The Effect of Baclofen on the Urinary Bladder Contraction Accompanying Micturition in Anesthetized Rats

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Abstract—We studied the effects of baclofen on the bladder contraction induced by infusion of Tyrode's solution into the urinary bladder in anesthetized rats. Baclofen (5 mg/kg, i.v.) completely inhibited bladder contraction and abolished the efferent discharges recorded from the left pelvic nerve, causing the bladder pressure to rise until solution leaked from the penis. The inhibitory effect of baclofen (5 mg/kg, i.v.) could not be reversed by picrotoxin (1 mg/kg, i.v., twice with an interval of 10 min) or naloxone (1 mg/kg, i.v.). In parallel with convulsion, strychnine (1 mg/kg, i.v.) contracted the bladder which had been inhibited by baclofen and generated electrical activities consisting of efferent discharges and electromyograms. The dose of intracerebroventricularly or intrathecally injected baclofen which completely inhibited the bladder contraction was 0.1 or 10 µg, respectively. After the inhibition of bladder contraction by i.v. injection of baclofen, electrical stimulation of the sacral cord could contract the bladder and cause a fall in bladder pressure to around the level existing after micturition. From these results, the active site of baclofen which is related to the inhibition of bladder contraction is thought to be the micturition center in the brain stem.

Baclofen (p-chlorophenyl-GABA), a widely used antispastic agent, is also known to be clinically useful for the treatment of detrusor-sphincter dyssynergia and pelvic floor spasticity in patients with spinal lesions (1-4) and for improving the diurnal and nocturnal high frequency of micturition and the severity of incontinence in patients with unstable urinary bladder syndrome (5). Recently, Sillén et al. (6) reported that baclofen was able to inhibit hyperactive bladder induced by I-dopa administration, and the inhibitory effects were related to interference with an opioid mechanism. With regard to the muscle relaxant activity of baclofen, the results of a large number of studies suggest that it is due to a reduction in the excitability of the spinal cord (7). Ono et al. (8) found that baclofen reduced the mono- and poly-synaptic reflex potentials and dorsal root – dorsal root reflex potential in anesthetized and spinal rats. We (9) recently reported the presence of GABA<sub>A</sub> receptors in the micturition center in the sacral cord, as well as in the brain stem, and that GABA<sub>A</sub> agonists inhibited bladder motility through the GABA receptors in both of these micturition centers. However, the relation between the inhibitory effects of baclofen on the urinary bladder contraction and the action on the spinal cord is still not clear. In the present experiment, we investigated the sites of action of baclofen using the method which were used to study the effect of diazepam on urinary bladder contraction (9). Baclofen is a selective agonist for GABA<sub>B</sub> receptor, and the location of GABA<sub>B</sub> receptors in the spinal cord is distinct from that of GABA<sub>A</sub> (10, 11). There is no known specific GABA<sub>B</sub> receptor antagonist. We studied the effects of strychnine after the inhibition of urinary bladder contraction by baclofen in order to clarify that the inhibition was due to the reduction of excitability of the spinal cord, since strychnine has an excitatory effect on the central nervous system, especially the spinal cord. The results
obtained in the present experiment indicated that the main site of action of baclofen was the micturition center in the brain stem and that the participation of the action on the spinal cord was small, in contrast with the antispastic activity. A preliminary report on this work has already been published (12).

Materials and Methods

The preparations used and the method of recording bladder contractions were described in our previous paper (9, 13). Male Wistar rats (weighing 250–350 g) were anesthetized with urethane (1.0 g/kg, s.c.) and α-chloralose (25 mg/kg, s.c.). The bladder was exposed through a midline incision in the abdomen, and the abdominal muscle was transected. The bladder was then prepared for the recording of pressure within the bladder as follows: a needle (1/4) attached to one end of a silicone tube (O.D., 1.0 mm and I.D., 0.5 mm, 30–40 cm in length) was inserted into the bladder through the left ureter. The left ureter was then ligated around the needle so that the urine from the left kidney flowed out from the incision of the ureter through which the needle was inserted. The right ureter was kept intact. The other end of the silicone tube was connected to a syringe and a pressure transducer (Nihon Kohden, MPU-0.5) by means of a T-tube. The whole system was filled with Tyrode’s solution lacking glucose. In order to infuse the Tyrode’s solution from the syringe into the urinary bladder at a constant rate (0.8–1.0 ml/10 min), the plunger of the syringe was continuously pushed by that of another syringe into which water was infused with a peristaltic pump (Atto, SJ-1211). The pressure signals were delivered by an amplifier (Nihon Kohden, RP-5) and recorded by a D.C. recorder (Watanabe Sokki, SR 6204). Cotton-wool swabs soaked with Tyrode’s solution were laid on the bladder to keep it moist, and the swabs were warmed with a lamp.

