE METHODS

Quantitative methods of assay are essential in any study of the occurrence and liberation of hormones in the body, and in the present instance it is also important to have methods which distinguish between the two catechol compounds. Simple extraction of the suprarenal glands and other chromaffin tissue can be effected with dilute acids, but with

all other organs and with nerves the extracts must first be purified. This is usually carried out by adsorption on aluminium hydroxide, followed by elution and concentration. The resulting solutions may then be subjected to bioassay. The activity can be suitably tested on the blood pressure of the cat, where noradrenaline is usually 2 to 5 times as effective as adrenaline, and on the isolated uterus of a non-pregnant rat, where by contrast adrenaline is far more effective. Using a simple calculation[13] the adrenaline and noradrenaline contents of the extract can be determined separately from the results of these two biological tests. Separate estimation is also possible using paper and other chromatographic methods. These techniques are generally not so sensitive as the biological tests but specificity is increased[14]. In place of biological estimations, fluorimetric and colorimetric methods may be used. A combination of paper chromatography and fluorimetry has succeeded in the difficult problem in which one catechol amine is present in concentrations far in excess of the other. For example, a mixture of 99 parts of adrenaline and 1 part of noradrenaline can be measured after development using a mixture of potassium ferricyanide and formaldehyde as the spray reagent[15], whereas a mixture of 1 part of adrenaline and 99 parts of noradrenaline can be measured using the ethylenediamine reagent[16]. These methods are possible since the two amines differ in sensitivity to the two spray reagents and in the shade of fluorescence seen in ultra-violet light.

Biological Occurrence

As far as we know, noradrenaline is the predominant transmitter substance liberated at adrenergic nerve endings. Although the greater part of the evidence for this has been obtained from animal experiments, it is unlikely that man differs from other mammals in this respect. Noradrenaline can be shown to be present in extracts of nearly all organs except the placenta. Since the placenta is devoid of nerves, it is probable that the nerves in the organs are responsible for the presence of noradrenaline. The nerves of the spleen of the sheep, for example, contain noradrenaline in large amounts (up to 15 μg./g. fresh tissue) and consist almost exclusively of post-ganglionic sympathetic adrenergic fibres; the total spleen of the sheep, however, contains only 2 to 3 μg./g. There appears to be a distinct correlation in nerves between the noradrenaline content and the number of adrenergic fibres[17].

In addition to noradrenaline, a small quantity of adrenaline occurs in adrenergic nerves, and it is possible that this may be due to small areas of chromaffin tissue in the vicinity of these nerves, for after sympathetic denervation, noradrenaline disappears from the organs, whereas the amount of adrenaline is only moderately reduced[18,19]. However, the quantity of adrenaline that can be extracted from various organs and nerves (other than the suprarenal medulla) rarely exceeds a few per cent. of the total amount of catechol amines. After stimulation of the nerves, the two substances are released apparently in about the same proportions as they occur in the nerves.

The first intimation of the presence of noradrenaline in the suprarenal

glands was made in 1947 by Holtz and his co-workers[9]. Their findings were soon confirmed and extended by various workers throughout the world. Using paper chromatography, James[20] reported the separation of the two amines and showed that adrenaline and noradrenaline were the only catechol amines to be found in many normal suprarenal extracts. It is now known that the relative amounts of adrenaline and noradrenaline

TABLE I

THE AMOUNTS OF ADRENALINE AND NORADRENALINE IN THE SUPRARENAL GLANDS OF VARIOUS ADULT ANIMALS (FROM THE LITERATURE)

Species	Adrenaline (mg./g.)	Noradrenaline (mg./g.)	Noradrenaline per cent.
Whale	0·15	1·50	91
Fowl	2·02	8·08	80
Dogfish	0·90	2·40	73
Lion	0·20	0·30	60
Cat	0·60	0·37	38
Sheep	0·50	0·25	33
Dog	1·16	0·40	26
Ox	1·20	0·42	26
Mouse	0·75	0·25	25
Man	0·52	0·10	16
Rat	0·91	0·10	9
Rabbit	0·48	traces	2
Guinea-pig	0·21	traces	2
Baboon	0·83	0	0

vary greatly in different animals and also in the same animal in different conditions. In man, noradrenaline constitutes about 20 per cent. of the total catechol amines; in the rabbit, almost all of the catechol amine content is adrenaline; and yet in the whale, noradrenaline may constitute more than 80 per cent. of the total. Table I records the amounts of these amines found in the suprarenal glands of various animals. In addition, mention must be made of the recent observations of Östlund[21], who used extracts of several insects (earwig, butterfly, beetle, mealworm, housefly and honey bee). He obtained evidence that another catechol amine was present in large amounts with adrenaline and noradrenaline in these lower forms of life. This was hydroxytyramine (dopamine), an amine previously shown to be present only in the suprarenal medulla of sheep and ox[18,22].

