Effects of Adrenergic α_2 -Receptor Agonists on Urinary Bladder Contraction in Conscious Rats

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Received May 19, 2000 Accepted August 21, 2000

ABSTRACT—We investigated the effects of the adrenergic α_2 -receptor agonists clonidine, oxymetazoline and tizanidine on bladder contractions induced by infusing fluid into the bladders of conscious male rats. I.v. clonidine and oxymetazoline (both 0.01 to 0.1 mg/kg) caused bladder hyperactivity, expressed by shortening of the intercontraction interval. Tizanidine (0.1 mg/kg, i.v.) caused slight shortening of the intercontraction interval. The rank order of potency was clonidine = oxymetazoline >> tizanidine. Intrathecal (i.t.) injection of 10 μ g clonidine and oxymetazoline, and intracerebroventricular (i.c.v.) injection at 15 μ g, produced almost the same pattern of bladder hyperactivity as that observed after i.v. injection of these drugs (0.03 mg/kg, i.v.). For all three administration routes of clonidine and oxymetazoline, i.v. idazoxan (0.3 mg/kg) exerted an inhibitory effect on the bladder hyperactivity induced by these drugs, except i.c.v. injection of oxymetazoline. I.t. phenylephrine (30 μ g) did not change the intercontraction interval. Although i.c.v. phenylephrine (15 μ g) shortened the intercontraction interval, the potency was weaker than those of i.c.v. clonidine and oxymetazoline (15 μ g). These results suggest that clonidine and oxymetazoline cause bladder hyperactivity by acting at adrenergic α_2 receptors in the micturition centers of the lumbosacral and supraspinal regions.

Keywords: Rat micturition reflex, Bladder hyperactivity, Clonidine, Oxymetazoline, Adrenergic α_2 -receptor agonist

We have previously reported the possibility that transmission in the neurons descending from the pontine micturition center to the sacral parasympathetic neurons that elicit bladder motility is mediated by adrenergic α_2 receptors in rats (1). This conclusion was drawn from results demonstrating that intrathecally injected clonidine caused shortening of the interval between bladder contractions, accompanied by micturition, and that yohimbine injected via the same route inhibited the bladder contractions induced by electrical stimulation near the locus coeruleus in anesthetized rats. However, Maggi et al. (2) reported that systemically injected clonidine exerted inhibitory effects on bladder contractions in anesthetized rats. On the other hand, Morikawa et al. (3) indicated that clonidine shortened and prolonged the time to micturition in conscious and anesthetized rats, respectively, but there was no explanation concerning the shortening of the time. Therefore, in the present experiment, we studied the effects of clonidine on the cystometrogram in conscious rats and compared its effects on bladder motility with those of other adrenergic

α_2 -receptor agonists (oxymetazoline and tizanidine) to investigate any differences in the pattern of bladder hyperactivity. Oxymetazoline acts on adrenergic α_1 as well as α_2 receptors, while tizanidine exerts muscle relaxant activity via adrenergic α_2 receptors in the locus coeruleus (4), which lies near to Barrington's nucleus, a micturition center.

MATERIALS AND METHODS

Animals

Male Wistar rats (Japan SLC, Shizuoka), aged 9–10 weeks and with a body weight of about 250–270 g, were used.

The animals were kept in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. All procedures used in this study were approved by the University Animal Care and Use Committee. The study was carried out under the control of the committee and in line with the "Guidelines to Animal Experiments in Hokuriku University Faculty of Pharmaceutical Sciences".

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Recording of cystometrograms in conscious rats

