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Influence of cerebral infarction on both bladder and urethral activities and changes after tramadol administration in rats

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Abstract

Aims: We investigated the changes in bladder and urethral function after cerebral infarction (CI) and the influence of tramadol on these functions.

Methods: Twenty-eight female Sprague Dawley rats were divided into normal and CI groups. In the awake condition, metabolic cage study and blood pressure were evaluated. Under urethane anesthesia, the intravenous effect of tramadol (0.01–1 mg/kg), which has both μ-opioid receptor stimulation and inhibition of norepinephrine and serotonin reuptake, on continuous cystometry, and simultaneous measurements of bladder and urethral perfusion pressure (UPP) were recorded. Infarcted lesions were examined by staining with triphenyltetrazolium chloride, a marker of mitochondrial enzyme activity.

Results: CI rats showed impaired sympathetic activity with Horner's syndrome and lower blood pressure. In metabolic cage study, urinary frequency during the dark phase was increased in CI rats. On bladder activity, in CI rats, the baseline pressure threshold for inducing bladder contractions was significantly lower ($p < 0.01$), and the intercontraction interval was prolonged after tramadol administration. On urethral activity, the baseline UPP was significantly lower in CI rats than in normal rats and it did not change after tramadol administration. Residual urine rate was significantly increased in normal rats, but not in CI rats. CI rats showed brain infarction including the cortex and hypothalamus, which is a center of the autonomic nervous system.

Conclusions: CI-induced ischemic brain damage results in impairment of both bladder and urethral functions, in addition to decreased sympathetic activity. Bladder overactivity after CI can be improved by tramadol; however, urethral activity cannot be improved by it.

KEYWORDS
bladder, infarction, stress urinary incontinence, sympathetic activity, urethra

1 | INTRODUCTION

The micturition function consists of two phases: the storage phase, in which urine is collected in the bladder, and the voiding phase, in which urine is drained through the urethra. These coordinated actions are intricately controlled not only through the peripheral nervous system (PNS, parasympathetic, sympathetic, and somatic) but also through the central nervous system (CNS).[1]

Disorders in the urinary storage phase include overactive bladder accompanied by detrusor overactivity (DO) and stress urinary incontinence (SUI), whereas in the voiding phase, it includes an underactive bladder with residual urine.[2] These lower urinary tract symptoms impair the quality of life.[3,4] Numerous neurological deficits, such as cerebral infarction (CI), damage neural control of the lower urinary tract.[5] In our recent human study, 23.5% of chronic stroke patients reported nocturia; 9.8%, urgency incontinence; and 17.6%, SUI.[6] Moreover, previous studies have reported that half and one-third of acute and chronic CI patients, respectively, have overactive bladder symptoms.[3,7] However, CI pathophysiology in the reciprocal function of the bladder and urethra has not been completely elucidated.

Glutamic acid, the major excitatory neurotransmitter in the CNS, facilitates bladder voiding function; whereas, noradrenaline and serotonin enhance the urethral continence reflex.[8–10] In our previous study using microtip-transducer catheter methods in CI rats, the urethral baseline pressure was lower than that in normal rats, and it did not change after intravenous administration of duloxetine, a norepinephrine- and serotonin-reuptake inhibitor.[11] Among the CI patients, 28%–79% experienced urinary incontinence associated with DO.[12] These results suggest that urinary incontinence after CI has an SUI component (i.e., impaired urethral continence mechanism) and DO in relation to bladder storage function. However, few studies have examined the changes in urethra after CI. Recently, we reported that intravenous administration of tramadol, which has dual effects of μ-opioid receptor stimulation and inhibition of norepinephrine and serotonin reuptake, enhanced both sympathetic innervated urethral smooth muscle and brain stem-derived external urethral sphincter continence reflexes in normal rats.[13] Tramadol has an inhibitory effect on DO in CI rats.[14] Thus, tramadol has dual effects on bladder and urethral functions. In this study, we investigated the changes in bladder and urethral function by continuous cystometry and simultaneous recordings of these functions after CI and the intravenous administration of tramadol in both normal and CI rats. In addition, to confirm the ischemic changes and the possibility of impaired projection to the sympathetic pathway and lower urinary tract after CI, 2,3,5-triphenyltetrazoliumchloride (TTC; a marker of mitochondrial enzyme activity) staining in the brain and heart rate and blood pressure as sympathetic activity were evaluated.

