Role of spinal $\alpha_1$-adrenoceptor subtypes in the bladder reflex in anesthetized rats

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Yoshiyama, Mitsuharu, and William C. de Groat. Role of spinal $\alpha_1$-adrenoceptor subtypes in the bladder reflex in anesthetized rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1414–R1419, 2001.—The contribution of different subtypes of $\alpha_1$-adrenoceptors in the lumbosacral spinal cord to the control of the urinary bladder was examined in urethane-anesthetized rats. Bladder pressure was recorded via a transurethral catheter under isovolumetric conditions. Drugs were administered intrathecally at the L6-S1 segmental level of spinal cord. RS-100329 (an $\alpha_{1A}$-antagonist) in doses of 25, 50, and 100 nmol significantly decreased bladder-contraction amplitude by 38%, 52%, and 95%, respectively, whereas (+)-cyclazosin (an $\alpha_{1B}$-antagonist) significantly decreased bladder-contraction amplitude (48% reduction) only in a 50-nmol but not a 100-nmol dose. Fifty nanomoles of RS-100329 and (+)-cyclazosin increased bladder-contraction frequency by 54% and 44%, respectively. BMY7378 (an $\alpha_{1D}$-antagonist), in doses of 25, 50, and 100 nmol, did not change bladder activity. These studies suggest that reflex-bladder activity is modulated by two types of spinal $\alpha_1$-adrenergic mechanisms: 1) $\alpha_{1A}$ or $\alpha_{1B}$-inhibitory control of the frequency of voiding reflexes presumably mediated by an alteration in the processing of bladder afferent input and 2) $\alpha_{1A}$-facilitatory modulation of the descending effenter limb of the micturition-reflex pathway. Spinal $\alpha_{1D}$-adrenoceptors do not appear to have a significant role at either site.

MODULATION OF MICTURITION by central noradrenergic pathways has been a topic of interest because it was reported that sympathetic and parasympathetic nuclei in the lumbosacral cord receive inputs from noradrenergic neurons in the brain stem (2). A large part of this input arises from neurons in the locus ceruleus (LC) (2, 16, 18, 19, 25), which has been implicated in the supraspinal control of micturition (3, 29, 30). In anesthetized cats, electrical stimulation of the LC induced bladder contractions that were blocked by the intrathecal injection of prazosin, an $\alpha_1$-adrenoceptor antagonist (29, 30). In addition, destruction of noradrenergic cells in the LC by microinjection of 6-hydroxydopamine, a toxin for catecholaminergic neurons, produced a hypotensive bladder, and this effect was partially reversed by the intrathecal injection of phenylephrine, an $\alpha_1$-adrenergic agonist (30). On the basis of these studies, it was proposed that bulbo-spasmodic noradrenergic inputs to the sacral parasympathetic nucleus played an essential role in voiding function. Although these findings were not confirmed in conscious cats (5, 6), they indicated that under certain conditions, $\alpha_1$-adrenergic mechanisms in the spinal cord could modulate voiding function. Studies in anesthetized (4, 35) and conscious (11) rats also support this conclusion.

Our previous studies in anesthetized rats revealed that reflex-bladder activity is modulated by two types of spinal $\alpha_1$-adrenergic mechanisms: 1) inhibitory control of the frequency of reflex-bladder contractions presumably due to modulation of afferent processing in the spinal cord and 2) excitatory modulation of the amplitude of bladder contractions due to regulation of the descending glutamatergic limb of the spinobulbo-spasmodic bladder-reflex pathway (4, 35). These mechanisms could involve activation of three subtypes of $\alpha_1$-adrenoceptors: $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ (10). This was examined in the present experiments by studying the effects on reflex-bladder activity of intrathecal administration of drugs that selectively block different subtypes of $\alpha_1$-adrenoceptors.

A preliminary account of this work has been presented in an abstract (34).

