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GABAergic contribution to rat bladder hyperactivity after middle cerebral artery occlusion

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Kanie, Sayoko, Osamu Yokoyama, Kazuto Komatsu, Koichi Kodama, Satoshi Yotsuyanagi, Susumu Niikura, Yasuhiro Nagasaka, Ken-Ichi Miyamoto, and Mikio Namiki. GABAergic contribution to rat bladder hyperactivity after middle cerebral artery occlusion. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R1230–R1238, 2000.—To evaluate the influences of γ-aminobutyric acid (GABA) mechanisms on bladder hyperactivity after left middle cerebral artery occlusion, cystometric recordings were obtained from unanesthetized female rats. Intracerebroventricular administration of both muscimol ($GABA_A$ receptor agonist; 0.1–10 nmol) and baclofen ($GABA_B$ receptor agonist; 0.1–3 nmol) produced dose-dependent inhibitions of micturition with increases in bladder capacity (BC). The effects of high doses (1–10 nmol) were similar in sham-operated (SO) and cerebral-infarcted (CI) rats. However, lower doses of muscimol (0.1 or 0.3 nmol) and baclofen (0.1 nmol) reduced BC in CI rats. After bicuculline ($GABA_A$ receptor antagonist; 1 or 3 nmol) administration, BC in both SO and CI rats first decreased and subsequently increased. An increase in urethral pressure was observed after administration of bicuculline (3 nmol) but not with either muscimol or baclofen. Infarct volumes in muscimol-, bicuculline-, or baclofen-treated rats were not significantly different from those of vehicle-treated rats. These results suggest that GABAergic mechanisms inhibit the micturition reflex at the supraspinal level but that this can change as a result of CI.

GABA or γ-aminobutyric acid; micturition reflex; cerebral infarction

THE LOWER URINARY TRACT has two main functions: storage and periodic elimination of urine. This control system performs like a simple switching circuit to maintain a reciprocal relationship between the reservoir (bladder) and the outlet components (urethra and urethral sphincter) of the urinary tract. These functions are regulated by a complex neural control system located in the brain and spinal cord. The switching circuit is modulated by several neurotransmitter systems and is therefore sensitive to a variety of drugs and neurological diseases (2).

Reflex bladder activity in the neurally intact rat is dependent on an integrative center in the pons that has been referred to as Barrington's nucleus or the pontine micturition center (PMC) (22, 24). Furthermore, the pontine urine storage center (PSC) located in the pons suppresses bladder contraction and regulates the external sphincter muscle activity during urine storage (25). Griffiths et al. (4), in a report on the results of stimulation and lesion experiments in the pontine tegmentum of cats, suggest that the M (medial) region forms a true micturition center, facilitating the detrusor voiding contraction and also ensuring synergic sphincter relaxation. The L (lateral) region appears not only to relay this voiding sphincter relaxation but also to be responsible for control of the pelvic floor and its sphincters in general, as well as for helping to maintain urinary continence.

γ-Aminobutyric acid (GABA) and glycine have been identified as inhibitory transmitters at various sites in the mammalian central nervous system. The inhibitory effects of GABA on urinary bladder motility have been widely investigated in rats, and, in the micturition reflex pathway, these agents appear to have an inhibitory function at both the spinal and supraspinal synapses (2). There is considerable evidence indicating that injections of muscimol (a $GABA_A$ receptor agonist) or GABA intrathecally, intracerebroventricularly, or directly into the PMC inhibit reflex bladder contractions in normal rats with or without anesthesia (urethan) (8, 10, 16). Furthermore, baclofen (a $GABA_B$ receptor agonist) suppresses the micturition reflex when injected intrathecally, intracerebroventricularly, intravenously, or intraperitoneally in normal rats with or without anesthesia (urethan) (11, 12, 18–20).

These findings suggest that $GABA_A$ and $GABA_B$ receptors are likely to be involved in supraspinal inhibitory mechanisms in the micturition pathway. We hypothesized that GABAergic agents [muscimol, bicuculline (a $GABA_A$ receptor antagonist), and baclofen] may also be related to the overactive bladder after

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cerebral infarction. To test this hypothesis, the effect of intracerebroventricular administration of GABAergic agents on bladder hyperactivity induced by left middle cerebral artery (MCA) occlusion was investigated.

MATERIALS AND METHODS

All experiments were performed in strict compliance with the guidelines of the Institutional Animal Care and Use Committee of the University of Kanazawa.

For this study, 79 female Sprague-Dawley rats weighing 210–280 g (mean = 240 g) were used.

Cystometrography in conscious rats. We adopted the method of Yaksh et al. (27) for cystometrography (CMG) in conscious rats. Rats were anesthetized with halothane (1.5%), and the bladder was exposed via a midline incision in the abdomen. The bladder end of a polyethylene catheter (size 4: ID 0.8 mm, OD 1.3 mm; Kunii, Tokyo, Japan) was heated to create a collar and passed through a small incision at the apex of the bladder dome, after which a suture was tightened around the collar of the catheter. The other end of the catheter was passed through the subcutaneous tissue and exited through the skin at the back of the neck. After the abdominal skin was sutured, the rat was allowed to recover from the anesthesia. During the CMG recording, the rat was placed in a restraining cage (Ballman Cage, KN-326 type 3; Natsume Seisakusho, Tokyo, Japan). The cystostomy catheter was connected to a pump (TE-311; Terumo, Tokyo, Japan), for the continuous infusion of saline, and to a pressure transducer (TP-200T; Nihon-Kohden, Tokyo, Japan) by means of a polyethylene T tube. Cystometry was performed with physiological saline at room temperature at a rate of 0.04 ml/min. Saline voided from the urethral meatus was collected and measured to determine the voided volume. Three parameters were established from the CMGs: bladder capacity (voiding and residual volume), threshold pressure (bladder pressure immediately before micturition), and micturition pressure (the maximum bladder pressure during micturition minus threshold pressure). The postadministration volume was expressed as a percentage of the preadministration volume, and threshold pressure and micturition pressure were similarly expressed.

