A CORTICOSTEROID MECHANISM AS THE KEY STAGE IN THE THYMOTROPHIC EFFECT OF OXYTHIAMINE IN RATS

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The writers showed previously [1] that injection of oxythiamine (OT; antivitamin B_1) causes acute involution of the thymus in rats, as a result of the more rapid death of cortical thymocytes, in which OT sharply reduces tolerance to exogenous hydrocortisone (HC). The hypocorticoid nature of this thymolytic effect was studied by producing hypovitaminosis-B_1 with OT in adrenalectomized (AE) rats.

EXPERIMENTAL METHOD

Adrenalectomy was performed on six-week-old male albino rats under ether anesthesia. Between the 8th and 11th days after the operation 48 animals each received OT in a dose of 40 mg/100 g body weight subcutaneously and ten rats each received 1 ml of 0.85% sodium chloride. On the 12th days the rats were decapitated; half of them were given an intraperitoneal injection of 2.5 mg HC/100 g body weight 2 h before sacrifice. In the control series 80 rats of both sexes aged four weeks underwent adrenalectomy or a mock operation (MO). After seventeen days they were decapitated at the same time of day as in the experimental series (between noon and 1 p.m.). The AE rats were kept at 22-24°C and given 0.85% sodium chloride solution to drink. Hypovitaminosis was tested by determining the transketolase activity of the liver. Histological treatment of the material and morphometry were carried out by the method described in [1].

EXPERIMENTAL RESULTS

The weight of the thymus in control AE animals was 55% greater than that of the MO rats (755 ± 17 mg compared with 488 ± 16 mg, $P < 0.001$). The increase in weight was accounted for by the cortex: the number of cells in the cortex and the corticomedullary ratio were increased (Table 1). Despite the greater weight of the thymus in males ($P < 0.01$) the mitotic index of the outer cortex (MI_{oc}) in males was comparable with MI_{oc} in females, whereas the mitotic index of the cambium (MI_{ca}) was paradoxically low. This fact will be seen more strikingly if it is recalled that MI_{ca} and MI_{oc} in AE males were one-third lower than in MO rats. The difference between MI_{ca} and MI_{oc}, determining the proliferative potential of the thymus,

TABLE 1. Morphometric Parameters of Thymus in Control AE Males (A) and AE Females (B)

Group of animals	Weight, mg		MI_{ca}		MI_{oc}		Diff. between MI values		Number of cells in 0.0169 mm²	Cortico-medullary ratio
	A	B	A	B	A	B	A	B		
AE	809 ± 23	711 ± 20	14,2 ± 0,3	21,8 ± 1,7	7,7 ± 0,3	8,5 ± 0,8	6,3 ± 0,2	13,2 ± 1,7	608 ± 6	3,419 ± 0,145
MO	511 ± 21	462 ± 26	22,3 ± 2,0	23,7 ± 1,7	12,3 ± 0,4	10,7 ± 0,5	10,0 ± 2,0	12,9 ± 1,3	512 ± 8	2,604 ± 0,148
P	< 0,001	< 0,001	< 0,01	< 0,5	< 0,001	< 0,05	< 0,1	> 0,5	< 0,001	< 0,001

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TABLE 2. Morphometric Parameters of Thymus in OT-Induced Hypovitaminosis B_1 in AE Rats

Days of hypovitaminosis	Weight, mg	Number of mitoses in 0.338 mm²	MI_{oc}	Number of cells in 0.0169 mm²
1st	472±34	85,2±2,3	7,4±0,2	569±15
P	>0,5	<0,02	<0,001	<0,01
2nd	464±16	89,8±2,4	7,9±0,2	570±6
P	<0,5	<0,1	<0,01	<0,001
3rd	488±58	96,4±6,5	9,2±0,6	525±19
P	>0,5	>0,5	<0,5	<0,5
4th	512±62	108,5±4,5	10,4±0,5	522±7
P	>0,5	<0,5	>0,5	<0,2
Control	518±64	100,2±4,7	10,0±0,5	503±10

TABLE 3. Morphometric Parameters of Thymus in OT-Induced Hypovitaminosis B_1 in AE Rats (treatment with HC in a dose of 2.5 mg/100 g body weight)

Days of hypovitaminosis	Weight, mg	Number of mitoses in 0.338 m² of cortical section	Pycnotic thymocytes per 0.0256 mm² of cortical section	Cortico-medullary ratio
1st	404±29	60,6±3,8	45,1±1,8	3,331±0,272
P	<0,05	<0,001	—	>0,5
2nd	437±33	56,8±7,3	56,2±4,0	3,731±0,567
P	<0,2	<0,001	<0,05	>0,5
3rd	383±35	82,5±5,0	70,5±3,7	3,081±0,383
P	<0,05	<0,01	<0,01	>0,5
4th	415±37	124,5±4,3	60,8±2,5	3,398±0,293
P	<0,1	<0,01	<0,01	>0,5
Control	552±57	103,3±1,9	46,5±1,7	3,392±0,201

was only 82% of MI_{oc} in AE males compared with 167% in AE females ($P < 0.01$); this difference, moreover, was equal to that of MI_{ca} in MO females and MO males.

