Institute of Experimental Morphology and Anthropology with Museum

Acta morphologica et anthropologica, 11
Sofia • 2006

Effect of Oxytocin on Leydig Cell Steroidogenic Enzyme Activity in Rat Testis – *in Vivo* and *in Vitro* Studies

Yvetta Koeva, Elisaveta Petrova-Souvandjieva, Lilia Popova

Department of Anatomy, Histology and Embryology, Medical University, Plovdiv, Bulgaria

The present study aimed to establish the change in the activity of the key steroidogenic enzyme 3β hydroxysteroid dehydrogenase (3β HSD) in the Leydig cells following in vivo and in vitro exposure with oxytocin. The short-term effect of oxytocin was studied in mature male Wistar rats that received single injection of 0,25 IU/100 g, and the long-term effect – after a 10-day period of injections of 0,25 IU/100 g per day. Fragments of rat testes were cultured in diffusion chambers with medium 199 and oxytocin (0,005 IU/ml) for 72 h. In vivo treatment with oxytocin increases 3β HSD activity in rat Leydig cells and the rise in enzyme activity after a short-term administration is more pronounced compared to the prolonged treatment. The enzyme-histochemical study showed that oxytocin supplementation upregulates 3β HSD enzyme activity in cultured in vitro rat Leydig cells.The results obtained, showing changes in steroidogenic enzyme activity in rat Leydig cells after in vivo and in vitro treatment with oxytocin, allow us to suggest that this neuropeptide may act as a local modulator of testicular steroidogenesis.

Key words: Oxytocin, testis, Leydig cells, 3 beta HSD.

Introduction

It is now well recognized that neuropeptide oxytocin appears to be both an auto/paracrine and endocrine factor. The intratesticular regulatory role of oxytocin has recently aroused considerable interest. Oxytocin is localized to the testis of several species where it is demonstrated to play an important role in steroidogenesis and seminiferous tubule contractility [6, 11, 12]. The peptide is also produced locally within the testis where it modulates the steroid metabolism and the functional activity of the reproductive tracts [12, 21]. Autoradiographical and immunocytochemical localization show that both oxytocin and oxytocin receptor are present in the mammalian testis, and both are markedly expressed in the Leydig cells (LC) [1, 2, 4, 21, 22, 23]. In vitro studies indicate that mature rat LC are capable of synthesizing and secreting oxytocin in response to stimulation with luteinizing hormone [5]. There is a growing body of evidence that oxytocin may act as a gonadal hormone affecting the LC steroidogenic activity [17]. Although the effects of oxytocin on LC functional activity have recently become a subject of several investigations, the mechanisms of this action are poorly investigated. Data about

the changes in the activity of specific enzymes that are requisite for testosterone biosynthesis in testis following oxytocin exposure are still very insufficient.

In this respect the aim of the present study was to examine the effect of oxytocin on the 3β hydroxysteroid dehydrogenase enzyme activity in rat LC under in vitro conditions and to establish whether comparable effects occurred in vivo.

Material and Methods

Sexually mature Wistar rats (n=8), weighing approximately 180–200 g were housed under conditions of 24-hour light and dark cycles. Foods and water were provided ad libitum. The short-term effect of oxytocin (Gedeon Richter, Hungary) was studied in rats that received single subcutaneous injection of 0,25 IU/100 g, and the long-term effect in animals that received injections of 0,25 IU/100 g per day for a 10-day period. Rats injected only with saline solution were used as control groups. Experimental animals were killed under ether narcosis, following the requirements of the International Convention for Protection of Animals in Experiments Conditions. For the enzymehistochemical study, testicular fragments was frozen and enzyme reaction was performed on fresh cryostat sections (6 μm thick) according to L e v y et al., [13] for visualization of 3β HSD enzyme activity with substrate dehydroepiandrosterone (Fluka). Control sections were incubated in medium without the specific substrate for the corresponding enzyme. For the in vitro investigation testes were removed under sterile conditions and immediately placed on ice in a buffer salt solution containing 100 IU/ml of penicillin, 100 mg/ml streptomycin and 50 IU/ml mycostatin. Fragments of testes were cultured in diffusion chambers with vitelline membranes [7] for 72 hours at 37°C under a water-saturated atmosphere of 95% air and 5% CO_2. Culture medium 199 (Sigma) was supplemented with 50 IU/ml penicillin, 50 μg/ml streptomycin and 2,5 μg/ml fungizone. Oxytocin (obtained in aqueous solution containing 0,9% NaCl) was added to cultures at concentration 0,005 IU/ml, and media was changed every 24h for 3 days. Cultures with hormone free-medium were used as controls. After cultivating enzymehistochemical reaction for 3β HSD was performed on the whole fresh cultivated samples (own modification) with following fixation in 10% neutral formaldehyde solution for 18–20h at room temperature and embedding in paraffin. Part of the cultures were fixed in 10% formaldehyde solution and stained with hematoxylin-eosin (H-E) for routine microscopical analysis. The light microscopical observation and documentation were done with Nikon Microphot CA.