MATERIALS AND METHODS

Animal preparation. Experiments were performed on urethane-anesthetized (1.2 g/kg sc) female Sprague-Dawley rats weighing 250–300 g. The trachea was cannulated with a polyethylene tube (PE-240) to facilitate respiration, and an intrathecal catheter was inserted according to the technique of Yaksh and Rudy (28). The occipital crest of the skull was exposed and the atlanto-occipital membrane was incised at the midline using the tip of a 16-gauge needle as a cutting edge. A catheter (PE-10) filled with artificial cerebrospinal fluid was inserted into the right lateral and left bladder and connected to a transducer via a polyethylene tube (PE-240) for measurement of bladder pressure. Bladder pressure was recorded via a transurethral catheter connected to a transducer under isovolumetric conditions.

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fluid (CSF) (7, 17) was inserted through the slit and passed caudally to the L4 level of the spinal cord. At the end of the experiment, a laminectomy was performed to verify the location of the catheter tip.

A transurethral bladder catheter (PE-90) connected to a pressure transducer was used to record the bladder pressure isovolumetrically with the urethral outlet ligated. The bladder was filled via the bladder catheter by incremental volumes of physiological saline until spontaneous bladder contractions occurred (total volume: 0.8–1.5 ml). For isovolumetric recording, the ureters were tied distally, cut, and the proximal ends cannulated (PE-10) and drained externally. This procedure prevented the bladder from filling with urine during the experiment.

The protocols in these studies were approved by the Animal Care and Use Committee of the University of Pittsburgh.

Drugs. Drugs used in these studies included urethane (ethyl carbamate, Sigma, St. Louis, MO), N-[2-(3-thiophenethoxy)phenyl], N’-[3-thyminylpropyl]piperazine hydrochloride (1, 6, 100329, Roche Bioscience, Palo Alto, CA) (12, 26), 4-[4-(amin-6,7-dimethoxyquinazolin-2-yl)-cys-octahydroquin- noxalin-1-yl]furam-2-ylmethanone [(+)-cyclozanocine, Roche Biosciences] (8, 12, 21), and 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl][ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride (BMY 7378 Research Biochemicals International, Natick, MA) (9). Urethane was dissolved in distilled water (0.5 g/ml solution). RS-100329 and (+)-cyclozanocine were dissolved in 100% DMSO (20 mM solutions), and BMY 7378 HCl was dissolved in artificial CSF (7.5 m). Drug doses were calculated for the base of each compound. Drugs were administered in small volumes (<5 µl), and then the intrathecal catheter was flushed by artificial CSF (7.5 µl).

Multiple doses of drugs or vehicles starting with the smallest amounts were injected in each animal. Increasing amounts were administered after bladder contractions recovered to control. Injections were spaced at intervals of at least 30 min even when bladder activity was not altered.

Evaluation and statistical analysis. The effects of RS-100329, (+)-cyclozanocine, and BMY 7378 were evaluated on the amplitude and frequency of reflex-bladder contractions recorded under isovolumetric conditions. The effects of vehicle solutions [100% DMSO for RS-100329 and (+)-cyclozanocine and artificial CSF with pH adjusted to 1.6 or 4.0 for BMY 7378] were also examined. All values are expressed as means ± SE. For statistical data analysis, ANOVA and paired t-test were used to compare the values before and after drug administration. Two-way ANOVA and unpaired t-test were applied to compare the differences between the effects of drug and vehicle solution. For all statistical tests, P < 0.05 was considered significant.

RESULTS

Animals with implanted intrathecal catheters (n = 30) exhibited rhythmic bladder contractions (mean amplitude: 33 ± 2 cmH2O; range: 17–51 cmH2O) at a mean frequency of 0.84 ± 0.04 contractions/min (range: 0.43–1.34 contractions/min) under isovolumetric conditions when the bladder was filled with 0.8–1.5 ml of saline.