Cystometrograms were recorded in conscious rats as described previously (5). Under ether anesthesia, the bladder was exposed through a midline incision in the abdomen. A silicone tube fitted with a 25 G needle was used as a bladder catheter. The needle was inserted into the bladder through the left ureter, which was then ligated onto the needle. The right ureter was maintained intact. An intravenous (i.v.) injection catheter was inserted through the femoral vein, and then the incisions in the abdomen and femur were closed with sutures. The rats were restricted in a Ballman cage (Type III; Natsume, Tokyo) during the experiment and were allowed to become accustomed to the cage for at least 2 h before measuring the cystometrogram. Bladder contraction was induced by infusing fluid into the bladder through the bladder catheter at a constant rate (0.8 ml/10 min). Tyrode's solution with the following composition: 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl_2 , 1.1 mM MgCl_2 , 0.42 mM NaH_2PO_4 and 11.9 mM NaHCO_3 , adjusted to about pH 7.4 with diluted HCl, was used as the infusion fluid. The bladder pressure signals were measured with a pressure transducer (Gould P23 ID; Statham, Georgia, USA) connected to the silicone tube via a T-tube, delivered through an amplifier (AP 601G; Nihon Kohden, Tokyo) and recorded with a D.C. recorder (SR 6204; Graphtec, Tokyo). Fluid excreted from the urethra was collected in a small, light cup, which was mounted to the hook of a force-displacement transducer (TB 611-T, Nihon Kohden). The amount of fluid was recorded simultaneously with the bladder pressure signals.

Implantation of cannulae for intracerebroventricular (i.c.v.) and intrathecal (i.t.) drug injections

I.c.v. and i.t. injections were performed according to our previously described method (6), modified for application to conscious rats. Implantation of the cannulae was performed under pentobarbital (40 mg/kg, i.p.) anesthesia.

For the implantation of the i.c.v. injection cannula, the rats were placed on a stereotaxic apparatus (ST-7; Narishige Co., Ltd., Tokyo). A scalp incision was made over the sagittal suture, and a hole (diameter 1.0 mm) was drilled in the parietal bone to expose the dural surface 1.0-mm lateral and 0.3-mm anterior to the bregma. A sterile stainless steel cannula (23 G) was then implanted as a guide and was fixed to the small anchoring screw previously placed on the skull with dental acrylic. A sterile obturator (30 G) was inserted into the cannula to maintain patency. The skin was then sutured and disinfected with 1% benzalkonium chloride, and the injection cannula was inserted through the guide cannula to a depth of 5.6 mm from the skull surface. I.c.v. injections were performed by means of a microsyringe. The volume of each injection was 5 μl .

For i.t. injection, a polyethylene tube (SP-8, Natsume) was inserted into the subarachnoid space to a depth of approximately 8.5 cm through the space between the first and second cerebral vertebrae, thus placing the end of the tube at the level of the T13 to S1 vertebrae. The other end of the catheter was sealed and left in the subcutaneous tissue at the back of the neck. The injection volume was 10 μl , delivered via a microsyringe. Before the experiments, the limb movement of each rat was checked, and rats with any dysfunction were not used.

After the experiments, the rats were deeply anesthetized with ether, and trypan blue solution was injected through the i.c.v. guide cannula. The rats were then killed with an i.v. injection of pentobarbital sodium and the distribution of the trypan blue in the cerebral ventricular space was studied. The location of the polyethylene i.t. injection tube was also checked by laminectomy.

One rat escaped from its cage during the postoperative recovery period and was used in the experiments 3 days after the operation.

Drugs

Clonidine hydrochloride, oxymetazoline hydrochloride and idazoxan hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). L-Phenylephrine was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). Tizanidine and prazosin were gifts from Sandoz Ltd. (Basle, Switzerland) and Pfizer Inc. (Groton, CT, USA), respectively. All drugs except prazosin were dissolved in saline. Prazosin was first dissolved in distilled water to give a 1 mg/ml solution and then further diluted with saline. All drug concentrations are expressed as the concentrations of their respective salts.

Procedure for drug injection and estimation of the bladder hyperactivity induced by each drug

Drug effects were studied once the fluid infusion had been started and the micturition interval (MI) had become almost constant. The drug was injected when micturition had occurred and the bladder pressure had fallen back almost to the baseline level. When the antagonistic effect of an adrenergic α -receptor antagonist on the action of α_2 -receptor agonist was being investigated, the antagonist was injected quickly after micturition and the α_2 -receptor agonist was injected 3 min after the antagonist.