Consequently the greater increase in size of the thymus in AE males despite their lower cortical mitotic activity than AE females (and MO animals of both sexes) indicates that regulation of lymphopoiesis by endogenous thymus hormones is not as autonomous as Metcalf once considered [5]. In the absence of the adrenals the number and nature of the cells repopulating the thymus are firmly controlled by the gonads. Since despite the decline in proliferative potential, the weight of the thymus increased in the AE males, it can be tentatively suggested that immigration of precursors from the bone marrow into the thymus was sharply intensified in these animals under the influence of testosterone. This may perhaps explain the long familiar fact, discovered in mice, that after adrenalectomy the thymus in males increases in size at all ages, but in females, only in young animals [6]. In AE females with a lower MI_{oc} than MO animals growth of the cortex was considerable. This indicates increased immigration of precursors into the thymus, also on account of adrenalectomy itself. In MO rats sex difference with respect to MI_{oc} were opposite to those in AE animals: lower in females than in males ($P < 0.05$), although, however, this did not lead to any difference in weight of the thymus. The relationships between the four processes controlling the size of the thymus — immigration of precursors, their proliferative differentiation, emigration of thymocytes, and their death in the organ itself — thus differ in females and males.

The question of which thymoctyes are responsible for growth of the thymus after adrenalectomy is very important. The properties of this subpopulation can be judged indirectly from the weight of the spleen and lymph nodes. For instance, in AE animals the weight of the mediastinal lymph nodes was 56.3 ± 4.3 mg compared with 29.6 ± 1.1 mg in MO rats ($P < 0.001$), in the absence of sex differences which, however, were present in the enlarged spleens (746.6 ±

25.9 mg in females compared with 629.5 ± 22.9 mg in males, $P < 0.001$). No sex differences in the weight of the spleen were found in MO rats, but correlation was found between the size of the spleen and the thymus ($r = +0.377 \pm 0.153$; $P < 0.05$), whereas no correlation was found between the weight of the lymph nodes and of the thymus in either MO or AE rats. Sex differences in morphometric parameters of the thymus and spleen in AE animals, coupled with their absence in the lymph nodes and in MO rats, indicate that mainly the subpopulation of thymocytes with ecotaxis for the spleen increased after adrenalectomy. Judging from the immunologic consequences of adrenalectomy and thymectomy in adult mice [2], this subpopulation belongs to the T_1 lymphocytes — less mature, short-living T lymphocytes which recirculate to only a slight degree and are most sensitive to HC [7].

In the experimental series (in AE males with hypovitaminosis) inhibition of liver transketolase activity on the 3rd day of hypovitaminosis amounted to 40.2% ($P < 0.01$) in rats killed without HC (group 1) and 38.8% ($P < 0.001$) in animals receiving HC before sacrifice (group 2). No significant changes in weight of the thymus were found in group 1 (Table 2). Although the number of mitoses in the cortex on the 1st and 2nd days of hypovitaminosis was reduced, the number of thymocytes during this period was greater than in the control; conversely, during recovery of the level of mitoses (3rd and 4th days of hypovitaminosis) the number of thymocytes fell to the control level. Unlike the data for hypovitaminosis in rats not undergoing operations (NO rats) [1], a decrease neither in size of the thymus nor in the number of thymocytes in the organ could be observed in AE animals. Nevertheless the weakened mitostatic effect of OT was still present, but its peak was shifted to the first day of hypovitaminosis (inhibition of mitosis in AE rats on the 1st, 2nd, and 3rd days of hypovitaminosis was 15, 10, and 4%, compared with 25, 48, and 28% respectively in NO rats). The increase in the number of cells in the cortex (1st and 2nd days of hypovitaminosis), however, despite inhibition of mitosis, was probably due to delay in thymocyte migration.

In group 2 (Table 3) the weight of the thymus decreased. The number of mitoses was 28.9% lower ($P < 0.001$) on the first day of hypovitaminosis and 36.7% lower ($P < 0.01$) than in group 1. However, on the 4th day of hypovitaminosis mitotic activity exceeded the control and was 14.7% higher ($P < 0.05$) than in group 1. This jump was undoubtedly the result of exposure in HC for 2 h; a similar increase in the intensity of proliferation was observed during the same 4th day of hypovitaminosis in NO rats. However, whereas the more intensive injury to the cells by the hormone, induced by OT in NO rats amounted to 96, 226, and 128% on the 2nd, 3rd, and 4th days of hypovitaminosis respectively, in AE animals it was only 24, 55, and 35%. However, even this reduced disintegration led to an extremely acute decrease in weight of the thymus (before any collapse of the cortex, and without any decrease in the corticomedullary ratio), which did not take place in AE rats with hypovitaminosis untreated with HC (Table 2 and 3). In the rats of group 2 HC also caused a decrease in weight of the spleen (633.4 ± 29.0 mg compared with 721.8 ± 30.4 mg in group 1, $P < 0.05$), although there was no difference in the control.

