Neuroendocrinology Letters No.6 December Vol.26, 2005
 ISSN 0172–780X **www.nel.edu**

ORIGINAL ARTICLE

Testosterone levels and histological features of reproductive glands in adult male rats treated neonatally with tamoxifen

Dr. Adriana Morales-Otal, Socorro Retana-Márquez, Armando Ferreira-Nuño & Javier Velázquez-Moctezuma

Department of Reproductive Biology. Universidad Autónoma Metropolitana-Iztapalapa. AP 09340, D. F. México.

Correspondence to: Dr. Javier Velázquez Moctezuma
Departamento de Biología de la Reproducción.
Universidad Autónoma Metropolitana-Iztapalapa
C.P. 09340. Iztapalapa. D.F. MÉXICO
PHONE: (525)8044701
FAX: (525) 8044704
EMAIL: jvm@xanum.uam.mx

Submitted: February 7, 2004 *Accepted: May 15, 2004*

Key words: **tamoxifen; sexual differentiation; testis; rats; testosterone**

Neuroendocrinol Lett 2005; **26**(6):729–732 **PMID:** 16380686 NEL260605A26

Abstract

OBJECTIVE: In this study we examined the possibility that demasculinization produced by the neonatal administration of tamoxifen is accompanied by a decline in plasma levels of testosterone during adulthood.
METHODS: Wistar male rats received either a treatment with 12.5 μg/kg of tamoxifen during the first eight days of age or a treatment with 100 μg/kg of tamoxifen for five days. Each treatment had their respective control group. During adulthood their masculine sexual behavior was analyzed. At 8 months of age, males were killed by decapitation, trunk blood was collected and peripheral glands were dissected and weighed. Testosterone levels were measured by HPLC. Histological analysis of peripheral glands was performed.
RESULTS: Both neonatal tamoxifen treatments significantly decreased male sexual behavior when compared to control values. In addition, both treatments also showed a significant decrease in testicular weight when compared to control groups, as well as a decrease in seminal vesicle weight. In the microscopic analysis, a significant decrease in the diameter of the seminiferous tubules was observed, especially in the animals treated with 100 μg/kg of tamoxifen. However, no differences were observed between tamoxifen treated and control animals concerning plasma levels of testosterone.
CONCLUSION: The present results indicate that behavioral manifestations and changes in peripheral reproductive organs that accompanied demasculinization are not due to a deficit in testosterone secretion.

Introduction

It is widely accepted that brain sexual differentiation is highly dependent upon the levels of androgens during the perinatal period [For review see: 1]. Under normal physiological conditions, male rats show high serum levels of androgens before birth [2, 3]. The levels of androgens remain elevated for several days postnatally [4]. In addition, it has been shown that neonatal castration

[5, 6] or neonatal injection of the estrogen antagonist tamoxifen (Tx) [7] permanently reduces the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA). Male rats treated pre- or postnatally with Tx show a reduction of their masculine sexual behavior during adulthood [8, 9,10].

Moreover, the main source of fetal serum androgens during the perinatal period is the fetus testicles [6]. Thus, one possible mechanism by which Tx affects brain sexual differentiation could be through an inhibitory action on the testicular secretion of testosterone. However, there are only a few studies concerning the influence of Tx upon the testicular secretion of testosterone and most of them have been done in adult rats [11, 12, 13, 14]. In 1978, Bartke et al [15] reported the effects of repeated administration of Tx on testicular function of adult rats. These authors observed a significant drop of plasma testosterone and LH levels in rats treated for 5 days with 2 or 10 mg Tx subcutaneously daily.

The purpose of the present study was to determine if perinatal administration of Tx affects brain sexual differentiation through a permanent inhibitory action on the fetal testicular secretion of testosterone. Therefore, in the present study, testosterone levels were measured in adult rats treated with Tx neonatally. The neonatal treatment with Tx used two treatment schedules that have been shown to reliably affect adult masculine behavior.

In addition, the morphological characteristics of the peripheral reproductive organs were determined.

Material and Methods

Adult Sprague Dawley rats from our own vivarium were used in this study. Pregnant females were kept in individual cages until delivery, in a room with light (12L:12D, lights on 9 am) and temperature controlled and with free access to food and water. Upon delivery, female offspring were eliminated from this study and males were cross-fostered keeping the same size of the litters (4) with each mother. Male offspring were randomly assigned to two different treatment schedules, each of which has been shown to affect adult masculine behaviors.

Group A (Tx12.5) (n=8). Subjects (Ss) were treated daily with subcutaneous administration of 12.5 µg of Tx dissolved in a volume of 0.05 ml corn oil. Injections were administered from day 1 to day 8 of age.