Induction of cerebral infarction. Two hours after implantation of the cystometry catheter, the rats were anesthetized with halothane (1%). The left carotid bifurcation was exposed through a midline incision in the neck. After ligation of the left common carotid artery, the left internal carotid artery (ICA) was isolated and carefully separated from the adjacent vagus nerve, followed by ligation of the pterygopalatine branch of the left ICA close to its origin. A 4–0 monofilament nylon thread, with the tip rounded by exposure to a flame, was inserted into the left ICA and advanced up to 17 mm from the carotid bifurcation on the left side of the brain. This procedure occluded blood flow in the MCA and induced infarction on the left side of the brain (13). In sham-operated rats, the left carotid bifurcation was exposed through a midline incision in the neck, but no further procedures were performed.

Intracerebroventricular administration of drugs. After implantation of the cystometry catheter, the rats were positioned in a stereotaxic apparatus (ST-7; Narishige, Tokyo, Japan). A scalp incision was made over the sagital suture, and the brain map by Paxinos and Watson (23) was consulted for implantation of a stainless steel cannula (OD 0.6 mm, ID 0.3 mm, length 10.5 mm). It was implanted into the right lateral ventricle by using the following coordinates with reference to the bregma: 0.3 mm anterior, 1.0 mm lateral to the midline, and 5.3 mm below the skull surface (10). With the aid of a small screw placed in the skull as an anchor, the cannula was fixed to the skull with dental acrylic.

Evaluation of the corrected infarction volume. After evaluation of the drug's effects, the rat brain was stained by means of perfusion with 2% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, St. Louis, MO) (5). We then performed a thoracotomy, inserted a catheter into the ascending aorta via the left ventricle, perfused it with heparinized saline, and incised the right atrium. After 2 min, the right atrium was clamped, and 2% TTC in saline was infused over a period of 7 min. After completion of the perfusion, the rat brains were removed and the cerebral hemispheres cut into five coronal slices, each 2 mm thick. The rostral surface of the TTC-stained sections was photographed with color slide film, and the volume of the infarctions was measured with the method of Golanov and Reis (3).

Evaluation of effects of muscimol, bicuculline, and baclofen on bladder activity during consciousness. One hour after MCA occlusion or sham operation, the effects of increasing doses of muscimol (0.1–10 nmol icv), bicuculline (0.1–3 nmol icv), baclofen (0.1–3 nmol icv), or vehicle (artificial cerebrospinal fluid, icv) on bladder activity were examined in awake rats after control cystometric recording. Increasing doses of the drugs were administered cumulatively at 60-min intervals.

Evaluation of effects of bicuculline on bladder and external urethral sphincter activities in urethan-anesthetized rats. Two hours after MCA occlusion or sham operation, rats were anesthetized with urethan (1 g/kg ip). Two insulated fine wire electrodes (80-μm diameter) were inserted into the periurethral musculature for electromyogram (EMG) recording of the external urethral sphincter (EUS). The amplitudes of EMG potentials were displayed and recorded on a storage oscilloscope (9). One hour after urethan anesthesia, bicuculline (0.1 or 3 nmol icv) was administered. During CMG and EMG recording, the rats were placed in a restraining cage.

Evaluation of effects of muscimol, bicuculline, and baclofen on bladder and urethral pressures in urethan-anesthetized rats. Two hours after MCA occlusion or sham operation, rats were anesthetized with urethan (1 g/kg ip). The bladder and proximal urethra were exposed through a midline abdominal incision, and the ureters were ligated. A polyethylene catheter (size 4; Kunii) was inserted through the bladder dome, secured with a ligature, and connected to a pump. The bladder was first filled with saline to induce rhythmic bladder contractions, after which isovolumetric bladder pressure was recorded. Urethral activity, measured as urethral perfusion pressure, was monitored with a double-lumen catheter (made of PE-160 and PE-50; Clay-Adams, Parsippany, NJ), with its tip embedded in a cone-shaped plug, which was introduced transvesically through a separate bladder incision in the bladder dome and then wedged in the bladder neck. The outer lumen of the catheter was connected to a pump for continuous saline infusion (0.075 ml/min), and the inner lumen was connected to a transducer for monitoring urethral perfusion pressure (9). One hour after measurement was started, muscimol, bicuculline, and baclofen (0.1 or 3 nmol icv) were administered, and bladder pressure and urethral pressure (UP) were recorded.

Drugs. Drugs used in this study were 3-hydroxy-5-aminomethylisoxazole (muscimol; Research Organics, Cleveland, OH), (−)-bicuculline methiodide (Research Biochemicals International, Natick, MA), and (±)-baclofen (Research Biochemicals International). All drugs were dissolved in artificial cerebrospinal fluid (138.6 nM NaCl, 3.35 nM KCl, 1.26

nM $CaCl_2$, 1.16 nM $MgCl_2$, 11.9 nM $NaHCO_3$, pH = 7.0–7.2) for icv administration (10).

Statistical analysis. The results are presented as means ± SE. Statistical comparisons used two-way repeated-measures ANOVA, with subsequent individual comparisons by Fisher's protected least significant difference test, a paired *t*-test, or an unpaired *t*-test. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Bladder capacities (BC) were 0.40 ± 0.03 ml before, and 0.41 ± 0.03 ml 1 h after sham operation. BCs were 0.51 ± 0.02 ml before, and 0.24 ± 0.01 ml 1 h after MCA occlusion. The rats with cerebral infarction showed a significant reduction in BC ($P < 0.01$) that was consistently less than that of sham-operated rats.

Effects of muscimol in sham-operated and cerebral-infarcted rats. Low doses of muscimol (0.1 and 0.3 nmol icv) produced significant reductions in BC (74.2 ± 5.8 and 61.1 ± 7.1%, respectively; both $P < 0.01$) in cerebral-infarcted rats ($n = 5$; Figs. 1*A* and 2*A*) but had no effect in sham-operated rats ($n = 9$) compared with rats administered vehicle (sham-operated rats: $n = 6$; cerebral-infarcted rats: $n = 5$). The percentage increases in BC at 1 nmol (icv) of muscimol in cerebral-infarcted rats were 122.0 ± 19.9% (Figs. 1*B* and 2*A*, $P < 0.01$) and 51.8 ± 12.3% in sham-operated rats compared with vehicle-administered rats. In both sham-operated and cerebral-infarcted rats, higher doses of muscimol (3 and 10 nmol icv) completely inhibited bladder contraction. BC in these rats was defined as the residual volume when saline leaked from the urethral meatus (Fig. 1*C*). The respective percentage increases in BC at 3 and 10 nmol of muscimol were 162.1 ± 19.8 and 203.8 ± 24.4% in sham-operated rats (both $P < 0.01$) and 317.0 ± 30.7 and 393.2 ± 52.1% in cerebral-infarcted rats (both $P < 0.01$) compared with rats administered vehicle (Fig. 2*A*).