When fluid infusion into the bladder was continued, MI was prolonged. In our previous paper, the ratio of the MI after injection of saline to that before injection was calculated at some time points and the calculation indicated that the ratio remained unchanged 1 h after injection of saline (7). In the present experiment, the intensity of drug activity was expressed as the ratio of the shortest MI after drug injection to that before injection. This ratio was arbitrarily

termed the "R value". However, after injection of the drug used in this experiment, the interval between two bladder contractions shortened considerably and the amount of fluid excreted from the urethra was minute. Therefore, the interval that was measured after drug administration was termed the "intercontraction interval", as opposed to the MI, which was used to describe the interval before drug injection. The interval between two bladder contractions

accompanied by excretion of fluid from urethra was measured, as the intercontraction interval, and the R value was calculated as the ratio of the shortest intercontraction interval to the MI before drug injection.

Statistics

All experimental data are expressed as the mean $\pm$ S.E.M. Statistical analyses of the differences between the R

Fig. 1. Representative tracings of cystometrograms obtained after i.v. injection of clonidine and oxymetazoline in conscious rats. Panels A and B indicate the effects of clonidine and oxymetazoline, respectively. Reflex bladder contractions were induced by infusing fluid into the bladder at a constant rate (0.8 ml/10 min). The drug was injected when the micturition interval had become almost constant. The curved line and the straight line underneath it represent the cystometrogram and the quantity of fluid excreted from the urethra (expressed as the urine weight [UW]), respectively.

Fig. 2. Representative tracings of cystometrograms obtained after i.t. and i.c.v. injection of clonidine in conscious rats. Panels A and B indicate the effects of intrathecal (i.t.) and intracerebroventricular (i.c.v.) injection of clonidine, respectively. For details, see the legend of Fig. 1.

value and 1 for each drug were performed by Student's *t*-test. The Mann-Whitney test was used to compare between the R values in the group treated with antagonist and the control group. These differences were considered to be statistically significant when *P* was <0.05.

RESULTS

Effects of injection of clonidine, oxymetazoline and tizanidine on bladder contraction

Representative traces of the cystometrograms obtained after i.v. injection of clonidine and oxymetazoline are shown in Fig. 1, while those obtained after i.t. and i.c.v.

injection of these drugs are shown in Figs. 2 and 3, respectively. The resulting R values after i.v. injection and i.t. and i.c.v. injection are shown in Figs. 4 (A) and 5 (A and B), respectively. Clonidine and oxymetazoline (both 0.03 to 0.1 mg/kg, i.v.) caused shortening of the intercontraction interval and the shortening was progressive, reaching a maximum 10 to 30 min after injection of drug. Reversal of intercontraction interval was observed 60 min after i.v. injection of drug (hatched columns in Fig. 4A). The activity of tizanidine was very weak in comparison with the other two drugs, and a dose of 0.1 mg/kg, i.v. was necessary for tizanidine to cause shortening of the intercontraction interval (Fig. 4A). Therefore, only clonidine and oxymetazoline

Fig. 3. Representative tracings of cystometrograms obtained after i.t. and i.c.v. injection of oxymetazoline in conscious rats. Panels A and B indicate the effects of intrathecal (i.t.) and intracerebroventricular (i.c.v.) injection of oxymetazoline, respectively. For details, see the legend of Fig. 1.

were used to study activity after administration via the i.c.v. and i.t. routes. Doses of 10 and 30 μ g, i.t. and of 0.15 to 15 μ g, i.c.v. clonidine and oxymetazoline caused progressive shortening of the intercontraction interval, and the shortening reached a maximum within 60 min after injection of drug. The pattern of bladder pressure change during the shortening was similar to that after i.v. injection of the drug. Bladder contraction pattern changed by i.c.v. injection of oxymetazoline did not always reverse itself even 120 min after injection.

Effects of idazoxan and prazosin on the shortening of the intercontraction interval induced by clonidine and oxymetazoline

Idazoxan (1 mg/kg, i.v.) significantly prolonged the intercontraction interval after injection, the ratio of the first intercontraction interval after injection to the MI before injection was 1.32 ± 0.08 ($n=6$, $P<0.05$), and the ratio 60 min after injection of idazoxan was 1.33 ± 0.16 . There was no significant difference in the ratio at lower doses of idazoxan.