A vitaminosis B_1 induced by OT in AE rats is thus not accompanied by acute involution of the thymus, due not only to the hypocorticism, but also to the slight decrease in tolerance of the thymoctyes to HC. Metabolic disturbances forming the basis of the thymotrophic effect are evidently created by OT with the participation of corticosteroids. The remarkably low degree of injury to the thymoctyes in group 2 is evidence that under conditions of hypocorticism OT does not cause any gross change in tolerance of the cells to exogenous HC. If OT acted by itself, without the participation of endogenous glucocorticoids, the same widespread pycnosis and karyorrhexis in the thymus would arise as in NO rats. Even greater injury would be expected because of accumulation of cells particularly sensitive to HC in the enlarged thymus. The abolition of the thymolytic effect of OT (group 1) was thus probably associated not so much with the hypocorticism itself as with inhibition of the metabolic potential of OT against the background of hypocorticism, mainly relative to the liver, through which HC exerts its action on the thymus [4].

An unambigous indication of a corticosteroid mechanism is the similarity of the time course and parameters of OT-induced involution of the thymus [1] with those occurring when the thymus is injured in rats by HC alone [3]. Since the thymolytic effect of HC in OT-induced hypovitaminosis B_1 in NO rats is intensified by more than 200%, further study of OT as a synergist of HC is called for with a view to alleviating the side effects of immunodepressive hormone therapy.

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ANTIAGGREGATING ACTIVITY OF SOME SYNTHETIC PROSTAGLANDINS

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Prostaglandins (PG) play an important role in the regulation of the circulation. Some of them are able to inhibit platelet aggregation and to cause dissociation of aggregated platelets, thereby regulating thrombus formation and the lumen of blood vessels. The most effective inhibitors of platelet aggregation are prostacycline (PGI_2) and prostaglandin E_1 (PGE_1) [12]. Natural PG are very labile (especially PGI_2) and they quickly lose their activity *in vivo*. Since some synthetic analogs of PG are more stable and have a prolonged action, the preparation of these compounds and the study of their biological properties have recently acquired great importance.

The 11-deoxyprostaglandins are among the simplest and most accessible analogs of natural PG. Absence of the 11-hydroxyl group in these compounds usually causes no change in the direction of their biological action [4]. For instance, the 11-deoxyprostaglandins largely preserve their ability to stimulate contraction of smooth muscles [6], and the antisecretory [10] and bronchodilator [7] activity of the PG. However, there are no data in the literature on their effect on platelet aggregation. In addition, in the case of several PG cases are known when the two opposite poles of the same PG are biologically active [11]. Since only the antiaggregating activity of PG of the natural stereochemical series is known, it was interesting to obtain data on the activity of their analogs not found in nature. In the investigation described below the antiaggregating activity of certain synthetic 11-deoxyprostaglandins and of racemic PGE_1 was studied.

EXPERIMENTAL METHOD

Samples of racemic PG synthesized previously at the Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Sciences of the USSR — rac-11-deoxy PGE_1 (m.p. 83-85°C) [1], rac-15-methyl-11-deoxy-PGE_1 (m.p. 97.5-98.5°C), rac-PGE_1 (m.p. 110-111°C) [2], rac-11-deoxy-PGE_2 (m.p. 48.5-50°C) [3], the sodium salt of rac-11-deoxy-PGI_2 (aqueous solution; obtained from rac-11-deoxy-$PGE_{2\alpha}$ [3] by analogy with [9]), and optically active PG — the sodium salt of nat-PGI_2 (from Upjohn, USA), nat-PGE_1 (Institute of Chemistry, Academy of Sciences of the Estonian SSR, Tallin), and nat-PGE_2 (from Upjohn, USA) were investigated. Immediately before the work the substances were dissolved in 50 mM of Tris-buffer (pH 10.0) and several dilutions of these solutions were prepared. Blood was taken from the left ventrical of a male rabbit under pentobarbital anesthesia. Sodium citrate (one part of a 3.8% solution of sodium citrate to nine parts of blood) was used as anticoagulant. To obtain platelet-enriched plasma blood was centrifuged at 160g for 10 min at room temperature. Platelet-deprived plasma was obtained by centrifugation of the lower layer at 4000g for 6 min. Platelets were counted under the microscope in a Goryaev's chamber. Platelet-enriched plasma was diluted with platelet-deprived plasma to a concentration of 600,000 platelets/μl. Platelet

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