Intracerebroventricular administration of muscimol (1–10 nmol) produced a dose-dependent inhibition of micturition and increased BC. The percentage increase in BC of cerebral-infarcted rats was significantly higher than that of sham-operated rats ($P < 0.01$).

With the increase in BC, the threshold pressure increased (Fig. 2*B*) and the micturition pressure decreased in both sham-operated and cerebral-infarcted rats (Fig. 2*C*).

Effects of bicuculline in sham-operated and cerebral-infarcted rats. In both sham-operated rats ($n = 6$) and cerebral-infarcted rats ($n = 5$), low doses of bicuculline (0.1 and 0.3 nmol icv) did not change BC (Fig. 3*A*), threshold pressure (Fig. 3*B*), or micturition pressure (Fig. 3*C*) compared with vehicle-administered rats (sham-operated rats: $n = 6$; cerebral-infarcted rats: $n = 5$). On the other hand, higher doses of bicuculline (1 and 3 nmol icv) produced a transient facilitation of the micturition reflex immediately after administration. This facilitatory effect was always followed by convulsions lasting a few minutes, after which BC increased (Fig. 4, *A* and *B*). In sham-operated rats, BC

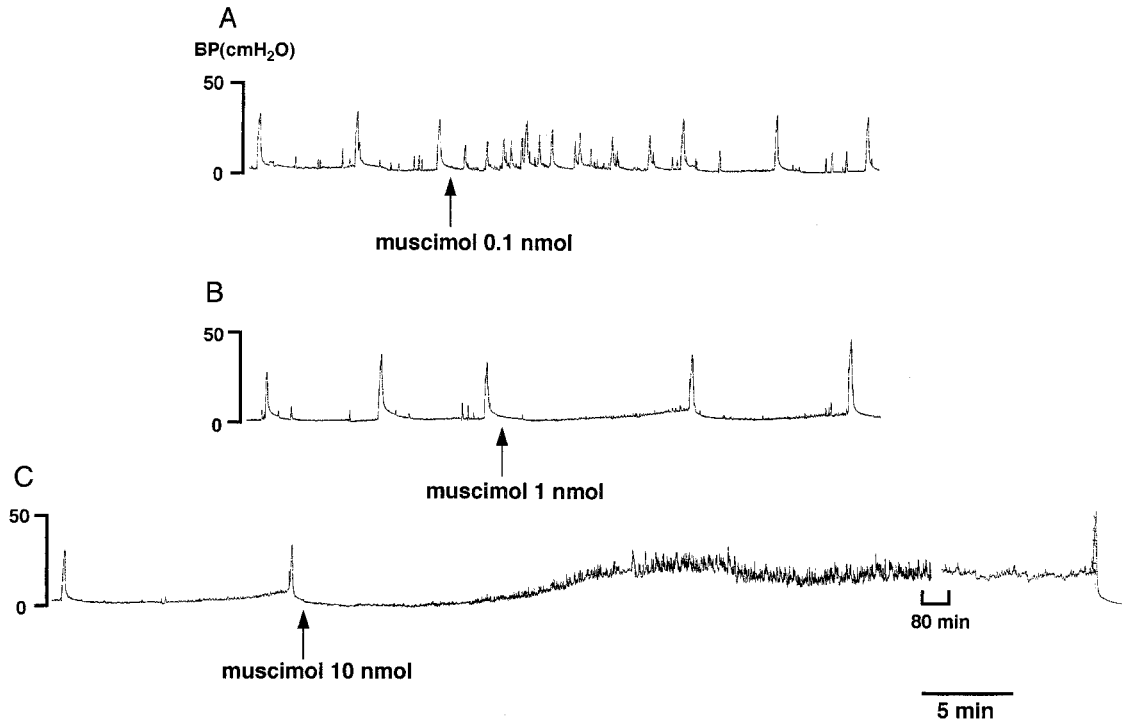

Fig. 1. Original recording of bladder pressure (BP) during cystometry performed on cerebral-infarcted rat before and after administration of muscimol. Administration of muscimol (0.1 nmol icv, arrow) significantly reduced bladder capacity (*A*). Bladder capacity at 1 nmol of muscimol (arrow) significantly increased (*B*). Muscimol (10 nmol, arrow) completely inhibited bladder contraction for 120 min accompanied by progressive increase in bladder capacity (*C*).