When the influence of aminooxyacetic acid (10 mg/kg, i.v.) and semicarbazide (200 mg/kg, i.v.) on the effects of baclofen were studied, baclofen was injected at least 2 or 4 hr after injection of aminooxyacetic acid or semicarbazide, respectively.

When the efferent activities were recorded from the urinary branches of left pelvic nerve fibers, the urinary branch of the left pelvic nerve fibers was isolated from the connective tissue for as long a distance as possible and separated from the fibers innervating the rectum. The central ends of the fibers were placed on a pair of platinum electrodes and covered with paraffin oil. Efferent impulses were amplified with an amplifier (Nihon Kohden, AVB-10) and displayed on an oscilloscope (Nihon Kohden, VC-10). As the amplitudes of these efferent impulses were very small and the spikes could not be completely separated from background noise, only those spikes with a voltage higher than the background noise were counted by a tachometer (Sanei Sokki, Model 1332). The output voltage of the tachometer was recorded on a D.C. recorder together with the bladder pressure measured with a pressure transducer (Nihon Kohden, LPU-0.1).

Intracerebroventricular (i.c.v.) injection was performed as follows. The rats were anesthetized with pentobarbital (40 mg/kg, i.p.) and placed on a stereotaxic apparatus. A catheter was implanted into the lateral ventricle using the following coordinates with reference to the bregma: 0.3 mm anterior, 1.0 mm lateral to the midline and 5.6 mm deep, from the skull surface. Four or five days after implantation, i.c.v. injection was performed by means of a microsyringe. Each injection volume was 5 μl. The position of the catheter was checked in all animals after the experiments by studying the distribution of injected trypan blue.

When intrathecal (i.t.) injection was performed, the drug was injected into the spinal subarachnoid space according to the method of Yaksh and Rudy (14). A polyethylene tube (SP-8, Natsume) was inserted to a depth of approximately 8.5 cm through a split between the first and second cerebral vertebrae into the subarachnoid space. At the rat head side, the board onto which the rat was fixed was raised by about 1 cm to flow the injected solution caudally. The injection volume was 20 μl (9). After the experiments, the location of the tube in the subarachnoid space was checked by laminectomy. In order to perform electrical stimulation of the spinal cord at the
site of drug injection, a polyethylene tube, through which an insulated copper wire (0.1 mm in diameter) with 1 mm of the tip exposed had been passed, was inserted in the same way as for i.t. injection, but into the space between the vertebrae and the dura mater. The exposed tip of the copper wire was pushed out of the tube once it had been placed in the spinal cord. A silver wire was then placed subcutaneously over the site of the copper wire tip, and the copper and silver wires were connected to a stimulator (Nihon Kohden, SEN-1101). Under artificial respiration and after injection of d-tubocurarine, rectangular pulses of 1 msec duration and 10 mA strength were applied at 5 Hz for 10 sec, and the change in bladder pressure was recorded. During stimulation, the heart rate was measured with a pulse rate tachometer (Nihon Kohden, RT-5) which was triggered by the R wave of the electrocardiogram lead I. In some experiments, the site of the tube tip was checked using a soft X-ray fluoroscope (Softex).

Baclofen was dissolved in saline (pH=6.5–7.0) and injected into the femoral vein (i.v.). Baclofen was dissolved in artificial cerebrospinal fluid (a.c.s.f., g/l: NaCl, 8.1; KCl, 0.25; CaCl₂, 0.14; MgCl₂, 0.11; and NaHCO₃, 1.0, pH=7.0–7.2) for i.c.v. injection or in saline (pH 4.5–5.0) for i.t. injection. After it had been confirmed that the bladder contraction was not influenced by i.c.v. and i.t. injection of a.c.s.f. or saline for at least 10 min, the solution containing baclofen was injected.

