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Role of adrenal hormones in the synthesis of noradrenaline in cardiac sympathetic neurones

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1. Adrenalectomy or adrenal demedullation affected neither the levels of endogenous catecholamines in the rat heart nor the accumulation of ^{3}H-noradrenaline 1 hr after its intravenous administration.

2. Twenty-four hours after intravenous administration of labelled amine, however, its retention was markedly reduced in the heart of adrenalectomized or demedullated rats. Ganglionic blockade prevented this reduction.

3. Rate calculations from the decline of catecholamine levels after blockade of synthesis with α-methyl-tyrosine showed that cardiac synthesis of noradrenaline increased about four-fold after demedullation and about three-fold after adrenalectomy. This increase in synthesis may compensate for the loss of circulating catecholamines.

4. There was no change in catechol-*o*-methyl-transferase activity, but monoamine oxidase activity was increased in the homogenates of the heart of adrenalectomized and demedullated rats. The increase in the cardiac monoamine oxidase activity was markedly greater in the adrenalectomized rats than in the demedullated rats.

5. It is suggested that adrenal cortex insufficiency may modulate the rate of synthesis of noradrenaline and monoamine oxidase activity in cardiac sympathetic neurones.

Noradrenaline is a normal constituent of the mammalian heart (von Euler, 1956); it is stored in the adrenergic nerves in dense core vesicles. Since large amounts of noradrenaline can be taken up by the heart, it is assumed that catecholamines discharged into the blood stream from the adrenal glands and from sympathetic nerves (as a result of normal flow of tonic impulses from the central nervous system) can contribute to the maintenance of transmitter stores in the adrenergic nerves. More recently, it has been shown that the myocardium contains all the enzymes necessary for synthesis of noradrenaline from its dietary precursors (Levitt, Spector, Sjoerdsma & Udenfriend, 1965) and that the maintenance of myocardial catecholamines is not totally dependent on the uptake of catecholamines of adrenal origin or on the presence of a functional adrenal gland (Bhagat, 1963; Bhagat & Shideman, 1964; Avakian & Vogt, 1966). In addition Kopin & Gordon (1963) calculated that 80% of the heart noradrenaline is synthesized in the tissue while 20% is taken up from circulating amine, some of which might be of adrenal origin.

Normally, tissue catecholamine remains at a steady level characteristic of each organ. There is considerable evidence that prolonged sympathetic nerve stimulation fails to deplete, and in some cases even increases, the endogenous concentrations of noradrenaline (Luco & Goni, 1948 ; von Euler & Hellner-Bjorkman, 1955). Increased sympathetic activity results in increased synthesis of noradrenaline (Oliverio & Stjärne, 1965 ; Bhagat, 1967 ; Bhagat & Friedman, 1969). Thus it seems logical that cardiac catecholamine concentrations could be maintained after removal of the adrenal medulla by increased catecholamine synthesis in peripheral sympathetic neurones. This would compensate for the lack of noradrenaline which is normally available for uptake by the heart after its release into the circulation from the adrenal gland (Westfall & Osada, 1968 ; Neff, Ngai, Wang & Costa, 1969).

The present work was done to gain further information on this point. We found that the increase in the turnover rate of cardiac noradrenaline in adrenal demedullated rats was greater than that in adrenalectomized rats. We also found that the monoamine oxidase activity of homogenates of the heart of adrenalectomized or demedullated rats was increased.

Methods

Male albino rats of the Holtzman strain weighing 200–225 g were used in all the experiments. Adrenal demedullation was performed under pentobarbitone sodium anaesthesia (50 mg/kg injected intraperitoneally). The capsule of the adrenal was incised at the upper pole of the gland and the medulla and much of the cortical parenchyma were then removed by gently squeezing the organ. Sham operation differed only in that the glands were not disturbed. Bilateral adrenalectomy was performed under pentobarbitone anaesthesia. Sham-operated rats received tap water containing 0·5% NaCl for drinking purposes. All animals were used for the experiments 240 hr after the operations. Systolic blood pressure was measured in unanaesthetized animals with a pulse transducer applied to the tail.

Estimation of catecholamines. Animals were killed by a blow at the base of the neck and decapitated. The heart was removed, dried between filter paper and weighed. It was homogenized in ice-cold 0·4 N perchloric acid. The protein-free supernatant solution obtained after centrifugation was absorbed on alumina at pH 8·6. The catecholamines were eluted with 0·05 N perchloric acid (Anton & Sayre, 1962). The endogenous noradrenaline was converted to its trihydroxyindole derivative by oxidation with potassium ferricyanide at pH 6·5 according to the method of von Euler & Lishajko (1961). A 1·0 ml. aliquot of the alumina eluate was counted in a liquid scintillation counter.

