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ROLE OF THE HYPOTHALAMUS IN THE RESPONSE OF THE RAT PITUITARY AND TESTIS TO INJECTION OF THE ANTIANDROGEN 4-NITRO-3-TRIFLUOROMETHYLISOBUTYRANILIDE

A. G. Reznikov and N. N. Volkova

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The effect of the antiandrogen 4-nitro-3-trifluoromethylisobutyranilide (flutamide, NFBA) on the plasma FSH, LH, and testosterone levels and on activity of steroid-Δ^5-3β-ol dehydrogenase activity in the testes was studied in intact male rats and rats with destruction of the median eminence of the hypothalamus. In rats undergoing a mock operation and with the hypothalamus intact, NFBA increased the LH, FSH, and testosterone levels by five, two, and four times respectively, and enzyme activity by three to four times. After destruction of the median eminence the secretion of FSH was unchanged by NFBA, and the LH and testosterone levels and enzyme activity were increased by only 1.5-2 times, i.e., much less than in animals undergoing the mock operation. The results of these experiments indicate a leading role of the hypothalamus in the response of the pituitary and testis to the antiandrogen.

KEY WORDS: antiandrogens; median eminence of hypothalamus; lutinizing hormone (LH); follicle-stimulating hormone (FSH).

During a study of the effect of the peripheral nonsteroid antiandrogen 4-nitro-3-trifluoromethylisobutyranilide (NFBA, niftolid, flutamide, Sch-13521) on the reproductive system of male rats a phenomenon of activation of the pituitary—testicular complex was observed, manifested by increased secretion of LH and testosterone [2]. The writers postulated that the primary link in the chain of these changes was the blocking action of antiandrogen on feedback receptors in the hypothalamic centers regulating gonadotropin secretion [2].

The obtaining of experimental data confirming this hypothesis is of great importance for establishment of a theoretical basis for the use of NFBA in order to study the functional reserves of the hypothalamic—pituitary—testicular system [1]. The object of the present investigation was to compare the response of the pituitary and testis of rats, both intact and with destruction of the median eminence (ME) of the hypothalamus, to injection of NFBA.

METHODS

Experiments were carried out on male Wistar rats weighing 200-250 g. Electrolytic destruction of ME of the hypothalamus was carried out on the animals by application of an anodal

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TABLE 1. Effect of NFBA on Plasma Gonadotropin and Testosterone Levels and SD* Activity in Testes of Rats with Intact and Destroyed ME of Hypothalamus (M ± m)

Index studied	Destruction of ME			Mock operation		
	group 1 (control, oil, n = 8)	group 2 (experiment, NFBA, n = 7)	P	group 3 (control, oil, n = 7	group 4 (experiment, NFBA, n = 8	P
FSH level, ng/ml	367±19,42	383±21,71	>0,5	542±60,42	983±80,47	<0,01
LH level, ng/ml	45±5,06	98±10,22	<0,001	73±7,73	372±22,16	<0,001
Testosterone level, ng/100 ml	278±24,79	470±45,04	<0,01	325±64,25	1286±146,62	<0,001
SD activity per testis	262±68,81	444±78,96	>0,1	239±48,82	764±93,26	<0,01
SD activity per gram tissue	120±38,94	287±42,33	<0,05	149±27,79	564±107,42	<0,001
SD activity per milligram protein	2,94±0,50	4,35±1,19	>0,2	1,99±0,44	8,91±1,19	<0,001

*Expressed in micrograms Δ^4-3-ketosteroids formed in homogenate of testis during incubation for 90 min.

current (1-2 mA) for 30-40 sec by means of nichrome electrodes. The electrodes were inserted by means of a stereotaxic apparatus at coordinates Ant 5.4, Lat 0.4, and Vert 4.0 [5]. One week after the operation some of the animals (group 2) were treated with 1.5% NFBA solution in peach oil in a dose of 25 mg/kg body weight daily for 10 days by means of a metal gastric tube. The other rats, with destruction of ME (group 1), received peach oil in corresponding doses. Animals undergoing a mock operation also were divided into two groups, one of which (group 4) received the NFBA solution in the above doses, the other (group 3) received peach oil. The animals were decapitated 24 h after the last dose of the antiandrogen. Activity of steroid-Δ^5-3β-ol dehydrogenase (SD) in fresh testicular tissue was determined by a modified spectrophotometric method, using dehydroepiandrosterone as the substrate [3]. The appropriate parts of the brain were fixed in Bouin's solution and the region of destruction was verified by examination of paraffin sections.