Drugs used were: baclofen (a gift from Daiichi Seiyaku), semicarbazide HCl, aminoxyacetic acid HCl and strychnine HNO₃ (Wako Pure Chem.), pentobarbital Na (Nembutal, Daiichi Seiyaku) and d-tubocurarine (Amerizole, Yoshitomi Pharm. Co.). All drug concentrations in this report are expressed as those of each respective salt.

Results

1) The effects of baclofen on bladder contraction induced by infusion of solution into the bladder: Baclofen (1 mg/kg, i.v.) slightly increased the level of bladder pressure at which the pressure rose steeply (micturition threshold) in only one out of four rats (Fig. 1A). Baclofen (5 mg/kg, i.v.) completely inhibited the bladder contraction. As infusion of solution into the bladder continued after the inhibition of bladder contraction by baclofen, the bladder pressure rose to the level at which solution leaked from the penis, and this high bladder pressure was maintained (Fig. 1B). The bladder pressure after inhibition of bladder contraction was significantly higher than the pressure at which the solution was excreted from the bladder by bladder contraction, and the percentage of former pressure relative to latter one was 119.1±4.0% (mean±S.E., n=9). In one out of five rats, the bladder contraction spontaneously resumed about 30 min after the injection of baclofen (5 mg/kg, i.v.), but in the other four rats, the bladder contraction had been inhibited. In the four rats infusion of solution was interrupted, and the solution in the bladder was drained about 40 min after injection of baclofen. When infusion of solution was resumed about 20 min after interruption of infusion in these four rats, the bladder contraction accompanying micturition occurred. The effects of picrotoxin, naloxone and strychnine were studied in the bladder whose motility had been inhibited by baclofen (5 mg/kg, i.v.). When picrotoxin (1 mg/kg, i.v., twice with an interval of 10 min) and naloxone (1 mg/kg, i.v.) were injected 20 min after baclofen injection, these drugs were unable to induce contraction in any of the four rats. However strychnine (1 mg/kg, i.v.) contracted the bladder along with convulsion immediately after injection. At that time, solution was excreted from the penis by the bladder contraction, and the bladder pressure was reduced. Thereafter, the bladder pressure gradually rose to the level existing before injection of strychnine (Fig. 1C). However, the bladder contraction was not repeated. The second injection of strychnine (1 mg/kg, i.v.) 10 min after the first injection was also able to contract the bladder immediately after injection in four out of six rats, while in the other two rats, bladder contraction was repeated after the second injection of strychnine. Even in the bladder whose motility was inhibited by diazepam (1 mg/kg, i.p.), strychnine (1 mg/kg, i.v.) was also capable of contracting the bladder in parallel with convulsion im-
2) The influence of pretreatment with semicarbazide or aminooxyacetic acid on the inhibitory effects of baclofen: Pretreatment with semicarbazide (200 mg/kg, i.v.) did not attenuate the inhibitory effect of baclofen (5 mg/kg, i.v.) on the bladder contraction, and pretreatment with aminooxyacetic acid (10 mg/kg, i.v.) did not potentiate the effect of baclofen (1 mg/kg, i.v.). Even in the preparation with a high micturition threshold after pretreatment with aminooxyacetic acid, baclofen (1 mg/kg, i.v.) did not interrupt the bladder contraction (Fig. 2).

3) The effects of baclofen on the efferent activities recorded from the left pelvic nerves: Baclofen (5 mg/kg, i.v.) abolished both efferent discharges and bladder contraction simultaneously (Fig. 3). After disappearance of efferent discharges, strychnine (1 mg/kg, i.v.) generated high-frequency, high-amplitude spikes which appeared to be a mixture of efferent discharges and electromyograms (Fig. 3).

4) The effects of i.c.v. or i.t. injection of baclofen on the bladder contraction induced immediately after injection.
by infusion of solution: After i.c.v. injection of baclofen (0.1 μg), bladder contraction was completely inhibited (Fig. 4A). Strychnine (1 mg/kg, i.v.) contracted the bladder which had been inhibited by i.c.v. injection of baclofen immediately after its injection (Fig. 4A). On the other hand, a high dose of baclofen (10 μg) was necessary to cause inhibition of the bladder contraction following i.t. injection (Fig. 4B). However, bladder contraction resumed in 3 rats, and the interruption time of the bladder contraction was 20.5±3.5 min (mean±S.E.). In the other 2 rats, the bladder contraction had been inhibited and the bladder contraction resumed when strychnine (1 mg/kg, i.v.) was injected 40 min after i.t. injection of baclofen (Fig. 4B).