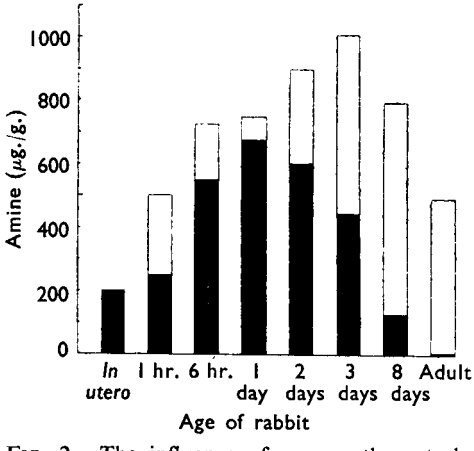


FIG. 2. The influence of age on the catechol amine content (μg./g.) of suprarenal glands of rabbit. Shaded area is noradrenaline: plain area is adrenaline (after Shepherd and West,[14]).

What are we to conclude from these varying proportions of adrenaline and noradrenaline in the suprarenal medulla? As will be mentioned later, adrenaline appears to be formed from noradrenaline so that the

latter substance may be present as a precursor of the former. However, it is more than probable that both have a function to perform in the body. In the fœtus, for example, the suprarenal glands contain noradrenaline and very little adrenaline, the capacity for synthesising adrenaline being developed only gradually (Fig. 2). The body at this stage of life appears to require only noradrenaline to be available in the suprarenal gland, ready for the possible function of helping to maintain vasomotor tone. This finding, however, does not in any way help us to understand why varying amounts of the two catechol amines occur in the adult suprarenal gland. We thought at one time that their relative quantities were dependent on the ratio of the size of the suprarenal cortex to that of the suprarenal medulla. This ratio is high (over 40) for the guinea-pig and rabbit (chiefly adrenaline), but near to unity for the fowl and whale where methylation is so incomplete. Intermediate ratio values fit well into this scheme[14]. Besides, in patients dying of Addison's disease (where this ratio has been as low as 0·01), there is less methylation in their suprarenals than is normally found[23]. If this hypothesis had been proved, then the degree of methylation in the medulla would have been related to the relative size of the cortex, and the methylating enzyme, transmethylase, could conceivably have been a constituent of, or be activated by, cortical tissue.

Further support was forthcoming when we examined the organs of Zuckerkandl of children at birth and also the retroperitoneal tissue of many young mammals. Both of these abdominal accessory chromaffin structures lack connection with the suprarenal cortical cells and were found to contain relatively large amounts of noradrenaline and no adrenaline[24]. However, when tested in lower vertebrates where the interrenal bodies representing the suprarenal cortex remain separated throughout life from the chromaffin bodies of rudimentary suprarenal medulla, adrenaline as well as noradrenaline was clearly identified and estimated. Two types of dogfish and the torpedo were used for these experiments which were carried out simultaneously in Bari, and in Dundee[25]. The results leave no doubt that methylation of noradrenaline does not require the immediate presence of cortical tissue, although a hormonal factor of cortical or pituitary origin may be necessary. A recent finding that the organs of Zuckerkandl in children aged more than 1 year contain both adrenaline and noradrenaline supports this conclusion[26]. The transient fœtal adrenal cortex has by this time been replaced by the permanent adult cortex with its supply of cortical hormones.

It is now widely accepted that provided the sympathetic innervation is intact adrenaline and noradrenaline are discharged continuously from the suprarenal medulla of many species. In the cat, for example, Kaindl and Euler[27] found a basal discharge of total catechol amines of 0·03 to 0·06 μg./kg./min. The venous blood was collected from the left adrenal and assayed biologically. This secretion may however be considerably altered by various experimental procedures. Direct electrical stimulation of the splanchnic nerve produces a hundredfold increase of the mixed

secretion. Reflex stimulation, resulting from electrical excitation of the central end of the cut sciatic nerve or the brachial plexus, increases the adrenaline (but not the noradrenaline) output[28], and similar effects have been noted during emotional stimuli, in hypoglycæmia, and after trauma. The noradrenaline output appears to be increased in conditions involving some kind of circulatory stress, such as activation of the carotid sinus pressor reflex, or heavy muscular work. After electrical stimulation of the hypothalamus in cats, the output can be varied depending on the site of stimulation[29]. When the electrodes, for example, are located in the anterior hypothalamus just posterior to the optic chiasma noradrenaline is said to be preferentially liberated, whereas when the electrodes are immediately before and behind the mamillary bodies adrenaline is produced in larger quantity[30]. It may be therefore that the secretion of the individual hormones is activated by specific nerves, and this differentiated secretion, depending on the functional requirements of the organism, suggests the possibility of specific adrenaline and noradrenaline producing cells. Already it has been demonstrated that specific cells in the suprarenal medulla show pigment formation on oxidation with potassium iodate in the same way as noradrenaline does *in vitro*[31]. It may be that noradrenaline is selectively stored in certain cells in the medulla, and besides being a precursor of adrenaline may act as an independent hormone, helping to raise the blood pressure in times of circulatory stress.