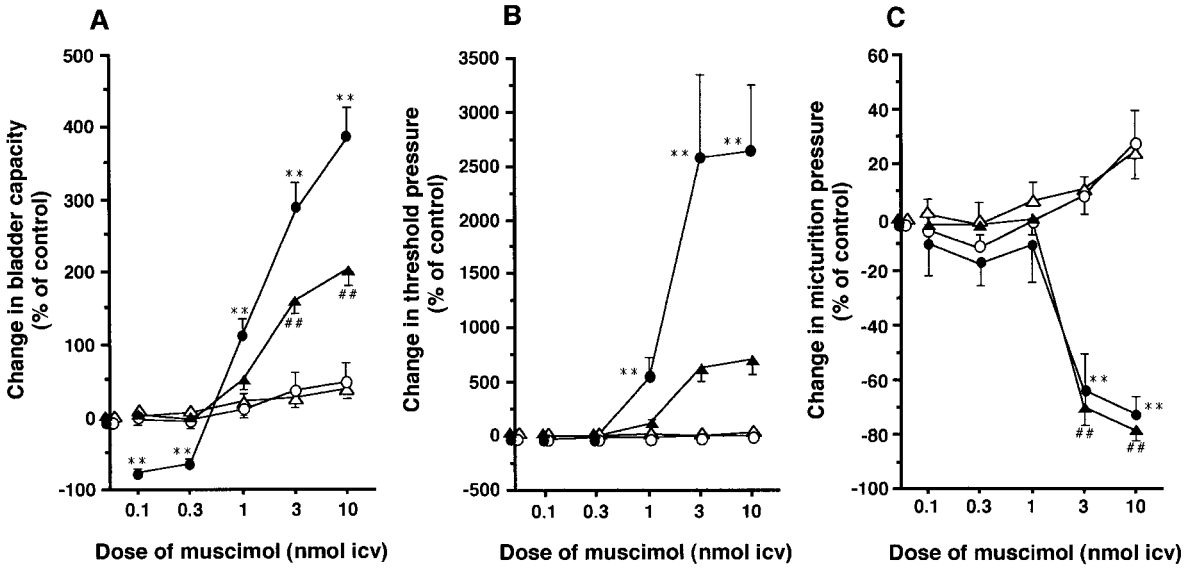

Fig. 2. Log dose-response curves showing effects of increasing doses of muscimol (0.1–10 nmol icv) or vehicle on percentage changes in bladder capacity (*A*), threshold pressure (*B*), and micturition pressure (*C*) in sham-operated rats (△: vehicle, $n = 6$; ▲: muscimol, $n = 9$) and cerebral-infarcted rats (○: vehicle, $n = 5$; ●: muscimol, $n = 5$). Values are means ± SE. $^{**}P < 0.01$ vs. cerebral-infarcted rats (vehicle); $^{\#\#}P < 0.01$ vs. sham-operated rats (vehicle) determined by 2-way ANOVA and post hoc tests.

just after administration of bicuculline (3 nmol icv) was significantly reduced from 0.48 ± 0.06 to 0.11 ± 0.02 ml (Fig. 5, $P < 0.01$), whereas BC increased to 0.79 ± 0.04 ml after convulsions. In cerebral-infarcted rats, BC just after administration of bicuculline (3 nmol icv) was also significantly reduced from 0.29 ± 0.02 to 0.09 ± 0.01 ml ($P < 0.01$) and then increased to 0.79 ± 0.05 ml after convulsions. As for BC after convulsions, the percentage increases in BC at 1 and 3 nmol were 94.6 ± 24.3% ($P < 0.05$) and 170.7 ± 26.2% ($P < 0.01$) in sham-operated rats and 130.6 ± 28.2 and 231.7 ± 39.1% (both $P < 0.01$) in cerebral-infarcted rats compared with vehicle-administered rats (Fig. 3*A*). The effects of bicuculline on bladder capacity were not significantly different in sham-operated and cerebral-infarcted rats.

In sham operated rats, large doses of bicuculline (1 and 3 nmol icv) significantly increased the percentage

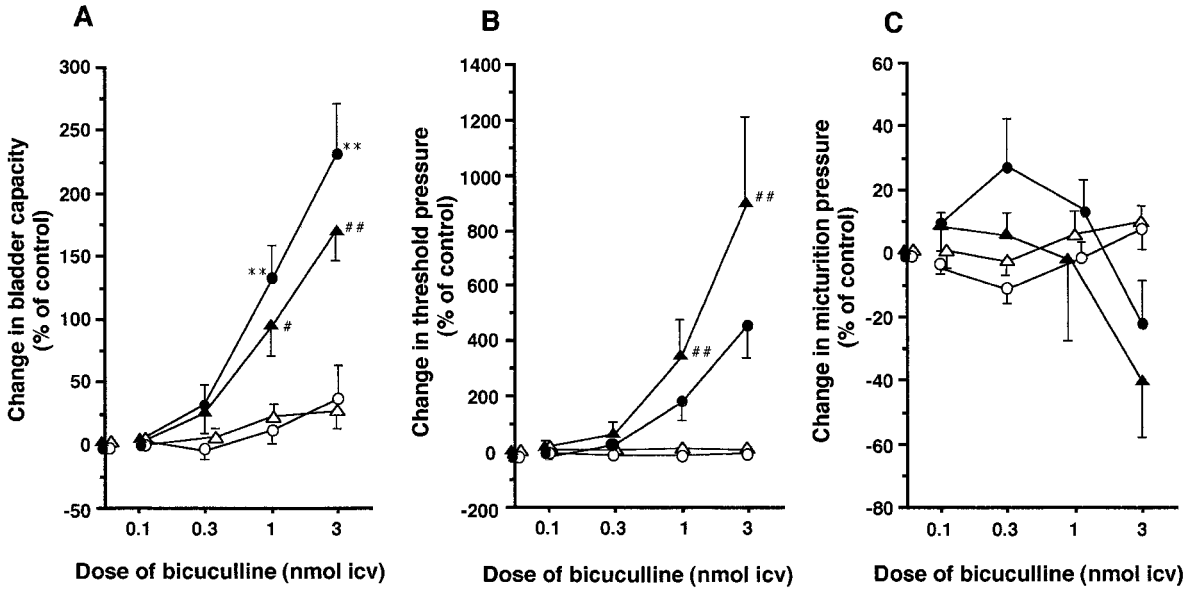

Fig. 3. Log dose-response curves showing the effects of increasing doses of bicuculline (0.1–3 nmol icv) or vehicle on percentage changes in bladder capacity (*A*), threshold pressure (*B*), and micturition pressure (*C*) in sham-operated rats (△: vehicle, $n = 6$; ▲: bicuculline, $n = 6$) and cerebral-infarcted rats (○: vehicle, $n = 5$; ●: bicuculline, $n = 5$). Values are means ± SE. $^{**}P < 0.01$ vs. cerebral-infarcted rats (vehicle); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. sham-operated rats (vehicle), determined by 2-way ANOVA and post hoc tests.

