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THE INHIBITORY EFFECT OF OXYTOCIN AND COLCHICINE ON THE VENTRAL PROSTATE OF ADULT RATS

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Oxytocin (OT) was previously reported to have a tropic effect on the epithelial component of the ventral prostate in orchiectomized rats, while in intact rats it was ineffective. The goal of the present study was to obtain more information about the factors interfering with the prostate response to this peptide.

Wistar rats received daily s. c. injections of synthetic OT (0. 25 IU/100 g) for 3 consecutive days and were killed 6 hours later. For one group of animals the last injection was supplemented with colchicine (0.1 mg/100 g).

In intact rats OT affected the prostate gland only when applied in combination with colchicine. This treatment caused a reduction of epithelial height, the volume density and the total volume of the epithelium, whereas an enhancement of the volume density and the mean diameter of the acionar lumen was recorded. Glandular noradrenaline content was decreased. These changes demonstrated a diminished secretory activity of the glandular epithelium and suppression of expelling the secretion. It seems that this effect was achieved by synergistic action of OT and colchicine at the level of the sympathetic control of the gland.

Key-words: Rat ventral prostate, oxytocin, colchicine.

INTRODUCTION

Our previous studies concerning the effects of oxytocin (OT) on the rat ventral prostate showed that systemically applied OT was effective only in orchiectomized animals. It partially prevented the postcastrational involution of the gland (Popović et al., 1990) by decreasing the apoptotic degeneration and by increasing the proliferative activity of the secretory epithelial cells (Plećaš et al., 1992). We assumed that the failure of OT to induce changes in the prostate gland of intact rats, when administered for 5 and 10 days, might be due to desensitization of the OT affected structures, or to inability to evoke a

distinct prostate response under the normal function of its hormonal and neural control systems. Another possibility was that OT acted via different targets which exerted antagonistic effects resulting in an unaltered status of the glandular epithelium. One pathway of inhibitory action of OT could be through the noradrenergic innervation (Carvalho et al., 1990), tropic for the epithelial cells (Wang et al., 1991), since a suppressory effect of OT on sympathetic preganglionic neurons has been found (Coote et al., 1982; Gilbey et al., 1982). To provide more information about factors interfering with the prostate response to OT in this study we applied a shorter, 3-day OT treatment. The presumed interaction of OT with the sympathetic innervation of the gland was examined using colchicine, an antimitotic agent known to inhibit axonal transport of noradrenaline containing granules (Dahlström, 1968). Prostate response was evaluated by stereological parameters, the content of catecholamines and the metaphase index of epithelial cells. A morphometric analysis of the seminal vesicle was also made because both the accessory glands are innervated by the same, pelvic ganglia (Langworthy, 1965).

MATERIAL AND METHODS

Adult Wistar rats (300-350 g) were maintained under controlled conditions of light (12 hours on/12 hours off) and temperature (21 ± 2^0C), with constant access to standard food and water. They received daily subcutaneous injections of synthetic OT (0.25 IU/100 g; Sandoz) diluted with saline or saline alone for 3 consecutive days. Alternatively, the final injection was supplemented with colchicine (0.1 mg/100 g; The British Drug Houses). Six hours later the animals were killed by cervical dislocation.

Morphometry

Ventral prostates and seminal vesicles from 5 animals per group were isolated, individually weighed, immersed in Bouin's fluid, dehydrated in a graded series of alcohols and embedded in paraffin. Five-μm-sections, random for the ventral prostate and transversal for the seminal vesicle, were cut and stained with haematoxylin and eosin.

Stereological measurements of the ventral prostate were perfomed by the point and intersection counting method (Weibel, 1979), with the aid of the multipurpose test system (42 points, 21 lines) in 50 randomly selected test areas per animal at a magnification of 125 x, as detailed elsewhere (Popović et al., 1990; Plećaš et al., 1992).

The height of the seminal vesicle epithelium was measured using an ocular micrometer on 15 randomly selected regions surrounding the main acini of each gland, at a final magnification of 1000 x.

Metaphase-arrest count

The metaphase-arrested glandular cells of the ventral prostate were counted in two sections at a magnification of 400 x. The total number of glandular

cells in those sections was calculated stereologically (Plećaš et al., 1992). For determination of metaphase index of seminal vesicle epithelial cells, 1500 glandular cells of the central acinus were counted. The metaphase indices were expressed per 1000 glandular cells.

