ROLE OF SEXUAL DIMORPHISM IN THE PHARMACOLOGIC EFFECT OF ETHIMIZOLE

A. F. Leshchinskii

UDC 615.214.31.015.4:[612.43].45.018+612.015.1]-055.1].2

KEY WORDS: ethimizole; sexual dimorphism; microsomal, creatine phosphokinase, and endocrine systems

Sex differences in metabolic processes in man and animals affect the functional state of individual organs and systems and may also affect the action of drugs, especially drugs such as ethimizole, which has a broad spectrum of action on the body [1, 3, 4].

With the assistance of T. A. Zolotareva, Z. I. Zuza, V. L. Konovalenko, and E. V. Chernetskaya, our aim was to study the effect of sexual dimorphism on the pharmacologic action of ethimizole on the pituitary–adrenal, pituitary–thyroid, and myocardial creatine phosphokinase and hepatic microsomal enzyme systems.

EXPERIMENTAL METHOD

Experiments were carried out on adult male and female Wistar rats weighing 180-220 g. The plasma 11-hydroxycorticosteroid (11-OHCS) was studied by the method of Pankov and Usvatova, and the corticosterone level by competitive protein binding of tritium-labeled and native hormone, extracted with methylene chloride (Volchek and Smirnov). Consumption of cholesterol (by the Liebermann–Burchard method) and ascorbic acid (using Thielmann's stain), utilized in steroid hormone synthesis, in the adrenals and the weight of the adrenals and thymus also were determined. Plasma ACTH was determined by radioimmunoassay using the ACTH K-IPR test system (CEA-IRE-Sorin, France), TSH, thyroxine (T_4), and tri-iodothyronine (T_3) were determined by the kits Ria-mat TSH (West Germany) RIO-T_4-PG and RIO-T_3-PG (USSR), and SPAC-T_3 and SPAC-T_4 (West Germany) and testosterone and estradiol with the aid of antiserum obtained from the Institute of Experimental Pharmacology and Therapy, Academy of Medical Sciences of the USSR (Sukhumi). The creatine phosphate concentration in the myocardium was studied by Alekseeva's method and creatine phosphokinase activity (EC 2.7.3.2) by Maksimova's method. Activity of microsomal enzyme systems in the soluble fraction of the liver was assessed by the velocity of N-demethylation of aminopyrine (by the method of McMahon and Cochin), and indirectly by the velocity of biotransformation of hexobarbital after injection intraperitoneally in a dose of 100 mg/kg (the length of time spent by the animals in the side position, and determination of the blood level of its activity by the method of Brodie et al., in the modification of Zolotareva and Vovchuk). Ethimizole was injected intraperitoneally in the form of a 1.5% solution and in a dose of 10 mg/kg daily for ten days. The parameters studied were determined before administration of ethimizole began and 1 and 10 days after its ending.

EXPERIMENTAL RESULTS

Comparison of the test parameters in animals of different sexes (Table 1) shows that blood levels of ACTH and corticosterone and the relative weight of the adrenals were statistically significantly higher in female than in male rats. The TSH level, however, was higher in the latter, and the T_4 and T_3 (especially T_3) levels in the serum and liver were significantly lower in females. In male rats the velocity of N-demethylation of aminopyrine was 1.5 times greater, and inactivation of hexobarbital was 2.5 times faster (concentrations of the drug were lower and the duration of sleep shorter) than in females. No significant differences were found in values of parameters of the creatine phosphokinase system in the myocardium of rats of different sexes.

Laboratory of Experimental Therapy, Odessa Research Institute of Balneology, Ministry of Health of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 91-93, July, 1989. Original article submitted October 10, 1988.

