EFFECT OF GLUCOCORTICOIDS ON CATECHOLAMINE SYNTHESIS IN THE ADRENALS AND HEART OF RATS DURING PHYSICAL FATIGUE

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The effect of adrenocortical hormones on catecholamine synthesis in the adrenals and heart of rats was studied after prolonged swimming (8 h). Catecholamine synthesis during incubation of the adrenals with L-tyrosine was sharply depressed after swimming. Addition of hydrocortisone or prednisolone in vitro (50 μg per sample) and also injection of these hormones in vivo (50 mg/kg intramuscularly, 3 h before decapitation) increased catecholamine synthesis in the adrenals of the swimming rats but not of intact rats. On incubation of the adrenals of swimming rats in the presence of L-dopa and L-noradrenalin catecholamine synthesis was reduced compared with that in intact animals and was not restored on the addition of glucocorticoids. No stimulating effect of aldosterone on catecholamine synthesis in the adrenals could be detected in the presence of L-tyrosine. On incubation of the heart tissue of swimming rats in the presence of L-tyrosine and L-dopa, catecholamine synthesis was depressed and was not restored by glucocorticoids in vitro or in vivo. It is concluded that glucocorticoids can restore catecholamine synthesis when depressed by intensive physical fatigue by acting on the tyrosine hydroxylase stage.

KEY WORDS: *catecholamines; corticosteroids; stress; swimming; physical fatigue.*

It was shown previously that during intensive physical fatigue in rats the catecholamine (CA) content in the adrenals is reduced and their level is not restored after administration of L-dopa to the animals; the ability of the adrenal tissue to synthesize CA is also depressed in vitro [1, 3-5, 8]. Detailed analysis has shown that activity of phenylethanolamine-N-methyltransferase, dopa decarboxylase, and, possibly, tyrosine hydroxylase is inhibited under these conditions [7].

In connection with data in the literature [6] on the action of ACTH and corticosteroids on phenylethanolamine-N-methyltransferase and tyrosine hydroxylase activity it was decided to study the effect of natural and synthetic corticosteroids on CA synthesis in the adrenals and heart of rats after prolonged swimming.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 200-250 g, some intact and others after swimming for 8 h in water at a temperature of 30-32°C. At the end of the experiment the rats were decapitated, the adrenals and heart were quickly removed and minced, and one adrenal or the heart was incubated for 60 min in Krebs–Ringer–bicarbonate solution in the presence of L-tyrosine (1000 μg per sample) and L-dopa (4 μg per sample). The adrenals also were incubated in the presence of L-nordadrenalin (4 μg per sample). The reaction was stopped by the addition of perchloric acid. The control samples had the same composition but perchloric acid was added to them before incubation began. The CA content in the tissues was determined by a fluorometric trihydroxyindole method [9]. The effect of corticosteroids on CA synthesis was studied in experiments in vitro and in vivo. In the experiments in vivo

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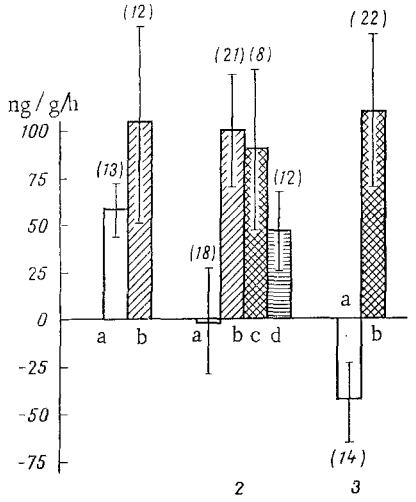

Fig. 1. Effect of glucocorticoids on adrenalin synthesis from L-tyrosine in adrenals of intact and swimming rats: a) absence of exogenous corticosteroids; b) in presence of 50 μg hydrocortisone; c) in presence of 50 μg prednisolone (in vitro) or after injection of 50 mg/kg prednisolone (in vivo); d) in presence of 150 μg corticosterone (in vitro). 1) Intact rats; 2) swimming rats (corticosteroids added in vitro); 3) swimming rats (corticosteroids injected in vivo). Number of animals given in parentheses. Ordinate, charge in adrenalin concentration (in ng/g/h) in incubation medium.