Catecholamine measurement

Ventral prostates from 9 animals per group were quickly removed, immediately frozen and kept at -20^0 C. Noradrenaline and dopamine contents were determined fluorimetrically (Laverty & Taylor, 1968).

Testosterone measurement

At the time of sacrifice, trunk blood from 11 animals per group was collected and serum specimens were frozen and stored at -20^0 C. Testosterone concentrations were determined by RIA (Testosterone MAIA; Biodata).

Statistical analysis

The results are presented as means ± SEM. Statistical analysis of the data was performed using the Wilcoxon test, except for the catecholamine content data, which were analysed by the aid of one-way analysis of variance followed by the Tukey test.

RESULTS

Ventral prostate responded to OT only when the last injection was combined with colchicine.

Stereological analysis showed (Table 1,2,3) that the height of the epithelium was reduced by 19%, the volume density of the epithelium by 22%, while the volume density of the acinar lumen was enhanced by 22%. The 21% decrease in the total volume of the epithelial component was accompanied by a 21% increase in the mean diameter of the acinar lumen. There was no change in the number of the epithelial cells. It was 106.8 x 10^6 ± 3.3 x 10^6 in colchicine-injected controls and 101.1 x 10^6 ± 6.2 x 10^6 in rats subjected to the combined OT and colchicine treatment.

Table 1. Volume fractions of glandular epithelium (V_{vep}), acinar lumen (V_{vlu}) and interacinar stroma (V_{vst}), surface density of epithelium (S_{vep}) and length density of glandular tubules (L_{vtu}) in ventral prostates of rats receiving OT or OT and colchicine (Co).

Treatment	V_{vep} (mm^3/mm^3)	V_{vlu} (mm^3/mm^3)	V_{vst} (mm^3/mm^3)	S_{vep} (mm^2/mm^3)	L_{vtu} (mm/mm^3)
Saline	0.24±0.03	0.56±0.02	0.20±0.01	13.3±0.4	18.9±0.4
OT	0.19±0.02	0.58±0.02	0.22±0.03	13.1±1.8	19.9±2.4
Saline + Co	0.27±0.02	0.48±0.03	0.25±0.02	12.9±0.4	22.3±2.0
OT + Co	0.21±0.01**	0.57±0.03*	0.22±0.02	12.5±0.4	20.5±0.9

Data are expressed as mean±SEM. *P<0.05; **P<0.01 compared to the appropriate control.

Table 2. Absolute volumes of glandular epithelium (V_{ep}), acinar lumen (V_{lu}) and interacinar stroma (V_{st}), total surface of epithelium (S_{ep}) and total length of glandular tubuli (L_{tu}) in ventral prostates of rats receiving OT or OT and colchicine (Co).

Treatment	Vep (mm^3)	V_{lu} (mm^3)	V_{st} (mm^3)	S_{ep} (mm^2)	L_{tu} (mm)
Saline	82±8	193+14	70±5	4587±68	6504±123
OT	61±8	185±19	69±6	4075±265	6124±472
Saline + Co	107±7	188±14	98+8	5047±226	8639±540
OT + Co	84±7*	221±11	88±14	4883±226	8072+530

Data are expressed as mean+SEM. *P<0.05 compared to the appropriate control.

Table 3. Epithelial height (H), diameters of acinar lumen (D_{lu}) and acini (D_{ac}), and free distance between acini (l) in ventral prostates of rats receiving OT or OT and colchicine (Co).

Treatment	H (μm)	Dlu (μm)	λ (μm)
Saline	18.1±1.8	168±11	97±3
OT	15.0±2.0	181±11	105±4
Saline + Co	21.3±1.6	149±10	118±7
OT + Co	17.2±1.1*	181± 8*	107±6

Data are expressed as mean+SEM. *P<0.05 compared to the appropriate control.

The metaphase index revealed that the treatment applied did not affect the mitotic activity of the epithelium. In colchicine-treated controls there were 0.18+0.07 mitoses per 1000 cells and 0.23±0.07 in rats receiving OT and colchicine.

