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EFFECT OF THYMOSIN (FRACTION 5) ON TESTICULAR ENDOCRINE FUNCTION IN MICE

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Much attention has been paid in recent years to the study of the connection between the immune and endocrine systems of the body. The idea has developed that there exists a hypothalamus–pituitary–gonad–thymus axis, responsible for the central modulation of many immunological functions [4]. Direct participation of sex steroids in reactions of cellular and humoral immunity is now known to take place; this problem has been reviewed by Grossman [5]. However, the effect of the immune system on the gonads and, in particular, on the testes, has not yet been adequately studied. Among the principal regulatory factors capable of realizing this effect may be included the polypeptide hormones of the thymus. The active substances of the thymus (thymosin, T-activin) are widely used in clinical practice to stimulate immunological functions, but data on their action on other vitally important systems, including the endocrine system, are extremely scanty. The aim of this investigation was to study the effect of thymosin (fraction 5), a thymus hormone, on the hormonal function of the testes.

EXPERIMENTAL METHOD

Male BALB/c mice aged 2 months, kept under standard animal house conditions, were used. Thymosin (fraction 5) was obtained at the Research Institute of Technology of Blood Substitutes and Hormonal Preparations, Moscow, by Goldstein's method [3] and was given in doses of 0.1 and 1 μg; control animals received bovine serum albumin (BSA) in equimolar doses. Indomethacin ("Serva"), an inhibitor of prostaglandin synthesis, was used in a dose of 100 μg per mouse. All preparations were injected intraperitoneally in 0.1 ml of sterile physiological saline. The animals were killed by decapitation. The blood was centrifuged to obtain plasma. The gonads were removed and placed in bicarbonate buffer (Krebs–Ringer solution saturated with carbogen) and incubated for 3 h at 37°C. Testicular hormonal function was characterized by the plasma testosterone level and by the specific production of this hormone by the testes in vitro, determined by radioimmunoassay using standard kits for testosterone determination (Minsk). The results were subjected to statistical analysis by monofactorial dispersion analysis, using Fisher's test.

EXPERIMENTAL RESULTS

A single injection of thymosin into male mice led to a fall in the peripheral blood plasma testosterone level of the experimental animals compared with the controls. A reduced

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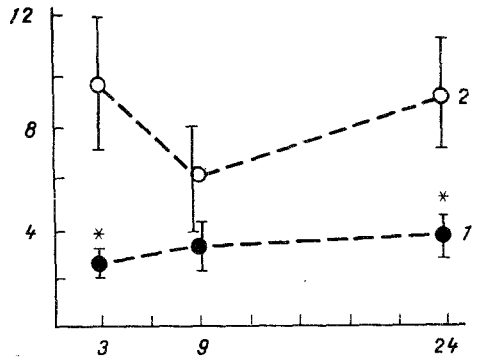

Fig. 1

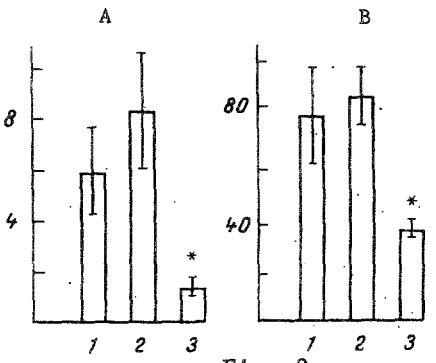

Fig. 2

Fig. 1. Dependence of blood plasma testosterone concentration of mice on time after injection of thymosin and BSA. Ordinate, testosterone concentration (in ng/ml); abscissa, time after injection (in h). Here and in Fig. 2, *p<0.05 compared with control.

Fig. 2. Endocrine function of mouse gonads 24 h after injection of various doses of thymosin. Ordinate: a) blood testosterone concentration (in ng/ml), b) specific testosterone production by gonads in vitro (in ng/100 mg/h). 1) BSA, 2 and 3) thymosin in doses of 0.1 and 1 μg per mouse respectively.

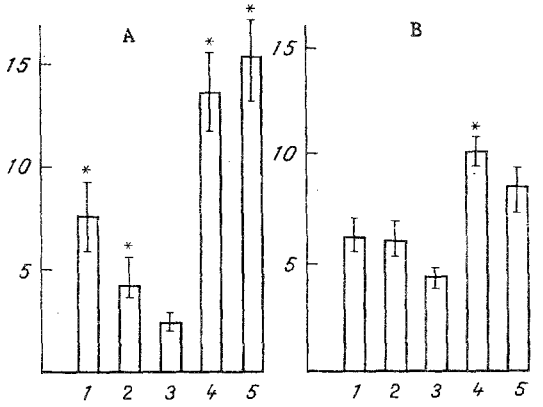

Fig. 3. Hormonal activity of testes 24 h after injection of thymosin preceded by indomethacin. Ordinate, testosterone concentration (in ng/ml). 1) Physiological saline, 2) BSA, 3) thymosin (1 μg per mouse), 4) indomethacin (100 μg per mouse) + thymosin (1 μg per mouse), 5) indomethacin (100 μg per mouse). *p < 0.05 compared with thymosin.