Results

Histological analysis showed that short-term administration of oxytocin caused a significant increase of the 3β HSD activity in rat LC compared to the control animals. Abundant dark-blue granules localized in the cytoplasm of the interstitial LC presented the staining product (Fig. 1, 2). Histochemical reaction positive for 3β HSD was not observed in the seminiferous tubules. No specific staining was found in the control sections (not shown). The prolonged in vivo oxytocin treatment slightly increased the activity of 3β HSD in LC in comparison to the control pattern. The interstitial and peritubular rat LC exhibited enzyme histochemical reaction for 3β HSD with moderate to strong staining intensity (Fig. 3).

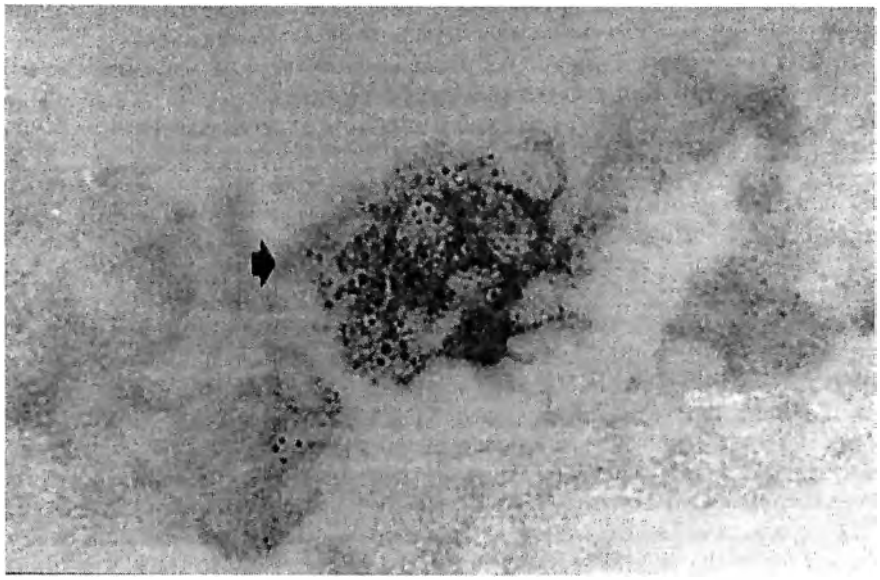

Fig. 1. Rat control testis. 3β HSD-enzyme activity in the cytoplasm of interstitial Leydig cells (arrow) (× 400)

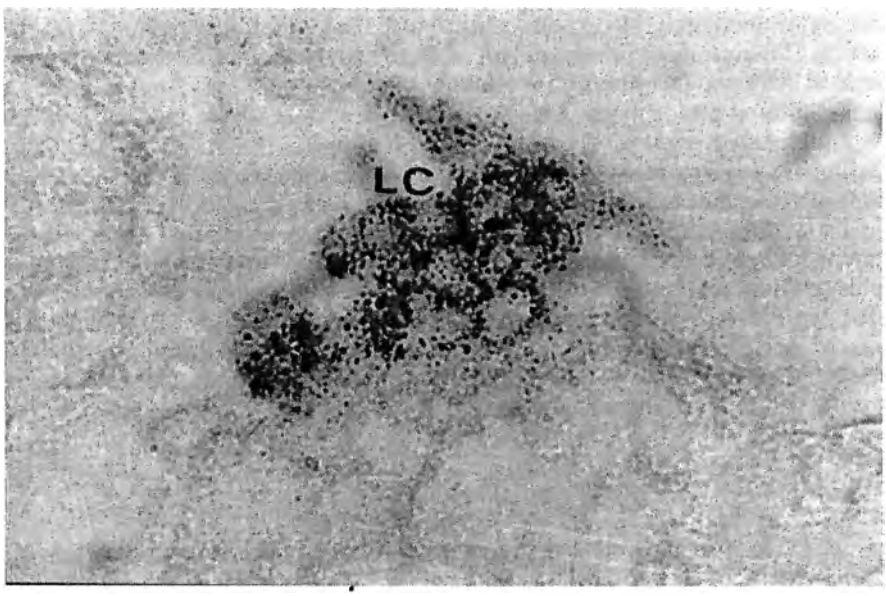

Fig. 2. Rat testis after short-term in vivo oxytocin treatment. Strong 3β HSD-enzyme activity was evident in the Leydig cells (LC) with cytoplasmic localization (× 400)