Monoamine oxidase (MAO) was assayed by determining the conversion of ^{14}C-tryptamine to ^{14}C-indoleacetic acid according to the method of Wurtman & Axelrod (1963). Catechol-*o*-methyl transferase (COMT) was assayed by measuring the formation of ^{14}C-normetanephrine on incubation with (−)-noradrenaline and ^{14}C-methyl-*s*-adenosylmethionine (specific activity 50 mc/m-mole, New England Nuclear) as described by Axelrod (1959). The MAO and COMT activity were expressed as mμ-moles of product formed per gram wet weight during 1 hr of incubation at 37° C. Turnover rates of noradrenaline in the heart were estimated from the decline in its concentration after blockade of synthesis by α-methyl-*para*tyrosine according to the method of Brodie, Costa, Dlabac, Neff & Smookler (1966).

The following substances were used: DL-7-^{3}H-noradrenaline hydrochloride (specific activity 5 c/m-mole, obtained from New England Nuclear Corporation, Boston, Mass.) and chlorisondamine hydrochloride. All doses refer to the salt used. Tests of significance were performed according to Student's t test (Snedecor, 1956). $P<0.05$ was regarded as significant.

Results

Effect of adrenalectomy or adrenal demedullation on cardiac storage of noradrenaline and on blood pressure

Adrenalectomy or adrenal demedullation affected neither the levels of endogenous catecholamines in the heart nor the heart weight. The blood pressure of adrenalectomized rats was significantly lower ($P<0.05$) than that of demedullated or control sham-operated rats. The results are summarized in Table 1. It seems that low blood pressure in the adrenalectomized rats is due to the lack of cortical hormones.

The accumulation of ^{3}H-noradrenaline in the heart, 1 hr after its intravenous administration, was the same in the adrenalectomized, the demedullated and the control sham-operated rats (Table 1). However, 24 hr after intravenous administration of ^{3}H-noradrenaline (10 μc/100 g), the retention of this amine in the heart of adrenalectomized or demedullated rats was significantly less than in control sham-operated rats (Table 3).

Since accumulation of labelled catecholamine in the heart at 1 hr was normal in both adrenalectomized and demedullated rat, the reduced retention of ^{3}H-noradrenaline observed in these animals at 24 hr may be due to an increased turnover rate of the amine.

TABLE 1. *Endogenous catecholamine, initial accumulation of ^{3}H-noradrenaline, heart weight and systolic blood pressure following adrenalectomy or demedullation*

Group	Rat weight (g)	Heart weight (mg)	Systolic blood pressure (mm Hg)	Noradrenaline (wet weight of tissue)	
				Endogenous (μg/g)	Labelled (mμc/heart)
Sham	200±4	590±5	118±2·5	0·96±0·05	109±7
Adrenalectomized	205±3	592±6	104±3·6	0·92±0·06	106±8
Demedullated	202±2	600±6	113±3·1	0·96±0·06	101±11

Sham-operated, demedullated or adrenalectomized rats were injected intravenously with ^{3}H-noradrenaline, 10 μc/rat. One hour later, these animals were killed and their hearts were assayed for endogenous and labelled noradrenaline. All values are means ±standard error of six to eight animals.

TABLE 2. *Turnover rate of noradrenaline in rat heart*

Group	Steady state noradrenaline levels (in fresh tissue) (μg/g±S.E.)	Rate constant (hr^{-1})	Turnover rate (μg/g per hr)
Sham-operated	0·98±0·04 (8)	0·041	0·042
Adrenalectomized	1·02±0·03 (6)	0·102	0·10
Demedullated	1·04±0·06 (10)	0·164	0·158

Rats were given α-methyl-tyrosine 100 mg/kg intravenously and the rats were killed 1, 2, 4 or 8 hr later. A second dose of 100 mg/kg was administered 2 hr after the first to the animals to be killed at 4 and 8 hr. Catecholamines in the heart were determined and expressed in terms of noradrenaline. Rate constant was calculated by the method of least squares.

