Effects of perinatal exposure to diazepam on the development of sexual behavior and neuroendocrine functions in male rats.

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Abstract: Developmental exposure with diazepam when coinciding with the ontogenesis of its receptors may be crucial for the induction of long-term functional disturbances in the rat brain. To investigate the possibility of intervention in the sexually dimorphic behavior of reproduction and the under lying morphological and hormonal basis the time-mated female rats were treated with 2.5 mg/kg, 5 mg/kg and 10 mg/kg, i.p.(n = 6-8) separately. Treatment was continued from gestation day 15 till delivery and further continued to neonates 5 days, postnatally. Control group (n= 5-8) given saline also followed the same treatment protocol. Sexual behavior in the male offspring at adulthood born to diazepam-exposed rats was enhanced, both in dose-dependent and time-dependent manner (p<0.01). Accordingly plasma LH and testosterone concentration both in plasma and testis was also influenced. Compared to controls treated animals also showed complete abolition of spermatogenesis and drastically reduced testicular steroidogenesis. Our findings, thus support the hypothesis that like psychoactive drugs benzodiazepines sedatives that crosses the blood brain barrier during the critical period of brain sexual differentiation offer serious risk of abnormal post-pubertal reproductive functions in the male rat.

Key Words: Diazepam, reproduction, pregnancy, mounting, intromission, and testosterone

Introduction

It has been established that benzodiazepines can influence the development of the brain and cause long-lasting teratological effects on several brain functions and behavior [1]. Exposure of rat offspring to diazepam through the placenta or through the mother's milk resulted in extensive morphological changes, including gliosis and perivascular cuffing, in the brain of mature rats [2].These changes depended upon the period of exposure to diazepam, since the postnatally exposed pups showed very few brain lesions [2].Furthermore, offspring that are perinatally exposed to diazepam show severe deficits in motor activity[1], open-field behavior and lordotic behavior [3-5] during adulthood. With respect to long-lasting diazepam-induced learning deficits, male rats appear to show more vulnerability than females [6]. Guillaman *et al* have demonstrated that a daily intraperitoneal administration of diazepam (2.5 mg/kg) to rat pups, from the day of birth to day 16, feminizes the continuously reinforced free-operant response in the adult male, while adult female rats were unaffected by the early postnatal diazepam treatment [3]. These observations are substantiated by the fact that the time course of appearance of benzodiazepines receptors in the developing rat brain shows a caudorostral gradient [7], and their maximum concentration in the brain is reached in the first postnatal week [8]. This

period of time, overlaps the periods of sexually dimorphic differentiation of several hypothalamic nuclei in the rat [9,10]. It has been established that benzodiazepines exert their CNS action via specific receptors located on the GABA receptor complex [11]. A major portion of the benzodiazepines receptors in the CNS appear to be part of a polymolecular complex which contains in addition to benzodiazepine receptors, the recognition sites for the inhibitory neurotransmitter GABA and the chloride ionophore protein[12]. Interaction of benzodiazepine at specific binding sites facilitates the action of GABA on the chloride ionophore. Since the sensitivity of GABA receptors in the brain has been shown to be significantly modulated by benzodiazepine receptors and since GABA is thought to mediate neuroendocrine functions [13-14], the present study was designed to investigate whether organization of neuronal basis of sexually dimorphic reproductive behavior is disrupted as a result of perinatal diazepam exposure in the rat.