The catecholamine content was not altered in rats injected with colchine alone, but in those treated with OT and colchicine the noradrenaline content was significantly decreased, whereas the dopamine content was unchanged (Figure 1).

Prostate weight was not influenced by any of the treatments (Table 4).

Table 4. Ventral prostate weights and serum testosterone concentrations in rats receiving OT or OT and colchicine (Co).

Treatment	Ventral prostate (mg)	Testosterone (ng/ml)
Saline	345 ± 14	2.86 ± 0.85
OT	315 ± 21	5.17 ± 1.69
Saline + Co	393 ± 15	0.24 ± 0.02
OT + Co	393 ± 21	0.27 ± 0.03

Data are expressed as mean±SEM.

Seminal vesicle, like the prostate gland, responded to OT only when the joint treatment of OT and colchicine was applied (Table 5). The height of the glandular epithelium was decreased by 17% but the number of dividing epithelial cells was not significantly different. It was 0.04±0.01 and 0.11±0.04 per 1000

cells in OT-treated rats and their controls, respectively. Significant changes were not found in the weight of the gland.

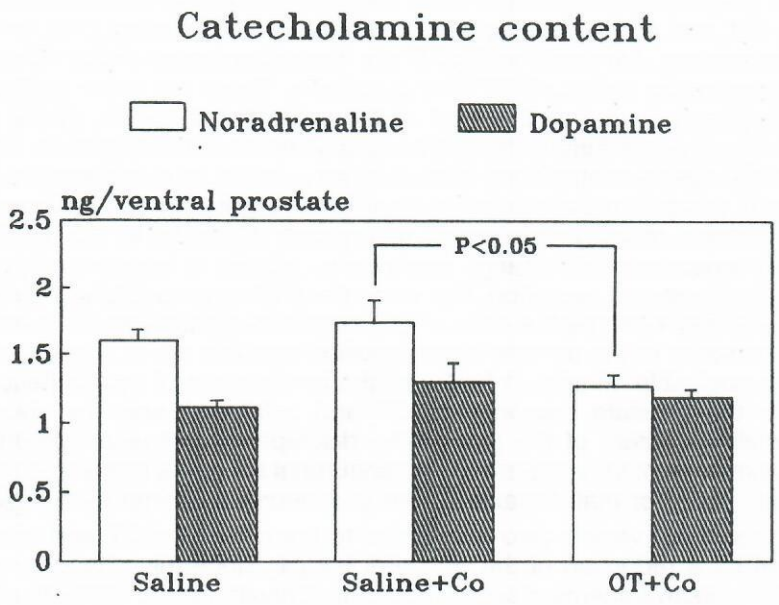

Figure 1. Ventral prostate noradrenaline and dopamine content in rats subjected to OT and colchicine (Co) treatment. Data are expressed as mean values for 9 animals per group ± SEM.

Table 5. Seminal vesicle weights and epithelial height (H) in rats receiving OT or OT and colchicine (Co).

Treatment	Seminal vesicle (mg)	H (μm)
Saline	936 ± 64	19.0 ± 1.0
OT	813 ± 71	16.6 ± 1.4
Saline + Co	653 ± 52	18.1 ± 0.5
OT + Co	561 ± 22	15.1 ± 0.3*

Data are expressed as mean ± SEM. *P<0.005 compared to the appropriate control.

Serum testosterone level was not affected by OT, while colchicine alone caused a 10-fold decrease in its concentration. OT was ineffective and when applied in combination with colchicine (Table 4).

DISCUSSION

The present data clearly demonstrate that OT was effective in causing changes in the ventral prostate only when the last injection was combined with colchicine. That treatment significantly decreased the volume density of the

epithelial component, the absolute volume of the glandular epithelium and its height, while plasma testosterone level was the same as in the colchicine-treated controls. A tendency towards the same changes was pronounced in rats receiving only OT, the corresponding parameters being reduced by 21%, 26% and 17%, respectively. Although not significant, these decreases indicated a synergistic suppressory action of OT and colchicine. There are some indices that this synergism occured at the level of the sympathetic control of the gland. The enhanced acinar lumen showed an accumulation of the secretion, normally expelled by muscle contractions evoked by an α_1 effect of noradrenaline (Wang et al., 199l). In addition, the reduction in epithelial height pointed to on attenuated tropic influence of its noradrenergic innervation (Carvalho et al., 1990). The possibility exists that this change was due to increased intraluminal pressure caused by the stored secretion. However, the finding (unpublished data) that, in rats receiving 4-fold higher dose of OT, such a decrease was not associated with an increase in the acionar lumen showed that this effect, if present at all, could be negligible. Finally, in favour of the involvement of sympathetic innervation in the prostate response to OT and colchicine was the decreased noradrenaline content of the gland. The discrepancy between noradrenaline and dopamine content suggests an extraneuronal source of dopamine (Adams-Ray et al., 1966) or that dopamine itself is a neurotransmitter in this gland.