Determinations of the catechol amines in the peripheral blood are possible only in exceptional circumstances and hardly ever under physiological conditions. Even using the fluorimetric method of Lund[32] which is one of the most sensitive, it has not been possible to estimate with any certainty either noradrenaline or adrenaline in the circulating blood. Recently, Weil-Malherbe and Bone[16] have estimated both amines in peripheral venous blood in man, using a modified fluorimetric method based on reactions with ethylenediamine. These authors say that the adrenaline arteriovenous difference is of the order of 0·5 μg./l. of blood. Their values have been questioned by Holzbauer and Vogt[33], who prefer lower basal values determined by biological assay. It may be that the conditions under which the blood samples were taken allowed in some cases disintegration of blood platelets; already it has been shown that adrenaline and noradrenaline are associated to a large extent with the blood platelets[34].

Although it is difficult, therefore, to give the concentrations of adrenaline and noradrenaline in the blood with much accuracy, one can measure the amounts in the urine. As previously mentioned, Holtz and his coworkers in 1947 were the first to establish that endogenous catechol amines are normally excreted in the urine. Later investigators showed that about 1 to 3 per cent. of adrenaline or noradrenaline given by intravenous infusion is excreted during the infusion[35], the largest proportion being recovered as the free amines. Later methods of adsorption on aluminium hydroxide before biological and chromatographic estimation, indicated a normal excretion in man of about 5 μg. of adrenaline

and 20 to 40 μg. of noradrenaline per 24 hours. Under certain conditions, considerable variations in the amounts excreted are found. For example, a ten-fold increase in the excretion of both amines may occur during muscular exercise[36]. A moderate dose of insulin in man may increase the urinary excretion of adrenaline 10 times, with little effect on the noradrenaline excretion; presumably stimulation of the suprarenal medulla is pronounced[37]. 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. Consequently, the urinary excretion of the catechol amines (particularly of noradrenaline) has been studied in many countries in various clinical conditions of hypertension. In the majority of cases of essential hypertension, however, excretion is within normal limits, though it may be considerably raised in cases of tumours of the adrenal gland (phæochromocytoma).

Biological Effects

In attempting to arrive at a conclusion about the functions of the adrenal medullary hormones in the body, it is instructive to observe their actions in tissues after injection. However, attention has not always been paid to the fact that in life the natural secretion is similar to a continuous infusion and unlike a single injection. Results may therefore be confusing on this count alone. In addition, the question of reflex actions of a compensatory nature coming into play bring about certain obvious difficulties when comparisons are made between the results of human and of animal experiments. Even the conclusions reached as a result of the experiments on several species of animal are equivocal. Again, some of the discrepancies between the results of different workers may be accounted for simply by dosage differences, equimolar amounts not always being used for comparisons. Thus there are numerous discrepancies to be found in the literature concerning the relative biological activities of the catechol amines.

The important differences between the actions of adrenaline and noradrenaline on the circulation in man must first be stressed. It is well known, for example, that adrenaline produces tachycardia and an increase in cardiac output. On the other hand, noradrenaline produces bradycardia brought about by a reflex vagal action, with scarcely any effect on cardiac output. Although adrenaline generally produces a rise in the systolic blood pressure, physiological amounts have hardly any effect on the diastolic blood pressure, which may even decrease; in consequence, the mean pressure increases relatively little[38]. On the other hand, correspondingly small doses of noradrenaline (up to 0·3 μg./kg./min. by intravenous drip) raise both systolic and diastolic pressures to about the same extent (see Fig. 3) so that the mean blood pressure is raised. Since the increased blood flow produced by adrenaline is not associated with a corresponding increase in blood pressure in man, adrenaline is considered by some workers[39] to be an over-all vasodilator and cardiac accelerator. Noradrenaline is said to be almost exclusively a vasoconstrictor and pressor agent. The reason why the total action of

adrenaline in man manifests itself as vasodilatation may be found in the blood flow changes in skeletal muscle[40]. Whereas both adrenaline and noradrenaline considerably reduce the blood flow through the skin, they differ in their effects on skeletal muscle. Thus, while noradrenaline