Effects of vehicles (100% DMSO or acidic CSF) on bladder activity. The vehicle for RS-100329 and (+)-cyclozanocine (up to 5 µl of 100% DMSO followed by 7.5 µl artificial CSF injection) did not alter the frequency of bladder contractions, but a large volume of vehicle decreased the amplitude of bladder contractions (see Fig. 2). The volume of vehicle for 50- and 100-nmol doses reduced bladder-contraction amplitude by 4.1 ± 1.2% (n = 10) and by 30.1 ± 11.0% (n = 11), respectively. Therefore, two-way ANOVA (followed by unpaired t-test) was used to compare the dose-response curves for vehicle (100% DMSO) with the dose-response curves for RS-100329 or (+)-cyclozanocine. The dose-response curves for BMY 7378 and its vehicle (artificial CSF adjusted to pH 1.6 or 4) were compared in the same manner, although the vehicle for BMY 7378 did not change bladder activity at any volume (n = 3–6).

Effects of RS-100329, (+)-cyclozanocine, or BMY 7378 on bladder activity. RS-100329 in 25-, 50-, and 100-nmol doses decreased the amplitude of bladder contractions by 16–85% (average: 38 ± 9%), 18–100% (average: 52 ± 10%), and 84–100% (average: 95 ± 2%), respectively, whereas smaller doses of the drug (6.25 and 12.5 nmol) had no effect (Figs. 1 and 2A). The depressant effect occurred within 1 min after the administration of the drug and persisted for 7–53 min depending on the dose (25 nmol, average: 15 ± 2 min; 50 nmol, average: 18 ± 3 min; 100 nmol, average: 34 ± 4 min). RS-100329 in the 50-nmol dose significantly increased (average: 51 ± 14%, range: 4–131%) the frequency of bladder contractions; however, smaller doses (6.25, 12.5, and 25 nmol) and a larger dose (100 nmol) had no significant effect (Figs. 1 and 2B).

The effects of (+)-cyclozanocine were variable between animals and not dose dependent (Fig. 3). The 50-nmol dose significantly decreased the amplitude of bladder contractions (average: 45 ± 11%, range: 6–79%) and increased the bladder-contraction frequency (average: 57 ± 20%, range: 19–145%) for periods ranging from 5 to 14 min (average: 9 ± 1 min); whereas smaller and larger doses (25 and 100 nmol) had no significant effect (Fig. 2, A and B) compared with vehicle. However, it should be noted that there was considerable variation in the effect of the 100-nmol dose that in three experiments completely blocked bladder contractions and in other experiments had minimal effects on bladder-contraction amplitude. Thus, the effects of the 100-nmol dose of (+)-cyclozanocine on bladder-contraction frequency could be evaluated in only three rats (Fig. 2B).

In these animals, the drug produced 35% increase in frequency, which was not statistically significant (P = 0.1361).

BMY 7378 (25, 50, and 100 nmol) did not significantly alter the amplitude or the frequency of bladder contractions (Fig. 4).

DISCUSSION

In our previous studies in anesthetized rats (4, 35), phenylephrine (an α1-adrenoceptor agonist) increased the intercontraction interval (i.e., the time between bladder contractions) and pressure threshold for inducing micturition during continuous infusion cystometrograms. Under isovolumetric conditions, the drug abolished bladder activity. On the other hand, doxazosin (a
nonselective α1-adrenoceptor antagonist) decreased intercontraction intervals during cystometrograms and increased the bladder-contraction frequency and decreased bladder-contraction amplitude under isovolumetric conditions. These results indicated that two types of spinal α1-adrenergic mechanisms are involved in reflex-bladder activity: 1) inhibitory control of the bladder-contraction frequency presumably due to modulation of afferent processing in the spinal cord and 2) excitatory modulation of bladder-contraction amplitude due to regulation of the descending limb of the spinobulbospinal bladder-reflex pathway. The present experiments have provided insights into the α1-adrenoceptor subtypes involved in these modulatory mechanisms.

