INHIBIN ACTIVITY IN MALE RAT REPRODUCTIVE ORGANS DURING TREATMENT WITH DIHYDROTESTOSTERONE

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Several organs have been shown to contain inhibin, a protein which inhibits biosynthesis and secretion of follicle-stimulating hormone (FSH) and secretion of luteinizing hormone [7, 13]. Since FSH is necessary for maintaining certain stages of spermatogenesis, inhibition of its production is accompanied ultimately by sterility. This state of affairs has attracted the attention of research workers to inhibin as a substance for the controlled regulation of fertility. Moreover, the development of some hormone-dependent tumors is accompanied by raised FSH levels.

Inhibin acts at the hypothalamic, pituitary, and gonadal levels [2]. The question of relations between inhibin and the steroids produced in the testis accordingly arises. We know that testosterone raises the inhibin level in the seminal plasma and peripheral blood [6], and in cultures of Sertoli cells [9]. Relations between inhibin and dihydrotestosterone (DHT), one of the most active metabolites of testosterone, increased production of which is linked with neoplastic degeneration of the prostate, have virtually not been studied. The few data available are contradictory [9, 14].

Considering the important role of DHT at the organization stage in the formation of the testis and prostate [4], in adults in the performance of the copulatory act, and also in the pathogenesis of neoplasms, the writer decided to study the effect of DHT on the inhibin level in the male reproductive system.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature Wistar rats. Control animals received a single dose of 0.2 ml of peach oil, and the experimental animals received 25 μg of DHT in the same volume of oil. The animals were killed 24 h after the injection and blood was taken for hormone assay. The testes and prostate were weighed, examined macroscopically, and homogenized for subsequent determination of inhibin, by biotesting *in vivo* [3] on sexually immature female mice. The method is based on removal of steroid hormones from the test objects (testes, prostate) and injection of the protein extract into mice after a preliminary injection of chorionic gonadotrophin (CG). The combined weight of the uterus and ovaries of the recipient 15 h after the last injection of CG was used as the indicator of its activity.

Hormones were determined in the peripheral blood of the donor rats by radioimmunoassay using standard commercial kits from CIS (France).

EXPERIMENTAL RESULTS

The weight of the ovaries was increased in the rats receiving DHT, and accordingly its ratio to body weight was 14.9 ± 0.5 mg/g compared with 10.0 ± 0.4 mg/g in the control ($P < 0.001$). Although the weight of the prostate gland was increased under these conditions (from 2.09 ± 0.39 to 3.07 ± 0.55 mg/g) the increase was not significant.

In rats receiving DHT the blood level of 17-α-hydroxyprogesterone, the precursor of testosterone on the Δ^{-4} pathway of biosynthesis, was raised 24 h after the injection (from 0.53 ± 0.16 to 1.25 ± 0.55 nmole/liter) and a tendency was observed for the testosterone concentration to rise (8.48 ± 1.00 nmole/liter compared with 5.33 ± 1.25 nmole/liter in the control),

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TABLE 1. Inhibin Activity in Male Rats (M ± m)

Group of animals	Experimental conditions	Substance injected into recipient mice	Body weight, g	Weight of uterus + ovaries, mg	Ratio of combined weight of uterus and ovaries to body weight	Inhibin activity, %	P
2	Control (n =5)	Oily solution	9,0±0,5	14,0±0,3	1,5±0,03	—	—
2	Control (n = 5)	CG	11,2±0,2	30,0±0,9	2,7±0,05	—	$P_{2-3}<0,001$
3	Control (n = 15)	Extractof testes	9,8±0,5	19,5±0,9	2,1±0,1	22,85	
4	Control (n = 15)	Extract of prostate	11,7±0,2	16,4±0,4	1,4±0,06	47,20	$P_{2-4}<0,001$
5	DHT (n = 26)	Extract of testes	10,8±0,3	17,3±0,6	1,7±0,03	37,83	$P_{3-4}<0,001$ $P_{5-3}<0,001$
6	DHT (n = 16)	Extract of prostate	10,6±0,4	15,6±0,8	1,4±0,07	45,70	$P_{5-2}<0,001$ $P_{6-4}>0,1$ $P_{6-2}<0,001$

and on the whole this evidently reflected stimulation of biosynthetic activity of the androgen-producing cells of the testis. The recipient mice were divided into six groups (Table 1), in which inhibin activity from various test objects was compared. After injection of CG (group 2) a significant increase was observed in the ratio of the combined weight of the uterus and ovaries to the body weight compared with this ratio in the control (group 1). Thus injection of CG stimulated growth of the ovaries and uterus of sexually immature mice. Subsequent calculations of inhibin activity involved comparison with the value obtained after injection of CG.