The R values of clonidine and oxymetazoline (both 0.03 mg/kg, i.v.) in the rats treated with idazoxan (0.03 to 0.3 mg/kg and 0.3 and 1 mg/kg, i.v., respectively) were sig-

Fig. 4. The effects of clonidine, oxymetazoline and tizanidine on the bladder intercontraction interval and the antagonistic effect of idazoxan in conscious rats. The responses to i.v. injection of clonidine, oxymetazoline and tizanidine are shown in the Panel A. The R value is the ratio of the shortest intercontraction interval measured after injection of a particular drug to the micturition interval (MI) before injection of the drug and is indicated by an open column. Hatched columns indicate the ratios of intercontraction interval 60 min after injection of drug to the MI before injection. The panel B indicates the inhibitory effect of idazoxan on the shortening of intercontraction interval induced by clonidine and oxymetazoline. Clonidine and oxymetazoline were injected 3 min after i.v. injection of idazoxan. Each column and bar represents the mean and S.E.M. for the number of animals indicated in parentheses. * $P < 0.05$, ** $P < 0.01$, for the difference between the R value and 1 (Student's *t*-test). In the panel B, a and b indicate statistical significance at $P < 0.05$ and $P < 0.01$, respectively, between the effects of clonidine and oxymetazoline in the group treated with idazoxan and those in the control group (Mann-Whitney test).

nificantly different from those in the control rats that were not injected with idazoxan (Fig. 4B). The R values of clonidine (15 μ g, i.c.v. and 10 μ g, i.t.) and oxymetazoline (10 μ g, i.t.) in the rats treated with idazoxan (0.3 mg/kg, i.v.) were also significantly different from those in the control rats that did not receive idazoxan (Fig. 5C). Idazoxan (0.3 mg/kg, i.v.) inhibited the shortening of the intercontraction interval induced by clonidine and oxymetazoline administered via the above-mentioned routes.

In the rats treated with idazoxan (0.3 mg/kg, i.v.), clonidine (10 μ g, i.t.) significantly prolonged the intercontraction interval of the post-dosing and the ratio of the interval to the MI before injection was 2.0 ± 0.27 ($n = 4$, $P < 0.05$).

Prazosin (0.3 mg/kg, i.v.) caused a reduction in peak pressure of bladder contraction and shortening of the MI in some rats. In the rats after injection of prazosin (0.3 mg/kg, i.v.) the shortening effects of clonidine and oxymetazoline (both 0.03 mg/kg, i.v.) on the intercontraction interval were weakened, and the R values of clonidine and

oxymetazoline in the rats treated with prazosin was significantly different from the control rats. However, the R values of clonidine and oxymetazoline (both 10 μ g, i.t. and 15 μ g, i.c.v.) in the rats treated with prazosin (0.3 mg/kg, i.v.) were not different from those in the control rats that did not receive idazoxan (Fig. 6).

Although phenylephrine (30 μ g, i.t.) prolonged the post-dosing intercontraction interval in some rats, the R value was not significant different from 1. Phenylephrine (15 μ g, i.c.v.) shortened the intercontraction interval (Fig. 7). Potency and duration were similar to those after i.c.v. clonidine and oxymetazoline (0.15 μ g) (Fig. 5B).

DISCUSSION

Our results show that the adrenergic α_2 -receptor agonists tested during the present study caused bladder hyperactivity, characterized by shortening of the intercontraction interval (Figs. 1–3). We have previously reported that

Fig. 5. The shortening of the bladder intercontraction interval induced by i.t. and i.c.v. clonidine and oxymetazoline and the antagonistic effect of idazoxan on the effects in conscious rats. Responses to intrathecal (i.t.) and intracerebroventricular (i.c.v.) injections of clonidine and oxymetazoline are shown in panels A and B, respectively. The panel C indicates the inhibitory effect of idazoxan on the shortening of intercontraction interval induced by these drugs. For details of the experimental method and the R value, see the legend of Fig. 4. Idazoxan was injected i.v., and clonidine and oxymetazoline were administered via each of the above-mentioned injection routes 3 min after injection of idazoxan. Each column and bar represents the mean and S.E.M. for the number of animals indicated in parentheses. ** $P < 0.01$, for the difference between the R value and 1 (Student's *t*-test). In the panel C, b indicates statistical significance at $P < 0.01$ between the effects of clonidine and oxymetazoline in the group treated with idazoxan and those in the control group (Mann-Whitney test).

