STATE OF CATECHOLAMINE SYNTHESIS DURING THE DEVELOPMENT OF MUSCLE FATIGUE

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The rate of formation of catecholamines from tyrosine in the rat adrenal gland and its relationship to the duration of running in a treadmill were studied in experiments in vitro. By adding together the increases in adrenalin, noradrenalin, dopamine, and DOPA in the tissues and incubation medium, a value reflecting tyrosine hydroxylase was obtained. The increase in DOPA and catecholamines in the adrenal and in the incubation medium after addition of tyrosine after running for 2.5 h was the same as in the control. After running for 6 h the synthesis of catecholamines and DOPA was sharply reduced. These results show that with an increase in the duration of muscular exertion the ability of the adrenals in rats to synthesize catecholamines decreases. The decrease in the formation of catecholamines and DOPA is evidently linked with depression of tyrosine hydroxylase activity.

The tissue catecholamine content is known to change during muscular exertion and to fall sharply during severe muscle fatigue [1, 2, 7]. The possible mechanisms of changes in the system of catecholamine synthesis during exposure to various factors have been discussed in the literature [3, 6, 8, 10, 12].

The object of this investigation was to study catecholamine synthesis in the adrenal tissues of rats undergoing muscular exertion. The substrate used was L-tyrosine, because its hydroxylation is the velocity-limiting stage of the process [11].

EXPERIMENTAL METHOD

Male rats weighing 180-200 g were subjected to muscular exertion in a treadmill as described previously [2]. The experimental animals were decapitated 2.5 and 6 h after starting to run. Control animals were decapitated at the same time. The adrenals were quickly removed, placed on ice, and freed from fat and connective tissue. To cancel out individual variations each sample was so composed that it contained eight half-adrenals obtained from four different rats, either control or experimental. Two control and two experimental samples were used in each experiment. The tissue for each sample was weighed and transferred into small vessels containing Krebs–Ringer bicarbonate solution (pH 7.4) containing glucose. Tyrosine (1000 μg) in 0.4 ml 0.01N HCl solution was added to one of the control and experimental samples, while the corresponding volume of solvent was added to the other two vessels. The final volume of incubation medium was 2 ml. All the samples were preincubated in 2 ml Krebs–Ringer solution for 30 min at 37.5°C in an atmosphere of 95% O_2 + 5% CO_2 in a Warburg apparatus. The incubation medium was then replaced by a fresh batch of buffer, tyrosine was added to the corresponding samples, and incubation was carried out for 2 h under the same conditions as preincubation.

After incubation the tissue and incubation medium were separated, and the concentrations of adrenalin, noradrenalin, DOPA, and dopamine in them were determined spectrofluorimetrically [4, 5].

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TABLE 1. Concentration of Catecholamines and DOPA in Adrenal (in μg/g) and Incubation Medium (in μg/g) after Incubation of Adrenals of Intact Rats and Rats Running in a Treadmill for 2.5 h (M±m)

Group of expts.	Substrate	Adrenal				Incubation medium			
		adrenalin	noradrenalin	dopamine	DOPA	adrenalin	noradrenalin	dopamine	DOPA
1	—	224±18	116±9,2	3,8±0,52	4,2±0,46	80±6,8	52±5,2	1,54±0,18	1,6±0,22
2	Tyrosine 1000 μg	295±13,9*	164±8,6*	3,9±0,6	5,6±0,4*	112±7,0*	82±6,0*	2,2±0,17	2,56±0,2*
3	—	109±8,0	90±11	4,5±0,6	3,4±0,32	40±4,0	33±3,2	1,7±0,16	0,86±0,12
4	Tyrosine 1000 μg	189±14,0*	148±13,0*	4,9±0,7	4,4±0,25*	79±9,0*	67±6,0*	2,84±0,2*	1,66±0,22*

Legend to Tables 1 and 2. Experiments of group 1) adrenals of intact animals; 2) adrenals of intact animals + 1000 μg tyrosine; 3) adrenals of experimental animals; 4) adrenals of experimental animals + 1000 μg tyrosine.
*Here and in Table 2, differences between groups 1 and 2, 3 and 4 significant ($P \leq 0.05$).