 0007-4888/89/0007-1012$12.50

TABLE 1. Parameters of State of Systems Studied in Intact Rats of Different Sexes (M ± m)

System and parameter	Males	Females	p
Pituitary–adrenal system			
Plasma ACTH, pmoles/liter	43.5 ± 3.9	59.7 ± 4.2	<0.01
Plasma corticosterone, nmoles/liter	191 ± 25	502 ± 45	<0.001
Corticosterone in liver, nmoles/kg	39.4 ± 3.5	38.9 ± 2.9	>0.05
Plasma 11-OHCS, nmoles/liter	482 ± 35	535 ± 17	>0.1
Concentrations in adrenals:			
cholesterol, mmoles/kg	91 ± 5.1	88.1 ± 3.0	>0.5
ascorbic acid, μmoles/kg	27.2 ± 1.1	25.5 ± 1.1	>0.2
Relative weight of adrenals, 10^{-3} g/kg body weight	17.9 ± 0.6	20.7 ± 1.0	<0.05
Weight of thymus, mg	363 ± 51	396 ± 36	>0.5
Pituitary–thyroid system			
Serum TSH, mIU/liter	2.58 ± 0.16	2.04 ± 0.10	<0.05
Serum T_4, nmoles/liter	68.3 ± 4.6	78 ± 6.4	>0.2
Serum T_3, nmoles/liter	1.20 ± 0.05	1.61 ± 0.10	<0.001
T_4 in liver, nmoles/kg	15.4 ± 1.4	58 ± 3.7	<0.001
T_3 in liver, nmoles/kg	5.34 ± 0.59	7.79 ± 0.37	<0.01
Microsomal system of liver			
Velocity of N-demethylation of aminopyridine, nmoles·min^{-1}·g^{-1}	33 ± 1.7	22 ± 1.2	<0.001
Plasma hexobarbital concentration, nmoles/liter	862 ± 134	1155 ± 34	<0.05
Duration of hexobarbital sleep, min	26 ± 1.4	65.0 ± 4.4	<0.001
Creatine phosphokinase system of myocardium			
Creatine phosphate, mmoles/kg	3.75 ± 0.12	3.99 ± 0.28	>0.2
Creatine phosphokinase activity, mmoles·kg^{-1}·sec^{-1}	34.8 ± 0.8	34.9 ± 0.8	>0.8

Consequently, sexual dimorphism affected mainly the functioning of the microsomal enzyme system of the liver in the direction of considerably limiting its activity in female rats, which was associated with higher activity of the pituitary–adrenal and pituitary–thyroid systems in these animals.

This fact probably determines differences in the response of the systems studied to ethimizole. It will be clear from Fig. 1 that activity of the microsomal enzyme was considerably enhanced by the action of ethimizole in female rats. The velocity of N-demethylation of aminopyrine was increased by 1.5 times ($p < 0.05$) and the concentration of hexobarbital injected was reduced and the duration of sleep induced by it was shortened (by 3.7 times, $p < 0.001$), i.e., biotransformation of hexobarbital was accelerated in the microsomal system, with associated loss of its pharmacologic activity. Induction of the microsomal enzymes in the liver also has been observed under the influence of caffeine [11], recalling that ethimizole was first produced by imitating the chemical structure of caffeine. In males, however, under the influence of ethimizole the velocity of N-demethylation of aminopyrine was reduced by 1.8 times ($p < 0.001$) and this was accompanied by minor changes in hexobarbital biotransformation. In females, a statistically significantly ($p < 0.05$) higher absolute plasma 11-OHCS level was observed under the influence of ethimizole and the weight of the thymus was sharply reduced (by 1.9 times), so that it was much less ($p < 0.02$) than in males receiving ethimizole. In females, moreover, the weight of the adrenals increased (from 20.7 ± 1.0 to 35.0 ± 0.22 mg/kg; $p < 0.001$) by a greater degree than in males (from 17.9 ± 0.6 to 29.8 ± 3.4 mg/kg; $p < 0.001$), and their ascorbic acid concentration fell (from 25.5 ± 1.1 to 16.5 ± 0.5 μmoles/kg, $p < 0.001$, and from 27.2 ± 1.1 to 20.4 ± 1.6 μmoles/kg, $p < 0.001$ respectively). Whereas in males under the influence of ethimizole the thyroid hormone level showed little change, in females the concentration of the more biologically active T_3 was reduced statistically significantly ($p < 0.01$)