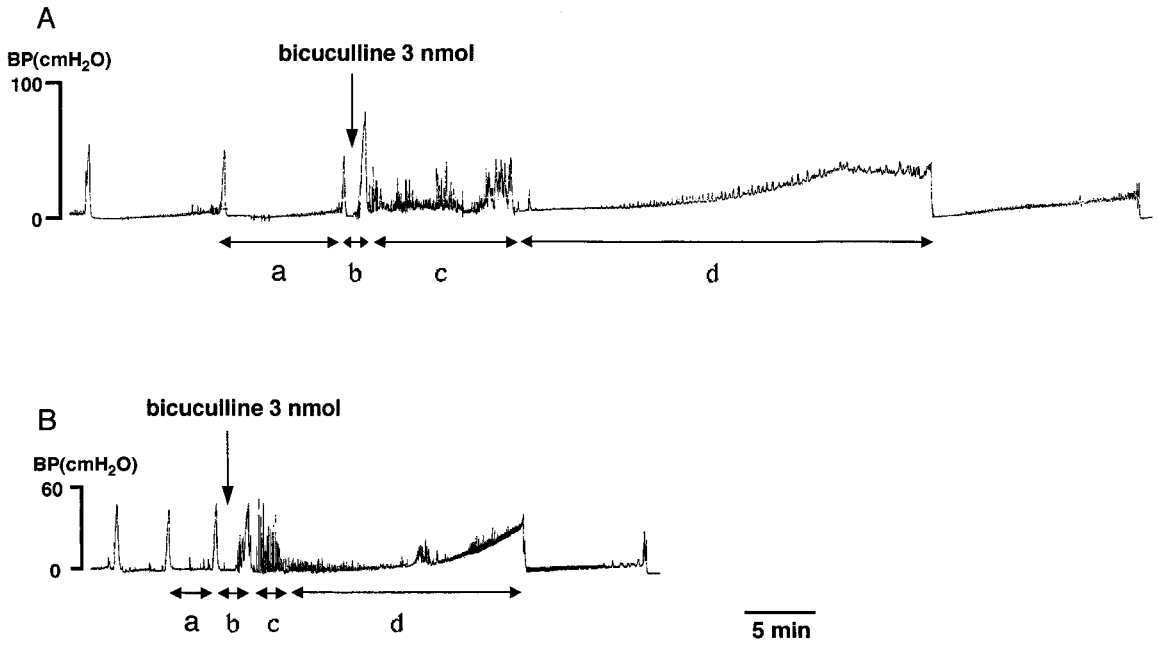

Fig. 4. Original recording of BP during cystometry performed on sham operated (*A*) and cerebral-infarcted (*B*) rats before and after administration of bicuculline. Bladder capacity just after administration of bicuculline (3 nmol icv, arrow) was significantly reduced and subsequently increased after convulsions (both *A* and *B*). a: before administration; b: just after administration; c: convulsion; d: after convulsion.

of threshold pressure (346.2 ± 134.4 and 899.6 ± 320.0%, both $P < 0.01$), and the same doses also resulted in an increase, but not a significant one, in cerebral-infarcted rats (185.0 ± 71.8 and 456.0 ± 114.0%) compared with vehicle-administered rats (Fig. 3*B*). A larger dose of bicuculline (3 nmol) produced a small and insignificant decrease in micturition pressure in both sham-operated and cerebral-infarcted rats (Fig. 3*C*).

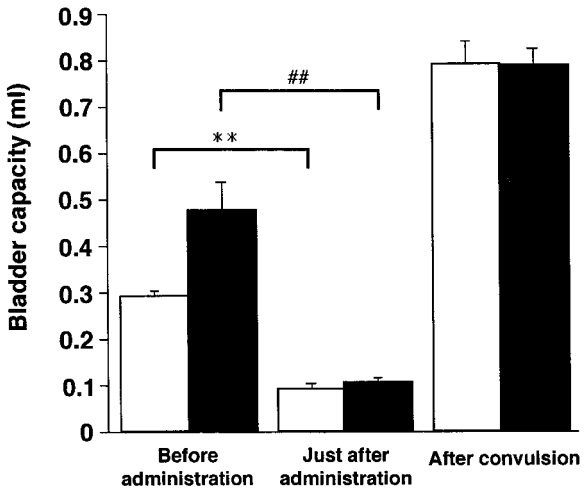

Fig. 5. Changes in bladder capacity just after administration of bicuculline (3 nmol icv) in sham-operated rats (solid bars, $n = 6$) and cerebral-infarcted rats (open bars, $n = 5$). Values are means ± SE. $^{**}P < 0.01$ and $^{\#\#}P < 0.01$ for indicated comparisons determined by paired *t*-test. Bladder capacity just after administration of bicuculline was significantly reduced and subsequently increased after convulsions.

Effects of baclofen in sham-operated and cerebral-infarcted rats. In cerebral-infarcted rats ($n = 5$), the percentage decrease in BC at 0.1 nmol (icv) of baclofen was 45.4 ± 20.1% ($P < 0.01$), whereas no change was observed in sham-operated rats ($n = 5$) compared with vehicle-administered rats (sham-operated rats: $n = 6$; cerebral-infarcted rats: $n = 5$; Fig. 6*A*). The percentage increase in BC at 0.3 nmol (icv) of baclofen in sham-operated rats was 88.5 ± 23.9% ($P < 0.01$) and 5.9 ± 21.6% in cerebral-infarcted rats compared with vehicle-administered rats. In both sham-operated and cerebral-infarcted rats, higher doses of baclofen (1 and 3 nmol icv) completely inhibited bladder contraction, and the respective percentage increases in BC were 146.7 ± 35.9 and 233.7 ± 87.7% in sham-operated rats (both $P < 0.01$) and 123.4 ± 28.5 and 259.2 ± 55.7% in cerebral-infarcted rats (both $P < 0.01$) compared with vehicle-administered rats.

In sham-operated rats, the percentage of threshold pressure significantly increased at 0.3–3 nmol (251.5 ± 56.4% at 0.3 nmol, 560.0 ± 100.6% at 1 nmol, and 1,736.7 ± 796.3% at 3 nmol, all $P < 0.01$), and these doses produced a small and insignificant increase in cerebral-infarcted rats (Fig. 6*B*).

With the increase in threshold pressure, the micturition pressure decreased significantly at 0.3–3 nmol in sham-operated rats (0.3 nmol: $P < 0.05$; 1 and 3 nmol: $P < 0.01$, Fig. 6*C*). In cerebral-infarcted rats the threshold pressure decreased in a dose-dependent manner; it decreased significantly at 3 nmol ($P < 0.01$).