Materials and methods

Adult Wistar female rats weighing 180 to 200 g were procured from the Aga Khan University housing unit. Each of these females experiencing verified normal ovarian cyclicity on the day of oestrous were placed with a sexually active male rat. Vaginal smears were taken the following morning between 9:00 to 9:30 h. The day on which sperms were present, was designated day 1 of gestation. Females were then randomly assigned to the following experimental groups; 1) diazepam (2.5 mg/kg), 2) diazepam (5 mg/kg) and 3) diazepam (10 mg/kg) (n=6-8 in each group). Intraperitoneal injections of either diazepam reconstituted in 0.9% saline as vehicle (n=5-8) or saline were made. As per duration of diazepam treatment following two different experiments were designed. **Experiment I:** Pregnant females were treated with diazepam from 15th day of gestation till delivery, after which pups were treated for five days, postnatally. **Experiment II:** Procedure was the same as mentioned in experiment I except pups were treated for 20 days postnatally. Rats were housed separately in controlled housing conditions (temperature 23±1°C; humidity 42±10%) with a 12:12 hr light:dark cycle (lights on from 0600 h to 1800 h). Food and water were freely accessible. All litters at birth were observed for symptoms of hypothermia. Those appearing severely hypothermic were seen deprived of maternal care, thus could not survive till the end of first week of their life. Litters from the diazepam groups were assigned to saline treated control groups mothers whose pups were born on the same day. All pups were weighed once weekly and weaned 25 days after birth. Animals were then segregated and at adulthood were tested against ovarian optimized females that were primed with 50 μg oestradiol benzoate for 2 days continuously and 5 mg progesterone. Fifteen days prior to the behavior testing on day 120 days both experimental and control male rats were tested against steroids primed females rats for 3 times over a period of three weeks with a gap of 4-6 days between each test for parameters of male sexual behavior. For this purpose, the receptive females were allowed to adapt to the testing arena for at least 5 min prior to the introduction of an experimental male. Males were permitted to mount female rats

for 5 min and the number of mounts as well as the quality of mount was recorded. Number of intromissions was also recorded till the animal ejaculated. One testis from each animal was decapsulated, cut into small pieces (diameter: 2 to 3 mm), and fixed in 5.5% glutaraldehyde in Sorensen phosphate buffer(PBS, pH -7.4).After brief wash in the same buffer solution, the samples were post fixed in 1% osmium tetraoxide in 0.1M PBS for150 min (pH-7.4 at 4˚C).After gradual dehydration in ethanol and finally in toluol (3×20 min), the tissue was embedded in Epon 812.Serial semithin sections were prepared with an LKB ultramikrotom (Ultradome III), stained with 1% borax-buffered toluidine blue, and examined under the light microscope. Testosterone concentrations in the testiscular tissue and serum were detected by radioimmunoassay.

Data for individual test between groups was compared and subjected to analysis of variance followed by Duncan's multiple range t test.

Results

The effects of perinatal diazepam exposure on male sexual activity were observed for three occasions with a gap of 10-14 days performed over a duration of 5-6 consecutive weeks. The sexual activity of male rats was recorded in terms of number of mounts and intromissions made by each rat per five-minute observation test period. The observations recorded are presented in Table 1. In experiment I where perinatal diazepam exposure (2.5mg/kg) continued from gestation day 15 till 5-days postnatally showed a decrease in mounting frequency in the first week but the effect was found reversed. There was an increase in frequency of mounting as a result of all the treatment doses in second and third tests, respectively ($p<0.5$ and $p<0.01$). Like, mounting the frequency of intromission was reduced in first test as compared to controls ($p<0.05$) however, it was also reversed as observed in the second and third test ($p<0.01$), respectively. Increasing the dose to 5mg/kg, diazepam caused an increase both in mounting and intromission frequencies and intensity ($p<0.001$). In 10mg/kg diazepam exposed male rats, increase in frequency of mounting was recorded in all the three tests ($p<0.01$), whereas the intromission frequency was seen relatively less significant ($p<0.05$). In experiment II where diazepam exposure to male rats continued from gestation day 15 through 20 days postnatally, the effects of all the three dose regimens on mounting frequency were noted more consistent. Extended treatment over with lowest dose regimen i.e. 2.5 mg/kg showed an increase in mounting frequency against OVX steroids primed female rats ($p<0.05$). The increase remained not only consistent with the smallest dose regimen used, it rather appeared more intense with the increase in the dose (Table I). Intromission frequency as compared to frequency of mounting showed a decrease ($p<0.05$) in the first test with the dose of 2.5 mg/kg which then found reversed in second and third test. Exposure to higher doses of diazepam showed an increase which was mild initially but turned more pronounced in the subsequent tests ($p<0.01$ and $p<0.001$), respectively.