Turnover rate of cardiac noradrenaline in adrenalectomized and adrenal demedullated rats

In order to assess the turnover rate of noradrenaline, rats were injected with α-methyl-*para*-tyrosine to inhibit the conversion of tyrosine to dihydroxyphenylalanine, the rate-limiting step in the biosynthesis of noradrenaline (Levitt *et al.*, 1965). The animals were killed at various times afterwards. It was found that there was a much greater decrease in the endogenous noradrenaline in the adrenalectomized or demedullated rats than in control rats with sham operation. The calculations, summarized in Table 2, indicate that demedullation caused about a four-fold increase while adrenalectomy caused about a three-fold increase in the cardiac turnover rate of noradrenaline.

Effect of ganglionic blockade on cardiac noradrenaline turnover in adrenalectomized or adrenal demedullated rats

To examine the influence of centrally mediated sympathetic activity on the increased turnover of cardiac noradrenaline in the operated rats, the effect of decentralization by chlorisondamine (a long-acting ganglionic blocking agent) was studied. Ganglionic blockade increased the retention of ^{3}H-noradrenaline more in the hearts of adrenalectomized or demedullated rats than in the hearts of sham-operated control rats. Twenty-four hours after the administration of ^{3}H-noradrenaline to groups of rats not receiving chlorisondamine, the adrenalectomized and demedullated rats had lower ^{3}H-noradrenaline levels in the heart than the sham-operated controls, consistent with increased turnover. There were, however, no differences between groups in the retention of ^{3}H-noradrenaline at 24 hr when the rats had been given chlorisondamine (Table 3).

TABLE 3. *Effect of ganglionic blockade on ^{3}H-noradrenaline storage in rat heart*

Group	^{3}H-Noradrenaline retention (mμc/heart)
Sham	65·5±2·5
Adrenalectomized	36·8±1·15*
Demedullated	25·6±2·0*
Sham+chlorisondamine	116·8±7·2
Adrenalectomized+chlorisondamine	110·5±8·7
Demedullation+chlorisondamine	114·6±6·8

Rats received 10 μc/100 g body weight of ^{3}H-noradrenaline. 15 min later some rats were given chlorisondamine 10 mg/kg every 8 hr subcutaneously. All animals were killed 24 hr after the injection of ^{3}H-noradrenaline and their hearts were analysed for ^{3}H-noradrenaline. Each group contained six to eight animals and the results are expressed as the mean±standard error. *$P<0·01$ for difference between operated and sham-operated rats.

TABLE 4. *Monoamine oxidase (MAO) and catechol-o-methyl transferase (COMT) activity in rat heart*

Group	MAO activity	COMT activity
Sham operated	2540±180	1650±140
Adrenalectomized	4372±210*	1560±170
Demedullated	2756±165	1610±155

The activity of MAO and COMT are expressed as mμ-moles of product formed per gram of tissue during 1 hr of incubation at 37° C. Product refers to ^{14}C-indoleacetic acid or ^{14}C-normetanephrine. Each group contains five to six rats. The results are given as the mean±S.E.M. *$P<0·01$ for difference between operated and sham-operated rats.

Effect of adrenalectomy or adrenal demedullation on cardiac monoamine oxidase activity and on catechol-o-methyl transferase activity

Since there was an increased turnover rate of cardiac noradrenaline and since there was no change in endogenous catecholamine levels following adrenalectomy or adrenal demedullation, it is conceivable that there was increased catabolism of noradrenaline. It was therefore of interest to determine whether or not there were any alterations in the activity of enzymes responsible for inactivation of noradrenaline. The results of these experiments are presented in Table 4. There were no changes in catechol-*o*-methyl transferase activity but there was a significant increase in the monoamine oxidase activity ($P<0{\cdot}01$) after adrenalectomy. However, after demedullation the difference was not statistically significant.

Discussion

The effect of either total adrenalectomy or demedullation in rats was to leave unchanged both the noradrenaline content of the heart and the accumulation of ^{3}H-noradrenaline 1 hr after its intravenous administration. At 24 hr, however, the retention of ^{3}H-noradrenaline was markedly less than that in sham operated controls. Since the initial accumulation of ^{3}H-noradrenaline was normal, the decreased labelled amine in the hearts of adrenalectomized or adrenal-demedullated rats at 24 hr is unlikely to result from a defect in delivery or uptake of amine, but more probably from increased release of ^{3}H-noradrenaline over 24 hr, once the stores had been labelled.