2 | MATERIALS AND METHODS

2.1 | Animals

A total of 28 female Sprague Dawley rats (12-week-old normal and CI rats) were used in this study. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of the Ryukyus (Protocol No. A2019234) in compliance with ARRIVE guideline (https://arriveguidelines.org/).

2.2 | Cerebral infarction

CI was performed as previously described.[10] Briefly, under 2% isoflurane anesthesia, the right carotid bifurcation was exposed through a paramedian incision on the neck. The distal side of the common carotid artery and external carotid artery close to bifurcation were ligated. A 4-0 monofilament nylon thread with the tip rounded by a flame was introduced into the internal carotid artery and advanced 17 mm from the carotid bifurcation to the arterial circle of Willis. Experiment 1 was performed 2 days after CI induction, followed by experiments 2 and 3 days after CI induction.

2.3 | Surgical procedures

In experiments 2 and 3 with the rat under 2% isoflurane, a polyethylene catheter (PE-10, Clay Adams) was inserted in the jugular vein for tramadol injection. Through an abdominal incision, the ureters were incised bilaterally; then, a PE-90 catheter (Clay Adams) was inserted into the bladder through the dome. In experiment 3, the bladder neck was tied to allow functional separation of bladder and urethral activities. A PE-50 catheter (Clay Adams) was inserted from the urethra to record urethral perfusion pressure (UPP). After these procedures, isoflurane anesthesia was turned off and replaced with 1.0 g/kg of intraperitoneal and subcutaneous urethane (Sigma-Aldrich Co.). After abdominal closure, catheters were connected to a pressure transducer (Transbridge 4M; World Precision Instruments).

2.4 | Experiment 1: Metabolic cage study and measurements of blood pressure

Six normal and six CI rats were placed in a metabolic cage (MATABOLICA, Sugiyama-gen Co. Ltd.) for 24 h to evaluate their voiding behavior. The room was maintained at standard temperature (24°C–26°C) and humidity (37%–40%) with a 12:12-h light-dark cycle (lighting at 8 a.m.). Food and water were provided ad libitum, and each animal was free to move around in the cage. The mean urine volume and the number of voidings were recorded in light and dark cycles separately. Twenty-four-hour urine output was estimated. Mean urine volume was calculated as [total urine volume (g)/number of voidings]. Furthermore, noninvasive measurements of heart rate and systolic and diastolic blood pressures were performed using the tail-cuff method (MK-2000MU, Muromachi Kikai Co., Ltd.) before and after metabolic caging.

2.5 | Experiment 2: Effects of intravenous tramadol on continuous cystometry in normal and CI rats

In eight normal and nine CI rats (including those in experiment 1), bladder activity was monitored by the intravesical PE-90 catheter under urethane anesthesia. Physiological saline at room temperature was infused into the bladder at a rate of 0.05 ml/min. A Power Lab (AD Instruments Pty, Ltd.) was used for data acquisition and manipulation. Cystometry was continued for at least 120 min, and the maximum voiding contraction (MVC), intercontraction interval (ICI), bladder pressure for threshold (PT), and baseline pressure (BP) were evaluated during the final 30 min as the predrug control values (Figure 1A).

The number and amplitude of nonvoiding contractions (NVCs) were measured at 1–2 min before each voiding contraction (Figure 1A, shaded area). NVCs were defined as contractions of >4 cmH_2O from BP, occurring during the filling phase. Thereafter, tramadol was injected intravenously and cumulatively administered at doses of 0.01, 0.1, and 1 mg/kg. Bladder activity was recorded for approximately 30 min after each concentration of drug was administered and compared to controls. Additionally, the voiding volume (VV) before and after each drug administration was measured, and the residual urine rate (%) was calculated as [residual urine volume (ml)/VV (ml) + residual urine volume (ml)] × 100 after single cystometry at the final dose.

