EFFECTS OF $\alpha_1$-ADRENERGIC RECEPTOR SUBTYPE SELECTIVE ANTAGONISTS ON LOWER URINARY TRACT FUNCTION IN RATS WITH BLADDER OUTLET OBSTRUCTION

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ABSTRACT

Purpose: Antagonists of $\alpha_1$-adrenergic receptors ($\alpha_1$ARs) relieve obstructive and irritative symptoms in patients with bladder outlet obstruction. However, to our knowledge mechanisms underlying the relief of irritative symptoms remain unknown. Because bladder $\alpha_{1d}$ARs are up-regulated in some rats with bladder outlet obstruction, we investigated the effect of the $\alpha_1$AR antagonist 5-methyl urapidil (5MU) vs the $\alpha_{1a}/\alpha_{1d}$AR antagonist tamsulosin on urinary frequency in obstructed rats.

Materials and Methods: Baseline frequency was measured using a chronic micturition recording system and then obstruction (40 rats) or sham obstruction surgery (11 rats) was performed. After 6 weeks frequency was reassessed, followed by subcutaneous implantation of osmotic pumps to deliver 5MU, tamsulosin or vehicle for 1 week. Upon the completion of drug treatment urinary frequency was again measured and the pressor response to the $\alpha_1$AR agonist phenylephrine was documented.

Results: Obstructed bladder mass was an average of 4.9 times greater than bladder mass in sham operated rats ($p < 0.001$). Urinary frequency was elevated in obstructed rats with a bladder mass of greater than 500 mg vs all rats with a bladder mass of under 255 mg ($p = 0.01$). Of rats with a bladder mass of greater than 500 mg frequency was decreased in those treated with tamsulosin ($p = 0.03$) but not in those treated with 5MU. Tamsulosin and 5MU inhibited the pressor response to phenylephrine.

Conclusions: Urinary frequency is increased in rats with a bladder mass of greater than 500 mg. The combined $\alpha_{1d}/\alpha_{1a}$AR antagonist tamsulosin decreases urinary frequency more than the $\alpha_{1d}$AR selective antagonist 5MU. This finding supports the hypothesis that the $\alpha_{1d}$AR is important for mediating irritative symptoms.

Key Words: bladder; bladder neck obstruction; rats, Sprague-Dawley; receptors, adrenergic, alpha-1; urination

Bladder outlet obstruction (BOO), such as that which occurs with benign prostatic hyperplasia, produces 2 types of symptoms, namely obstructive (hesitancy, poor stream, prolonged urination and feelings of incomplete emptying) and irritative (frequency, urgency, nocturia and unstable bladder contractions). Irritative symptoms may persist after removal of the obstruction.

The involvement of $\alpha_1$-adrenergic receptors ($\alpha_1$ARs) in BOO is coming under increasing scrutiny. $\alpha_1$ARs mediate actions of norepinephrine and epinephrine through 3 $\alpha_1$AR subtypes ($\alpha_{1a}$, $\alpha_{1b}$, and $\alpha_{1d}$). Nonsubtype selective $\alpha_1$AR antagonists relax prostate smooth muscle and relieve obstructive and irritative symptoms. Prostate smooth muscle relaxation is mediated by $\alpha_{1a}$ARs and consequently subtype selective $\alpha_{1a}$AR antagonists increase urine flow in benign prostatic hyperplasia. However, $\alpha_{1d}$AR antagonists do not appear to relieve irritative symptoms. We recently identified $\alpha_{1d}$AR mRNA and protein in the human bladder and reported that bladder $\alpha_{1d}$AR mRNA and protein are elevated in a rat model of BOO. We examined the role of $\alpha_{1d}$ARs in the development of 1 irritative symptom, that is high urinary frequency, in the rat model of BOO.

MATERIALS AND METHODS

Figure 1 shows the study design. Our chronic micturition recording system (CMRS) was used to document the frequency and volume of voiding preceding (CMRS1) and 6 weeks following (CMRS2) surgical intervention to establish obstruction and sham obstruction models. One week following the implantation of osmotic pumps delivering $\alpha_1$AR antagonists or their vehicles a final round of CMRS (CMRS3) was done, followed by study of the pressor response to the $\alpha_1$AR agonist phenylephrine.