apomorphine also causes bladder hyperactivity in anesthetized rats through the activation of dopamine D_2 receptors (8). After injection of apomorphine, the bladder contraction pattern consisted of many repetitive contractions with occasional quiescent periods, and this pattern was different from that after injection of the α_2 -receptor agonists during the present study. Although tizanidine shortened the intercontraction interval, its effect on bladder motility was very weak, so we did not investigate the antagonism of this activity by idazoxan. As the shortening of the intercontraction interval induced by systemically injected clonidine and oxymetazoline was inhibited by idazoxan and the inhibitory effect was dependent on the dose of idazoxan (Fig. 4B), we consider the bladder hyperactivity induced by these drugs, including tizanidine, to be mediated via stimulation of α_2 receptors. According to the IUPHAR compendium of receptor characterization and classification (9), clonidine

is an agonist for three adrenergic receptor subtypes (α_{2A} , α_{2B} and α_{2C}), while oxymetazoline is classified as a partial but selective agonist for α_{2A} receptors. The pharmacological activities of tizanidine have been reported to be exerted through the activation of adrenergic α_{2B} receptors (10, 11). This classification suggests that the receptor subtype through which these drugs mediate bladder hyperactivity is the adrenergic α_{2A} receptor. However, further experiments using selective adrenergic α_2 -receptor subtype antagonists will be necessary to determine which α_2 adrenoceptor subtype is the main contributor to the generation of bladder hyperactivity.

I.c.v. and i.t. injection of clonidine and oxymetazoline also caused shortening of the intercontraction interval, and the pattern was similar to that observed after i.v. injection of these drugs (Figs. 1–3). We therefore consider that these drugs act on the micturition centers in the supraspinal

Fig. 6. The antagonistic effect of prazosin on the shortening of the bladder intercontraction interval induced by clonidine and oxymetazoline in conscious rats. Responses to i.v., intrathecal (i.t.) and intracerebroventricular (i.c.v.) injection of clonidine and oxymetazoline after i.v. injection of prazosin are shown in panels A, B and C, respectively. For details of the experimental method and the R value, see the legend of Fig. 4. Prazosin was injected i.v., and clonidine and oxymetazoline were administered via each of the above-mentioned injection routes 3 min after injection of prazosin. Each column and bar represents the mean and S.E.M. for the number of animals indicated in parentheses. * $P < 0.05$, ** $P < 0.01$, for the difference between the R value and 1 (Student's *t*-test). In the panel A, a and b indicate statistical significance at $P < 0.05$ and $P < 0.01$, respectively, between the effects of clonidine and oxymetazoline in the groups treated with idazoxan and those in the control group (Mann-Whitney test).

Fig. 7. The effects of phenylephrine on the bladder intercontraction interval in conscious rats. The responses to intrathecal (i.t.) and intracerebroventricular (i.c.v.) injection of phenylephrine are shown. For details of the experimental method and the R value, see the legend of Fig. 4. Each column and bar represents the mean and S.E.M. for the number of animals indicated in parentheses. ** $P < 0.01$, for the difference between the R value and 1 (Student's *t*-test).

and lumbosacral regions to cause shortening of the intercontraction interval. Maggi et al. (2) reported that, depending on certain factors, clonidine could produce either

inhibitory or excitatory effects on bladder motility. They suggested possible mechanisms through which clonidine might inhibit reflex bladder response to distention; namely inhibition of neural excitability at the level of the pontine micturition center, inhibition of excitatory neurotransmission at pelvic ganglion level and inhibition of excitatory neurotransmission at the postganglionic level. It has also been reported that intraperitoneally-injected ST-91, an adrenergic α_2 -receptor agonist that does not readily cross the blood-brain barrier, frequently produces an increase in bladder contraction, possibly due to sensitization of the urinary tract to cholinergic activation after peripheral blockade of adrenergic transmission (12). However, we have previously found that electrical stimulation of the hypogastric nerve does not inhibit the reflex bladder contractions induced by bladder distension and that the sympathetic nerve does not act as an inhibitory system for rat bladder motility (13). We have no direct evidence to neglect the participation of peripheral action of clonidine in the bladder hyperactivity and think that main active site is in the central nervous system. We also think that differences in the experimental conditions, as described by Maggi et al. (2), can explain why clonidine produces both excitatory and inhibitory effects on the micturition reflex. One of the reasons behind the apparent conflict between our results and those of Maggi et al. may be differences in the experimental methodology. Maggi et al. studied the effects of clonidine on the volume-evoked micturition reflex (iso-