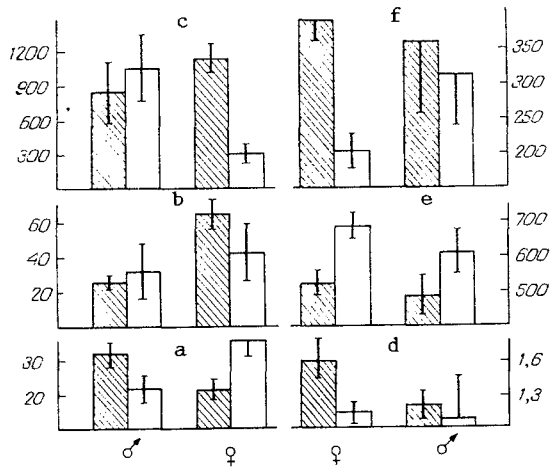

Fig. 1. Effect of ethimizole on parameters of thyroid, glucocorticoid, and microsomal enzymes activity in rats of different sexes. a) Velocity of N-demethylation of aminopyrine (in nmoles·min^{-1}·g^{-1}); b) duration of hexobarbital sleep (in min); c) hexobarbital concentration (in nmoles/liter); d) T_3 concentration (in nmoles/liter); e) weight of thymus (in mg). Shaded columns represent intact animals, unshaded — animals receiving ethimizole.

TABLE 2. Effect of Level of Thyroid Activity on Sex Hormone Levels in Rats

Thyroid activity	Plasma concentration, pmoles/liter (M ± m)			
	testosterone in males		estradiol in females	
		p		*p*
Normal	2019±125	—	299±31	—
Depressed	1598±117	<0,05	182±24	<0,02
Raised	2414±116	<0,05	128±24	<0,001

and abruptly. The rather greater decrease in creatine phosphokinase activity in the myocardium of females (from 34.9 ± 0.8 to 29.8 ± 1.2 mmoles·kg^{-1}·sec^{-1}) will be noted, with the same tendency toward an increase in the creatine phosphate concentration.

The changes induced by ethimizole could no longer be observed after ten days.

Importance of activation of the pituitary—adrenal axis could be identified in the effect of ethimizole on the microsomal system. A definite link between these systems (fall of the cytochrome P450 level and in the velocity of N-demethylation and hydroxylation, and of biotransformation of hexobarbital after adrenalectomy) was established previously [5]. However, no restoration of the microsomal enzymes was found in response to administration of glucocorticoids after adrenalectomy [8]. This indicated [10] that steroid hormones do not play fundamental role in the control of enzyme induction. Probably the changes in thyroid activity induced by ethimizole are more important. Considering that thyroid hormones prevent the androgenic rise in activity of the drug-metabolizing system [10], the sharp fall in the T_3 level induced by ethimizole in females can be explained by the simultaneous considerable increase of activity of microsomal enzymes which is depressed in them. In males, however, changes in thyroid hormone levels are mild in degree and activity of the microsomal enzymes undergo smaller changes. Changes in thyroid activity induced by ethimizole are reflected in blood levels of the sex hormones (Table 2). In males after thyroidectomy the estradiol level falls, i.e., androgenic influences begin to predominate, whereas in males it is lowered because of lowering of the testosterone level. In the presence of an excess of thyroxine (intramuscular injection in a dose of 1 mg/kg daily for 10 days), however, the androgen level rises in males, and in females the estradiol level falls.