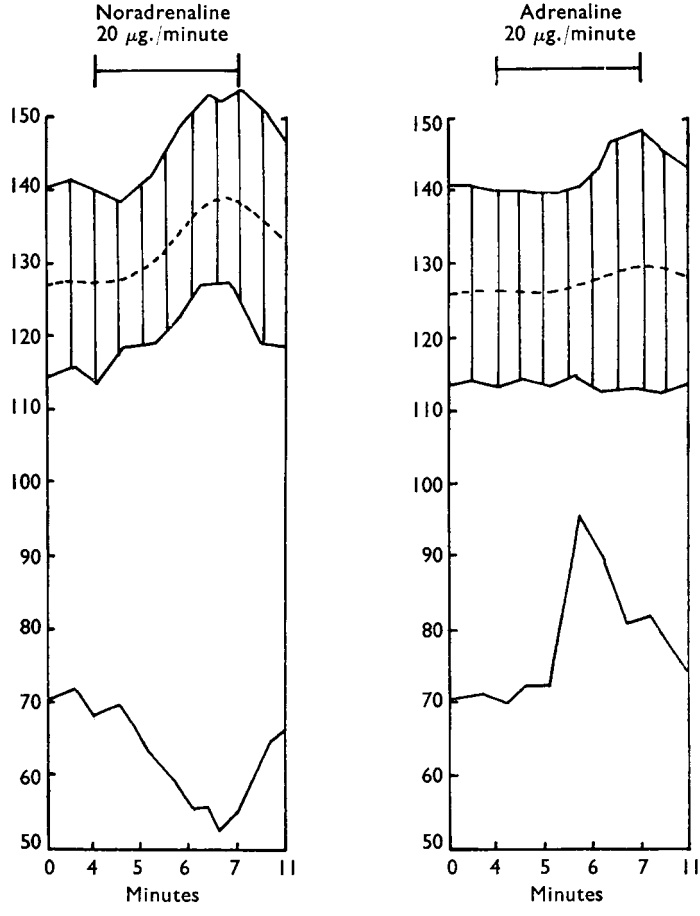


FIG. 3. Effect of noradrenaline and adrenaline infusions on the blood pressure (upper record) and heart rate (lower record) in man (after Barcroft and Konzett[38]).

produces vasoconstriction over a wide range of concentrations[41], adrenaline in physiological concentrations causes marked vasodilatation[42]. Hence, adrenaline causes a decrease of general peripheral resistance in man, whereas noradrenaline causes an increase.

Another school of thought, however, considers that both amines are more or less qualitatively identical in action, if results on animals are taken into account[43]. As early as 1949, Burn and Hutcheon[44] showed that noradrenaline may be dilator in action on the intestinal vessels of the cat and on the vessels of the rabbit ear during perfusion with 2-benzylimidazoline. More recently, Ahlquist, Taylor, Rawson and

Sydow[45] determined the comparative effects of equimolar doses of the two catechol amines in dogs under pentobarbital anæsthesia. Their results clearly show that noradrenaline is more effective than adrenaline in increasing the systolic, diastolic and mean arterial pressures, and in producing vagal bradycardia. However, adrenaline is more effective in diminishing urine output (a reflection of increased renal vascular resistance) and in causing splenic contraction and inhibition of the rhythmic activity of the gut. The fact that noradrenaline is usually a more potent pressor agent than adrenaline in the anæsthetised dog does not mean that the former is always a more potent vasoconstrictor, for a blood pressure record represents the sum of several opposing agencies. In the kidney, for example, adrenaline is 2 to 10 times more active than noradrenaline as a vasoconstrictor[45,46]. In the total femoral bed, the difference in vasoconstrictor activity of the two amines after intra-arterial injection is not so marked, although adrenaline produces a secondary dilatation which is not seen with noradrenaline[47]. Thus, the two amines may be similar in action in these experiments but quantitatively different.

The underlying mechanism responsible for the differences seen in man and in animals might be found in the relative amount of blood normally reaching the skeletal muscles and other areas in which adrenaline produces vasodilatation and noradrenaline causes vasoconstriction. Another obvious factor which must be considered is the use of anæsthetics (particularly in the animal tests) and their effect on the tone of certain blood vessels. Yet another factor is that the muscular vascular beds in different species may contain both cholinergic and adrenergic vasodilator mechanisms, one of which may play a dominant role in the control of vascular tonus. For example, the sympathetic vasodilator fibres in the cat[48] are cholinergic in nature. In the dog[49], they are also in part cholinergic, but small doses of adrenaline after ergotoxine still produce vasodilatation in the presence of atropine. There may be both cholinergic and adrenergic receptor mechanisms in the muscle vascular beds of the dog hind limbs. More recently, other workers[50] have shown that the intra-arterial injection of adrenaline and noradrenaline causes marked vasoconstriction in the skinned hind legs of dogs after atropine.

Ahlquist[51] has suggested that variations in the activity of sympathetically innervated organs could result from the presence of different receptor mechanisms. He has proposed (*a*) an α-adrenotropic receptor for vasoconstriction in the viscera and skin, contraction of the nictitating membrane and uterus, relaxation of the intestine and ureter and stimulation of the dilator pupillæ, and (*b*) a β-adrenotropic receptor for vasodilatation in skeletal muscle, the coronary vessels and viscera, relaxation of the uterus and bronchioles and for stimulation of the myocardium. This assumption requires that stimulation of either receptor may cause either excitation or inhibition, that union with the cell receptor is determined by the structure of the sympathomimetic amine, but the nature of the response elicited after union is determined by the organ. This hypothesis was suggested by experimental results obtained with 6

sympathomimetic amines and by various experimental procedures giving results which are at variance with those obtained by many other investigators. Obviously there are differences in experimental procedures and in the responses in different species. However, if vasoconstriction and intestinal relaxation involve the same type of receptor (the α-receptor) as proposed by Ahlquist, adrenaline reversal after a sympatholytic agent should be associated with blockade of the intestinal response. But inhibitory responses of adrenaline and of noradrenaline are most difficult to reduce and block. Perhaps the receptors in these cases will be blocked when suitable experimental procedures are found out. It is certainly of interest to note here that pieces of intestine and uterus stored in the refrigerator for periods up to 5 days lose their sensitivity to adrenaline but do not lose it to noradrenaline[52]. Their sensitivity to isoprenaline is increased, suggesting on Ahlquist's theory a change-over from α-receptor combination to β-receptor combination. In this case however it is possible that two processes are going on side by side—(1) a general failure of the tissue metabolism measured by the loss of sensitivity to adrenaline, and (2) a failure of the mechanism of innervation leading to a denervation and therefore an increase in sensitivity to noradrenaline[44]. If one assumes excitation or inhibition to result from stimulation of a specific receptor, it becomes difficult to explain the action of sympathomimetic amines on the heart, since cardiac effects do not correlate well with vascular effects.

