Involvement of $\alpha_2$-Adrenoceptors in the Sacral Micturition Reflex in Rats

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ABSTRACT—We have studied the effects of intrathecally-injected drugs that act on $\alpha$-adrenoceptors in the urinary bladder reflex contractile activity evoked by continuous infusion of fluid into the bladder of anesthetized rats. Clonidine (10 and 30 $\mu$g) facilitated and yohimbine (100 $\mu$g) abolished the bladder contractile activity, and pretreatment with yohimbine (30 $\mu$g) inhibited the effect of clonidine (10 $\mu$g). Phenylephrine (60 $\mu$g) abolished the bladder contractile activity, but prazosin (40 $\mu$g) had no significant effect on it. The bladder contractions induced by electrical stimulation of the pontine micturition center were inhibited by yohimbine in a dose-dependent manner. These results suggest that transmission in the descending neurons from the pontine micturition center to the sacral parasympathetic neurons that control bladder motility is mediated by $\alpha_2$-adrenoceptors in rats.

Keywords: Clonidine, Yohimbine, Sacral micturition center (rat), Urinary bladder

Reflex contraction of the urinary bladder in various species is mediated by a spinobulbospinal reflex pathway that passes through the pontine micturition center (PMC). Recently, Yoshimura et al. (1-3) demonstrated that the neurons descending from the pontine micturition center of cats originate in the locus ceruleus and that norepinephrine derived from the neurons activate the sacral interneurons via $\alpha_2$-adrenoceptors, thereby inducing excitation of the parasympathetic neurons that receive impulses from the interneurons. In this experiment, we intrathecally injected various drugs that act on adrenoceptors and studied their effects on the sacral micturition center in rats. Our results indicate that excitation of the descending neurons from the pontine micturition center to the sacral parasympathetic neurons innervating the bladder is mediated by $\alpha_2$-adrenoceptors in rats.

MATERIALS AND METHODS

The preparations used and the method of recording bladder contractions have been described in our previous paper (4). Male Wistar rats (weighing 250–350 g) were anesthetized with urethane (1.0 g/kg, s.c.) and $\alpha$-chloralose (25 mg/kg, s.c.). The bladder was exposed through a midline abdominal incision, and a needle (1/4) attached to a silicone tube was inserted into the bladder through the left ureter, which was ligated around the needle. The right ureter was left intact. The bladder was replaced in the abdominal cavity, and then the incision was sutured. Reflex bladder contractions were induced by infusing glucose-free Tyrode's solution into the bladder through the silicone tube at a constant rate (0.8–1.0 ml/10 min). A pressure transducer (Gould P23 ID, Statham, U.S.A.) was used to measure the pressure signals, which were then amplified (AP 601G, Nihon Kohden) and recorded by a D.C. recorder (SR 6204, Watanabe Sokki). Throughout the experiment, glucose-free Tyrode's solution was continuously infused into the bladder.

All drugs, except for the anesthetics, were injected intrathecally (i.t.) into the subarachnoid space of the sacral cord, as described in our previous paper (4). For the experiment in which the effects of drugs on the reflex bladder motility induced by infusion of fluid into the bladder were studied, the rats were placed on their backs. For the experiments in which bladder contraction was evoked by electrical stimulation of the pontine micturition center, a bipolar electrode (UB 9004, Unique Medica) was advanced stereotaxically to a site on the locus ceruleus, determined according to the atlas of Paxinos and Watson (5), using the following coordi-
nates with reference to the bregma: 9.5 mm posterior, and 1.0 mm lateral to the midline and 7.0 mm deep from the skull surface. The rats were held still in the stereotaxic apparatus (ST-7, Narishige); glucose-free Tyrode's solution was infused into the bladder, as described above; and then the animals were subjected to supramaximal electrical stimulation (rectangular pulses, 50–70 Hz, 0.4 msec duration, for 10 sec, SEN-1101, Nihon Kohden). When the electrically induced bladder pressure elevation was very small, the electrode was further inserted in a ventral direction or moved rostrally to induce a greater response. After an almost constant level of bladder contractile activity and accompanying micturition were established, the drug injection was performed.

On completion of this experiment, pentobarbital (50 mg/kg, i.p.) was administered to some rats and then these rats were perfused with 10% v/v formalin solution via the left ventricle. Their brains were removed and soaked in 10% v/v formalin solution which also contained 30% w/v sucrose, for about a week, after which frozen sections (50-μm-thick) of the brainstem were prepared and stained with cresyl violet for histological confirmation of the electrode position.

The following drugs were used: clonidine HCl (Sigma Chemical Company), yohimbine HCl (Wako Pure Chem.), phenylephrine HCl (Wako Pure Chem.) and prazosin (gift from Pfizer Co., Ltd.). Clonidine and phenylephrine were dissolved in normal saline, and all the other drugs used were dissolved in distilled water (pH = approximately 5.5). The drug concentrations were expressed in terms of the respective salts. Each drug concentration was tested in at least four different rats. The results were expressed as means ± S.E., and statistical analyses were carried out by Student’s t-test for paired samples; differences at P values of less than 0.05 were considered to be significant.