2.6 | Experiment 3: Effects of intravenous tramadol on simultaneous recordings of intravesical pressure and UPP in normal and CI rats

To confirm the urethral activity concomitant with bladder activity before and after CI/tramadol, simultaneous recordings of intravesical pressure and UPP were performed. In five normal and six CI rats (including those from experiment 1), the bladder was infused with physiological saline at a rate of 0.05 ml/min to exceed the threshold volume, inducing isovolumetric bladder contractions. A urethral catheter was continuously infused with 0.05 ml/min of physiological saline. Under stabilized bladder contractions, the amplitude of the isovolumetric contractions and the intravesical pressure threshold for inducing urethral relaxation were measured. UPP nadir during reflex urethral relaxation, baseline UPP between reflex bladder contractions, and UPP change, calculated as the difference between the UPP nadir and the baseline UPP, were measured. Moreover, the mean rate and amplitude of high-frequency oscillations (HFOs) of the urethral striated muscle during reflex bladder contractions were measured (Figure 1B). These parameters were averaged over 30 min and used as controls. Following a control period, the rats were injected intravenously with tramadol (1 mg/kg) at the final dose used in experiment 1. Bladder and UPP parameters after tramadol were averaged and compared with the pretramadol-treatment control values. To evaluate the effect of tramadol on the urethral basal tone, Δ baseline UPP ratio, calculated as (baseline UPP after tramadol minus baseline UPP before tramadol/baseline UPP before tramadol), was estimated.

2.7 | Experiment 4: Evaluation of the ischemic damage

After experiments 1 and 2, normal rats ($n = 4$) and CI rats ($n = 4$) were anesthetized with additional intraperitoneal pentobarbital (50 mg/kg; NAKALAI TESQUE, Inc.) and immediately decapitated to remove brain tissue. The brains were immediately frozen in ice-cold acetone (FUJIFILM Wako Pure Chemical Co., Ltd.) at −80°C until further processing. A 2-mm coronal brain slice using brain matrices (EM Japan Co., Ltd.) was used to make six sections for each tissue. Staining with 2% TTC (Sigma-Aldrich), a marker of mitochondrial enzyme activity, was conducted with 20-min immersion at room temperature. After washing, the TTC-stained brain slices