I think the problem is even more complex, since these oversimplified hypotheses describe rather than explain the mechanism of adrenergic transmission. Burn and Hutcheon, for example, also showed in 1949 that adrenaline causes vasodilatation in two instances in the cat, (*a*) when the blood pressure is high because the vagi have been cut and ether anæsthesia has been used, and (*b*) when sympatholytic agents have been administered. We must ask if these two conditions are the same. There has been much discussion on this point, and it appears that they are not. For example, noradrenaline was shown to cause no vasodilatation in (*a*), and yet it dilated the rabbit ear vessels in the presence of a reversing agent. It is however possible that noradrenaline may produce vasodilatation when conditions are as near physiological as possible (e.g., very early in experiments on spinal cats when the blood pressure is high), but this result is exceptional and quickly passes to vasoconstriction so that it is easily missed or not shown[53].

The observations of Meier and Bein[54,55] on the vasodilator action of adrenaline in muscles may supply an answer to the foregoing problem. They recorded the blood flow through the femoral artery of anæsthetised cats, and found that intravenous doses of adrenaline caused increased blood flow through the artery without raising the systemic blood pressure. There was no doubt that the increased flow was through skeletal muscle. After adrenalectomy and a delay of 2 hours or so, the injection ceased to increase flow but caused diminution. The dilator action of adrenaline could then be recovered by infusing minute quantities of noradrenaline into the veins. Thus, the normal vasodilator action of small doses of adrenaline in muscle probably depends on the presence of noradrenaline

in the blood stream. The suprarenal medulla of cats may therefore be continuously secreting this minute amount of noradrenaline. This gives further support for the hypothesis that noradrenaline may act as an independent hormone besides being a precursor of adrenaline. It also indicates how the results of blood flow studies in animals have to be treated with some reserve. Further work has clearly shown that adrenaline can potentiate both excitor and inhibitory actions of noradrenaline[56]. Still more important, very minute amounts of both amines may play a role in the maintenance and regulation of the intrinsic tone and resistance to stretch of the carotid arteries[57].

The effect of the two catechol amines on the heart and coronary circulation has been the subject of many investigations, but results are mostly variable. Several authors, for example, have shown that both substances increase coronary flow[44,58], but the effect may be due in part to the simultaneous increase in cardiac output. However, the coronary vessels contain noradrenaline[59] so that it is logical for the substance liberated from the nerves of the coronary vessels under physiological conditions to produce vasodilatation. The rate of the isolated heart is increased by both amines but effects are different in the intact animal as mentioned earlier.

TABLE II

RATIO OF DOSE OF (±)-NORADRENALINE TO EQUI-ACTIVE DOSE OF (−)-ADRENALINE ON VARIOUS FRESH PREPARATIONS (WEST[52])

Test object	Ratio	Excitor (E) or inhibitor (I) action
Cat blood pressure	0·8	E
Cat, pregnant uterus	0·8	E
Cat, ileum	1·0	I
Cat, nictitating membrane	1·25	E
Rabbit, ileum	2·0	I
Rat, ileum	3·0	I
Rabbit, pregnant uterus	4·0	E
Rabbit, non-pregnant uterus	5·0	E
Frog, blood vessels	5·0	E
Frog, Straub heart	8·0	E
Cat, non-pregnant uterus	10·0	I
Frog, perfused heat	33·0	E
Rat, non-pregnant uterus	100·0	I

The effects of the compounds on organs with smooth muscle are qualitatively similar[52], although adrenaline is generally more active in cases when its action is inhibitory (see Table II). After denervation, the sensitivity of some tissues (e.g., the nictitating membrane of the cat) to noradrenaline is greatly increased whilst that to adrenaline is only slightly affected[60]. This may be due in part to a decline in the amine oxidase content of the membrane. Both adrenaline and noradrenaline are inactivated in the body by the widely distributed amine oxidase, but it is possible that noradrenaline is much more readily inactivated on incubation with the enzyme than is adrenaline. Bain and Batty[61] have already shown that the amine oxidase of human liver destroys noradrenaline more rapidly than it does adrenaline.

Adrenaline is usually much more powerful than noradrenaline in its effects on metabolic processes. This difference may be seen, for example,

in the action on the total oxygen consumption in man[39], on blood sugar[62], on blood potassium[63], and on eosinophil counts[64]. It is also more potent in inhibiting autonomic ganglia in dogs[65] and the peristaltic reflex in guinea-pigs[66]. The strong metabolic effects of adrenaline on the one hand and the pronounced rise in blood pressure produced by noradrenaline on the other reveal yet again functional differences between the two hormones.