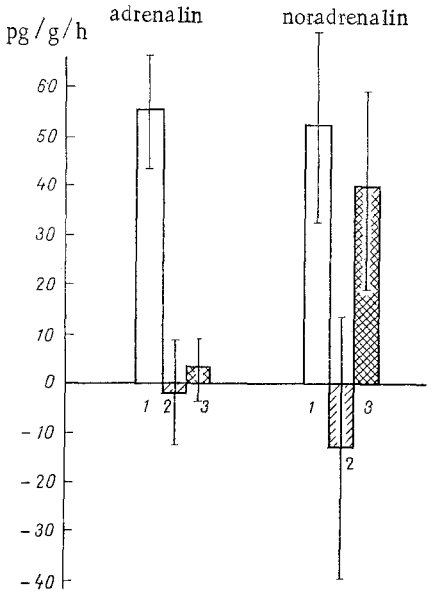

Fig. 2. Effect of prednisolone on catecholamine synthesis in heart of swimming rats: 1) intact rats (27 experiments); 2) swimming rats (10 experiments); 3) swimming rats, prednisolone added in vitro (14 experiments). Ordinate, change in adrenalin concentration (in pg/g/h) in incubation medium.

prednisolone (50 μg), hydrocortisone (50 mg), corticosterone (150 μg), or aldosterone (4 μg) was added to the samples. In the experiments in vivo glucocorticoids were injected intramuscularly in a dose of 50 or 12.5 mg/kg 3 h or 1 h before decapitation. The corresponding volumes of physiological saline were injected into the control animals.

EXPERIMENTAL RESULTS

In agreement with results obtained previously [7] it was found that the adrenals of the swimming rats formed less CA in the presence of L-tyrosine than the adrenals of intact animals. On the addition of hydrocortisone to the incubation medium of the adrenals of intact rats, CA synthesis was unchanged. On incubation of the adrenals of the swimming rats and addition of hydrocortisone, adrenalin synthesis was sharply increased, exceeding its production during incubation of the adrenals of intact animals (Fig. 1). Similar changes were found in the presence of prednisolone also. Activation of adrenalin synthesis was rather less marked in the presence of corticosterone. Adrenalin synthesis was not stimulated by the addition of aldosterone. No regular noradrenalin synthesis was observed in the adrenals of intact rats, and during incubation a decrease in its concentration was often observed. No significant changes in noradrenalin synthesis were discovered under the influence of either swimming or injection of corticosteroids in both groups of rats.

In the adrenals of the intact rats adrenalin synthesis took place in the presence of L-dopa or L-noradrenalin. It was inhibited in the swimming rats. Prednisolone (50 μg per sample) had no effect on the depressed synthesis of catecholamines from L-dopa or L-noradrenalin in the swimming rats.

In the next series of experiments the effect of prednisolone, injected intramuscularly (50 mg/kg) 3 h before decapitation, i.e., 5 h after the rat began to swim, was studied. CA synthesis in the adrenals was investigated in the presence of L-tyrosine. The injected prednisolone, like glucocorticoids added in vitro, completely restored adrenalin synthesis. After injection of the same doses of prednisolone 1 h before decapitation of the animals or after injection of one-quarter of this dose of prednisolone (12.5 mg/kg) 3 h before decapitation, stimulation of synthesis also was observed but the effect was weaker.

During incubation of the heart tissue of intact rats in the presence of L-dopa or L-tyrosine synthesis of noradrenalin and adrenalin was discovered, but it was inhibited after the rats had swum for 8 h. Prednisolone in vitro did not prevent this reduction (Fig. 2). The absence of activation of CA synthesis also was discovered on the addition of hydrocortisone in vitro and on injection of prednisolone in vivo (50 mg/kg 3 h before decapitation).

Glucocorticoids can thus restore depressed CA synthesis in the adrenals during intensive physical fatigue. The main hormone of the rat adrenal cortex, corticosterone, which has both glucocorticoid and mineralocorticoid properties, had a similar action. The mineralocorticoid aldosterone did not stimulate this process.

