Catecholamines

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The L-isomers of adrenaline and noradrenaline are the two catecholamines that have been shown to occur in blood. They are reasonably stable in plasma if there is no delay in freezing the sample, but are very unstable in whole blood. In view of this it is essential, if accurate plasma levels are to be obtained, to spin off the red cells at the bedside immediately the blood samples are collected and to freeze the plasma samples as quickly as possible (Carruthers *et al.*, 1970). In contrast both adrenaline and noradrenaline are stable in urine (Mann, 1953). If acidified and kept below 4°C, there is said to be virtually no loss, even over periods of several months.

Adrenaline and noradrenaline are distributed throughout the body in the adrenal gland and other chromaffin tissue and also in other tissues in relation to their postganglionic adrenergic innervation (Iversen, 1967). The highest concentrations are found in the vas deferens, ciliary body, heart, salivary gland, and spleen. Low amounts are found in skeletal muscle and none has been detected in bone marrow or placenta (Iversen, 1967). Physiological action of noradrenaline and adrenaline differ in many important aspects (Euler, 1951). Noradrenaline is a more powerful vasoconstrictor whereas adrenaline has greater metabolic effects. The main function of noradrenaline seems to be the normal control of the circulation, while adrenaline produces various effects in conditions of stress and emergency.

Euler (1946) was the first to show that noradrenaline and not adrenaline was the main neurotransmitter of the adrenergic nervous system. Ahlquist (1948) postulated that there were two types of receptor responding to catecholamines, the α and β . Activation of the α receptors produces vasoconstriction, stimulation of the uterus, nictitating membrane, ureter and dilator pupillae, and also intestinal relaxation. On the other hand activation of

the β receptors produces myocardial stimulation (both inotropic and chronotropic), vasodilatation, and inhibition of the uterine and bronchial musculature. Though the development of specific α and β adrenergic blockers has given considerable support for this concept, the individual α and β receptors have not as yet been identified. In addition to the effects of catecholamines already mentioned, they also produce important metabolic changes (Ellis, 1956). Oxygen consumption is increased, hyperglycaemia is produced with increased glycogenolysis in the liver and peripheral tissues, and there is increased fat mobilization with a rise of free fatty acids. It has also been shown that insulin release is blocked from the pancreas by adrenaline (Porte *et al.*, 1966), another way in which hyperglycaemia may be produced, in addition to increased glycogenolysis.

The mechanism by which the catecholamines stimulate the myocardium has produced much speculation. Haugaard (1963) considered that activation of phosphorylase 'a' was the important factor, whereas Sutherland, Robison, and Butcher (1968) thought that the positive inotropic effects of catecholamines were mediated by stimulating production of 3', 5'-cyclic adenosine monophosphate. Another view was that the catecholamines act directly on myosin B to facilitate the response to the calcium ions (Honig, 1968). However, Nayler (1970) considers that the primary mechanism explaining the effect of catecholamines on the myocardium at a subcellular level was that the sympathomimetic amines increase the rate at which the sarcoplasmic reticulum accumulates ionized calcium. This author states that possibly the other biochemical events known to be associated with the inotropic action of the sympathomimetic amine, e.g. the altered percentage of the phosphorylase enzyme present in the 'a' form and the activation of adenyl cyclase enzyme, reflect this altered intracellular concentration of

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ionized calcium and therefore may be regarded as secondary rather than primary changes.