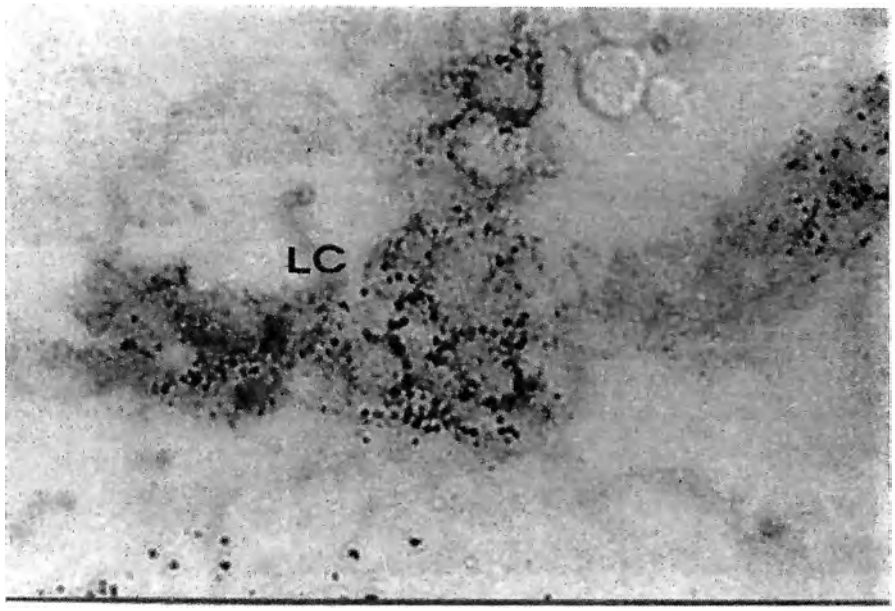

Fig. 3. Rat testis after long-term in vivo oxytocin administration. A slight increase in the 3β HSD-enzyme activity was found in the Leydig cells (LC) (× 400)

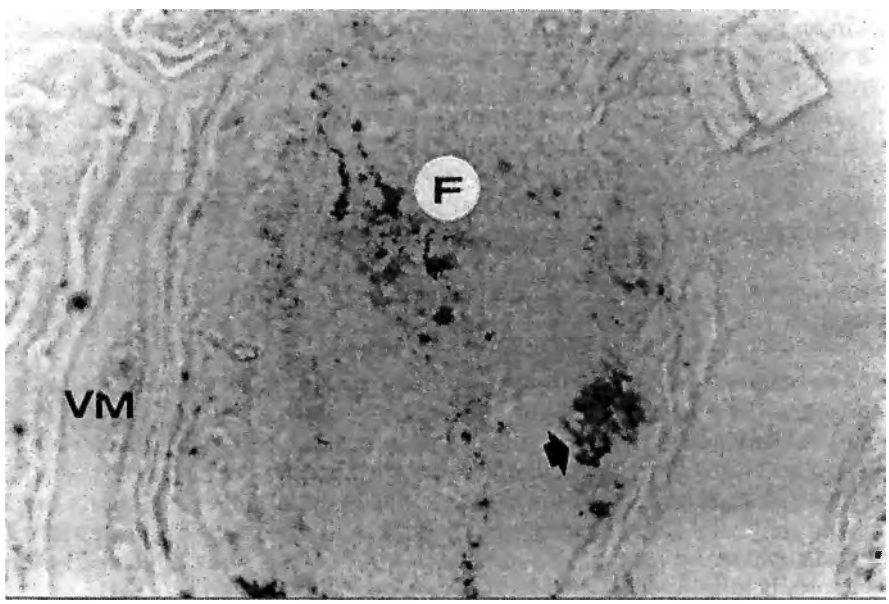

Fig. 4. Fragments of rat testes (F) cultured in diffusion chambers with vitelline membranes (VM). In vitro control culture (72h). Histochemical reaction for 3β HSD in the Leydig cells (arrow) (× 400)