Alterations in testicular weight, testicular histology, and testicular testosterone along with plasma LH and testosterone were observed following exposure to different doses of diazepam in male offspring which are presented in Table 2.

Table 1
Percentage differences of various doses of treatment on male sexual activity vs saline during Experiment I and Experiment II

Week of Tests	Experimental treatment	Frequencies			
		Experiment I		Experiment II	
		Mounting	Intromission	Mounting	Intromission
1	DZ_1	20.5[*] ↓	9.8[*] ↓	15.0[*] ↑	7.2[*] ↓
	DZ_2	65.6[**] ↑	95.5[**] ↑	38.5[**] ↑	4.1 ↑
	DZ_3	57.7[**] ↑	45.5[*] ↑	38.6[**] ↑	5.4 ↑
2	DZ_1	34.0[*] ↑	65.6[**] ↑	90.0[*] ↑	53.0[**] ↑
	DZ_2	91.0[**] ↑	64.9[**] ↑	83.8[***] ↑	49.3[**] ↑
	DZ_3	46.3[**] ↑	53.0[*] ↑	80.0[**] ↑	58.0[**] ↑
3	DZ_1	78.9[**] ↑	44.3[**] ↑	51.0[**] ↑	69.0[**] ↑
	DZ_2	75.8[**] ↑	75.6[**] ↑	70.0[**] ↑	80.3[***] ↑
	DZ_3	62.0[**] ↑	43.0[*] ↑	73.4[**] ↑	67.7[**] ↑

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 2
Percentage differences of various doses of treatment on testicular weight and hormonal profile in male offspring vs saline during Experiment I and Experiment II.

Experimental treatment	Testicular weight	Plasma LH	Testosterone	
Experiment I			Plasma	Tissue
DZ_1	0.99 ↑	2.0 ↓	131.0[**] ↑	3.6 ↑
DZ_2	1.8 ↑	3.0 ↓	35.0[**] ↑	21.1[*] ↑
DZ_3	4.6 ↓	62[***] ↓	387.0 ↑	41.0 ↑
Experiment II				
DZ_1	52.5[***] ↓	5.7 ↓	113.7[**] ↑	1.2 ↑
DZ_2	51.8[***] ↓	9.8 ↓	109[**] ↑	38.0 ↑
DZ_3	49.3[***] ↓	16.0[*] ↓	115.5[**] ↑	102.0 ↑

*$p<0.05$ compared to controls; ** $p<0.01$ and *** $p<0.001$.

Testicular weight remained unaffected following exposure to different doses of drug. Testicular concentration of testosterone remained unaffected in 2.5 mg/kg diazepam treated males. However, significant rise ($p<0.01$) in tissue content was found in both 5 and 10mg/kg diazepam exposed males. Plasma LH which was estimated to monitor any pituitary related effect did not show any change in its concentration except at highest dose where reduction occurred was significant ($p<0.001$) as compared to control. Furthermore, significantly elevated (2.5 mg/kg: $p<0.01$, 5mg/kg: $p<0.05$ and 10mg/kg: $p<0.00l$) mean plasma levels of testosterone at all doses were also noted.