Group B (CON12.5) (n=8). Ss received a daily injection of corn oil in the same volume and in the same period as group A.

Group C (Tx100) (n=8). Ss were treated daily with subcutaneous administration of 100 µg of Tx dissolved in a volume of 0.05 ml of corn oil. Injections were applied from day 1 to 5 of age.

Group D (CON100) (n=8). Ss received a daily injection of corn oil in the same volume and in the same period as group C.

All injections were given between 11:00 and 12:00 h. After the treatment period, the animals were left undisturbed with their mothers. At 30 days of age, the male offspring were separated from their mothers and were housed 4 per cage with males in like treatment conditions housed together. In addition, Ss were checked daily for testicular descent. Between 5 and 6 months of age, the males were tested for their spontaneous masculine sexual behavior.

Hormonal Assessment

At eight months of age, males were decapitated and trunk blood was collected. Testosterone was extracted from the plasma and quantified by HPLC using a modification of the method reported by Woodward and Emery [16]. Blood samples were centrifuged and plasma (1 ml) was mixed with 100 µl 19-nortestosterone solution (5 mg/ml in methanol) as an internal standard. Testosterone was extracted into 5 ml of diethyl ether-dichloromethane (60/40 v/v) mixed by vortex and immediately centrifuged for 5 min. The supernatant was vortex mixed with 1 ml HPLC-grade water. After centrifugation, the supernatant (3ml) was evaporated at room temperature under nitrogen. The residue was re-dissolved in 100 µl of methanol-water (60:40 v/v). Results were analyzed using the Maxima 820 Chromatography Workstation, obtained from Waters (Milford, Massachusetts). Inter-assay and intra-assay coefficients of variation (C.V.) were determined using four plasma pools in the 0.2–8 ng/ml range, covering the normal ranges in rat plasma.

Histological analysis

In addition, peripheral reproductive glands were dissected, weighted and microscopically analyzed. The whole testis, fixed in formaldehyde 10% and embedded in paraffin, were serially sectioned to 6 µm. The total number of sections was recorded; every section was mounted and stained with hematoxylin-eosin for histologic and morphologic studies, as described by Vergouwen et al [17], and examined by light microscopy on an Axioscop KS 300.

Statistical analysis

Table 1. Effect of neonatal administration of tamoxifen on sexual behavior parameters of rats.

Treatment (µg/kg)	Mount latency	Intromission latency	Ejaculation latency	Number of Mounts	Number of Intromissions	Ejaculatory Frequency
Control, n=29	16.6±5.3	29.3±6.2	320.3±50.61	6.6±1.2	6±1	3.2±0.2
Tx12.5 n= 25	413.4±69.2**	854±49**	804**	17.3±5.6**	4.8±1.4	006**
Tx100 n=33	188.9 ± 50.4**	248 ± 59*	531*	30 ± 3.3**	7.25 ± 0.91	0.08**

*p < 0.01, **p < 0.001

Latencies and frequencies in sexual behavior were analyzed using a Student T Test. Steroids plasma levels were analyzed using a Tukey test. Proportions in sexual behavior were analyzed using a Chi square test.

Results

Results concerning sexual activity corroborate previous reports about the permanent damage to masculine sexual behavior induced by the neonatal treatment with Tx. Table 1 summarizes the main findings observed in masculine sexual behavior. When compared to oil treated groups (pooled as control group), both treatment schedules induce significant differences in several parameters of sexual behavior. In addition, no significant differences were observed between Groups A and C indicating that both treatment schedules induce similar effects on sexual behavior.

Although sexual behavior was greatly impaired in Tx treated males, plasma testosterone levels in the treated animals were not different from control levels. Figure 1 shows the levels of testosterone in control groups as in Tx treated males.

As can be seen in Figure 2A, both doses of Tx produced a significant reduction in testicular weight. Perinatal treatment with 12.5 µg or 100 µg of Tx produced a reduction in testicular weight of about 25% and 50%, respectively, of control weights. When the diameter of the seminiferous tubules was analyzed, the results showed a significant reduction with both doses of Tx. However, the dose of 12.5 µg of Tx induce only a slight decrease, while the dose of 100 µg of Tx induced a marked decrease of about 50% compared to control values (Figure 2B). Concerning the weight of the seminal vesicles, both doses induce a trend to decrease, but this reduction reached statistical significance only in the dose of 100 µg with a reduction of almost 50% compared to control groups (Figure 2C).

Discussion

The present results corroborate previous findings concerning the deleterious effect of neonatal treatment with Tx on masculine sexual behavior during adulthood [9, 10]. On the other hand, the present results clearly indicate that there is an effect on peripheral reproductive organs, mainly in the testicles and in the seminal vesicles. However, despite these marked changes in the testicles, there were no detectable differences in the testosterone levels of the experimental groups. Thus, the impairments of masculine sexual behavior observed after the neonatal treatment with Tx cannot be explained by a deficit in circulating levels of testosterone.