TABLE 2. Concentration of Catecholamines and DOPA in Adrenal (in μg/g) and Incubation Medium (in μg/g) after Incubation of Adrenals of Intact Rats and Rats Running in a Treadmill for 6 h (M±m)

Group of expts.	Substrate	Adrenal				Incubation medium			
		adrenalin	noradrenalin	dopamine	DOPA	adrenalin	noradrenalin	dopamine	DOPA
1	—	211±20	105±12,3	4,1±0,5	4,0±0,38	80±10,0	56±8,2	1,68±0,18	1,82±0,18
2	Tyrosine 1000 μg	291±15*	157±17*	4,1±0,5	5,5±0,36*	120±9,6*	90±7,0*	2,44±0,2*	2,98±0,22*
3	—	88±5,8	74±3,2	3,7±0,57	2,2±0,16	33,0±2,6	30±4,8	1,58±0,13	0,7±0,076
4	Tyrosine 1000 μg	74±4,7	75±6,5	4,5±0,34	2,6±0,2	27,0±1,2	30±4,0	1,94±0,2	1,02±0,1*

Trial determinations showed that during incubation of the tissue without tyrosine only traces of catecholamines were formed. The absolute increase in catecholamines and DOPA obtained by comparing samples to which tyrosine had or had not been added was accordingly taken to reflect the intensity of synthesis. By adding together the increases in all the compounds studied in the tissue and incubation medium, a value was obtained which characterizes tyrosine hydroxylase activity to a certain degree; this activity was expressed in nmoles/g per hour. The tyrosine concentration was determined in the adrenal tissues [14].

EXPERIMENTAL RESULTS AND DISCUSSION

The results are given in Tables 1 and 2. A comparison of the results of the experiments of groups 1 and 3 shows that muscular exertion lowered the concentrations of catecholamines and DOPA in the adrenal; this is in agreement with the results of the previous investigations [2]. The fact that on incubation of the intact adrenals with the addition of tyrosine an increase in the concentration of these compounds was observed is evidence that the enzyme systems of synthesis are preserved under the chosen conditions of incubation.

During incubation of the adrenals of the intact and experimental rats in the presence of tyrosine, the changes in the synthesis and secretion of catecholamines into the medium were generally in the same direction although marked to a different degree.

Tyrosine led to different changes in the catecholamine and DOPA levels in the adrenals of the experimental animals after running for different durations. For instance, during incubation of the adrenals of rats which had run for 2.5 h some slight acceleration of catecholamine synthesis was observed. The increase in adrenalin was 9, in noradrenalin 10, and in dopamine 0.4 μg/g compared with the control. The increase in DOPA was less than in the control (1.4 in the control, 1 μg/g in the experiment). When tyrosine hydroxylase activity was determined by the method described above, a tendency toward an increase was found: 2.5 h after the beginning of exposure its activity in the control was 520±28, but in the experimental series it was 608±37 nmoles/g per hour ($0.1 > P > 0.05$). On incubation of the adrenals of rats which had run on the treadmill for 6 h (Table 2), on the other hand, the increase in concentration of these compounds was less than in the control (no adrenalin and noradrenalin in general were formed); tyrosine hydroxylase activity was sharply reduced (control 590±70 nmoles/g per hour, experiment 31±10 nmoles/g per hour; $P < 0.001$).

Special experiments (the results of which will be described in another communication) showed that tyrosine, injected intraperitoneally, did not change the catecholamine concentration in the adrenal of the intact animals but raised their lowered level in the adrenal of rats which had run for 2.5 h on the treadmill. An increase in the rate of catecholamine synthesis in the relatively early stages of exposure to various factors or during adaptation to them has been observed by several investigators [6, 8, 9]; this increase in rats has been shown to be associated with increased activity of the enzymes of catecholamine synthesis [9, 13]. However, during more prolonged exposure to unfavorable factors (in particular, during prolonged running) the opposite changes can evidently occur. Bearing in mind the fact that the tyrosine concentration in the adrenal was unchanged after running for 6 h (control 72±4.7 μg/g, experiment 67±6.5 μg/g it can be postulated that the cause for the sharp decline in the level of catecholamines and DOPA in the gland under these circumstances was a general lowering of the synthetic ability of the tissue, principally on account of a decrease in tyrosine hydroxylase activity.

In the discussion of the mechanism of this decreased activity it may be assumed that the synthesis of the enzyme itself is disturbed during the development of muscle fatigue. Another more likely cause of the observed decrease may be the appearance of products formed during increased muscular activity and inhibiting the enzyme. A decrease in the level of coenzymes of catecholamine synthesis may also take place during prolonged physical exertion.

The decrease in the intensity of catecholamine synthesis discovered in these experiments (probably associated with inhibition of tyrosine hydroxylase) is evidently characteristic of the adrenal alone, for in none of the other organs tested was so marked a decrease in the level of the various compounds discovered [2, 7].

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