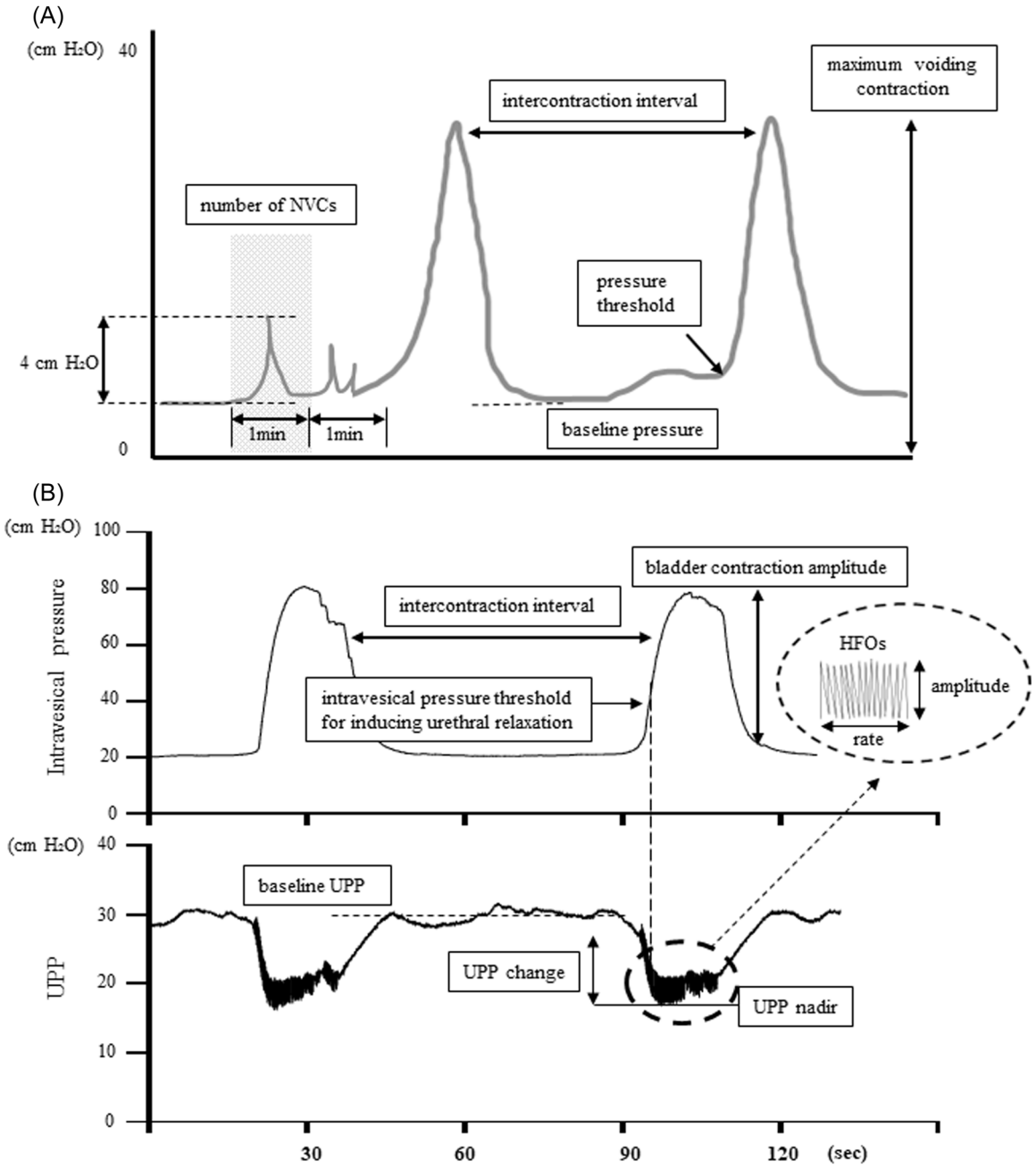

FIGURE 1 (A) Evaluation of continuous cystometry. (B) Evaluation of simultaneous recording of intravesical pressure and UPP under isovolumetric conditions. HFOs, high-frequency oscillations; NVCs, nonvoiding contractions; UPP, urethral perfusion pressure

were scanned for examination of the size and location of the CI with an image analysis software (ImageJ, National Institutes of Health, Bethesda, MD, USA).

2.8 | Drugs and assessment of infarction

Tramadol was dissolved in physiological saline and administered at 0.01, 0.1, and 1 mg/kg at a volume of 0.1 ml/rat. The dosage was determined based on our previous report[14] and preliminary experiments. TTC was diluted in physiological saline to a concentration of 2%. Both drugs were diluted on the day of the experiment or before it.

2.9 | Statistical analysis

The data were expressed as mean ± standard error, which was calculated for each group of animals from the average value of each rat before or after tramadol. Wilcoxon signed-rank test was used to compare these data before and after tramadol administration. The

nonparametric Mann–Whitney *U* test with Bonferroni correction for multiple comparisons was used for comparison of normal and CI rats, with threshold of statistical significance at $p < 0.05$. All statistical analyses were two-sided and performed using GraphPad Prism 8 for Windows (GraphPad Software).

3 | RESULTS

3.1 | Animals

Ptosis and drooping of the right upper eyelid were observed in all the CI rats (Figure 2). CI rats leaned towards the left paralytic side. No significant difference in body weight was found between normal and CI rats (248.4 ± 3.6 g vs. 239.8 ± 2.5 g, respectively; $p = 0.054$).

3.2 | Experiment 1: Voiding behaviors and sympathetic activity

In the metabolic cage study, the mean voided volume had a tendency to decrease in CI rats compared with normal rats during either the light ($p = 0.113$) or dark cycle ($p = 0.106$) (Table 1).

The number of voiding was significantly increased in CI rats than in normal rats during dark phase (25.2 ± 0.2 vs. 14.4 ± 1.6 times, respectively, $p = 0.014$) and during 24 h (38.2 ± 0.0 vs. 22.6 ± 0.2 times, respectively, $p = 0.031$). However, no differences were observed during light phase. The 24-h urine volume did not differ between normal and CI rats.