The administration of antiestrogens in adult subjects interferes with the normal negative feedback of sex steroids at hypothalamic and pituitary levels. Antiestrogens administration results in an increase of endogenous gonadotropin-releasing hormone

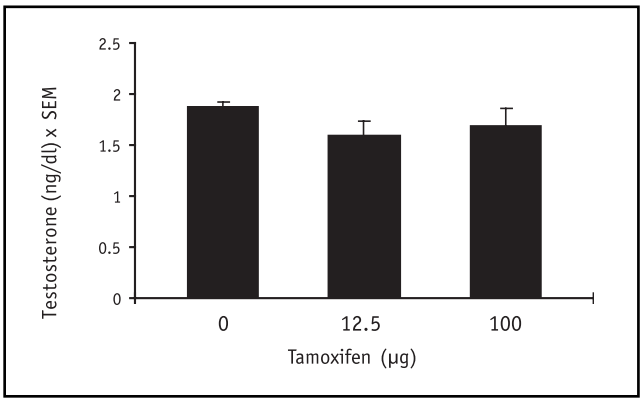

Figure 1. Plasma levels of testosterone in adult males neonatally treated with two doses of tamoxifen. No significant differences were observed between the experimental and control groups.

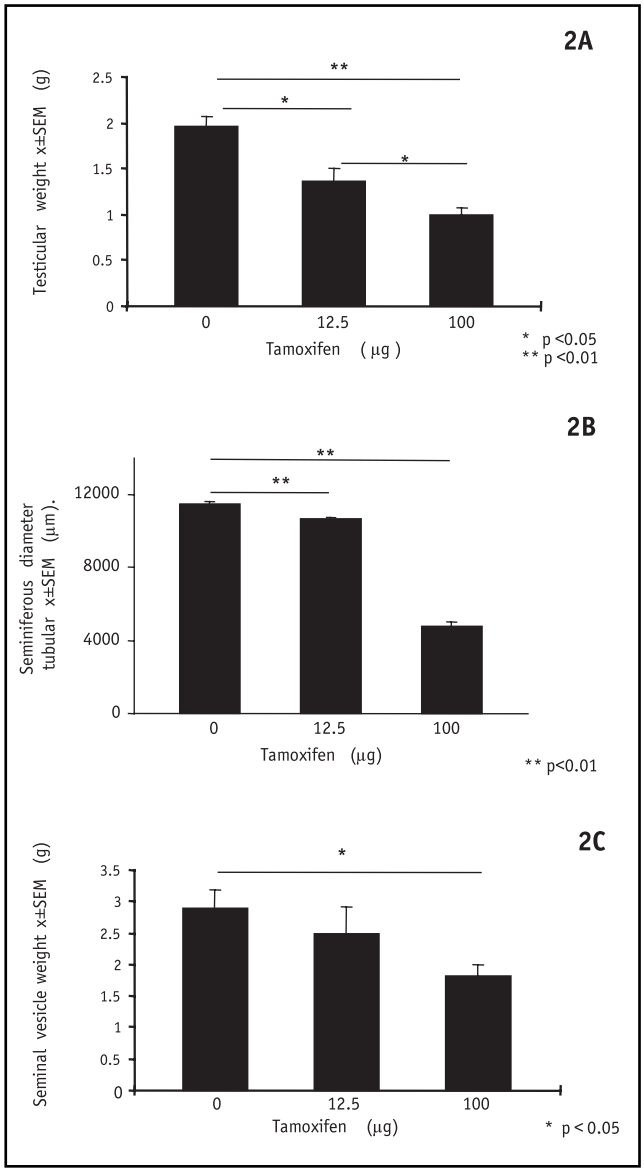

Figure 2. Testicular weight, diameter of seminiferous tubules and weight of seminal vesicles of adult rats neonatally treated with two doses of tamoxifen. * = p < 0.05; ** = p < 0.01. Student T test.

secretion from the hypothalamus and also an increase of FSH and LH secretion directly from the pituitary. In turn, FSH and LH stimulate the testes eliciting an increase of spermatogenesis or steroidogenesis [For a review see: 18].

Neonatal administration of Tx seems to induce permanent organizational changes not only at cerebral levels but also in peripheral glands. Male rats treated neonatally with Tx display during adulthood deficiencies both in sexual behavior and in fertility [10]. Thus, it is possible that, besides cerebral impairments, Sertoli cells could also be permanently altered by neonatal Tx administration, resulting in a decrease of fertilization capabilities.

