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STEREOLOGIC STUDY ON THE EFFECT OF PROLONGED URAPIDIL-TREATMENT ON THE RAT VENTRAL PROSTATE

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This work was performed in order to examine the long-term effect of urapidil, an α_1 - adrenoceptor antagonist, on the structure of the rat ventral prostate. Adult rats were injected s. c. with urapidil in a daily dose of 0.02 mg/100 g for 15 or 30 days, or with 0.04 mg/100 g during 30 days. Stereological analysis was done on paraffin sections stained with hematoxylin and eosin. In rats receiving urapidil for 15 days the relative and absolute volumes, as well as the height of the epithelium, were significantly increased, while the diameter of the acinar lumen and the percentage of acini containing cuboidal epithelium were decreased. These changes were not found following 30 days of treatment, but a tendency towards an increase in epithelial volume was observed. The higher dose of urapidil was ineffective. These results indicate that prolonged administration of urapidil may evoke a contractile response of the ventral prostate that is followed by a mild hyperplasia of the glandular epithelium.

Key words: Rat ventral prostate, urapidil, α_1- adrenoceptor antagonist, stereology.

INTRODUCTION

In the recent literature much emphasis is put on the role of autonomic innervation in the regulation of growth and function of the prostate.

The rat prostate is innervated by both sympathetic and parasympathetic nerve fibers (Langworthy, 1965; Purinton et al., 1972; Vaalasti and Hervonen, 1979). The sympathetic input is supplied by the hypogastric nerve, and the parasympathetic input is provided by the pelvic nerve. Adrenergic fibers are in close proximity to the smooth muscle cells surrounding the prostatic ducts, whereas the cholinergic fibers innervate mainly the glandular epithelial cells. Adrenergic stimulation of the gland evokes a large secretory response by con-

traction of prostatic smooth muscle, while the cholinergic stimulation has a secretory effect that appears to be due to direct stimulation of epithelial secretion (Wang et al., 1991a).

The distribution of adrenoceptors indicates, however, that sympathetic innervation also influences the epithelial component of the gland. Radioligand binding and autoradiographic studies have characterized several types of adrenergic receptors in prostatic tissue. α_1-Adrenoceptors are predominantly located in the prostatic stroma and mediate the contractile response (James et al., 1989; Killam et al., 1995). α_2-Adrenergic receptors are present mainly in the region of the blood vessels and glandular epithelium (James et al., 1989; Felsen et al., 1994), but their role is still unknown. β-Adrenoceptors are found in the vicinity of prostatic epithelial cells and a possible influence on epithelial activity has been suggested (Dube et al., 1986).

Although α_1-adrenoceptor antagonists are broadly used in therapy (Ruffolo and Hieble, 1994), there have been no reports on their chronic effects on normal prostatic tissue. The aim of the present paper was, therefore, to examine whether a prolonged α_1-adrenoceptor blockade affects the structure of the rat ventral prostate. Urapidil, an antihypertensive drug, was chosen as a selective α_1-adrenoceptor antagonist (Testa et al., 1993; Ruffolo and Hieble, 1994).

MATERIAL AND METHODS

Adult Wistar rats (250 - 300 g) were housed under controlled conditions (12 hours light, 12 hours dark) with food and water ad libitum. They were treated with urapidil (Ebrantil, Byk Gulden) for 15 or 30 consecutive days. The doses of urapidil were: 0.02 mg/100 g/day for the shorter and 0.02 or 0.04 mg/100 g/d for the longer tratment. Urapidil solutions, diluted with saline, were prepared fresh every day and injected subcutaneously. Control animals received saline by the same route.

One hour after the last injection the rats were killed by decapitation in light ether narcosis. The ventral lobe of the prostate was immediately dissected, weighed, fixed in Bouin's fluid and processed for standard light microscopy. Six-micrometer sections were cut, mounted on glass slides and stained with hematoxylin and eosin.

Morphometric analysis. For each animal 10 sections from the three representative regions of the gand (tnat is distal, intermediate and proximal) were chosen. Fifty test areas per gland were stereologically analyzed using the multipurpose test system M_{42} (Weibel, 1979) at a final magnification of 100 x.