volume contraction) after ligation of the urethra and their rats were anesthetized with urethane. We studied the effects on bladder contraction accompanied by micturition in conscious rats with intact urethra function. Urethane has been reported to mask the micturition reflex induced by excitatory glutamatergic transmission in the micturition centers and their efferent pathways (14–16) and might therefore have masked the excitatory effect of clonidine on the micturition reflex.

Although clonidine and oxymetazoline exerted similar stimulatory effects on bladder motility, some differences in action were noted. I.t. injection of clonidine resulted in prolongation of intercontraction interval after injection of idazoxan. Clonidine may therefore have a suppressive effect on the excitability of the micturition center in the lumbosacral region besides a stimulatory effect on the adrenergic α_2 receptors within the center. The stimulatory effect of i.v. clonidine was almost completely inhibited by idazoxan (0.3 mg/kg, i.v.), but that of i.v. oxymetazoline still remained after treatment with idazoxan (1 mg/kg, i.v.). Since oxymetazoline acts on adrenergic α_1 receptors as well as on α_2 receptors, the possibility that the stimulatory effect of oxymetazoline on the α_1 receptor may also contribute to shortening of the intercontraction interval was examined. Prazosin (0.3 mg/kg, i.v.) exerted an inhibitory effect on the shortening of intercontraction interval induced by i.v. injection of clonidine and oxymetazoline (Fig. 6). However, i.t. phenylephrine did not shorten the intercontraction interval. Although i.c.v. phenylephrine caused shortening of the intercontraction interval (Fig. 7), the potency was weaker than that of i.c.v. oxymetazoline (Fig. 5B). The results indicate that the possibility described above can be ruled out and oxymetazoline also shortens the intercontraction interval through the activation of the α_2 receptors.

Prazosin did not attenuate the shortening of intercontraction interval induced by i.t. and i.c.v. injection of these drugs. The inhibitory effect of prazosin on the shortening of intercontraction interval induced by these drugs may be due to the peripheral action of prazosin. Prazosin reduces the contractile force of the internal urethral sphincter muscle and may increase the amount of fluid excreted from the bladder per bladder contraction. As a result, longer time is required to build up an adequate amount of fluid in the bladder to elicit a micturition reflex than that before injection of prazosin. However, the hypothesis is correct, the intercontraction interval shortened by i.t. and i.c.v. injection of these drugs may be prolonged. Furthermore, MI was shortened when prazosin reduced the peak pressure. It has been reported that prazosin inhibited the urethral contraction induced by electrical stimulation of the pudendal afferent nerve and a tonic facilitation to the pudendal nerve system through the blocking action on adrenergic α_1 recep-

tors in the central nervous system (16, 17). We can not explain the inhibitory effect of prazosin on the shortening of intercontraction interval by i.v. clonidine and oxymetazoline now.

Further experiments will be necessary to explore the mechanisms by which stimulation of the adrenergic α_2 receptors of the neurons in the micturition centers generates bladder hyperactivity and to investigate the effects of adrenergic α_2 -receptor agonists on bladder motility in other experimental animals. For example, in cats, transmission in the pathway descending from the pontine micturition center to the preganglionic neurons controlling the pelvic nerves in the lumbosacral region is mediated via adrenergic α_1 receptors (18–20). Such experiments will hopefully clarify the usefulness of adrenergic α_2 -receptor blockers for the treatment of pollakiuria.

In conclusion, adrenergic α_2 -receptor agonists cause bladder hyperactivity in rats, characterized by shortening of the intercontraction interval. The sites of action of these drugs are in the central nervous system; i.e., the micturition centers in the lumbosacral and the supraspinal regions.

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