The original study design was for 48 rats, including 12 sham obstructed rats with pumps delivering vehicle (water in
RESULTS

The mean bladder mass ± SEM of rats that underwent obstruction surgery was significantly greater than that of sham obstructed rats (663 ± 88 vs 136 ± 15 mg, p < 0.001). However, many bladders in the obstructed group were not hypertrophied. Figure 2 shows bladder mass at sacrifice after CMRS3 on a log scale to show indicate wide dispersion of bladder masses and the large number of low mass bladders found in the obstructed rat group, of which many were within the range of sham obstructed bladders. The dotted line at 255 mg marks the limit of sham obstructed rat bladders and that

![Graph showing bladder mass at sacrifice](image)

FIG. 2. Bladder mass at sacrifice plotted on log scale. Each point represents bladder mass at sacrifice of 1 rat. Dotted line at 255 mg represents limit of sham bladder mass. Dotted line at 500 mg represents threshold for bladders expected to show elevated \( \alpha_{1dAR} \) expression. *tam*, tamsulosin.
at 500 mg marks the approximate threshold for bladders expected to show elevated α1dAR expression based on previous studies.\textsuperscript{11}

Figure 3 shows the change in urinary frequency with obstruction in different subsets. Sham obstructed rats showed a decrease, as did obstructed bladders with a mass of less than 255 mg (small BOO). These 2 groups were considered together as all less than 255 mg (11 sham obstructed, range 92 to 255 mg, and 13 ostensibly obstructed, range 128 to 217). Obstructed rats overall (all BOO) showed almost no change in frequency, whereas obstructed rats with a bladder mass of above 255 mg (BOO greater than 255) showed an increase and those with a bladder mass of above 500 mg (BOO greater than 500) showed a larger increase. The change in frequency in the BOO greater than 500 group was significantly different from that in the all less than 255 group (vs all less than 255, p = 0.01).

Study of the effects of α1AR antagonists on urinary frequency was limited to rats with a bladder mass of greater than 500 mg because they showed elevated urinary frequency (fig. 3) and were expected to show elevations in α1dAR relative to α1aAR.\textsuperscript{11} Because obstruction surgery produced only 3 rats with bladders this large in each of the BOO water and BOO PG groups (fig. 2), these 2 vehicle groups were combined after statistical analysis showed no significant difference between them. There was a high correlation of urinary frequency with water intake (r = 0.62), a lower correlation with food intake (r = 0.24) and none with body mass (r = 0.08). Accordingly water intake was used as a predictor on multiple regression analysis. Urinary frequency at CMRS2 (1 week before) was also used as a predictor to help control for unspecified factors intrinsic to individual rats.

To ensure that no rats with anomalous urinary frequency values would distort the multiple regression analysis, we examined the relationship between urinary frequency at CMRS2 and CMRS3 in all rats (fig. 4). This plot showed a strong linear relationship between the 2 frequencies (slope 1.03). However, 1 vehicle rat departed strongly from that linear relationship increasing from 30 micturitions daily before pump insertion to 112 micturitions daily 1 week after pump insertion. The effect of this anomalous rat was to exaggerate CMRS3 frequency in the vehicle group. This rat also greatly increased the estimated effects of drugs on regression analysis. Accordingly this rat was removed from the data set.

The table lists results of multiple regression analysis. CMRS2 frequency was a strong predictor of CMRS3 frequency (p <0.0001), as was water intake (p = 0.108). The coefficients of the effects of tamsulosin relative to vehicle and 5MU relative to vehicle indicate the decrease in the number of micturitions events daily attributable to tamsulosin (23, p = 0.026) and 5MU (18, p = 0.066), when controlling for frequency at CMRS2 and water intake.

A dose-response study of the effect of phenylephrine on mean arterial pressure in rats was performed after CMRS3 (fig. 5). Phenylephrine induced marked transient elevations in mean arterial pressure in rats with vehicle pumps, whether sham obstructed or obstructed. Rats with 5MU pumps showed a much larger shift to the right (perhaps 40-fold, although the curve was so nearly flat as to leave considerable uncertainty).

**DISCUSSION**

While nonsubtype selective α1AR antagonists relieve obstructive and irritative symptoms of BOO, α1a selective antagonists relieve only obstructive symptoms in humans.\textsuperscript{8} This finding suggests the hypothesis that another α1AR subtype, most likely α1d, is important in mediating irritative symptoms. The central finding of the current study, that the combined α1a/α1dAR antagonist tamsulosin decreases urinary frequency more than the α1aAR selective antagonist 5MU in high mass obstructed rat bladders, supports that hypothesis.

The variability of bladder mass in the rat obstruction model is well known.\textsuperscript{12} Given that our study was designed to test the importance of α1dARs in the irritative symptom of high urinary frequency, it seemed prudent to examine hyper-
trophied bladders in which $\alpha_{1A}$AR protein expression would be expected. We previously noted a shift in predominance from $\alpha_{1A}$AR to $\alpha_{1D}$AR mRNA and de novo $\alpha_{1D}$AR protein expression in hypertrophied rat bladders after obstruction, particularly in bladders with a mass more than 5-fold greater than the mean of sham bladder masses. In the current study rats with similarly hypertrophied bladders also demonstrated significantly increased urinary frequency prior to drug therapy (fig. 3), further justifying our decision to focus on this group only.