testosterone concentration was observed in the animals throughout the period of investigation (24 h) (Fig. 1). The most significant differences were found 3 and 24 h after injection. This parameter in the experimental animals after 9 h was the same as in the controls because of a fall in the testosterone level of the latter at this period. The existence of a circadian rhythm of testosterone in laboratory rats with a minimum at 9 p.m. has been reported in the literature [9]. Since the present experiment began at 10 a.m. and since 9 h corresponds conventionally to 7 p.m., the testicular hormonal function was depressed at this time, and this evidently is the reason why no differences were found between the experimental and control animals.

A more detailed study of the effect of thymosin on testicular endocrine function was undertaken on animals receiving the preparation 24 h before sacrifice (Fig. 2). The results showed that injection of thymosin in a dose of 0.1 μg per mouse had no effect on the plasma testosterone level, whereas a dose of 1 μg depressed this function sharply (Fig. 2a). The fall in the concentration of the sex steroid was connected with a decrease in production of this hormone by the testes (Fig. 2b). In this case only a large dose of thymosin was effective. One of the first pieces of evidence that thymus hormones can affect the gonads was ob-

tained by Rebar and co-workers [10]. In their experiments perfusion of the hypothalamus–pituitary complex of female rats in vitro with thymosin (fraction 5) led to considerable secretion of gonadotrophin releasing factor from the mediobasal hypothalamus, followed by release of pituitary luteinizing hormone (LH). Our own data showing inhibition of testicular function in response to injection of thymosin do not agree with those given above. This may be due either to the fact that the experiments were carried out on males and not on females, or that the action of thymosin in vitro is not the same as its action in vivo. Hall et al. [7] showed that intraventricular injection of thymosin into rats, on the other hand, depresses the circulating LH level. The experiments of these workers are evidence of the central character of the influence of thymosin on gonad function. It can be tentatively suggested that the fall of the circulating LH level in response to injection of thymosin also led to inhibition of testicular activity in our own experiments, but the possibility of a direct influence of this hormone on the gonads cannot be ruled out.

In 1983, Garaci and co-workers [2] obtained evidence of the action of thymosin (fraction 5) on lymphocytes through stimulation of prostaglandin production. On the other hand, the mediator role of these compounds in the regulation of hormonal activity of the gonads has already been studied. For instance, prostaglandins of the A, E, and F groups, injected subcutaneously, depress the testosterone level and do not affect the plasma LH concentration in mice and rats [11]. A fall in the blood testosterone level was connected with the inhibitory action of these compounds on steroid production in the testes [1]. Prostaglandins of all known groups inhibit testosterone production by the testes, induced by LH, although they are not directly included in the mechanism of action of this gonadotrophin [6]. These facts suggest that the effect of thymosin on the gonads in the present experiments may be mediated by prostaglandins. To test this hypothesis, indomethacin, an inhibitor of prostaglandin synthesis, was injected into mice 30 min before receiving an injection of thymosin. Injection of indomethacin completely abolished the inhibitory action of thymosin on the gonads: the blood testosterone level not only did not fall in the presence of indomethacin, but it actually rose; this latter effect, moreover, is the result of intensification of steroid production by the testes (Fig. 3). Indomethacin itself also activates the endocrine function of the testes, and the level of function of the gonads was approximately the same as after injection of indomethacin and thymosin. This indicates that in animals receiving the thymus hormone after indomethacin, what occurred was not summation of the effects of indomethacin (activation of the gonads) and thymosin (inhibition of steroid production), but abolition of the action of the latter on the testes. Our own results suggest that prostaglandins are intermediaries in the mechanism of action of thymosin on the gonads, an action which probably has a direct influence on the testes. Another possibility is that if the thymus hormone is injected in vivo, summation of the central and peripheral effects of the hormone takes place, but further experiments are needed in order to distinguish between these effects.

It can thus be concluded that the thymus hormone thymosin (fraction 5), which promotes differentiation and maturation of T lymphocytes and plays an important role in the mechanism of immunological reactions, participates at the same time in the hormonal regulation of testicular function. This effect is evidently a reflection of the existence of negative feedback between the thymus and gonads, taking place in the hypothalamus–pituitary–gonad–thymus axis. This connection seems all the more likely because the genetic structures controlling blood antigen levels and genes of the immune response are spatially linked in mice in the major histocompatibiliby locus [8].

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