Effects of muscimol, bicuculline, or baclofen on the volume of cerebral infarction. The volumes of cerebral infarct in muscimol-, bicuculline-, or baclofen-treated

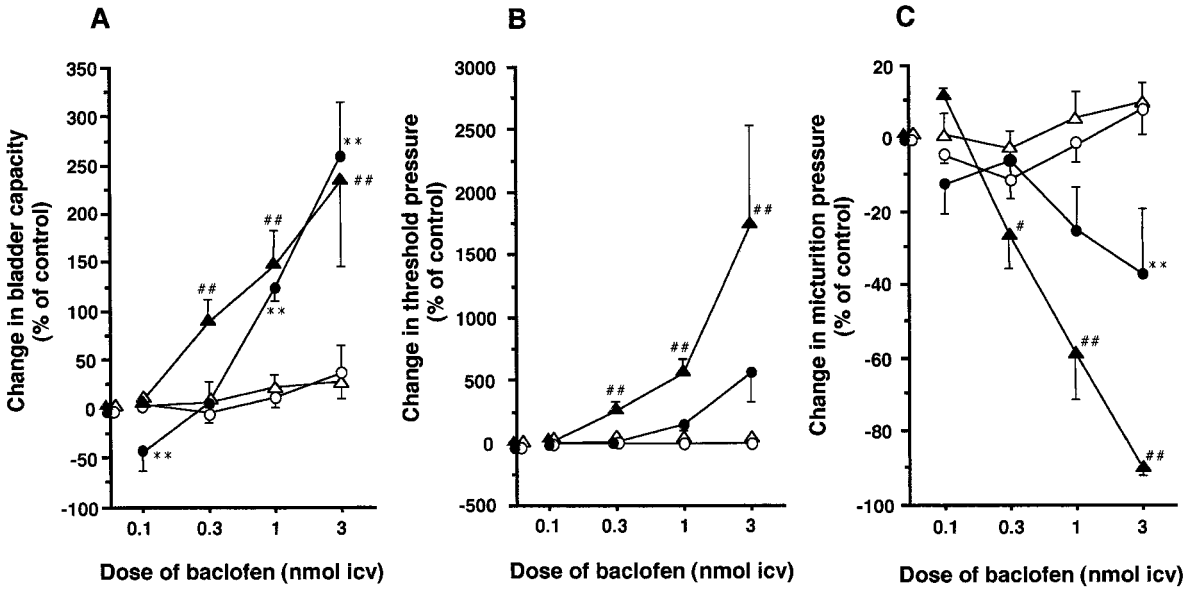

Fig. 6. Log dose-response curves showing effects of increasing doses of baclofen (0.1–3 nmol icv) or vehicle on percentage changes in bladder capacity (*A*), threshold pressure (*B*), and micturition pressure (*C*) in sham-operated rats (△: vehicle, $n = 6$; ▲: baclofen, $n = 5$) and cerebral-infarcted rats (○: vehicle, $n = 5$, ●: baclofen, $n = 5$). Values are means ± SE. $^{**}P < 0.01$ vs. cerebral-infarcted rats (vehicle); $\#P < 0.05$, $\#\#P < 0.01$ vs. sham-operated rats (vehicle), determined by 2-way ANOVA and post hoc tests.

rats were compared with those of vehicle-treated rats (Fig. 7). The mean infarction volumes (muscimol: 207.8 ± 8.1, bicuculline: 195.2 ± 14.2, baclofen: 202.6 ± 5.8 mm^3) were not statistically different from those of vehicle-treated rats (211.8 ± 5.8 mm^3).

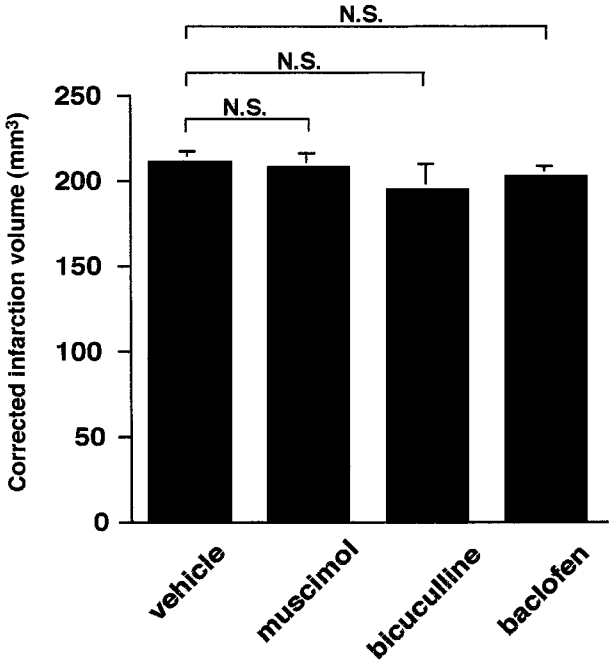

Fig. 7. Corrected infarction volume in the left hemisphere in cerebral-infarcted rats injected with vehicle ($n = 5$), increasing doses of muscimol ($n = 5$), increasing doses of bicuculline ($n = 5$), or increasing doses of baclofen ($n = 5$). Values are means ± SE. Comparisons of corrected infarction volumes between rats administered vehicle and muscimol, vehicle and bicuculline, and vehicle and baclofen were evaluated by unpaired *t*-tests. NS, not significant; the size of the infarct did not differ between drug-administered and vehicle-administered rats.

Effect of bicuculline on bladder and EUS activities in urethan-anesthetized rats. The EUS EMG of sham-operated rats exhibited high-frequency bursting activity during reflex bladder contractions (Fig. 8). This EUS activity, which is characterized by periods of high-amplitude spikes separated by periods of complete EMG silence, is similar to that observed in cerebral-infarcted rats. In both sham-operated and cerebral-infarcted rats, no significant changes in BC or EUS activity occurred in response to bicuculline (0.1 nmol). After 3 nmol of bicuculline administration, BC in both sham-operated and cerebral-infarcted rats first decreased and subsequently increased. This pattern was similar to that during consciousness. The EUS activity increased during suppression of bladder contractions, whereas high-frequency bursting activity was reduced during bicuculline-induced suppression of micturition.