Stereology was carried out following the procedure of Huttenen et al. (1981). By counting test points falling on various compartmens of the gland, the volume fractions of the epithelium, acinar lumen, interacinar stroma and blood vessels were determined, while counting the intersections of the test lines with

the apical surface of glandular epithelial cells, the surface density of the epithelium was estimated. Because of the striking regional heterogeneity of the epithelium within the rat ventral prostate with respect to cell (Hayashi et al., 1991), acini with cuboidal epithelium, as a site of programmed cell death (Sensibar et al., 1991), were additionally analyzed by determining the percentage of acini profiles containing this type of epithelial cell. Since the specific gravity of the rat ventral prostate is 1 (DeKlerk and Coffey, 1978), the total volume of each compartment was calculated by multiplying each fraction with the total tissue weight. Therefore total volumes of the epithelium, acinar lumen, interacinar stroma and blood vessels were obtained. The volume fractions and surface density of the epithelium were used also to calculate mean height of the epithelium, mean diameter of the acinar lumen and mean distance between acini.

Statistical analysis. All data are expressed as means for 5 animals per group $\pm$ SEM. Statistical analyses were carried out by the Wilcoxon test for comparison between two groups and one-way ANOVA followed by Duncan's multiple comparisons when more than two groups had to be compared.

RESULTS

Treatment with urapidil did not influence the body weight gain in any experimental group.

As can be seen from Tables 1 and 2, the 15-day treatment with the lower dose of urapidil significantly increased the relative (47%; $P<0.05$) and absolute (48%; $P<0.05$) volumes of glandular epithelium. These changes were due to an increase in the mean height of the epithelium (35%; $P<0.05$) (Table 3). Compared with the control animals, the ventral prostate of urapidil-treated rats contained a four times lower percentage of acini profiles with cuboidal epithelium (2.64 ± 0.7 vs. 10.85 ± 3.73 in controls; $P<0.05$). At the same time the mean diameter of the acinar lumen was significantly reduced (21%; $P<0.025$) (Table 3).

Table 1. Volume fractions (mm^3/mm^3) of glandular epithelium (Vvep), acinar lumen (Vvlu), interacinar stroma (Vvst) and blood vessels (Vvbv) in ventralprostates of urapidil-treated rats.

Treatment	Vvep	Vvlu	Vvst	Vvbv x 10^{-3}
15 days				
Saline	0.171 ± 0.021	0.617 ± 0.026	0.212 ± 0.017	6.76 ± 2.47
Urapidil 1	0.251 ± 0.022*	0.538 ± 0.036	0.216 ± 0.022	7.33 ± 1.48
30 days				
Saline	0.198 ± 0.007	0.603 ± 0.015	0.200 ± 0.014	6.38 ± 0.91
Urapidil 1	0.210 ± 0.016	0.613 ± 0.026	0.177 ± 0.016	6.10 ± 1.15
Urapidil 2	0.180 ± 0.013	0.641 ± 0.014	0.179 ± 0.016	7.05 ± 0.70

Urapidil 1: 0.02 mg/100 g/d; Urapidil 2: 0.04 mg/100 g/d. Data are expressed as mean $\pm$ SEM. *, $P<0.05$ compared with respective control.

Table 2. Absolute volumes (mm^3) of ventral prostate (Vovp), glandular epithelium (Voep), interacinar stroma (Vost) and blood vessels (Vobv) in urapidil-treated rats.

Treatment	Vovp	Voep	Volu	Vost	Vobv
15 days					
Saline	385 ±24	65 ± 7	238 ± 18	82 ± 9	2.52 ± 0.83
Urapidil1	383 ±25	96 ± 11*	206 ± 24	81 ± 5	2.83 ± 0.55
30 days					
Saline	359 ± 24	71 ± 5	217 ± 17	71 ± 6	2.34 ± 0.41
Urapidil1	454 ± 40	94 ± 7*	282 ± 34	78 ± 5	2.70 ± 0.49
Urapidil2	372 ± 34	69 ± 11	239 ± 23	65 ± 5	2.61 ± 0.35

Urapidil 1: 0.02 mg/100 g/d; Urapidil 2: 0.04 mg/100 g/d.Data are expressed as mean ± SEM. *, $P<0.05$ compared with respective control; *, $P<0.05$ compared with Urapidil 2.

Table 3. Epithelial height (h), diameter of acinar lumen (Dlu) and acini (Dac), and free distance between acini (g) in ventral prostates of urapidil-treated rats.