L-noradrenaline is synthesized from L-tyrosine via dihydroxyphenylalanine (DOPA) and dihydroxyphenylethylamine (dopamine) (Blaschko, 1939). L-adrenaline is produced from L-noradrenaline by the addition of a methyl group under the action of the enzyme phenylethylamine-N-methyltransferase. This enzyme is almost entirely restricted to the adrenal gland and only in the adrenal gland does adrenaline appear to be produced (Axelrod, 1962). The major pathway for the metabolism of noradrenaline and adrenaline involves O-methylation to yield physiologically inactive metanephrine and normetanephrine (Axelrod, 1959). Monoamine oxidase is mainly concerned with the deamination of the O-methylated metabolites rather than the adrenaline and noradrenaline themselves. Catechol-O-methyl transferase catalyses the O-methylation of adrenaline and noradrenaline and is widely distributed in the body. When noradrenaline is released at nerve endings, it is metabolized either by monoamine oxidase within the nerve, or by catechol-O-methyl transferase outside the nerve. However, a considerable proportion of the catecholamine is inactivated by being bound again or by diffusing into the circulation (Axelrod, 1965). The metabolic fate of adrenaline has been studied in man by the intravenous administration of physiological doses of the radioactive amine. After the administration of ^3H -labelled adrenaline the urine was collected for 48 hours and the metabolic products were separated and measured by specific procedures involving solvent extraction and column chromatography. Only 6 per cent of unchanged adrenaline was excreted; over 80 per cent of the administered adrenaline was excreted as O-methylated products: metanephrine (free and conjugated) 40 per cent, methoxy 4-hydroxymandelic acid (VMA) 41 per cent, and 3-methoxy-4-hydroxyphenylglycol sulphate (7%) (LaBrosse *et al.*, 1961). It seems likely that in view of the small percentage of catecholamine excreted unchanged in the urine that the urinary excretion rate of catecholamines is unlikely to be a sensitive index of catecholamine production. As regards the relation between the urinary and plasma catecholamine levels, this is also not simple. It has been found that the renal clearance of adrenaline is significantly greater than the glomerular filtration rate though less than the renal plasma flow. From this it was concluded that adrenaline is excreted both by tubular secretion and glomerular filtration (Jones and Blake, 1958). This work

suggests that the relation between urinary and plasma levels of catecholamine depends on renal blood flow and function and hence is unlikely to be constant, particularly between different individuals. This, of course, makes the interpretation of the urinary catecholamine levels in relation to the plasma levels somewhat uncertain.

It has been demonstrated that catecholamines are taken up by the tissues and that this is probably an important route in their inactivation (Axelrod, Weil-Malherbe, and Tomchick, 1959). Iversen (1967) has further shown that in this uptake process there are two separate mechanisms, uptake 1 and uptake 2. Uptake 2 differs in several respects from the uptake 1 process. Uptake 2 has a higher affinity for adrenaline and is entirely inoperative at low perfusion concentrations. In addition the catecholamines accumulated by the uptake 2 process appear to be more easily released than those taken up by the uptake 1 process.

The concentrations of adrenaline and noradrenaline in plasma are very low and require very sensitive methods for their determination. Chemical methods are available which depend upon the conversion of the amines into derivatives which emit a characteristic fluorescence greater than the parent amine (Crout, 1959; Udenfriend, 1959). However, though these methods have been used with success for urinary estimation (when the levels are much higher) they are at the limits of their sensitivity when used for plasma estimation. In addition these techniques do not usually employ internal standardization for each individual sample and values for adrenaline in most cases must be derived from total catecholamine and noradrenaline values rather than being measured directly. It has also been shown (Carruthers *et al.*, 1970) that several common dietary and therapeutic agents (including tea, coffee, ampicillin, vitamins, aminophylline, and lasix) cause moderate or striking fluorescence *in vitro* when put through the same extraction and analysis stages as the plasma sample. Hence these substances are liable to interfere with the catecholamine estimation. A double isotope method (Siggers, Salter, and Toseland, 1970) has been described which overcomes many of these problems. This method is a development of a double isotope technique produced by Engelman, Portnoy, and Lovenberg (1968). It employs a ^{14}C -labelled methionine donor in the presence of catechol O-methyltransferase to methylate adrenaline and noradrenaline. The estimation, performed by liquid scintillation counting, uses a tritium-labelled internal

standard to determine the recovery of catecholamine in each individual sample.

In the investigation of catecholamine metabolism in man both plasma and urinary levels have been measured. Vendsalu (1960) has shown that plasma levels vary at different sites of blood flow in healthy subjects. The plasma levels are liable to change quickly since it has been shown that when radioactive labelled adrenaline and noradrenaline are given intravenously, they disappear rapidly from the plasma (Axelrod *et al.*, 1959). The actual act of taking blood by venepuncture has been shown to increase significantly the adrenaline, though not the noradrenaline levels (Carruthers *et al.*, 1970). It has also been shown that a change in position from recumbent to the head-up tilt position is sufficient to raise the catecholamine plasma levels (Vendsalu, 1960). It is thus apparent that the interpretation of the significance of the plasma levels of catecholamine is likely to be difficult. Urinary catecholamines have been measured in a number of conditions, in the hope that they will reflect plasma levels. However, in view of the fact that changes and differences in renal blood flow and function (Jones and Blake, 1958) are likely to affect the relation between plasma and urine levels, the interpretation of urinary levels must also be judged with some caution.