The percentages of bladder pressure after inhibition of bladder contraction by i.c.v. or i.t. injection of baclofen relative to the pressure at which the excretion of solution from the bladder began before inhibition were 135.2±11.2 and 69.4±2.5 (mean±S.E., n=4), respectively, and i.t. injection significantly reduced the bladder pressure at which solution leaked from the penis after inhibition of bladder contraction (P<0.01).

5) The response to electrical stimulation of the spinal cord in the bladder showing complete inhibition of contraction by baclofen or diazepam: Figure 5 shows the response to electrical stimulation of sacral cord in the rats in which the bladder contraction was not induced by infusion of solution: After i.c.v. injection of baclofen (0.1 μg), bladder contraction was completely inhibited (Fig. 4A). Strychnine (1 mg/kg, i.v.) contracted the bladder which had been inhibited by i.c.v. injection of baclofen immediately after its injection (Fig. 4A). On the other hand, a high dose of baclofen (10 μg) was necessary to cause inhibition of the bladder contraction following i.t. injection (Fig. 4B). However, bladder contraction resumed in 3 rats, and the interruption time of the bladder contraction was 20.5±3.5 min (mean±S.E.). In the other 2 rats, the bladder contraction had been inhibited and the bladder contraction resumed when strychnine (1 mg/kg, i.v.) was injected 40 min after i.t. injection of baclofen (Fig. 4B).
Fig. 4. The effects of intracerebroventricular (i.c.v., A) or intrathecal (i.t., B) injection of baclofen on the urinary bladder contraction and the responses to i.v. injection of strychnine after the disappearance of bladder contraction by each injection of baclofen. Vertical bar: bladder pressure. Horizontal bar: time after i.c.v. injection of artificial cerebrospinal fluid (a.c.s.f.) or i.t. injection of saline. The solid circle indicates i.c.v. or i.t. injection of baclofen, and the open circle indicates i.c.v. injection of a.c.s.f., i.t. injection of saline or i.v. injection of strychnine. Dotted arrows (↓) indicate the appearance of convulsion.

Fig. 5. The response to electrical stimulation of the sacral part of the spinal cord in the urinary bladder. This experiment was performed in the rats whose bladders were not contracted by infusion of Tyrode's solution into the bladder. Vertical bar: bladder pressure. At the point indicated by the arrow, electrical stimulation at 5 Hz was performed for 10 sec. Top trace shows the heart rate. The artifact of electrical stimulation was superimposed during stimulation. d-Tubocurarine (0.1 mg/kg, i.v.) was injected 10 min before the first electrical stimulation.

solution into it. About 10 min after d-tubocurarine (0.1 mg/kg, i.v.) was injected for immobilization, electrical stimulation of the sacral cord was able to contract the bladder and excreted solution from the penis. Figure 6 shows the responses to electrical stimula-
tion of the sacral cord in the rats whose bladders were contracted by infusion of solution into it. After inhibition of bladder contraction by baclofen (5 mg/kg, i.v.) and diazepam (1 mg/kg, i.p.), electrical stimulation contracted the bladder, and % fall of bladder pressure upon electrical stimulation relative to the bladder pressure after inhibition of bladder contraction by baclofen or diazepam was 95.0±2.1 or 62.9±4.2 (mean±S.E.: n=4), respectively. Baclofen (5 mg/kg, i.v.) did not inhibit the response to electrical stimulation of the sacral cord.