Treatment	h (μm)	Dlu (μm)	Dac (μm)	λ (μm)
15 days				
Saline	16.7 ± 2.0	242 ± 10	276 ± 8	117 ± 9
Urapidil 1	22.5 ± 2.6*	190 ± 13**	235 ± 12	123 ± 13
30 days				
Saline	16.2 ± 1.1	197 ± 7	229 ± 9	97 ± 6
Urapidil 1	17.8 ± 1.5	207 ± 9	243 ± 8	95 ± 7
Urapidil 2	15.0 ± 1.6	213 ± 17	243 ± 19	88 ± 3

Urapidil 1: 0.02 mg/100 g/d; Urapidil 2: 0.04 mg/100 g/d. Data are expressed as mean ± SEM. *, $P<0.05$, **, $P<0.025$ compared with respective control values.

A cursory histological examination revealed that in the ventral prostate of urapidil-treated rats alveoli were contracted and convoluted throughout the whole gland (Figure 1b). In control rats most alveoli were round in shape, fully extended and filled with fluid (Figure 1a).

After 30 days of treatment with urapidil a significant change was found only in the total volume of the glandular epithelium of rats receiving the lower dose (Tables 1 to 3). It was increased (36%; $P<0.05$) in comparison with the value determined in animals treated with the higher dose of urapidil. In comparison with controls the increase (32%) was statistically significant ($P<0.06$) only when the Wilcoxon test was employed for testing the differences. Although increases in the glandular weight and total volume of acinar lumen were also found (27% and 30%, respectively), they did not reach statistical significance.

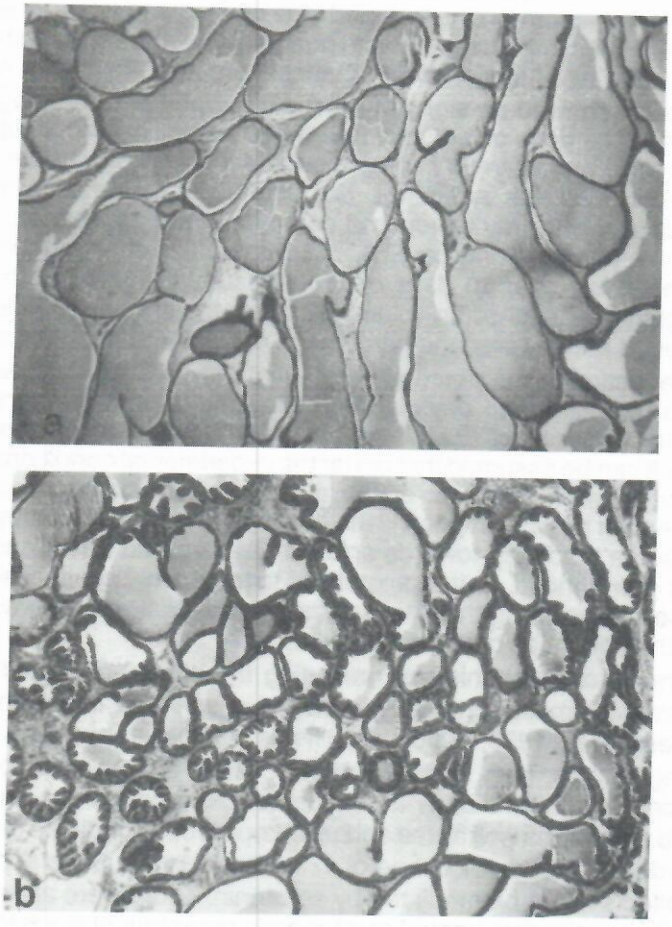

Figure 1. Ventral prostate of a) saline- and b) urapidil-injected rats. In animals receiving urapidil for 15 days alveoli are contracted and convoluted over the entire gland. x56.

DISCUSSION

The results of this work showed that prolonged administration of the selective α_1-adrenoceptor antagonist urapidil affected both the muscular and the epithelial component of the rat ventral prostate. The effect was stimulatory and dependent on the dose and duration of the treatment.

Sympathetic innervation appears to play a fundamental role in the regulation of prostate growth and function. Denervation in the rat ventral prostate is associated with a lower glandular weight, decreased cell height and reduced

secretory activity (Wang et al., 1991b). Similar changes were found following unilateral sympathectomy in the lesioned side of the gland (McVary et al., 1994). After total chemical sympathectomy with guanethidine, the rat prostate displayed structural atrophy and functional disturbance (Lamano Carvalho et al., 1990).

On results showed no signs of prostatic atrophy after prolonged urapidil administration. The dose of 0.02 mg/100 g/d of urapidil had a stimulatory effect on the contractile and secretory components of the ventral prostate, whereas a two-fold higher dose was ineffective. The contractile response, evident by the reduced diameter of acinar lumen and contracted and convoluted alveoli, was present only in rats receiving urapidil for 15 days. The effect on the epithelium persisted, however, over 30 days or urapidil administration. Both treatments enhanced glandular epithelium volume, but only the shorter one increased the epithelial height and lowered the percentage of acini with cuboidal epithelium.