Intrathecal administration of RS-100329, an α1A-adrenoceptor antagonist, suppressed bladder-contraction amplitude in a dose-dependent manner, indicating that the descending limb of the micturition-reflex pathway is facilitated by bulbo spinal noradrenergic inputs acting on α1A-adrenoceptors. Certain doses of RS-100329 or (+)-cyclazosin, an α1B-adrenoceptor antagonist, significantly increased the bladder-contraction frequency, indicating that α1A- or α1B-adrenoceptors modulate the spinal processing of afferent input from bladder mechanoreceptors. It is likely that these adrenergic modulatory mechanisms regulate N-methyl-D-aspartate (NMDA) and non-NMDA glutamatergic synapses that play an essential role in the micturition-reflex pathway (31–33).

Although RS-100329, which has a high affinity and selectivity for the α1A-adrenoceptor versus the α1B- and α1D-adrenoceptor subtypes (26), suppressed bladder-contraction amplitude in a dose-dependent manner, only the 50-nmol dose significantly increased the frequency of bladder contractions. Smaller and larger doses (25 and 100 nmol) were ineffective. The lack of effect on this parameter by the largest dose (100 nmol) raises the possibility that α1A-adrenoceptors are not involved or that the high dose nonselectively affected other transmitter mechanisms to negate the effects of the lower dose.

In contrast to the prominent effect of RS-100329 on bladder activity, the α1D-antagonist BMY 7378 had no significant effect. This indicates that α1D-adrenoceptors do not play an important role in controlling reflex-bladder activity under the conditions of our experiments. On the other hand, the role of α1B-adrenoceptors is less clear. The effects of (+)-cyclazosin were complicated. The 50-nmol dose had significant effects on bladder-contraction amplitude and frequency, whereas lower and higher doses did not produce significant changes. There may be several reasons for this unusual dose-response relationship. First, the vehicle (100% DMSO) may have interfered with the effect of the drug. Second, (+)-cyclazosin may not be sufficiently selective at α1B-adrenoceptors. Radioligand binding studies indicated that (+)-cyclazosin was a potent and selective ligand for the α1B-adrenoceptor subtype (8), whereas functional studies indicated that (+)-cyclazosin displayed low potency and did not act as a competitive antagonist (21). The lack of any effect on either parameter by 100 nmol may be due to the interaction with other receptor(s). Furthermore, in the present studies, the largest volume of the vehicle (100% DMSO) for RS-100329 and (+)-cyclazosin signif-
significantly decreased the amplitude of bladder contractions. Therefore, the vehicle could have interacted synergistically with the drugs to enhance the depression of bladder-contraction amplitude and conversely to antagonize the facilitatory drug effects on the frequency of bladder contractions.

The present results suggesting that α₁A-adrenoceptors are the most important and that α₁B-adrenoceptors are of lesser importance in the regulation of reflex-bladder activity are consistent with the previous studies using a radioligand binding assay (24), which revealed that in the rat lumbar spinal cord, the α₁A- and α₁B-adrenoceptor populations comprised 70% and 30%, respectively, of the total population of α₁-adrenoceptors in the spinal ventral and dorsal horns and that α₁D-adrenoceptors were expressed at very low levels.

In other spinal systems, α₁A-adrenoceptors also seem to play a major role. For example, in vivo experiments in rats indicated that spinal α₁A-adrenoceptors mediated the spontaneous tail flicks induced by 8-hydroxy-2-(di-n-propylamino)tetralin (1), and experiments on the rat lumbar spinal cord slice preparation revealed that α₁A-adrenoceptors were essential for adrenergic facilitation of spinal motoneuron activity (23). On the contrary, Wilson and Minneman (27) reported that in the in vitro cervical spinal cord preparation, 42% of α₁-adrenoceptors were inactivated by chloroethylclonidine, an α₁B/1D-adrenoceptor antagonist, suggesting a somewhat lower proportion of α₁A-adrenoceptors at this level of the cord.