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Semithin section (X 150) of testis of control group of rats showed that seminiferous tubules were completely intact (Fig. 1). Dividing spermatogonia and spermatocytes were observed and there was very little interstitial space between the adjacent spermatogonia and spermatocytes. Compared to the controls, the testis of diazepam exposed (2.5mg/kg) rats showed shrinkage of seminiferous tubules (Fig. 2)
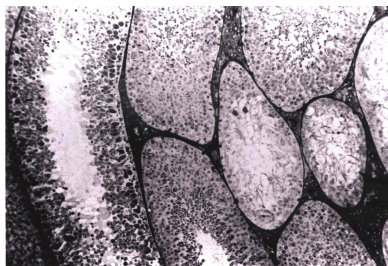

Fig. 1: Testis of a rat exposed to 2.5 mg/kg semithin section stained with toulidine blue showing shrinkage of seminiferous tubales. The network shows shrinkage and is broken partially (X580)
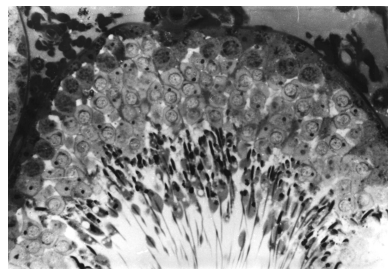

Fig. 2: Testis of a control rat (age 120 days) shows small leydig cells with largely condensed chromatin with dark cytoplasm. Intracellular bridges between the secondary spermatocytes are intact (X10000).

Wide spaces between the germinal cells were observed. The network was partially broken. There appeared significant reduction in the number of spermatocytes. Although basement membrane was intact as it was relatively thin but it was composed of only one to two layers. The Leydig cells were small and contained small nuclei with largely condensed chromatin with dark cytoplasm. The interstitial tissue contained fibrocytes, macrophages, and mast cells. The electron micrograph (X 10,000) of the testis of the control group shows that the intercellular bridges between the secondary spermatocytes are intact (Fig.3). Electron micrograph of similar magnification in the testis of the treated animals (2.5mg/kg) showed that there were wide gaps between the spermatocytes and the intercellular bridges were pushed apart. The network did not seen intact and the intercellular bridges showed marked thickening (Fig. 4).
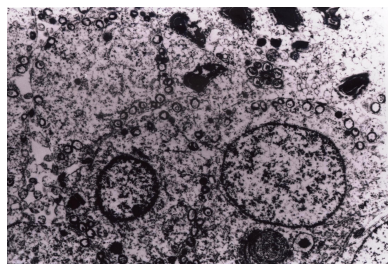

Fig. 3: Testis of a rat exposed to 2.5 mg/kg diazepam (120 days) stained with toulidine blue. Semithin cross section show dissolution of germ cells in the adluminal compartment (X150).
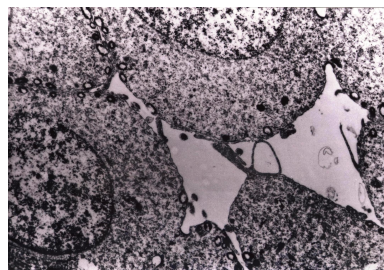

Fig. 4: Electron micrograph of a testis (120 days) exposed to diazepam (2.5 mg/kg) shows wide gaps between the spermatocytes. The network is broken and the intracellular bridges are markedly thickened (X10000)