Effects of muscimol, bicuculline, or baclofen on bladder and urethral pressures in urethan-anesthetized rats. A transient urethral relaxation was observed concomitant with each rhythmic bladder contraction in both sham-operated and cerebral-infarcted rats (Fig. 9, *A* and *B*). When the bladder contracted, a series of rapid, "twitch-like" intraluminal-pressure high-frequency oscillations appeared at the urethral site. This period of high-frequency oscillations of UP, caused by EUS bursting activity, was followed by a drop in UP below the baseline pressure. UP then gradually returned to the baseline pressure after the termination of EUS bursting. In both sham-operated and cerebral-infarcted rats, there were no significant changes in UP before and after the administration of low doses (0.1 nmol) of muscimol, bicuculline, or baclofen. Bicuculline (3 nmol) suppressed rhythmic bladder contraction,

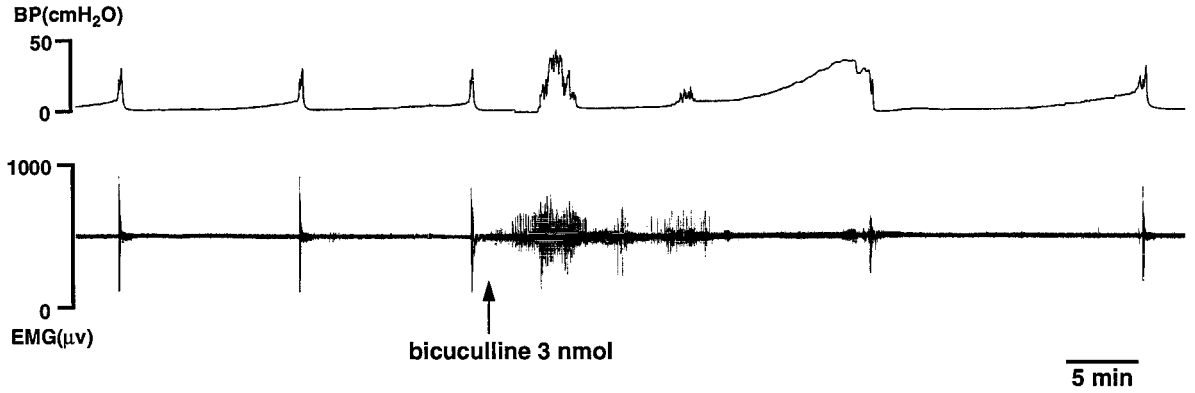

Fig. 8. Simultaneous electromyograms (EMG) of the external urethral sphincter (EUS) and BP in sham-operated rats. Each bladder contraction is associated with an increase in EUS-EMG activity. Bicuculline (3 nmol icv) first reduced and subsequently increased bladder capacity. EUS activity increased during suppression of bladder contractions, whereas high-frequency bursting activity was suppressed during micturition. Arrow shows administration of bicuculline (3 nmol icv).

and, during this period, UP increased tonically in both sham-operated and cerebral-infarcted rats (Fig. 9*A*), whereas muscimol and baclofen (both 3 nmol) inhibited rhythmic bladder contraction but did not increase UP in both sham-operated and cerebral-infarcted rats (Fig. 9*B*).

DISCUSSION

The present study evaluated the contribution of GABAergic mechanisms to bladder hyperactivity caused by cerebral infarction in conscious or anesthetized rats. As noted in recent experiments, muscimol and baclofen inhibit reflex bladder contractions (2), and injection of muscimol and baclofen, either intrathecally or intra-arterially, inhibits the micturition reflex, resulting in dribbling incontinence in conscious rats (8). Furthermore, intracerebroventricular administration of muscimol and baclofen was found to inhibit bladder contraction and to increase bladder capacity in urethan-anesthetized rats (10, 11). We also evaluated the effects of intracerebroventricular administration of muscimol and baclofen on bladder activity in conscious

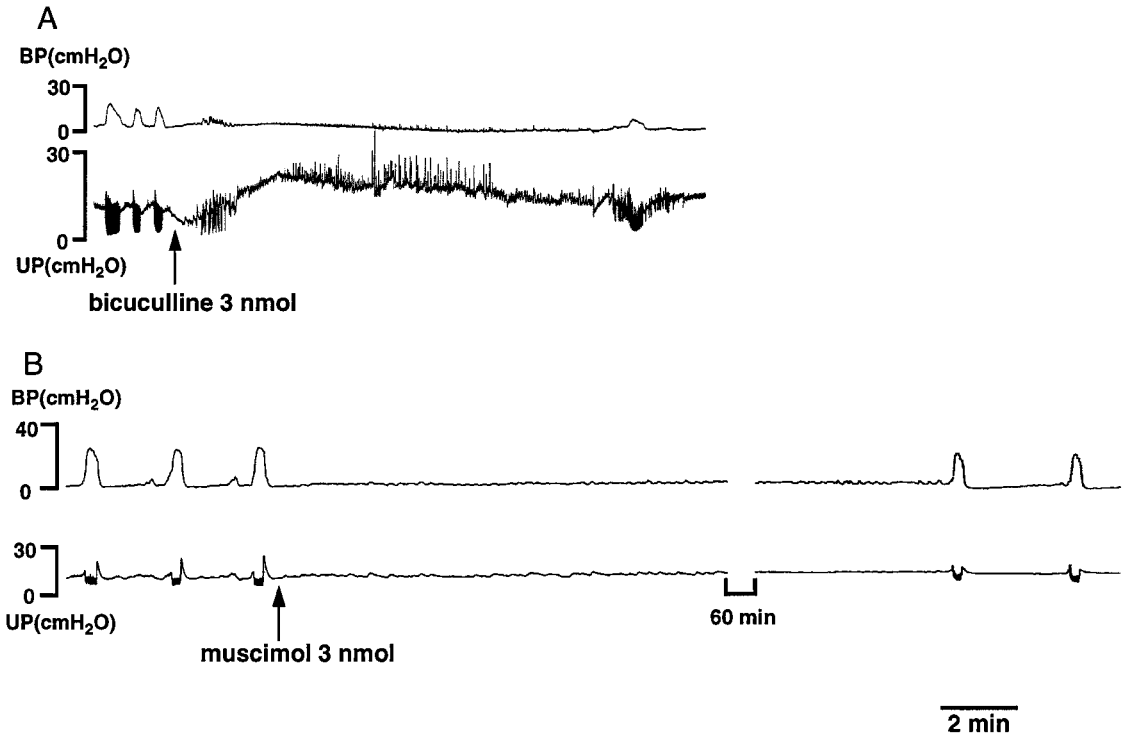

Fig. 9. Simultaneous recordings of urethral pressure (UP) and rhythmic BP in sham-operated rats. Each contraction is associated with a decrease in UP. Bicuculline (3 nmol) suppressed rhythmic bladder contraction, whereas UP increased tonically in cerebral-infarcted rats (*A*). Muscimol (3 nmol) inhibited rhythmic bladder contraction but did not increase UP (*B*). Arrow shows administration of bicuculline or muscimol.

rats. Muscimol and baclofen inhibited micturition reflexes dose dependently. High doses of muscimol (3 and 10 nmol) and baclofen (1 and 3 nmol) completely inhibited bladder contractions, ending with urinary retention in sham-operated rats. Mallory et al. (16) reported that, in either decerebrate unanesthetized or chloralose-anesthetized cats, direct injection of muscimol or GABA into the PMC depressed rhythmic bladder activity and increased the bladder capacity. These reports indicate the possibility that intracerebroventricular administration of muscimol can act directly on the PMC, resulting in an increase in bladder capacity. However, the possibility also exists that muscimol acts on other sites in the brain that project to the PMC.