Because bladder activity in the present studies was evaluated under isovolumetric conditions in which reflex-bladder contractions occurred against a closed outlet, it might be questioned whether this bladder activity is elicited via different mechanisms than “normal” voiding reflexes. For example, isovolumetric contractions might activate high-threshold bladder afferents that, in turn, stimulate nociceptive pathways in the spinal cord. Thus the effects of adrenergic drugs on this type of bladder activity might reflect adrenergic mod-

Fig. 2. Graphs showing the effects of intrathecal (i.t.) administration of RS-100329 (n = 7–10), (+)-cyclazosin (n = 3–9), and vehicles (100% DMSO, n = 8–11) for these drugs on amplitude and frequency of bladder contractions under isovolumetric conditions in urethane-anesthetized rats. *P < 0.05, **P < 0.01 (comparison between vehicle and each drug by unpaired t-test following 2-way ANOVA).

*Significant differences between measurements taken before and after each drug injection (by paired t-test).

Fig. 3. The effects of (+)-cyclazosin (25 and 50 nmol it) on bladder activity under isovolumetric conditions in an urethane-anesthetized rat. Note that 50 nmol of the drug increased the bladder-contraction frequency. The bladder-contraction amplitude was slightly reduced in this rat; however, the effect of the drug on amplitude varied in different animals. The 25 nmol did not change either the bladder-contraction amplitude or frequency.
ulation of visceral nociceptive mechanisms rather than the control of normal micturition. However, we believe that this is unlikely because isovolumetric bladder contractions, like voiding reflexes, are dependent on similar central mechanisms including glutamatergic transmission and a spinobulbospinal-reflex pathway (31–33). In addition, doxazosin, a nonselective $\alpha_1$-adrenoceptor antagonist, decreased micturition pressure in conscious rats during continuous-infusion cystometrograms (CMGs) with an open urethral outlet (11), and phenylephrine, a $\alpha_1$-adrenoceptor agonist, altered the profile of bladder contractions in anesthetized rats during continuous CMGs with the bladder catheter inserted through a ureter (14). Thus $\alpha_1$-adrenergic drugs are still effective in modulating the activity of rat bladder even under conditions in which voiding is unobstructed and bladder afferent activity is entirely nonnoxious.

In summary, the present results taken together with previous studies (4, 35) indicate that two types of spinal $\alpha_1$-adrenergic mechanisms are involved in the control of reflex activity in anesthetized rats: 1) $\alpha_{1A}$- or $\alpha_{1B}$-adrenergic inhibitory control of afferent processing in the spinal cord and 2) $\alpha_{1A}$-adrenergic excitatory modulation of the descending limb of bladder-reflex pathway (Fig. 5). These two mechanisms possibly involving $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptors acting in concert would facilitate urine storage by increasing bladder capacity and also enhance voiding efficiency by increasing parasympathetic nerve activity and the amplitude of bladder contractions.

Perspectives

In clinical urology, nonselective $\alpha_1$-adrenergic antagonists have been used in the treatment of benign prostatic hypertrophy (15). This therapy was initially designed to block adrenergic receptors in the proximal urethra and prostate gland and thereby reduce urethral resistance and increase urine flow. It was discovered that the drugs not only improved urine flow but also reduced irritative bladder symptoms. However, the changes in urinary flow rates were not correlated with the improvement in symptoms (15). This raises the possibility that the two effects might occur by different mechanisms. The reduction in abnormal bladder sensations could be mediated by a suppression of unstable bladder contractions due to an effect on efferent pathways to the bladder. This could occur as a result of actions at various sites including 1) the spinal cord, as suggested by the present experiments, 2) at...
presynaptic α1-adrenergic facilitatory receptors on efferent parasympathetic nerve terminals in the bladder wall (20, 22), or 3 at α1-adrenergic facilitatory receptors in bladder parasympathetic ganglia (13). Thus α1-adrenergic receptors at various sites in the peripheral and central nervous system, as well as in smooth muscle, may play a role in voiding function.

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