During intensive physical fatigue in rats the blood corticosterone concentration is known to fall [2]. This suggests that the cause of depression of catecholamine synthesis in the adrenals during intensive physical exertion is a decrease in the activating action of the glucocorticoids.

Since the stimulating effect of glucocorticoids on depressed CA synthesis was manifested only on incubation of the adrenals with L-tyrosine and not with L-noradrenalin or L-dopa, it can be concluded that the glucocorticoids restore CA synthesis when depressed during physical fatigue by their action on tyrosine hydroxylase.

During prolonged swimming CA synthesis from L-tyrosine was inhibited in the heart tissue of rats just as in the adrenals.

Considerable evidence has now been accumulated to show the effect of hormones of the pituitary-adrenocortical system on catecholamine synthesis in the adrenal gland. However, the activating effect of ACTH and glucocorticoids on the enzymes of CA synthesis, phenylethanolamine-N-methyltransferase and tyrosine hydroxylase, in the adrenals, according to data in the literature, is manifested predominantly after hypophysectomy [10-13, 15, 16].

The modulating action of glucocorticoids on the transsynaptic induction of tyrosine hydroxylase and dopamine-β-hydroxylase, mediated through the nervous system, has been postulated [14]. However, information on the direct effect of glucocorticoids on CA synthesis in the adrenals, when inhibited during stress, is not to be found in the literature. It can be concluded from the present experiments that one cause of the depression of CA synthesis during exposure to severe stress, as in the case of physical fatigue, is a reduction in the glucocorticoid activity of the adrenals.

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ROLE OF ENDOPEROXIDES OF THE PROSTAGLANDINS IN PLATELET AGGREGATION

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Phospholipase A and lysolecithin stimulate the reaction of liberation of thromboplastic factor and aggregation of erythrocytes and platelets. Polarographic investigations have shown that these aggregating agents cause absorption of O_2 in medium containing platelets, possible evidence of the formation of these conditions of intermediate products of prostaglandin synthesis, namely endoperoxides. Albumin does not prevent the liberation reaction and the absorption of O_2 caused by phospholipase and lysolecithin but it completely inhibits their aggregating action. Aspirin, on the other hand, blocks O_2 consumption by platelets although its action on the aggregating effect of lysolecithin is only very slight. It is suggested that the aggregation of the blood cells is connected with perturbation of the lipid–protein structure of their membranes and not with endoperoxide synthesis.

KEY WORDS: *blood cells; aggregation; liberation reaction; prostaglandins.*

Reports have recently been published on the important role of intermediate products of prostaglandin synthesis (endoperoxides, prostaglandins G_2 and H_2) in platelet aggregation [8]. The suggestion has been made that aggregating agents (such as collagen, ADP, thrombin) can activate phospholipase activity and liberate arachidonic acid, the common precursor of the prostaglandins [11, 12]. The endoperoxides subsequently formed "trigger" platelet aggregation [15].

The investigation described below was carried out to study this problem.

TABLE 1. Effect of Phospholipase and Lysolecithin on Aggregation of Blood Cells (M ± m)

Conditions of aggregation	Plasma enriched with platelets		Suspension of washed erythrocytes	
	Ma, mm	T, min	Ma, mm	T, min
Phospholipase	39,8±1,36	18,8±0,84	38,0±1,36	22,2±0,90
Phospholipase + albumin	5,5±1,37	11,3±2,89	3,8±1,08	11,9±2,68
P	<0,001	<0,01	<0,001	<0,02
Phospholipase + aspirin	10,9±1,71	14,5±1,80	11,4±1,91	18,9±1,92
P	<0,001	<0,05	<0,001	>0,1
Lysolecithin	46,0±2,48	22,6±1,25	41,7±2,74	24,7±1,47
Lysolecithin + albumin	2,5±1,04	7,2±3,05	4,0±1,87	6,8±2,94
P	<0,001	<0,01	<0,001	<0,02
Lysolecithin + aspirin	36,0±2,99	22,4±1,42	35,8±1,95	21,2±1,01
P	<0,05	>0,5	>0,05	>0,05

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