There was no significant difference in the heart rate between the normal and CI rats (Table 1). However,

TABLE 1 Comparisons in voiding behaviors and sympathetic activity between normal and CI rats

	Normal rats ($n = 6$)	CI rats ($n = 6$)
Metabolic cage study		
voiding volume per time (g)		
24 h	0.76 ± 0.06	0.54 ± 0.06
light phase	0.80 ± 0.08	0.60 ± 0.06
dark phase	0.75 ± 0.06	0.53 ± 0.10
number of voiding (times)		
24 h	22.6 ± 0.2	38.2 ± 0.0*
light phase	8.3 ± 0.8	12.8 ± 0.1
dark phase	14.4 ± 1.6	25.2 ± 0.2*
24-h urine output (g)	16.8 ± 4.7	20.2 ± 10.1
Sympathetic activity		
heart rate (bpm)	426.4 ± 7.9	420.8 ± 6.5
systolic blood pressure (mmHg)	141.8 ± 3.6	112.3 ± 6.2**
diastolic blood pressure (mmHg)	82.7 ± 3.4	58.0 ± 3.0**

Note: *$p < 0.05$ and **$p < 0.01$ (normal vs. CI). Mean ± standard error.
Abbreviation: CI, cerebral infarction.

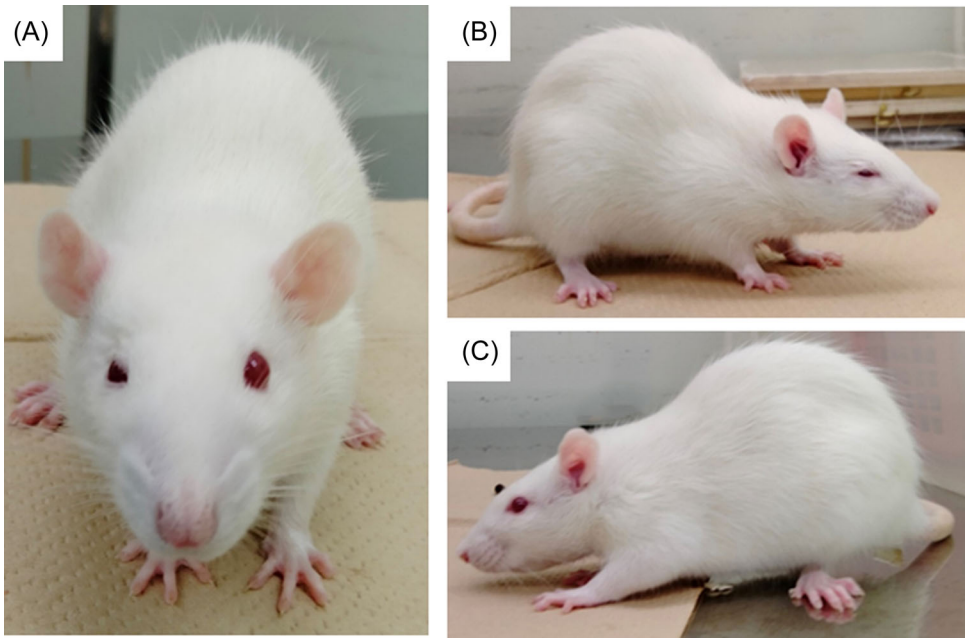

FIGURE 2 Right ptosis (i.e., Horner's syndrome) was shown after creation of CI by occlusion of right middle cerebral artery from frontal (A), right lateral (B), and left lateral (C) view. CI, cerebral infarction

systolic and diastolic blood pressures in CI rats (112.3 ± 6.2 mmHg and 58.0 ± 3.0 mmHg, respectively) were significantly lower ($p = 0.001$ in both) than those in normal rats (141.8 ± 3.6 and 82.7 ± 3.4 mmHg, respectively).

3.3 | Experiment 2: Changes in bladder activity after intravenous tramadol in normal and CI rats

PT was lower in CI rats than in normal rats (10.0 ± 0.8 cmH_2O vs. 16.6 ± 1.8 cmH_2O, $p = 0.008$). ICI tended to be shorter in CI rats (3.4 ± 0.3 min) than in normal rats (4.7 ± 0.8 min), but not significant ($p = 0.245$). However, NVCs (i.e., DO) were only observed in CI rats. In normal rats, after administration of tramadol, ICI tended to be prolonged (6.1 ± 1.2 min) at a low concentration (0.01 mg/kg) than that at the baseline (4.7 ± 0.8 min), whereas it returned to the pre-dose level at higher concentrations (0.1–1 mg/kg) (Table 2 and Figure 3A). However, in CI rats, ICI was prolonged in a dose-dependent manner ($p = 0.0195$ and 0.0098) after administration of tramadol at 0.1 and 1 mg/kg (4.6 ± 0.7 min and 6.4 ± 1.0 min, respectively) (Table 2 and Figure 3B). After the final dose of tramadol (1 mg/kg) was administered, the residual urine rate was higher in normal rats than in CI rats (53.5%, $p = 0.014$). In one out of eight normal rats, urinary retention was observed at the final dose. However, BP, MVC, and VV did not differ between normal and CI rats and did not change in either normal or CI rats after tramadol (0.01–1 mg/kg) administration.