The reduction in the percentage of acini with cuboidal epithelium in rats treated with urapidil for 15 days is the finding that deserves attention. As the programmed epithelial cell death takes place in the acini of this type (Sensibar et al., 1991), it can be assumed that under the experimental conditions described, the balance between glandular epithelial cell proliferation and death was disturbed. In favor of this assumption is the observation that after 30 days of urapidil administration a tendency towards epithelial hyperplasia and glandular hypertrophy was pronounced. In the prostate of these animals epithelial height was not increased, suggesting that the increase in epithelial volume was the consequence of the enhanced cell number.

The receptor mechanism(s) mediating the effects of urapidil on the rat ventral prostate remains unclear. Urapidil is a selective α_1-adrenoceptor antagonist (Testa et al., 1993; Ruffolo and Hieble, 1994) with a weak β_1-adrenoceptor blocking activity (van Zwieten, 1990). There are no data on its partial α_1-adrenoceptor agonistic or α_1-adrenoceptor blocking activity. The contractile response to urapidil, as well as the epithelial hypertrophy, indicate that the effects of urapidil do not involve blockade of α_1-adrenoceptors. Several data suggest that both effects might be mediated by serotonin. Thus, there are some indices that urapidil can stimulate central serotonergic receptors of the S HT_{1A}-subtype (Ramage, 1991). The prostate contains relatively high concentrations of serotonin (Abrahamsson and Di Sant' Agnese, 1993), and the presence of its receptors has been demonstrated in rat prostatic tissue (Killam et al., 1995). Serotonin was shown to induce prostatic contraction by activation of 5-HT_2 receptors and indirect activation of a_1-adrenoceptors (Killam et al., 1995). Finally, some reports indicate that it has growth factor activity (Noordzij et al., 1995). Alternatively, urapidil could stimulate the release of serotonin which could overcome the blocking effects of urapidil on α_1-adrenoceptors.

The absence of the contractile response in rats receiving urapidil for 30 days, shown by the unaltered diameter of acinar lumen, might be due to desensitization of the mechanism (s) mediating this effect, or to activation of intraglandular homeostatic mechanisms regulating expulsion of the secretion.

The ineffectiveness of the higher dose of urapidil in altering prostatic structure might include down-regulation of the receptors involved or possibly an interaction with adrenergic targets that prevent or negate its effects at the prostate level. Thus, it has been shown that only large doses of urapidil reduce preganglionic sympathetic nerve activity in rats (Gillis et al., 1987).

Surprisingly, there have been no reports on the chronic effects of α_1-adrenoceptor antagonists on normal prostatic tissue. Our results point to a possible difference between the chronic and acute effects of these agents on the prostate, when it is well known that acute effects are related to relaxation of prostate smooth muscle (Felsen et al., 1994; Killam et al., 1995). Since, several α_1-adrenoceptor blocking agents are currently under investigation for the treatment of benign prostatic hypertrophy (Ruffolo and Hieble, 1994), the data obtained in this work might have some useful medical implications.

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STEREOLOŠKO ISPITIVANJE UTICAJA PRODUŽENOG TRETMANA URAPIDILOM NA VENTRALNU PROSTATU PACOVA

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SADRŽAJ

U cilju ispitivanja uticaja produženog tretmana urapidilom, antagonistom α_1-adrenergičnih receptora, na strukturu ventralne prostate, polno zreli pacovi su primali urapidil s. c. u dnevnoj dozi od 0.02 mg/100 g tokom 15 ili 30 dana ili u dozi od 0.04 mg/100 g tokom 30 dana. Stereološka analiza ventralne prostate je rađena na parafinskim isečcima obojenim hematoksilinom i eozinom. U žlezdi pacova tretiranih urapidilom 15 dana značajno su povećane relativne i apsolutne zapremine, kao i visina epitela, dok je dijametar lumena acinusa i procenat acinusa sa kuboidnim epitelom smanjen. Posle tridesetodnevnog tretmana istom dozom urapidila zapaženo je samo izvesno povećanje ukupne zapremine epitela. Veća doza urapidila je bila neefektivna. Dobijeni rezultati ukazuju na to da produženi tretman urapidilom može da izazove kontraktilni odgovor ventralne prostate, posle koga sledi blaga hiperplazija epitela.