Biological Synthesis and Metabolism

The way in which noradrenaline is formed in the body is not yet known with certainty, though its biosynthesis through aromatic amino-acids or amines has been repeatedly assumed but never proved. It is true that adrenaline can be formed from noradrenaline by methylation, as Bülbring and Burn[67] demonstrated in the isolated adrenal gland of the dog perfused with blood. There is evidence, however, to show that this process is not always accomplished as quickly as noradrenaline is formed, and this suggests that methylation may be a reaction carried out with some difficulty. For example, when the adrenal gland is put under stress for long periods of time (following continuous splanchnic nerve stimulation or insulin overdosage), progressively more noradrenaline usually appears.

The discovery[68] that animal organs contain an enzyme capable of decarboxylating dihydroxyphenylalanine (dopa) into hydroxytyramine (dopamine) indicated one possible way of formation of noradrenaline. This enzyme, dihydroxyphenylalanine decarboxylase, is specific for (−)-dihydroxyphenylalanine and occurs in the suprarenal and renal tissue of many warm-blooded animals[69]. Hydroxytyramine seems to occur regularly in urine and has been found in the suprarenal glands of a few mammals, particularly the sheep[22]. When rats are fed on a diet deficient in pyridoxine (the co-enzyme), the amount of (−)-dihydroxyphenylalanine decarboxylase in the liver is greatly diminished[70] and given insulin they appear to lack the ability to replenish the store of pressor amines in the adrenal medulla. When normal rats are given insulin to exhaust their adrenal glands, only dihydroxyphenylalanine among many likely competitors can restore the store of catechol amines rapidly[71]. Thus it is possible that the enzyme is part of the normal mechanism of noradrenaline formation, a hypothesis put forward by Blaschko[72] in 1942. However, the process whereby the introduction of the hydroxy group in the side chain of hydroxytyramine is effected remains unknown although it has been observed to occur *in vitro*[73]. Many groups of workers in different parts of the world are busy working on inhibitors of dihydroxyphenylalanine decarboxylase in the hope that accumulation of dihydroxyphenylalanine will occur. Related compounds seem to be a favourite line of research[74], as also are derivatives of phenylalanine[75].

All this work has been concerned with (−)-dihydroxyphenylalanine decarboxylase, but we must ask ourselves "Where is the dihydroxyphenylalanine?" Two reports have appeared to show it to be present

in mammalian tissues, but confirmation has not been forthcoming so far. We have been looking for it by paper chromatography using animals treated with desoxypyridoxine (to inhibit the decarboxylase activity), but although the relative noradrenaline content of the adrenal gland has been altered, no dihydroxyphenylalanine has been found.

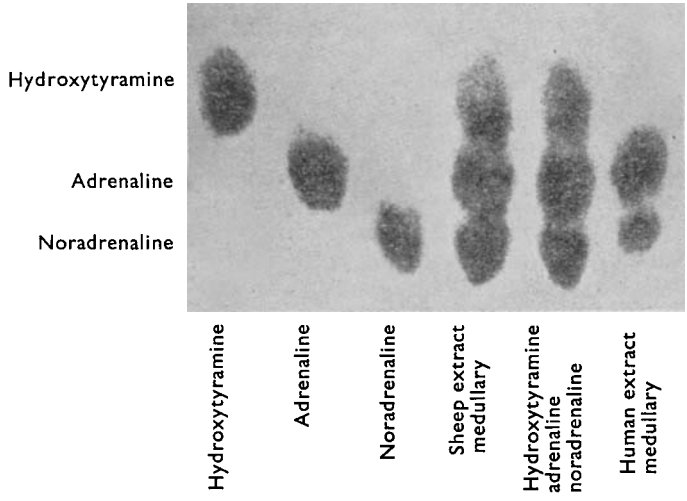


FIG. 4. Paper chromatogram, showing presence of adrenaline, noradrenaline and hydroxytyramine in adrenal medullary extracts of sheep, but hydroxytyramine absent from human medullary extracts (after Shepherd and West[22]).

Since hydroxytyramine has been found in the suprarenal glands of only the sheep and ox (see Figs. 4 and 5), we wonder by reason of its absence in other mammalian glands if it is not a by-product in the biosynthesis in these two species.