3.4 | Experiment 3: Changes in bladder activity and UPP after intravenous tramadol in normal and CI rats

The baseline UPP before tramadol administration was significantly lower in CI rats than in normal rats (25.0 ± 2.0 vs. 30.2 ± 0.9, $p = 0.018$). Other UPP parameters, such as UPP nadir, UPP changes, and HFOs, did not differ between normal and CI rats (Table 3 and Figure 4). After tramadol administration, there was a tendency of decrease ($p = 0.074$) in Δ baseline UPP ratio of CI rats compared with normal rats (−0.29 ± 0.15 vs. 0.00 ± 0.01). Bladder contraction amplitude was significantly decreased ($p = 0.029$) in CI rats, and it was further decreased after tramadol treatment ($p = 0.031$). Pressure threshold for bladder contraction was significantly decreased ($p = 0.031$) in CI rats. ICI and other UPP parameters did not change in either normal or CI rats after tramadol treatment.

3.5 | Experiment 4: Evaluation of the ischemic damage

Images of brain slices spaced 2 mm apart were recorded using ImageJ, and the area of infarct areas was measured. The average infarct area ($n = 4$) at the coronal section of the level of pituitary gland (thalamus) was 92.9 ± 56.9 mm^2 (9.2 ± 6.0% to the total area of this section) and the maximum infarct area 1230 mm^2 (33% to the total area of this section) in a severe CI rat (Figure 5). The brains of normal rats did not show infarcted areas on TTC staining.

TABLE 2 Effects of intravenous tramadol on continuous cystometry in normal and CI rats

	Normal rats ($n = 8$)				CI rats ($n = 9$)			
	Tramadol (mg/kg)				Tramadol (mg/kg)			
	Before	0.01	0.1	1	Before	0.01	0.1	1
BP (cmH2O)	9.2 ± 0.8	9.5 ± 0.7	10.4 ± 1.4	8.8 ± 0.8	7.6 ± 0.6	8.1 ± 0.7	7.4 ± 0.7	7.1 ± 0.7
PT (cmH2O)	16.6 ± 1.8	17.3 ± 1.7	18.3 ± 3.3	13.1 ± 1.5	10.0 ± 0.8**	13.1 ± 2.0	10.1 ± 1.1	10.8 ± 1.1
MVC (cmH2O)	23.6 ± 1.8	23.8 ± 1.8	24.6 ± 3.1	22.6 ± 1.1	26.3 ± 3.1	26.1 ± 2.5	23.5 ± 2.1	23.2 ± 1.8
ICI (min)	4.7 ± 0.8	6.1 ± 1.2	4.7 ± 0.9	5.1 ± 1.6	3.4 ± 0.3	5.9 ± 1.4	4.6 ± 0.7†	6.4 ± 1.0††
VV (ml)	0.2 ± 0.02	0.2 ± 0.05	0.2 ± 0.07	0.3 ± 0.10	0.2 ± 0.02	0.3 ± 0.06	0.3 ± 0.08	0.3 ± 0.07
RUR (%)				53.5*				12.7
NVCs (number)	0	0	0	0	1.0 ± 0.5	0	0	0

Note: *$p < 0.05$ and **$p < 0.01$ (normal vs. CI). †$p < 0.05$ and ††$p < 0.01$ (before vs. after tramadol). Mean ± standard error.

Abbreviations: BP, baseline pressure; CI, cerebral infarction; ICI, intercontraction interval; MVC, maximum voiding contraction; NVCs, nonvoiding contractions; PT, pressure threshold; RUR, residual urine rate; VV, voiding volume.