On the other hand, the normal levels of testosterone in adult rats neonatally treated with Tx suggest that Leydig cells were not permanently affected. It must be noted that some reports indicate that Leydig cells of the neonatal testicle are quite different from the Leydig cells that can be found in the adult testicle [19, 20]. Thus, it is possible that the neonatal effect of Tx is restricted only to the population of Leydig cells observed around birth without affecting the population of Leydig cells that are functionally active during adulthood.

Acknowledgements

Authors would like to express their gratitude to Edith Monroy and Donald Moss for her expert advice on manuscript language. This study was partly supported by CONACyT 400200-5-26442M (JVM).

REFERENCES

1 Gorski RA. Sexual dimorphisms of the brain. J Anim Sci. 1985; **61** Suppl 3:38–61.
2 Dorner G. Sex hormone dependent brain diferentiation and sexual behavior. Exp Brain Res. 1981; Suppl 3:238–245.
3 Weisz J, Ward IL. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinol. **106**(1):306–316.
4 Döhler KD, Wuttke W. Changes with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats, Endocrinol 1975; **97**(4):898–907.
5 Gorski RA, Gordon JH, Shryne JE, Southam AM. Evidence for a morphological sex difference within the medial preoptic area of the rat brain, Brain Res 1978; **148**(2):333–346.
6 Jacobson CD, Csernus VJ, Shryne JE, Gorski RA. The influence of gonadectomy, androgen exposure, or a gonadal graft in the neonatal rat on the volume of the sexually dimorphic nucleus of the preoptic area, J Neurosci 1981; **1**(10):1142–1147.
7 Döhler, K. D., Coquelin, A., Davis, F., Hines, M., Shryne, J. E. and Gorski, R. A., Differentiation of the sexually nucleus in the preoptic area of the rat brain is determined by the perinatal hormone environment, Neurosci Lett 1982; **33**(3):295–298
8 Döhler KD. The pre- and postnatal influence of hormones and neurotransmitters on sexual differentiation of the mammalian hypothalamus, Int Rev Citol 1991; **131**: 1–55.
9 Csaba G, Karabélyos C. The effect of a single neonatal treatment (hormonal imprinting) with tha antihormones, tamoxifen and mifepristone on the sexual behaviour of adult rats, Pharmacol Res 2001; **43**(6): 531–534.
10 Morales-Otal A, Ferreira-Nuño A. Velázquez-Moctezuma J. Monoaminergic and cholinergic stimulation of masculine sexual behavior in neonatally demasculinized male rats, Pharmacol Res 2002; **46**(1):61–66.
11 Donaldson MD, Saez JM, Forest MG. Effects of the aromatase inhibitor 1,4,6-androstatriene-3,17-dione and the anti-oestrogen tamoxifen on rat testicular function, J Steroid 1981; **14**:823–828.
12 Cigorraga SB, Sorrell S, Bator J, Catt J, Dufau ML. Estrogen dependence of a gonadotropin-induced steroidogenic lesion in rat testicular Leydig cells. J Clin Invest 1980; **65**:699–705.
13 Hodgson YM, Urquhart G. Kretser DM. Effect of oestradiol and tamoxifen on the testosterone response in male rats to a single injection of hCG. J Reprod Fert 1983; **68**:295–304.
14 Lin T, Murono EP, Osterman J, Nankin HR. Direct inhibition of rat Leydig cell function by tamoxifen, Metabolism 1981; **31**:543–547.
15 Bartke A, Mason M, Dalterio S, Bex F. 1978. Effects of tamoxifen on plasma concentrations of testosterone and gonadotrophins in the male rat. J Endocrinol 1978; **79**: 239–240.
16 Woodward JH, Emery PW. Determination of plasma corticosterone using high-performance liquid chromatography. J. Chromatogr. 1987; **419**:280–284.
17 Vergouwen RPFA, Huiskamp R, Bas RJ, Roepers-Gajadien HL, Davids JAG, de Rooij DG. Postnatal development of testicular cell populations in mice. J Reprod Fert 1993; **99**:479–485.
18 Vandekerckhove P, Lilford R, Vail A, Hughes E. Clomiphene or tamoxifen for idiophatic oligo/asthenospermia. Cochrane Database Syst Rev. 2000; (**2**): CD000151.
19 Huhtaniemi I, Pelliniemi LJ. Fetal Leydig cells: cellular origin, morphology, life span, and special functional features Proc Soc Exp Biol Med. 1992; **201**(2):125–40.
20 Kuopio T, Tapanainen J, Pelliniemi LJ, Huhtaniemi I. Developmental stages of fetal-type Leydig cells in prepubertal rats. Development. 1989; **107**(2):213–20.