Other routes of formation of noradrenaline are possible and these are shown in Figure 6. Dihydroxyphenylserine (or noradrenaline carboxylic acid) has not been found in nature so far, but it is decarboxylated when incubation proceeds anærobically with extracts of guinea-pig kidney[76], resulting in the formation of noradrenaline. The route *via* tyramine and *p*-hydroxyphenylethanolamine (*p*-norsynephrine) is of interest since both substances have been found to

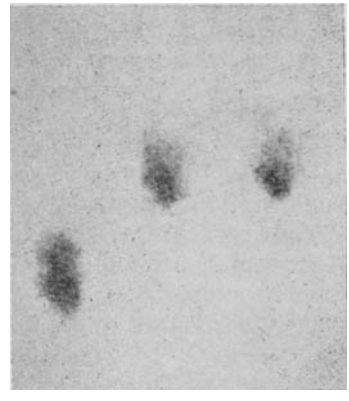
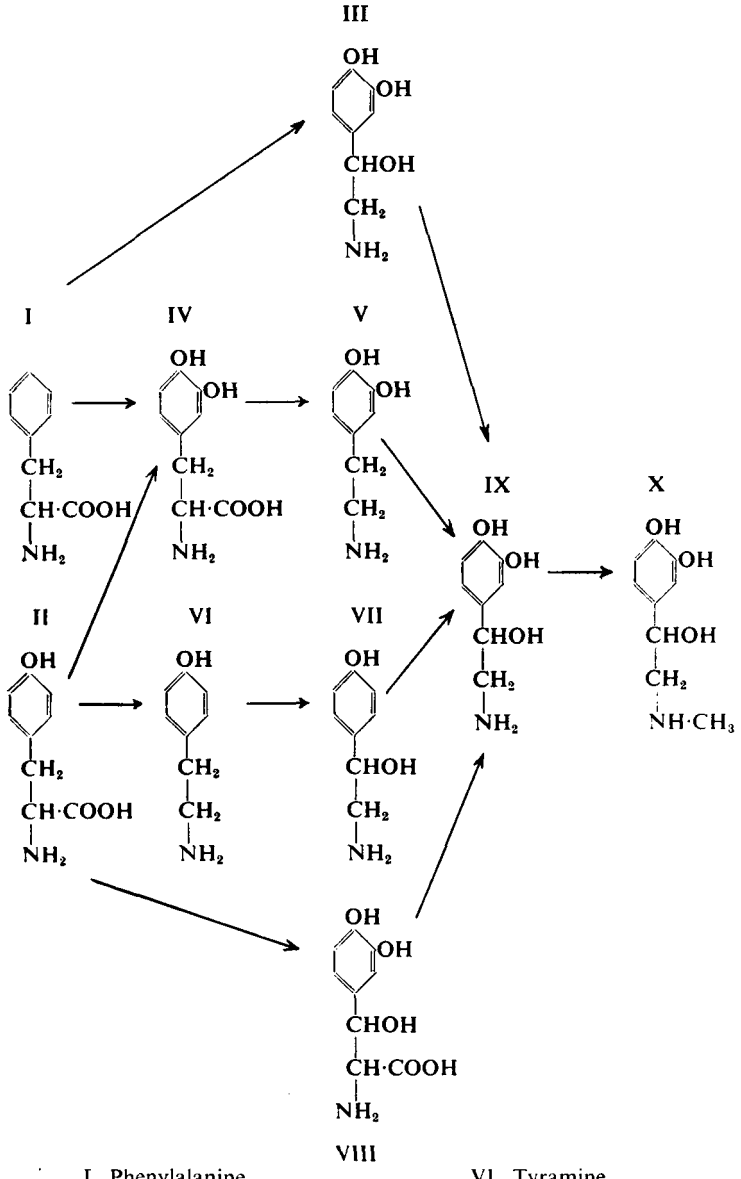


FIG. 5. Paper chromatogram of eluate of hydroxytyramine area from undeveloped chromatogram of sheep medullary extract re-chromatographed between standard adrenaline and hydroxytyramine solutions (after Shepherd and West[22]).

occur in nature, sometimes together as in the octopus[77]. If a solution of *p*-norsynephrine is subjected to ultra-violet light in the presence of oxygen, noradrenaline forms in good yield. Similarly, the *m*-compound



I, Phenylalanine.
II, Tyrosine.
III, *m*-Norsynephrine.
IV, Dihydroxyphenylalanine.
V, Hydroxytyramine.
VI, Tyramine.
VII, *p*-Norsynephrine.
VIII, Dihydroxyphenylserine.
IX, Noradrenaline.
X, Adrenaline.

FIG. 6. Possible routes of formation of adrenaline from noradrenaline.

can be transformed into noradrenaline. A scheme for the identification of these possible precursors by paper chromatography has been worked out, since many of them are biologically inactive or nearly so[78]. Yet, none of these intermediates has so far shown up in fair quantities, and the biosynthesis still appears to be an open question.

The use of radioactive isotopes to gain information about drug metabolism is becoming very widespread and obviously the schemes shown above should be tested by this means. For a long time, adrenaline was thought to be converted into inactive conjugated compounds such as the sulphate[79] or the glucuronide[80], because such conjugates appeared in the urine of animals which had ingested large amounts of this hormone. Schayer and his co-workers[81] administered *N*-methyl ^{14}C-labelled adrenaline to rats and found that only half of the radioactivity was contained in the adrenaline in urine. He therefore concluded that one of the metabolic pathways involved removal of the methyl group, and that amine oxidase is involved in the degradation of adrenaline to yield methylamine. Little work along similar lines has been reported for noradrenaline. There are indications that the rate of turnover of adrenaline in the adrenal medulla is very low[82]; nothing is known about noradrenaline but its turnover rate is probably higher. Until quantitative data for all catechol amines are available, the activities required of enzymes concerned in formation and breakdown cannot be estimated. It is of interest to record here that the bulk of the pressor amines in the adrenal medullary cell is located in the cytoplasmic granules, yet the cytoplasmic fluid contains the dihydroxyphenylalanine-decarboxylase. Thus it is possible that the freshly made amine (probably hydroxytyramine) passes on to or into the granules, and the finished product passes in the outward direction[83]. Further work with radio-isotopes is obviously required in this field. Knowledge about the passage of dihydroxyphenylalanine and hydroxytyramine into the suprarenal medulla, and information about their fate, would be very valuable and indicate lines to pursue.