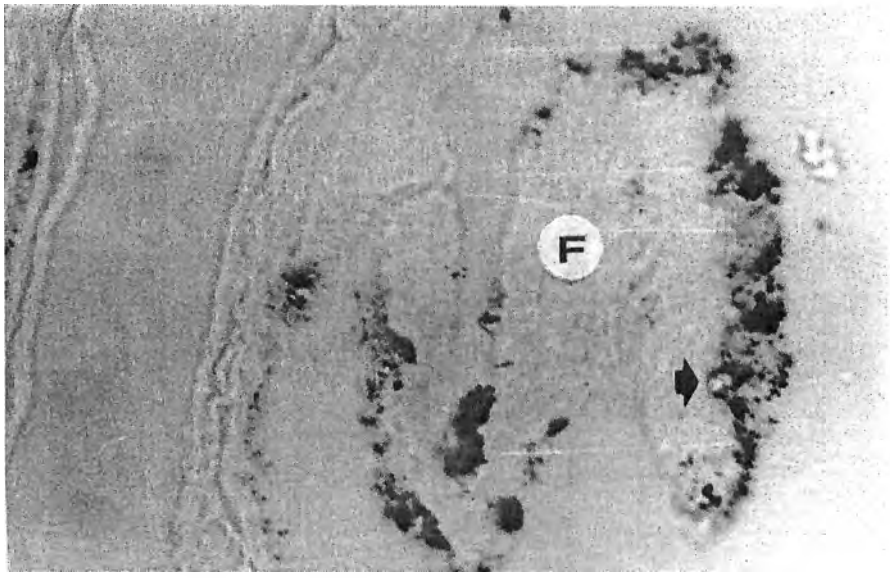

Fig. 5. In vitro culture of rat testicular fragments (F) with oxytocin supplemention (72h). 3β HSD- enzyme activity with strong intensity was observed in the Leydig cells (arrow) (× 400)

In vitro study – pieces of testicular parenchyma are used to evaluate the effect of oxytocin on rat LC steroidogenesis. By the routine light microscopical analysis, a preserved vitality of the testicular fragments under *in vitro* conditions was observed. Staining with H-E showed abundant interstitial LC with their specific morphological features – polygonal shape, eosinophilic cytoplasm and euchromatic round eccentric nuclei (*not shown*). Oxytocin added to culture medium induce rise in 3β HSD enzyme activity in the LC compared to the control cultures without hormone supplemention. The staining product was localized in the cytoplasm of the LC while no specific reaction was detected in the other testicular cell components (Fig. 4, 5).

Discussion

It has been established that oxytocin is present in the mammalian testis and affects steroidogenesis and sperm transport in male reproductive tracts [8, 11]. There are several lines of evidence that neuropeptide oxytocin can modify steroidogenesis in testis in vivo and in vitro exerting a dual effect on LC- stimulatory effect on basal testosterone accumulation during a short-term exposure and inhibitory effect after a long-term administration [5, 14, 20]. The enzyme 3β HSD catalyzes an essential step in the biosynthesis of all steroid hormones and is one of the key enzymes involved in the testosterone production in testis [18]. In the present study we establish that in vivo short-term oxytocin administration in rats results in increasing activity of 3β HSD, which is one of most relevant markers for LC steroidogenic capacity. These findings correspond with previous data indicating a stimulatory effect of oxytocin on the basal testosterone production in vitro and/or serum testosterone concentration during short-term exposure [3, 9, 10, 20]. Our histochemical analysis reveals an increasing 3β HSD enzyme activity in the LC following prolonged in vivo oxytocin treatment, with lower staining intensity compared to the group of single injected rats and in vitro study closely sup-

ports this finding. These results are in contrast to previously described reduction in testicular and plasma testosterone levels following long-term *in vivo* and *in vitro* oxytocin administration [14, 20]. An explanation of this discrepancy might be that the lower limits of testosterone production by LC after the long-lasting action of oxytocin is associated with the increase of 5α-reductase activity which converts testosterone to dihydrotestosterone, thereby decreasing the testosterone level [16]. The results obtained allow suggesting that the low measured levels of the basal testosterone accumulation in vitro and/or serum testosterone concentration do not exclude a stimulating effect of oxytocin on key steroidogenic enzymes in the LC and are a step towards elucidating the mechanisms of this action.

In conclusion, our results demonstrate rise in the steroidogenic enzyme activity in rat LC following in vivo and in vitro exposures with oxytocin and suggest that neuropeptide oxytocin may act as a local modulator in the fine-tuning control of testicular functions.