Discussion

Three different groups of female rats were subjected to diazepam exposure for two different durations at three different dose regimens. The treatment period as chosen so for the first experiment (i.e. from gestation day 15 to postnatal day 05) as it coincides not only with the timing of ontogenesis of benzodiazepine receptors but also with the androgen-dependent critical period of brain sexual differentiation in the fetal rat CNS [15-17] that continues to day five, postnatally. In rat, neuronal multiplication occurs during the third week prenatally, a period comparable to the second trimester of human pregnancy [7]. Further to that, postnatal development of benzodiazepine receptors is rapid and adult densities are reached between the second and fourth week in the rat [18]. Coen *et al* evaluated behavioral and neurochemical consequences in adult rats of prolonged exposure to chlordiazepoxide (a long-acting benzodiazepine) during the first three weeks of postnatal life which seems to be the period specific for the ontogenesis of central benzodiazepine and GABA receptors [19]. This was regarded as a firm reason for having the other treatment duration employed, i.e. from day 15 of gestation uptil 20 days of postnatal life; thus taking care of not only the period of brain sexual differentiation but also the attainment of the peak concentration of benzodiazepine receptors [20]. Although, the periods of exposure used in this study is challenged for the first time in such a research study of this nature, however, the data obtained as a result of exposure to both the agents for the two durations was found essentially similar and not very significantly different from each other. Therefore, the results will mainly be discussed in the context of the dose regimen employed and to some extent of two durations of exposure to these agents. For diazepam, dose regimen used were similar i.e. 2.5 mg, 5 mg, and 10 mg/kg/day. The three dose regimen were selected as an equivalent of therapeutic to largely a supra therapeutic benzodiazepine exposure. While the doses given in experimental studies may seem high when compared to clinical doses, however the clearance rate of the compound differs between species. Approximately 50% of the total injected drug was eliminated in the faeces and urine of the dams over 80 days of exposure to diazepam at 25mg/kg/day [21]. The effects observed in relation to sexual behavior in male offspring in the present study were pronounced. Monitoring of the male sexual behavior indices in this study demonstrate that male rat offspring exposed to any of the dose regimen of diazepam used, displayed significantly enhanced copulatory activity. This is in line with the previous observation of *in utero* exposure to diazepam a positive modulator of the GABA (A) receptor that exerts profound effects on the offspring that become most apparent after the maturation of the brain during puberty. The effects are often sex specific suggesting that the early exposure might have interfered with CNS organizing actions of sex steroids [22]. The decrease in LH to group exposed to highest dose used in our study could be attributed to the activation of the central GABAergic system because in an earlier finding Ro5-4864 a selective peripheral benzodiazepine agonist have been shown to cause a significant increase in hypothalamic LHRH and pituitary LH content concomitant with low serum LH levels suggesting diazepam treatment may have

impaired LH release at the hypothalamic level Furthermore, it is shown that the Leydig cells atrophies when release of LH from pituitary is eliminated following hypophysectomy or is suppressed by treatment with testosterone and estradiol releasing implants [23]. On the contrary, despite unaffected (in case of low doses) or low LH concentration in exposed rats (10mg/kg), testosterone concentration in the present study is significantly elevated in all groups. The increased testosterone concentration is possibly mediated via peripheral benzodiazepine receptors (PBRs), which are localized principally in steroidogenic tissues such as adrenal, testis and ovary [24,25]. Peripheral benzodiazepine receptors are mainly located on outer mitochondrial membrane where enzymes involved in steroidogenesis are strictly compartmentalized. In vitro studies using decapsulated testes or interstitial cell suspensions have shown that benzodiazepines (diazepam and Ro5-4864) stimulated androgen production [26]. The possible underlying mechanism as proposed by Vassilios, elaborates that steroidogenesis begins with the conversion of cholesterol to pregnenolone in the inner mitochondrial membrane, which then leaves the mitochondrion to undergo enzymatic transformation in the endoplasmic reticulum that finally gives rise to the final steroid products. This pathway is essentially regulated by LH in testicular and ovarian cells and the magnitude of the LH that binds to its receptor on the Leydig cell surfaces primarily control the synthesis and secretion of androgen by the adult Leydig cells [27].In the present study, histological examination of gonads of animals exposed to higher doses in experiment I revealed a marked increase in the number of Leydig cells in the interstitial compartment of the testicular tissue. This finding is in contrast with the previous observation that the Leydig cells atrophies in the absence of LH from pituitary [28]. It is reported that androgen secretion is modulated by various effectors that are synthesized and secreted in the testis which include benzodiazepine binding inhibitory protein besides enormous number of modulators of androgen production in Leydig cells. Preferential increase of plasma testosterone as compared to testicular testosterone in both experiment 1 and II may also be attributed to stimulation by the diazepam of the adrenals as PBRs have been demonstrated on adrenal cells [24,29]. Differential findings of testicular testosterone concentrations in this study could also be due to dose regimen or schedule of perinatal diazepam exposure employed. Previously, experimental design reported lowered serum testosterone levels following intraperitoneal administration of benzodiazepine [30] and it was suggested that diazepam acts directly on the testicular interstitial cells to inhibit testosterone production, as there was no difference in serum LH and FSH or in the hypothalamic LHRH content. Increase in testosterone concentration resulting is significantly increased sexual behavior in male rats by interfering positively with central neurotransmission has already been associated with LH-independent rise in circulatory testosterone concentrations which indicates that some central mechanism may possibly be responsible for enhanced motor execution of the copulatory activity. In fact, evidence indicates that testosterone acts on the brain through activation of the dopaminergic system and inhibition of the serotoninergic system[31].Later it has been shown that tuberoinfundibular dopaminergic neurones are activated by