Abnormal Production

Holton in Britain[84], and Goldenberg in the United States[85], were probably the first workers to demonstrate large amounts of noradrenaline in tumours of the suprarenal medulla. In these conditions the physiology of the suprarenal differs from normal in the following respects—the total amount of pressor hormone secreted in response to stimulus is far in excess of normal, and a high proportion of noradrenaline may be produced so that the effect of the mixtures of the amines is that of noradrenaline and not that of adrenaline. There is evidence too that in many instances the mixture of hormones is liberated from the gland continuously over long periods, in contrast to the transient response to stress in the normal individual.

It has already been stated that with phæochromocytoma the urinary excretion of noradrenaline is very considerably increased. It has been found that the proportion of the two catechol amines excreted in the urine and the relative content of these substances in the tumour is well

correlated. After removal of the tumour, the output of urinary catechol amines decreases to normal. Thus a reliable diagnosis of phæochromocytoma may be obtained by estimating the amines in the urine. Although most workers in this field use the biological methods of assay after adsorption on to aluminium hydroxide, it is possible that the photofluorimetric method possesses certain advantages[86].

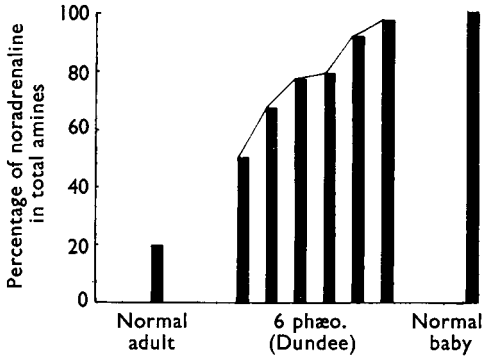


FIG. 7. Comparison of the relative noradrenaline content of the suprarenal glands of patients with phæochromocytoma and of the normal baby and adult.

Adrenal medullary tumours have now been assayed in many laboratories throughout the world. Whereas in the normal adult human suprarenal the relative content of noradrenaline rarely exceeds 20 per cent. of the total catechol amines, in the cases of the tumours it ranges from 50 to 99 per cent. (Fig. 7). Tumour tissue therefore resembles embryonic tissue in this respect, since the adrenal glands of the fœtus and baby contain predominantly noradrenaline. The full significance of these findings is not yet certain.

SUMMARY AND COMMENTS

In the normal subject, adrenaline as a physiological substance is not a specific hypertensive agent. It appears to possess the more general function of stimulating various metabolic processes in the body in conditions of stress. Only in abnormal states, with large amounts of circulating adrenaline and noradrenaline, is there an over-all vasoconstriction. Frequently in such cases the removal of a phæochromocytoma is followed by an acute hypotension due to the sudden removal of the constrictor hormones which by their presence in the circulation have maintained the over-all vascular tonus.

Noradrenaline is not normally an effective circulating agent for its action is localised at the adrenergic nerve endings, but when elaborated by medullary tumours and liberated into the circulation it causes effects similar to those seen during its infusion in man. Whereas adrenaline has enjoyed a prominent place in the therapy of hypotension for many years, on physiological grounds noradrenaline would appear to be the agent of choice in peripheral circulatory collapse. It has now been used with success in the treatment of such conditions in many countries. The advantages of noradrenaline over adrenaline are: it is less toxic; its pressor effects are usually more pronounced; it causes bradycardia and not tachycardia; it is less liable to precipitate extra systolic arrhythmias; and it does not cause the tension and agitation which sometimes occurs as a troublesome side-effect of adrenaline.

The occurrence of different cell types in the suprarenal medulla has often been described, and it is now probable that noradrenaline is exclusively or predominantly stored only in certain specific cells which may be separately innervated. If this proves to be so, then noradrenaline is not only a precursor of adrenaline but is also a hormone. The reason why varying amounts of each amine exist in the suprarenal glands of different species may be linked with these specific nerve fibres, but so far it has not been elucidated.

Since noradrenaline is liberated in very small amounts at the adrenergic nerve endings and maintains local vasomotor tone and the peripheral resistance, the sympathetic nervous system and the suprarenal medulla should be considered as separate entities from the functional point of view. It is probable that Cannon's view on the emergency function of adrenaline still holds good. Indeed, the recent work of investigators on the human subject has done much to indicate the role of adrenaline in normal physiology to be that which Cannon first propounded.

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