References

1. Baghate, R.A., C. Sernia. Characterization and localization of oxytocin receptors in the rat testis. – J. Endocrinol., **141**, 1994, No 2, 343-52.
2. Einspanier, A., R. Ivell. Oxytocin and oxytocin receptor expression in reproductive tissues of male marmoset monkey. – Biol. Reprod., **56**, 1997, No 2, 416-22.
3. Frayne, J., H. D. Nicholson. Effect of oxytocin on testosterone production by isolated rat Leydig cells is mediated via a specific oxytocin receptor. – Biol Reprod., **52**, 1995, No 6, 1268-73.
4. Frayne, J., H.D. Nicholson. Localization of oxytocin receptors in the human and macaque monkey male reproductive tracts: evidence for a physiological role of oxytocin in the male. – Mol. Hum. Reprod., **4**, 1998, No 6, 527-32.
5. Frayne, J., H. D. Nicholson. Regulation of oxytocin production by purified adult rat Leydig cells in vitro: effects of LH, testosterone and lipoproteins. – J. Endocrinol., **143**, 1994, No 2, 325-32.
6. Frayne, J., D. Townsend, H. D. Nicholson. Effects of oxytocin on sperm transport in the pubertal rat. – J. Reprod. Fertil., **107**, 1996, No 2, 299-306.
7. Georgiev, I., V. Berbenkova. Cultivation of human gonads in vitro in semipermeable chambers. – Folia Medica (Plovdiv), **19**, 1977, No 2, 101-7.
8. Gerendai, I. Fine-tuning control of testicular functions. Neurobiology-Bp., 5, 1997, No 3, 347-59.
9. Gerendai, I., Z. Csaba, V. Csernus. Testicular injection of 5,6-dihydroxytryptamine or vasectomy interferes with the local stimulatory effect of oxytocin on testicular steroidogenesis in immature rats. – Neuroendocrinology, **63**, 1996, No 3, 284-9.
10. Gerendai, I., V. Csernus. Effect of intratesticular administration of oxytocin on testicular steroidogenesis in immature rats. – Andrologia, **27**, 1995, No 5, 291-7.
11. Harris, G. S., H. D. Nicholson. Characterization of the biological effects of neurohypophysial peptides on seminiferous tubules. – J. Endocrinol., **156**, 1998, No 1, 35-42.
12. Ivell, R., M. Balvers, W. Rust, R. Baghate, A. Einspanier. Oxytocin and male reproductive function. – Adv. Exp. Med. Biol., **424**, 1997, 253-64.
13. Levy, H., H. W. Deane, B. L. Rubin. Visualization of steroid 3β of dehydrogenase activity of tissues of intact and hypophyisectomized rats. – Endocrinology, **65**, 1959, 932-43.
14. Nicholson, H. D., S. E. Guldenaar, G. J. Boer, B. T. Pickering. Testicular oxytocin: effects of intratesticular oxytocin in the rat. – J. Endocrinol., **130**, 1991, No 2, 231-8.
15. Nicholson, H. D, M. P. Hardy. Luteinzing hormone differentially regulates the secretion of testicular oxytocin and testosterone by purified adult rat Leydig cell in vitro. – Endocrinology, **130**, 1992, No 2, 671-7.
16. Nicholson, H. D., L. Jenkin. Oxytocin and prostatic function. – Adv. Exp. Med. Biol., **395**, 1995, 529-38.
17. Nicholson, H. D., B. T. Pickering. Oxytocin, a male gonadal hormone. – Regul. Pept., **45**, 1993, No 1-2, 253-6.
18. O'Shaugnessy, P.J., L. Murphy. Steroidogenic enzyme activity in the rat testis following Leydig cell destruction by ethylene – 1,2-dimethanesulphonateand during subsequent Leydig cell regeneration. – J. Endocrinol., **131**, 1991, 451-457.

19. Pointis, G., A. Tahri-Joutei. Intragonadal control of testicular function by neurohypophyseal-like peptides. – Ann. Endocrinol. (Paris), **51**, 1990, No 5-6, 9-17.
20. Tahri-Joutei, A., G. Pointis. Time-related effects of arginine vasopressin on steroidogenesis in cultured mouse Leydig cells. – J. Reprod. Fertil., **82**, 1988, No 1, 247-54.
21. Ungefroren, H., M. Davidof, R. Ivell. Post-transcriptional block in oxytocin gene expression within the seminiferous tubules of bovine testis. – J. Endocrinol., **140**, 1994, No 1, 63-72.
22. Whittington, K., S. J. Assinder, T. Parkinsson, K. R. Lapwood, H. D. Nicholson. Function and localization of oxytocin receptors in the reproductive tissue of rams. – Reproduction, **122**, 2001, No 2, 317-25.
23. Yeung, W. S., S. E. Guldenaar, R. T. Worley, J. Humphrys, B. T. Pickering. Oxytocin in Leydig cells: an immunocytochemical study of Percoll-purifed cells from rat testes. – Cell Tissue Res. **253**, 1998, No 2, 463-8.