The seminal vesicle also responded to the combined OT and colchicine treatment by a reduction of the epithelial height. Since this change was also seen after selective chemical sympathectomy (Carvalho et al., 1990), this finding and the fact that both accessory glands are innervated by the same ganglia (Langworthy, 1965) supported the view that sympathetic innervation was involved in the prostate response to OT and colchicine.

The possible site(s) of OT action might be the sympathetic preganglionic neuron which was shown to respond to OT by a diminished activity (Coote et al., 1982; Gilbey et al., 1982). However, for colchicine it could be the ganglionic neuron, because the related antimitotic drug vinblastine influenced the noradrenaline content of some viscera in the same way (Keen and Livingston, 1970). Taken together, these data strongly suggest that OT and colchicine induced a prostate response via neuronal pathways conveying sympathetic influences to the gland. The 12-hour and 30-hour delay in expression of the vinblastine effect might explain our finding of an unaltered prostatic noradrenaline content 6 hours after colchicine administration.

The lack of changes in the ventral prostate of rats subjected to the 5-day OT and colchicine treatment, revealed in our previous study (Plećaš et al., 1992), indicates that the mechanism(s) underlying the prostatic response to OT may undergo desensitization.

Judging by the failure of OT to induce significant changes in the epithelial component of the ventral prostate when applied alone, OT is not potent enough to disturb the balance among the factors of hormonal and neural regulation of its secretory activity. On the other hand, it might also mean that OT has a multiple action, involving structures with antagonistic influences, their sum effect

being the unaltered state of prostatic epithelium. In favour of the last assumption is the stimulatory effect found in castrated rats (Popović et al., 1990; Plećaš et al., 1992) and the suppressory one shown in this study.

The results of this and our previous study strongly suggest that OT is involved in the regulation of ventral prostate activity. There are much indices that the tropic effect obtained in orchiectomized rats (Popović et al., 1990; Plećaš et al., 1992) is achieved by direct action of the peptide, a finding which may contribute to understanding of the functional meaning of the high content of OT in the gland (Nicholson et al., 1985). The suppressory effect at the level of the adrenergic control of the gland possibly represents protection from over-stimulation during enhanced activity of the sympathetic system.

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INHIBITORNI UTICAJ OKSITOCINA I KOLHICINA NA VENTRALNU PROSTATU ADULTNIH PACOVA

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U ranijim publikacijama smo pokazali tropno delovanje oksitocina (OT) na epitelnu komponentu ventralne prostate orhidektomisanih pacova, dok intaktni pacovi nisu reagovali na primenjeni tretman. Svrha ovog rada je da pruži više informacija o faktorima od kojih zavisi reagovanje ove žlezde na OT.

Wistar pacovi su injicirani s.c. sintetskim OT (0.25 IU/100 g/d) u toku 3 dana i žrtvovani nakon 6 časova. Jednoj grupi životinja uz poslednju injekciju je dat i kolhicin (0.1 mg/100 g).

U intaktnih pacova OT izaziva promene u prostati samo kada se aplikuje u kombinaciji sa kolhicinom. Izražene stereološkim parametrima one se sastoje u smanjenju visine epitela, njegove volumenske gustine i ukupne zapremine, a povećanju volumenske gustine i prosečnog dijametra lumena acinusa. Sadržaj noradrenalina u žlezdi je smanjen. Ovi podaci pokazuju smanjenu aktivnost sekretnog epitela i supresiju evakuacije sekreta. Izloženi su argumenti u prilog gledištu da je dobijeni efekat proizvod sinergističkog inhibitornog delovanja OT i kolhicina na nivou simpatikusne kontrole žlezde.