benzodiazepines, that is entirely within the hypothalamus thereby enhancing the sexual behavior in male rats [32]. Percentage of rats that mate with receptive females was shown increased by DA. It is documented that psychotic patients who undergo long term treatment with dopaminergic blocking agent have reduced sexual activity while plasma prolactin, LH and testosterone concentrations are normal [31]. Treatment with benzodiazepine has indicated a trend towards recovery of sexual function i.e. restoration of libido and spontaneous erection. Significantly increased penile erectile reflexes and shortening of latency to onset of reflexes was already been shown [33]. Inhibition of sexual activity by diazepam and chlordiazepoxide has been reported [34] whereas benzodiazepine antagonist, Flumazenil was shown to be without effect. This indicates that normal development of the neuroendocrine components involved with gonadal function and reproductive behaviour of the animals appears to be highly sensitive to a critical steroid hormonal milieu during pregnancy [17,35-36]. Enhanced dopaminergic activity in the medial preoptic area results in an increased number of ejaculations, increased efficiency of the mounting performance and facilitated extra-copulatory penile reflexes [37-39] whereas DA antagonism is known to attenuate sex behavior at therapeutic doses in humans [40].

The histological changes (broken network, wide gaps between spermatocytes) produced as a result of drug exposure are in agreement with other studies stating various degrees of localized necrosis in the seminiferous epithelium and Leydig cells hyperplasia when nitrazepam was administered to male rats for four weeks [41]. Morphological changes in seminiferous tubules and necrotic changes in Leydig cell cytoplasm were observed after two weeks exposure to 1, 4 benzodiazepine, SC' 32855 in dogs [42].

Thus, our findings support the hypothesis that administration of psychoactive drugs including benzodiazepine sedatives that crosses blood brain barrier during the critical period of brain sexual differentiation offer serious risk for abnormal post-pubertal reproductive performance.

Acknowledgement

References

1. Kellog CK. Benzodiazepines: Influences on the developing brain. In: Boer GJ, Feenstra GP, Mirmiran M, Swaab DF, F Van Haaren (Eds.), Progress in Brain Research Vol 73, Amsterdam, Elsevier Science Publishers (Biomedical Div); 1988; pp 207-31
2. Frieder B, Epstein S, Grimm VE. The effects of exposure to diazepam during various stages of gestation or during lactation on the development and behavior of rat pups. Psychopharmacology 1984; 83: 51-55
3. Guillamon A, Cales JM, Rodriguez-Zafra M, Perez-Laso C, Caminero A, lzquierdo MAP, Segovia S. Effects of perinatal diazepam administration on two sexually dimorphic nonreproductive behaviors. Br Res Bull 1990; 25(6): 913-16
4. Diamond M, Binstoek T, Kohl JV. From fertilization to adult sexual behavior. Horm Behav 1996; 30: 333-353

5.   Mariam T, Shaharyar S, Siddiqui A. Changes in sexually dimorphic behaviour in the rat offspring following perinatal diazepam exposure. Pak J Zool 2001, 33(2): 145-50