The effects of muscimol or baclofen on micturition reflex in cerebral-infarcted rats were significantly different from those in sham-operated rats. Although high doses of muscimol and baclofen produced significant increases in bladder capacity, low doses of muscimol (0.1 and 0.3 nmol) and baclofen (0.1 nmol) reduced bladder capacity. How can this reversal in the actions of GABA receptor agonists be explained? The micturition circuit is controlled at the pontine level by the coordinated action of both the PMC and the PSC (7), the latter being involved in the storage of urine during continence. These neurons are located more ventrally and more laterally in the pontine tegmentum than the PMC, and this cell group, known as the L region, projects to the motoneurons of the EUS in the nucleus of Onuf. Direct injection of muscimol and GABA into the PSC is reported to reduce bladder capacity and urethral sphincter EMG activity (17, 21). These results indicate that GABAergic input into the PSC facilitates the micturition reflex. Therefore, the fact that low doses of muscimol and baclofen reduce bladder capacity in cerebral-infarcted rats can be explained by the change in pharmacological response in the PSC. It is likely that GABAergic mechanisms in the PSC change after the cerebral infarction and that the sensitivity to GABA agonists may well increase.

It has been suggested that glutamatergic and dopaminergic excitatory controls of micturition are upregulated by cerebral infarction (26). An *N*-methyl-D-aspartate (NMDA) glutamatergic antagonist (MK-801) increased BC in cerebral-infarcted rats but reduced it in sham-operated rats. A D_2-selective antagonist (sulpiride) or nonselective dopaminergic antagonist (haloperidol) produced an increase in BC in cerebral-infarcted rats but had no effect in sham-operated rats. These differences in responses to glutamatergic and dopaminergic agents between sham-operated and cerebral-infarcted rats seem to be induced by the upregulation of NMDA glutamatergic and D_2 dopaminergic excitatory mechanisms in the brain stem or spinal cord (29). For this reason, it seems reasonable to speculate that cerebral infarction causes changes in GABAergic mechanisms in the PSC.

Injection of bicuculline, intrathecally or intra-arterially, increased the micturition reflex in conscious rats (8). Mallory et al. (16) reported that injections of bicuculline (1 nmol) into the PMC in a decerebrate cat stimulated bladder activity and reduced BC. In another cat with a partially distended bladder, bicuculline (1.5 nmol) resulted in irregularly occurring bladder contractions as well as hindlimb twitching. A high dose of bicuculline (0.3 mg/kg iv) produced a transient increase in micturition reflex for 3–10 min in 7 of 11 urethan-anesthetized rats (14). However, within 15 min of bicuculline administration, bladder contractions became irregular and transiently ceased in 6 of the 11 rats. It was noted that, in an additional 4 rats that developed convulsions just after injection of bicuculline, bladder activity was suppressed after a very transient potentiation for, at most, 15–30 min. Lower doses of bicuculline (30–100 μg/kg) produced either no effect or a transient and slight potentiation of bladder contraction ($n = 6$). It was therefore assumed that this excitatory effect was of short duration.

We evaluated the effects of intracerebroventricular administration of bicuculline on bladder activity in conscious rats. Lower doses of bicuculline (0.1 and 0.3 nmol) produced either no effect or a small and insignificant increase in BC. Higher doses of bicuculline (1 and 3 nmol) produced a transient facilitation of the micturition reflex and subsequently increased BC. We also evaluated the effects of intracerebroventricular administration of bicuculline on EUS activity and UP in urethan-anesthetized rats. Urethan produces only minimal or no enhancement of GABAergic neurotransmission at the level of the central and, possibly, the peripheral nervous systems (15). We therefore used urethan to restrain rats during monitoring of UP and EUS activity. A high dose of bicuculline (3 nmol) increased UP, whereas neither muscimol nor baclofen resulted in an increase. It was reported that urethra and bladder neurons overlapped, as confirmed by the distribution of pseudo-rabies virus-labeled cells, which means that the central nervous control of bladder and urethral activity is coordinated (27). For this reason, it is possible that intracerebroventricular administration of bicuculline (3 nmol) acts on both the central nervous control of the bladder and on urethral activity. The transient facilitation of the micturition reflex may be caused by the direct action of bicuculline on the PMC. A high dose of bicuculline (3 nmol) increased EUS activity during suppression of bladder contractions. This indicates the possibility that bicuculline works directly on the PSC and subsequently increases BC through suppression of relaxation of the urethra.

Holstege and co-workers (1, 6) have shown that the cat has two regions of the dorsolateral pontine tegmentum, that is, the M and L regions, which are involved in bladder and urethral control. The M region produces micturition via direct projections to the sacral cord but not via an inhibitory projection to the L region. The L region, on the other hand, produces a continuous excitatory effect on the bladder sphincter but does not affect the M region. The conclusion was reached that the M and L regions are two separate functional systems that act independently at the supraspinal level. Furthermore, the M region inhibits the nucleus of Onuf through GABA interneurons in the sacral cord. These

findings and conclusions provide reasonable grounds for the hypothesis that the PMC (i.e., the M region) and the PSC (i.e., the L region) are both stimulated independently by a high dose of bicuculline, which produces a transient facilitation of the micturition reflex and, subsequently, an increase in BC.

Our study demonstrated that there was no difference in the size of the infarct between drug-treated and vehicle-treated rats. This suggests that these drugs increase BC not because they reduce the size of the infarct but because they act on the central nervous system during micturition.

In conclusion, $GABA_A$ and $GABA_B$ receptors appear to be involved in supraspinal inhibitory mechanisms in the micturition reflex. Our findings suggest that cerebral infarction causes a change in the GABAergic mechanisms in the PSC, resulting in an enhanced sensitivity to GABA agonists. Therefore, the therapeutic potential of GABAergic agents for the hyperactive bladder warrants careful evaluation.

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