Discussion
Sillén et al. (6) have studied the effects of baclofen on the bladder hyperactivity induced by administration of L-dopa and carbidopa in pentobarbital-anesthetized rats, and they suggested that the baclofen acted in the vicinity of the 4th ventricle, the pontine-mesencephalic brain part, and that the inhibitory effects of baclofen were mediated by interference with an opioid mechanism. In the present experiments, although the method by which bladder contraction was induced and the regions receiving systemic and i.c.v. administration of baclofen were different from those in the study of Sillén et al. (6), the dose ranges of baclofen in both administration routes which were able to exert an inhibitory effect on bladder contraction were very similar in both experiments (Ref. 6 and Figs. 1 and 4). We (9) reported that the dose ranges of i.p. administration of diazepam and i.c.v. administration of muscimol which could inhibit bladder contraction were very similar to those of Sillén et al. (15). The inhibitory effect of baclofen was not reversed by picrotoxin at the dose range which could
reverse the inhibitory effect of diazepam (9). In order to study the influence of the change in GABA concentration in rats on the inhibitory effect of baclofen, pretreatments of glutamic acid decarboxylase inhibitor semicarbazide and GABA transaminase inhibitor aminooxyacetic acid were performed at the dose ranges which could attenuate and potentiate the inhibitory effects of diazepam on the bladder contraction, respectively (9). However, these drug pretreatments did not influence the inhibitory effect of baclofen. It was reported that each pretreatment of these drugs attenuated or potentiated the inhibitory effects of diazepam on the anemic decerebrate rigidity, but did not influence that of baclofen (16). From these results, we also confirmed the suggestion of Sillén et al. (6) that the inhibitory effects of baclofen would be unrelated to the bicuculline sensitive neurotransmission. On the other hand, we did not obtain any evidence to suggest that the inhibitory effect of baclofen would be mediated through interference with the opioid mechanism (6). The inhibitory effect of baclofen (5 mg/kg, i.v.) was not reversed by naloxone (1 mg/kg, i.v.). Though the dose of naloxone in the present experiments was very low in comparison with the dose (25 mg/kg, i.v.) in the experiments of Sillén et al. (6), the dose of naloxone (1 mg/kg, i.v.) was about 10 times higher than that capable of reversing the inhibitory effect of morphine on bladder contraction and that it did not influence the generation of efferent discharges recorded from the left pelvic nerve (our unpublished data). We did not try a higher dose of naloxone, since naloxone itself was reported to have an excitatory effect on bladder contraction and to increase the frequency of efferent discharges from the pelvic nerves (17, 18).

The dose of i.c.v.-injected baclofen which was able to completely inhibit bladder contraction was about 100 times lower than that by i.t. injection (Fig. 4). The main site of action of baclofen would thus seem to be the micturition center in the brain stem. The disappearance of efferent discharges after injection of baclofen would therefore result from its inhibition of the micturition center in the brain stem (Fig. 3). After the inhibition of bladder contraction by i.v. injection of baclofen, electrical stimulation of the sacral cord reduced the bladder pressure to the level existing after micturition, but not after the inhibition by diazepam (Fig. 6). These results suggest that the inhibitory effect of baclofen on the micturition center of the sacral cord may be weaker than that of diazepam. The site and conditions of electrical stimulation of the sacral cord appeared to be adequate, since bladder preparations which could not be contracted by infusion of solution into the bladder were contracted by electrical stimulation without any significant influence on heart rate (Fig. 5). Electrical stimulation of the sacral cord could contract the bladder which was not contracted by infusion of solution into it in a reflex manner (Fig. 5). In the rats, anesthetics might reduce the excitability of the micturition centers, so that the afferent discharges from the bladder were unable to evoke excitation of the micturition reflex (19).

The bladder contraction which was induced immediately after injection of strychnine would result from nonspecific excitation of the neurone in the spinal cord, since efferent discharges and electromyograms would be observed (Fig. 3). The recovery of bladder contraction after i.t. injection of baclofen by strychnine would result from the physiological antagonism between the two drugs, rather than being due to competition.

In our preparation, both the pressures at which the solution was excreted from the bladder by bladder contraction and solution leaked from the penis after interruption of micturition were thought to indicate the pressure at which the outlet of the bladder became opened. The bladder pressure after i.t. injection of baclofen was significantly lower than that after i.c.v. or systemic administration. It was reported that baclofen depressed both pudendal-to-pudendal and pelvic-to-pudendal nerve reflexes and reduced the urethral pressure in patients with spinal injury and in dogs (3, 20). When baclofen was injected into the sacral cord at high concentration, it would cause relaxation of the muscle around the outlet of the bladder, as well as inhibition of detrusor muscle contraction due to inhibition of the micturition
center in the sacral cord.

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