6.   Grimm VE, Frieder B. Differential vulnerability of male and female rats to the timing of various perinatal insults. Intl J Neurosci 1985; 27: 155-64

7.   Schlumpf M, Richards JG, Lichtensteiger W, Mohier SG. An autoradiogaphic study of the development of benzodiazepines binding sites in rat brain. J Neurosci 1983; 3: 1478-87

8.   Braestrup, C, Nielson N. Ontogenetic development of benzodiazepine receptors in the rat brain. Brain Res 1978; 147(1): 170-73

9.   Gorski RA. Critical role for the medial preoptic area in the sexual differentiation of the brain. Brain Res 1984; 61: 129-46

10. Del Abril A, Segovia S, Guillamo'on A. Sexual dimorphism in the paraestrial nucleus of the rat preoptic area. Dev Brain Res 1990; 52: 11-15

11. Richards JG, Mohler H. Benzodiazepine receptors. Neuropharmacol 1984; 23: 233-42.

12. Schoch P, Richards JG, Haring P, Takacks B, Stahli C, Staehelin T, Haefely W, Mohler H. Co-localization of GABA receptors and benzodiazepine receptors in the brain shown by monoclonal antibodies. Nature 1985; 314 (6007): 168-71

13. Costa E, Guidotti A, Mao CC. A GABA hypothesis for the.action of benzodiazepines. In Robert E, Chase TN, Tower DB(Eds), GABA in Neurons system Function, New York, USA Raven Press; 1976; pp 413-26

14. Hegarty AA, Vogel WH. The effects of acute and chronic diazepam treatment on stress-induced changes in cortical DA in the rat. Pharmacol Biochem Behav 1995; 52(4): 771-78

15. Dohler KD. The pre-and postnatal influence of hormones and neurotransmitters on sexual differentiation of the mammalian hypothalamus. Cytol Rev 1991; 13: 1-57

16. Jacobson CD, Gorski RA. Neurogenesis of the sexually dimorphic muscle and pre-optic area of the rat. J Comp Neurol 1981; 196(3): 519-29

17. Siddiqui A, Gilmore DP. Regional differences in catecholamine content of the rat brain Effect of neonatal castration and androgenization. Acta Endocrinol 1988; 118: 483-94

18. Candy JM, Martin 1L. The postnatal development of the benzodiazepine receptor in the cerebral cortex and cerebellum of the rat. J Neurochem 1979; 32: 635-37

19. Coen E, Abbracctio MP, Balduini W, Cagiano R, Cuomo V, Lambardelli G, et al. Early postnatal chlordiazepoxide administration: permanent behavioral effects in the mature rats and possible involvement of the GABA-benzodiazepines system. Psychopharmacology 1983; 81: 261-66

20. Kellog CK. Benzodiazepines and the developing nervous system: Laboratory findings and clinical implications. in: Kellog CK (Ed.), Maternal substance Abuse and the Developing Nervous System, New York, USA, Academic Press Inc; 1992; pp 283-321

21. Simmons RD, Miller RK, Kellog CK. Prenatal diazepam Distribution and metabolism in prenatal rats. Teratology 1983; 28: 181-88

22. Kellogg CK, Olson VG, Pleger GL. Neurosteroid action at the GABAA receptor in fetal rat forebrain. Dev Brain Res 1998; 108(1-2): 131-37

23. Shah PG, Sheth AR, Sheth UK. Mode of action of phenobarbital and diazepam on hypothalamic pituitary function in adult male rats. Ind J Exp Biol 1979; 17(8): 776-78

24. D' Souza E, Anholt R, Murphy K, Snyder S, Kuhar M. Peripheral type benzodiazepines receptors in endocrine organs: autoradiographic localization in rat pituitary, adrenal and testis. Endocrinol 1985; 116: 567-73