In mice receiving extract of the testes of the control rats (group 3) the increase in combined weight of the uterus and ovaries was depressed by CG, and accordingly its ratio to body weight fell, as a result of the effect of inhibin on gonadotrophic hormones. An even more marked fall was observed after injection of prostatic extract (group 4). Preliminary injection of DHT into the rats followed by tests on recipient mice (group 5) revealed higher inhibin activity in the testes, as was shown by a decrease in the ratio of the combined weight of the uterus and ovaries to body weight. Inhibin activity in the prostate (group 6) was unaffected by analogous hormonal treatment.

The results thus point to the presence of inhibin not only in the testis, but also in the prostate. The fact that inhibin receptors exist in the prostate was demonstrated only recently [10], but the reactivity of this organ has not hitherto been investigated under conditions of preliminary hormonal treatment. As the present experiments showed, the inhibin level in the prostate is quite high — higher than in the ovary. The biological significance of this phenomenon is evidently connected with the role of the prostate in maintaining viability of the spermatozoa, and it confirms the presence of a direct link between the prostate and the pituitary. There is reason to suppose that the inhibin contained in the testes differs in molecular weight and, correspondingly, in its biological activity from the inhibin present in the prostate.

The inhibin level in the testes rose after injection of DHT. This increase, although significant, can be classed [1] as relatively small (by 15%), and less than that observed after injection of testosterone [6, 9]. In the writer's opinion, the increased inhibin activity in the testis after preliminary injection of DHT was largely due to the corresponding increase in the testosterone concentration which was discovered. This conclusion is supported by observations [11] which showed no change in the gonadotrophic hormone levels after injection of small doses of DHT, and in particular, the dose which was used in the present experiments.

DHT thus causes a very small increase in inhibin activity in the testis. Compounds which are derivatives of DHT (Stanolon, Neodrol, etc.) must therefore inhibit the follicle-stimulating function of the pituitary by a lesser degree than those obtained on the basis of testosterone, so that they have the advantage when used in andrologic practice.

The absence of differences in the inhibin activity of the prostate following injection of DHT can evidently be explained on the grounds that this gland, even before administration of exogenous DHT, contains a higher concentration of it than the other glands and organs of the nongonadal system.

The further study of relations between androgens and inhibin at peripheral and central levels will facilitate fertility control.

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USE OF A DIPLOID CELL LINE TO DETECT TOXIC COMPONENTS IN MEDICAL IMMUNOBIOLOGICAL PREPARATIONS

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Medical immunobiological preparations (MIBP) is the term used to describe vaccines and sera used for prevention and treatment of infectious diseases [11]. Control methods included in the requirements for biological preparations, although approved by an expert committee of the World Health Organization, are not laid down by law and require continuous updating [10].

The need to revise the safety regulations for MIBP has increased with the publication of new data on the properties of chemicals [9] present in the composition of MIBP and also by the increase in the number of allergic and autoimmune diseases [1, 2, 9].

WHO experts recommend the extensive use of cell cultures as a substitute for experiments on animals, not only on humanitarian and economic grounds, but also to obtain more objective and informative data on the quality of a preparation which may be administered to man [3, 5]. This is particularly important when studying the effects of small doses of substances, whose action on cells and their structural components may not be manifested immediately after administration of the preparation to a whole organism, but not until years later [5, 7, 8].

In the USSR [11] parameters of acute toxicity on animals and production of allergic reactions in man are used to characterize the safety of MIBP. This system for safety control does not give a complete evaluation of the quality of the MIBP.

The anti-Pertussis-Diphtheria-Tetanus vaccine (APDTV), which we used as the model for our original investigations [6, 12], is considered to be more prone to produce reactions than any other biological prophylactic agent used at the present time [2, 13]. Safety of APDTV is determined by giving a single injection of the ready prepared form to guinea pigs in a dose equal to four times the human dose [11]. WHO experts state that methods assessing the quality

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