It has been shown that there is an increased urinary excretion of noradrenaline with a depletion of atrial and left ventricular muscle levels in patients with heart failure (Chidsey, Braunwald, and Morrow, 1965). They concluded that heart failure was associated with an augmented activity of the sympathetic nervous system and suggested that there might be a relative deficiency of sympathetic function which adversely affects the contractile state of the myocardium. However, Braunwald *et al.* (1969) have shown that catecholamine depletion does not affect the intrinsic contractile state of the myocardium, but interferes with the augmentation of contractility provided by the adrenergic nervous system. Previously Lee and Shideman (1959) had stressed the importance of normal levels of myocardial catecholamines for maintaining normal cardiac contractility, and Braunwald, Harrison, and Chidsey (1964) have even suggested that the heart may act as a neuroendocrine organ, releasing catecholamines into the circulation.

On the other hand catecholamines may give rise to deleterious effects on the heart. Patients have been described in whom myocardial lesions have been produced by treatment with adrenaline (Piscatelli and Fox, 1968) and nor-

adrenaline (Szakacs and Cannon, 1958). Similar myocardial lesions have been produced in dogs with therapeutic doses of 1-noradrenaline (Szakacs and Mehlman, 1960) and also found in patients with phaeochromocytoma (Van Vliet, Burchell, and Titus, 1966). In addition, catecholamines have also been shown to be capable of directly producing arrhythmias in man (Katz, 1965).

Increased levels of catecholamines in the urine after acute myocardial infarction have been reported by many workers (Forssman, Hansson, and Jensen, 1952; Nuzum and Bischoff, 1953; Valori, Thomas, and Shillingford, 1967; Januszewicz *et al.*, 1968; Wallace, 1968; Jewitt *et al.*, 1969). In particular, high levels appeared to be associated with heart failure, cardiogenic shock, and arrhythmias. High plasma catecholamine levels have also been found in this condition (Gazes, Richardson, and Woods, 1959; McDonald *et al.*, 1969; Siggers, Salter, and Fluck, 1971) and it has been suggested that the high catecholamine levels may be a factor in the production of the arrhythmias that follow acute myocardial infarction (Maling and Moran, 1957; Ceremuzynski, Straszewska-Barczak, and Herbaczynska-Cedro, 1969). It has also been postulated that high levels produce this arrhythmic effect to some extent by raising the level of free fatty acid in the body, since Kurien and Oliver (1970) have shown that high fatty acid levels may be another stimulus to the onset of arrhythmias. Other workers, however (Opie *et al.*, 1971), were not able to confirm that high concentrations of circulatory free fatty acids played a major role in the genesis of serious arrhythmias after coronary artery occlusion. High plasma catecholamine levels have been found in patients during and after operations involving cardiopulmonary bypass (P. Taggart *et al.*, 1972, personal communication). In that study it was shown that the myocardial levels of noradrenaline progressively fell during the operations. Emotional stress is another situation found to be associated with high plasma catecholamine levels. The levels have been found to be high in racing drivers (Taggart, Gibbons, and Somerville, 1969) and also in speakers presenting scientific papers (Somerville, Taggart, and Carruthers, 1971).

The aetiological role of catecholamines in hypertension still remains controversial. Lorrimer *et al.* (1971) found there was no evidence that patients with sustained hypertension had an increased production of catecholamines either at rest or under stress. However, other workers (De Quattro and Chan, 1972) have found plasma catecholamine levels higher in the patients with hypertension than normal

subjects. Differences in methodology and selection of patients may well explain the discrepancy in these results.

Sarnoff (1960) has stressed that the physiological activity of catecholamines is an essential ingredient of a fully complete organism, whereas Raab (1960) has emphasized the key position that catecholamines play in functional and degenerative cardiovascular pathology. To resolve many of the problems that still remain, the use of more accurate techniques is essential. This particularly applied in the measurement of plasma samples. However, it must be realized also that the plasma levels of catecholamines only reflect the balance of biosynthesis, catabolism, uptake, and release at the time of sampling, and may vary rapidly. Similarly, though the concentration of catecholamine and their metabolites in the urine may represent to some extent an overall estimate of sympathetic activity, they are unlikely to reflect the influence of transient changes. Thus, the investigation of the various roles that catecholamines play in man, both in health and disease, still presents many difficult problems.

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