RESULTS

Effects of i.t.-injected drugs on the reflex bladder motility induced by infusion of fluid into the bladder

Before injection of clonidine or yohimbine, the mean interval between the bladder contractions was 5.6 ± 0.6 min (n = 18). Clonidine (10 or 30 μg) caused the intervals between the bladder contractions to become shorter and shorter, finally fusing them; this effect finally disappeared, returning to the preinjection levels after 66.2 ± 6.7 min (n = 5) and 93.2 ± 11.5 min (n = 5), respectively (Fig. 1). Yohimbine (30 μg) elevated the threshold pressure, above which the bladder pressure rose steeply and prolonged the interval between the bladder contractions in three rats. In another rat after injection of 30 μg yohimbine and in all four rats after injection of 100 μg yohimbine, the bladder pressure rose to a level at which fluid in the bladder leaked from the urethra, and this high pressure was maintained until micturition was resumed. The cystometrographic patterns returned to the pre-injection one by 20.6 ± 4.6 min (n = 4) and 53.7 ± 17.4 min (n = 4) after the injection of 30 and 100 μg yohimbine, respectively (Fig. 2). When the antagonism between clonidine and yohimbine was studied, yohimbine (30 μg) was first injected and then clonidine was administered after the establishment of a bladder contraction with a threshold approximately the same as that before yohimbine injection. Yohimbine (30 μg) suppressed the effect of clonidine (10 μg) on the bladder motility (Fig. 3), but not that of clonidine (30 μg) in all four rats. Phenylephrine (60 μg) elevated the threshold pressure and only prolonged the interval between the bladder contractions before and
after injection of phenylephrine (Fig. 4). Prazosin (40 μg) did not cause any significant change in the pattern of the cystometrogram (Fig. 5).

**Effect of i.t.-injected yohimbine on the response induced by electrical stimulation of the PMC**

Electrical stimulation of the PMC induced an almost immediate sharp increase in the bladder pressure. During electrical stimulation of the PMC, a concomitant tonic contraction or slight lifting of the tail were observed, but there were some rats in which tail lifting occurred in the absence of bladder pressure elevation. The bladder pressure fell steeply after evacuation of fluid from the urethra or cessation of the electrical stimulation of PMC. In some rats, the bladder contraction occurred after cessation of the electrical stimulation of PMC, and fluid was evacuated from the urethra. In order to perform electrical stimulation of the PMC when the volume of fluid in the bladder was virtually the same, electrical stimulation was carried out at near constant intervals of 3 or 5 min after the bladder contraction accompanying evacuation of fluid from the urethra. Yohimbine (30 and 100 μg) inhibited the electrical stimulation-induced bladder response in a dose-dependent manner (Fig. 6, A and B). As fluid was not evacuated from the urethra, bladder pressure was grad-
ually elevated to almost the peak pressure level during micturition before injection of yohimbine, and high bladder pressure was maintained until electrical stimulation of the PMC could evacuate fluid from the urethra. When fluid was evacuated from the urethra, bladder pressure fell almost to baseline levels (Fig. 6A).

**DISCUSSION**

The results of this study indicate that the excitation of the sacral neurons that provide the excitatory input to the bladder are mediated via $\alpha_2$-adrenoceptors in rats. In contrast, the excitation of the $\alpha_1$-adrenoceptors of the sacral neurons induced the suppression of the bladder motility. The bladder motility facilitation appeared gradually after i.t.-injection of clonidine and intensified with time, suggesting that it may take some time for clonidine to reach the neuronal site at which it acts. Yohimbine inhibited the bladder contraction induced by infusion of fluid into the bladder at almost the same dose at which it inhibited the effect of clonidine on the bladder motility. These results suggest that clonidine may stimulate the sacral neurons that are activated by the descending neurons from the PMC. Alternatively, clonidine may act on other interneurons in the sacral cord which induce the activation of the pelvic nerves. Therefore, we studied the sacral neurons that are activated by the descending neurons from the PMC to determine whether $\alpha_2$-adrenoceptors are involved in the transmission process. Also, in rats, it has been reported that the pontine noradrenergic cell groups including the locus ceruleus are the sole source of norad-
The effect of i.t.-injection of yohimbine on the urinary bladder response induced by electrical stimulation of the pontine micturition center in anesthetized rat. (A) Cystometrogram of urinary bladder response induced by electrical stimulation before and after i.t.-injection of yohimbine. At the point indicated by the star, the rat was subjected to electrical stimulation (rectangular pulses, 50–70 Hz, 0.4 msec duration for 10 sec). For details, see Fig. 1 legend. (B) The results are expressed as percentage of the response induced by electrical stimulation before injection of yohimbine (n = 5–6 per group). Solid column: before injection of yohimbine. Open column: after injection of yohimbine. The numbers below the column indicate the time (min) after drug injection. *P < 0.05, **P < 0.01: significant difference compared with the response before drug injection (paired t-test).
bladder pressure elevation was independent of the tail movement. We previously reported that reserpine treatment suppressed the micturition reflex in rats (9). The inhibitory effect on the bladder motility in rats given reserpine seems to be explained by the depletion of norepinephrine in the descending neurons from the PMC. Though in many species, the sympathetic pathway (hypogastric nerve) transmits an inhibitory effect on the bladder motility, in rats, the hypogastric nerve stimulation did not inhibit the bladder motility (10, 11). Therefore, in rats, the depletion of norepinephrine in the hypogastric nerve endings after treatment with reserpine does not affect the bladder motility. The excitatory transmission from the descending neurons from the PMC to the sacral neurons may be interrupted in the rats given reserpine and the bladder motility may be suppressed.

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