Sex differences in the function of the microsomal system are considered [10] to be due to the anabolic effect of androgen, aimed at increasing its activity. In fact, in the present

experiment, on the one hand, androgenization of females (daily administration of testosterone proprionate in a dose of 10 mg/kg for 10 days) was accompanied by an increase in the velocity of N-demethylation of aminopyrine from 22 ± 1.2 to 39 ± 3.3 nmoles·min^{-1}·g^{-1} ($p < 0.001$); on the other hand, in the case of estrogenization of males (5 injections of estradiol dipropionate in a dose of 5 μg/kg every other day) this parameter fell to 23.0 ± 1.8 nmoles·min^{-1}·g^{-1} ($p < 0.001$). Stimulation of enzyme activity of hepatic microsomes by testosterone and its inhibition by estrogens have been reported in the literature [7, 9].

Since sex differences in the rate of metabolism of the type I substrate which we used (hexobarbital, aminopyrine) are due to a difference in cytochrome P450 binding with them [12], and since proteins of microsomal membranes are the most powerful ethimizole acceptors [2], it can be postulated that the effect of the drug on the microsomal enzyme system which is determined by sexual dimorphism, is manifested at this level.

LITERATURE CITED

1. Yu. S. Borodkin, Antiphenes (Experimental and Clinical Data) [in Russian], Moscow (1966).
2. Yu. S. Borodkin, L. M. Belyaevskaya, O. G. Kulikova, et al., Byull. Éksp. Biol. Med., No. 10, 53 (1983).
3. I. S. Zavodskaya, É. A. Migas, and V. V. Bul'on, Byull. Éksp. Biol. Med., No. 3, 54 (1975).
4. I. S. Zavodskaya, E. V. Moreva, and N. A. Novikova, The Effect of Neurotropic Substances on Neurogenic Heart Damage [in Russian], Moscow (1977).
5. A. F. Leshchinskii, T. A. Zolotareva, O. T. Karpenchuk, and Z. I. Zuza, Farmakol. Toksikol., No. 3, 265 (1979).
6. A. F. Leshchinskii, V. L. Konovalenko, and Z. I. Zuza, Vopr. Kurortol., No. 2, 9 (1985).
7. P. V. Sergeev, N. N. Vedernikova, A. I. Maiskii, et al., Byull. Éksp. Biol. Med., No. 9, 43 (1975).
8. J. A. Castro, F. E. Green, P. Gigon, et al., Biochem. Pharmacol., 19, 2461 (1970).
9. A. H. Conney and J. J. Burnes, Acta Pharmacol. (Copenhagen), 19, 31 (1962).
10. R. Kato, Xenobiotica, 7, 25 (1977).
11. L. Lombrozo and C. Mitoma, Biochem. Pharmacol., 19, 2317 (1970).
12. D. M. Moor, Foreign Compound Metabolism in Mammals, Vol. 2, London (1972), pp. 398-411.

RETINOIC ACID MODIFIED CELL CULTURE FOR REPRODUCING ENTEROPATHOGENIC VIRUSES

V. N. Tatskaya, V. I. Nozdrin, and V. G. Kvachev

UDC 578.835.11.083.22

KEY WORDS: retinoic acid; cell culture; enteropathogenic viruses; infectivity titer

Among the various aspects of the biological action of retinoic acid (RA), namely its vitamin A, immunomodulating, and growth-stimulating actions [2, 4, 5], it is the ability of RA to exert a differentiating action on tissue cell cultures that has attracted particular attention.

It was suggested previously that the water-soluble form of RA can be used to increase the sensitivity of embryonic cells in vitro to viruses capable of reproducing only in mature differentiated cells [3]. The experimental verification of this phenomenon and its use in biotechnology has presented new opportunities for the study of cell biology in culture and for increasing the productivity of cultures during reproduction of different viruses.

The aim of this investigation was to study the efficacy of culture of enteropathogenic viruses in RA-modified cell culture.

EXPERIMENTAL METHOD

Reproduction of enteropathogenic porcine viruses was studied in a transplantable culture of hog kidney cells (HK), treated beforehand with RA. The growing cell culture was treated

Ukrainian Veterinary Research Institute, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. V. Kovanov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 93-95, July, 1989. Original article submitted December 26, 1988.

0007-4888/89/0007-1015$12.50