Plasma from the blood taken at decapitation was stored at −70°C until analysis. The concentration of FSH and LH in the plasma was determined by a radioimmunological method, using double antibodies. Rat LH-RP-1, and rat FSH-RP-1 from the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD), USA, were used as standard preparations. Iodination (with ^{125}I, from Radiochemical Centre, Amersham, England) was carried out by the chloramine method (with chloramine T, from Koch-Light Laboratories Ltd., England), followed by purification on a column with Sephadex G-75; NIAMDD-A Rat LH S-4 and A Rat FSH S-8 rabbit antisera served as the binding system. To precipitate the hormone—antibody complex donkey serum against rabbit globulins with a working dilution of 1 : 16 (from the N. F. Gamaleya Institute of Microbiology and Epidemiology, Academy of Medical Sciences of the USSR) was used. The radioactivity of the samples was counted in a NZ-310 gamma-counter (Gamma, Hungary). The plasma testosterone concentration was determined by a radioimmunological method, using "Testok" kits (from CEA-IRE-Sorin, France) and an Isocap-300 liquid scintillation counter (from Nuclear Chicago, USA). The results of the investigations on rats with destruction of ME were compared with the corresponding values obtained in animals undergoing the mock operation.

RESULTS

The experimental results are given in Table 1. Analysis of the consequences of electrolytic destruction of ME shows that this operation reduced the basal concentration of gonadotropins by one-third without any appreciable effect on the plasma testosterone concentration or SD activity in the testis. The degree of lowering of the basal gonadotropin level corresponded to data in the literature [4].

Injection of NFBA into rats undergoing the mock operation, i.e., animals with an intact hypothalamus (group 4), was accompanied by a fivefold increase in plasma LH concentration compared with the control (group 3). In rats with destruction of ME the hormone level was increased only twofold under the influence of the compound (groups 1 and 2). Injection of the antiandrogen combined with destruction of ME had no effect in general on the level of FSH secretion (groups 1 and 2), whereas in rats with an intact hypothalamus the corresponding procedure led to a twofold increase in the concentration of this hormone in the blood plasma (groups 3 and 4).

Gonadotropins are known to activate androgen biosynthesis in the testis and, in particular, to increase the rate of conversion of Δ^5 steroids into their Δ^4 derivatives. In rats with an intact ME stimulation of the gonads by endogenous gonadotropins led to a three- to fourfold increase in enzyme activity expressed per whole organ, per gram tissue, and per milligram protein. In the rats of group 2 (destruction of ME) there was only a tendency for the total and specific (in mg protein) enzyme activity to rise, but not by a significant level. SD activity increased significantly when calculated per gram tissue, but it was considerably lower than in animals with an intact ME.

The results of radioimmunologic determination of the plasma testosterone concentration were in good agreement with the data illustrating changes in enzyme activity. In response to administration of NFBA to rats undergoing the mock operation the testosterone concentration showed a three- to fourfold increase compared with the control. In animals with destruction of ME the hormone level was increased by only 1.5-2 times under the influence of the antiandrogen.

These experiments thus showed for the first time the key role of the hypothalamus in the response of endocrine organs of the male reproductive system to the action of this nonsteroid antiandrogen. Partial preservation of the stimulating effect of NFBA on LH and androgen secretion in rats with destruction of ME is not, in our opinion, the result of the direct action of the compound on the pituitary and gonads, although this is a matter for special study. A much more likely explanation is that the hypothalamus continues to control LH secretion to some degrees. An alternative explanation could be that when the effects of the hypothalamus are completely blocked, the feedback mechanism is realized at the pituitary level. However, there are sufficiently convincing facts which contradict this hypothesis [6-8].

It can be concluded from the data as a whole that NFBA is able to interrupt feedback between the testis and the hypothalamic—pituitary complex, as a result of which the secretion of pituitary gonadotropic hormones is disinhibited. In this respect NFBA is the most active antiandrogen so far known, and its unique properties can evidently be used to test the functional state of the hypothalamic centers regulating male reproductive function.

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