After intravenous administration, ^{3}H-noradrenaline is taken up by the axonal membrane of the sympathetic nerves, retained in storage vesicles, where it gradually equilibrates with endogenous amine, and is released in response to sympathetic nerve stimulation. The rate of decline of ^{3}H-noradrenaline in a labelled tissue, therefore, is a measure of turnover rate. If the endogenous stores remain constant, the turnover rate is a measure of noradrenaline synthesis. Thus the rapid decline in ^{3}H-noradrenaline in adrenalectomized or demedullated rats, at 24 hr after its administration, indicates increased noradrenline turnover and synthesis. These results are consistent with those of Landsberg & Axelrod (1968).

This rapid rate of ^{3}H-noradrenaline decline, in cardiac tissue of adrenalectomized and demedullated rats at 24 hr after injection, was prevented by ganglionic blockade. These results therefore implicate centrally mediated sympathetic activity in the genesis of the increased noradrenaline turnover and synthesis.

Rate calculations from the decline of endogenous catecholamine levels, after blockade of the biosynthesis at a rate limiting step by α-methyl-*para*-tyrosine, indicates that removal of the adrenal gland or adrenal demedullation in the rat caused a three–four-fold increase in heart noradrenaline synthesis. Thus, the catecholamines that are continuously released from the adrenal medulla into the circulation may modulate the rate of cardiac noradrenaline synthesis.

The increased turnover of noradrenaline observed in the present study may be limited to the heart, since the salivary glands of adrenalectomized or demedullated rats did not show the same decreased ^{3}H-noradrenaline accumulation at 24 hr after injection (Bhagat, unpublished).

Monoamine oxidase plays an important role in the regulation of endogenous amine levels. In normal conditions, a considerable amount of noradrenaline is

slowly released from the storage granules intraneuronally and is mostly deaminated and inactivated by monoamine oxidase present in the mitochondria of the sympathetic nerve. It has been suggested that free intraneuronal noradrenaline may control the rate of tyrosine conversion to noradrenaline (Alousi & Weiner, 1966). Inhibition of monoamine oxidase would preserve the free intraneuronal noradrenaline and may cause an inhibition noradrenaline synthesis (Costa & Neff, 1966). In the present study cardiac monoamine oxidase activity after adrenalectomy and demedullectomy was increased. This increase may reduce the intraneuronal noradrenaline resulting in the acceleration of the noradrenaline synthesis.

The increase in turnover of noradrenaline after adrenalectomy probably involves intraneuronal deamination to a greater degree than in the demedullated animals because of the insignificant change in monoamine oxidase activity in the latter group.

The increase in turnover rates of noradrenaline was lower in rats without entire adrenals than in rats without the medulla alone. It appears, therefore, that in the presence of the adrenal cortex synthesis of noradrenaline in the heart takes place more efficiently.

The monoamine oxidase activity increased more following adrenalectomy than after adrenal demedullation. These results therefore indicate that cortical hormones may modulate the heart monoamine oxidase activity. Adrenalectomized rats had a low blood pressure. In contrast, blood pressure was well maintained after adrenal demedullectomy. These results suggest that adrenal cortical insufficiency causes haemodynamic alterations responsible for the hypotension after total adrenalectomy in the rat.

Thus, adrenalectomized rats, when compared with demedullated animals, had (1) lower turnover rate of cardiac noradrenaline ; (2) increased cardiac monoamine oxidase activity ; (3) lower blood pressure. These findings suggest that adrenal cortical hormones influence the synthesis of noradrenaline and monoamine oxidase activity of cardiac sympathetic neurones. The hormones may also influence the haemodynamics of the cardiovascular system.

The present study demonstrates the ability of the heart of the adrenalectomized or demedullated rat to maintain its endogenous noradrenaline levels even in face of a loss of 20% circulating catecholamines, which are normally available for uptake into the heart after the release from the adrenal glands. It has been shown previously that vigorous sympathetic nervous activity produced by exposure to cold for 6 hr, administration of drugs such as histamine and β-tetrahydronapthylamine or direct sympathetic nerve stimulation for 1 hr fails to deplete cardiac catecholamine (Bhagat, 1967). In all these cases, synthesis acts to maintain endogenous levels of noradrenaline. It is increased to compensate for the loss and, consequently, there is only slight or no reduction in the catecholamine content of tissues. Not only the release of catecholamine from adrenal medulla, but also the adrenal cortical hormones may modulate the cardiac noradrenaline synthesis.

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