25. Antkiewicz-Michaluk, Guidotti A, Krueger KE. Molecular characterization and mitochondrial density of a recognition site for peripheral type benzodiazepine ligands. Mol Pharmacol 1988; 34(3): 272-78

26. Ritta MN, Calandra RS. Testicular interstitial cells as targets for peripheral benzodiazepines. Neuroendocrinol 1989; 49: 262-66

27. Vassilios P. Peripheral type benzodiazepine / diazepam binding inhibitor receptor: biological role in steroidogenic cell function. Endo Rev 1993; 14(92): 222-40

28. Wayne C, Mathew P, James F. In: Reproductive Endocrinology, Surgery and Technology. Eli Y Adashi, John, A. Rock and Zev Rosenwaks (Eds.) Lippincott-Raven Vol 1; 1996; pp: 506-525

29. Oke Bo, Suarez-Quain CA, Riond J, Ferrara P, Papadopoulos V. Cell surface localization of the peripheral type benzodiazepine receptor in adrenal cortex. Mol Cell Endocrinol 1992; 87: RI-R6
30. Cook PS, Notelovitz M, Kalra PS. Effect of diazepam on serum testosterone and the ventral prostate gland in male rats. Arch. Androl 1979; 3: 31-35
31. Gaetano F, Renato L, Armando M, Costanzo M, Vincenzo S, Dma N. Neurotransmitters, Opiodergic system, steroid hormone action and involvement in the replacement therapy of sexual disorders. J Steroid Biochem 1990; 37(3): 411-19
32. Fuxe K, Li XM, Tanganelli S, Hedlund P, O'Connor WT, Ferraro L, et al. Receptor-receptor interactions and their relevance for receptor diversity. Focus on neuropeptide/dopamine interactions. Ann NY Acad Sci 1995; 757: 365-76
33. Martino V, Mas M, Davidson JMC. Chlordiazepoxide facilitated erections and inhibits seminal emission in rats. Psychopharmacology 1997; 91: 85-89
34. Agmo A, Fernandez H. Benzodiazepine receptor ligands and sexual behavior in the male rat: the role of GABAergic mechanisms. Pharmacol Biochem Behav 1991; 38(4): 781-88
35. McEwen BS, Cameron H, Chao HM, Gould E, Magarinos AM, Watanabe Y, *et al.* Adrenal steroids and plasticity of hippocampal neurons: toward an understanding of underlying cellular and molecular mechanisms. Cell Mol Neurobiol 1993; 13(4): 457-82
36. Swaab DF, Hofman MA. Sexual differentiation of the human hypothalamus in relation to gender and sexual orientation. Trends Neurosci 1995; 18(6): 264-70
37. Pehek FA, Thompson JT, Hull EM. The effects of intracranial administration of the dopamine agonist apomorphine on penile reflexes and seminal emission in the rat. Brain Res 1989; 23(1-2): 325-32
38. Hull EM, Bazzett TJ, Warner RK, Eaton RC, Thompson JT. Dopamine receptors in the ventral tegmental area modulate male sexual behaviour in rats. Brain Res 1990; 512(1): 1-6
39. Bitran D, Kellogg C K, Hilvers RJ. Treatment with an anabolic-androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical GABAA receptors in the rat. Horm Behav 1993; 27(4):568-83
40. Tupala E, Haapalinna A, Viitamaa T, Mannisto PT, Saano V. Effects of repeated low dose administration and withdrawal of haloperidol on sexual behavior of male rats. Pharmacol Toxicol 1999; 84(6): 292-95
41. Sanbuissho A, Terada S, Suzuki K, Masuda H. Male reproductive toxicity study of nitrazepam in rats. J Toxicol Sc 1995; 20(3): 319-28
42. Means JR, Chengili CP, Jasty V. Testicular toxicity induced by oral administration of SC-32855, a l,4-benzodiazepine, in the dog. Res Commun Pathol Pharmacol 1982; 37: 3-17

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