Testing the importance of the $\alpha_{1D}$ARs in high urinary frequency presented 2 major difficulties. 1) There is no $\alpha_{1A}$AR antagonist available that is selective enough in vivo. (The standard highly selective $\alpha_{1A}$AR selective antagonist BMY7378 is a partial agonist at 5-HT$_{1A}$ receptors and 5-HT$_{1A}$ receptor agonists affect micturition in rats.) 2) Drug uroselectivity is different in rats than in other species (rabbits, dogs and humans). We chose the combined $\alpha_{1D}/\alpha_{1A}$AR antagonist tamsulosin and the $\alpha_{1D}$AR antagonist 5MU, each demonstrating a greater than 10-fold higher affinity and antagonist potency compared to other $\alpha_{1}$AR subtypes. With respect to the in vivo selectivity of these drugs in the rat, data on conscious rats showed that tamsulosin has higher efficacy and potency to decrease uterine pressure than 5MU but the selectivity of 5MU to decrease uterine pressure compared to blood pressure in rats was higher than for tamsulosin (7-fold vs nil). These data were confirmed for tamsulosin in a separate study.

To use these small selectivity differences of the 2 drugs in our rat model we selected dose levels for infusion of these drugs that were deduced from published studies. Verification of the adequacy of obtained drug exposure in the rats was done by analysis of the shift in the phenylephrine dose-response curve for blood pressure responses. In the rat $\alpha_{1A}$AR and $\alpha_{1D}$AR subtypes are present in the conduit and resistance vessels and, therefore, they may be important in hemodynamic responses. In this respect it is not surprising that tamsulosin with its affinity for $\alpha_{1D}$AR and $\alpha_{1A}$AR subtypes has more of a blood pressure effect than 5MU (fig. 5).

Apart from showing firm hemodynamic effects, tamsulosin also showed a statistically significant decrease in urinary frequency in rats with a high bladder mass. The relatively stronger effect of 5MU on the decrease in urinary frequency compared to hemodynamics in our study, which is in line with the in vivo uroselectivity data of Martin et al, unfortunately does not allow us to draw a firm conclusion about the identity of the $\alpha_{1A}$AR subtype functionally involved in the increased urinary frequency in our model. Assuming that the drug plasma levels in our study were in agreement with those obtained in the pharmacokinetic study, the steady state levels for tamsulosin and 5MU would translate into concentrations for the $\alpha_{1D}$AR that are about 1,200-fold the $pA_2$ for 5MU, about 770-fold the $pA_2$ for tamsulosin, to about 2 and 0.8-fold the $pA_2$ level for $\alpha_{1D}$AR, and to about 10 and 75-fold the $pA_2$ level for $\alpha_{1A}$AR, respectively. This shows that bladder frequency data in our study do not match with drug effects at $\alpha_{1A}$ARs (these ARs were indeed not found in the rat bladders in our prior study). In this reasoning potency at the $\alpha_{1D}$AR is higher for tamsulosin, while at the $\alpha_{1A}$AR 5MU is the more effective drug. Looking at the current results, this implies that there is a stronger involvement of $\alpha_{1D}$AR in hemodynamic and the bladder effects, while $\alpha_{1A}$AR is not likely to be significantly involved in hemodynamic and bladder effects.

This does not apply in humans, in whom $\alpha_{1A}$AR mediated pressor effects primarily depend on $\alpha_{1A}$AR in young individuals with increasing importance of the $\alpha_{1B}$ subtype with aging. Supporting this hypothesis, nonsubtype selective $\alpha_{1}$AR antagonists decrease mean arterial pressure in humans of all ages, whereas the $\alpha_{1A}/\alpha_{1D}$AR antagonist tamsulosin has shown to have minimal blood pressure effects in older individuals, presumably due to its lack of $\alpha_{1B}$AR antagonist effects. With respect to the role of $\alpha_{1D}$ARs in human bladders data are not yet available. Assuming some analogy between humans and rats in the bladder pathophysiology of BOO, there could be a role for $\alpha_{1D}$AR selective drugs to decrease urinary frequency in human BOO. In humans this should occur without an effect on hemodynamics.

**CONCLUSIONS**

Our previous finding of enhanced $\alpha_{1D}$AR expression in obstructed rats with markedly hypertrophied bladders and our current finding of a decrease in urinary frequency in such rats with tamsulosin more than with 5MU support the hypothesis that the $\alpha_{1D}$ARs are mechanistically involved in the development of irritative symptoms and they are plausible targets for therapeutic intervention. If these findings are confirmed in humans, targeting $\alpha_{1D}$AR may provide a new
therapeutic approach to controlling bladder irritative symptoms associated with BOO.

REFERENCES