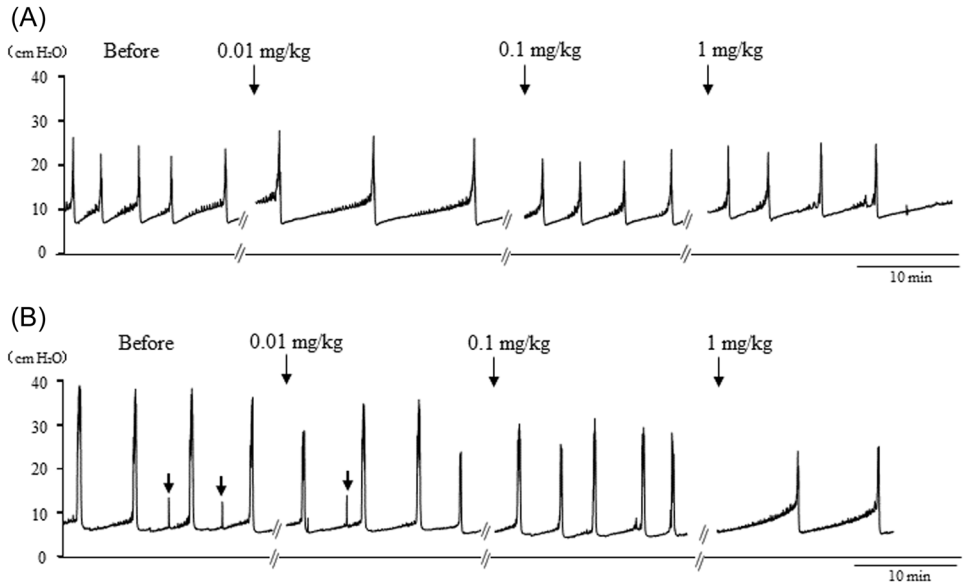

FIGURE 3 Continuous cystometry in a (A) normal and a (B) CI rat before and after intravenous tramadol (0.01–1 mg/kg). In a normal rat, the interval between bladder contraction did not change. However, in a CI rat, it was prolonged after final dose of tramadol (1 mg/kg) was administered. NVCs only shown in CI rats (black arrow) were inhibited after tramadol administration. CI, cerebral infarction; NVCs, nonvoiding contractions

TABLE 3 Effects of intravenous tramadol (1 mg/kg) on simultaneous recordings of intravesical pressure under isovolumetric conditions and UPP in normal and CI rats

	Normal rats ($n = 5$)		CI rats ($n = 6$)	
	Before	**After**	**Before**	**After**
Intravesical pressure				
bladder contraction amplitude (cmH2O)	49.0 ± 5.2	43.5 ± 6.0	31.7 ± 5.9*	17.4 ± 3.7†
pressure threshold (cmH2O)	26.1 ± 7.6	27.9 ± 7.1	17.2 ± 1.2	12.7 ± 2.8†
intercontraction interval (min)	1.9 ± 0.3	2.8 ± 0.6	1.5 ± 0.3	10.6 ± 6.1
UPP				
baseline (cmH2O)	30.2 ± 0.9	30.5 ± 1.1	25.0 ± 2.0*	18.0 ± 4.3
Δbaseline UPP ratio	–	0.00 ± 0.01	–	–0.29± 0.15
nadir (cmH2O)	17.5 ± 2.1	18.8 ± 1.7	13.7 ± 2.2	11.1 ± 3.7
change (cmH2O)	11.3 ± 0.7	11.6 ± 1.1	11.0 ± 1.6	7.2 ± 1.8
HFOs rate (Hz)	4.8 ± 0.1	4.7 ± 0.1	4.6 ± 0.3	3.8 ± 0.8
HFOs amplitude (cmH2O)	3.0 ± 0.2	3.5 ± 0.3	2.8 ± 0.4	2.5 ± 0.6

Note: *$p < 0.05$ (normal vs. CI), †$p < 0.05$ (before vs. after). Mean ± standard error

Abbreviations: CI, cerebral infarction; HFOs, high frequency oscillations; UPP, urethral perfusion pressure.

4 | DISCUSSION

The voluntary function of the bladder and urethra is regulated by the spinobulbospinal reflex that passes through the pontine micturition center and the brain.[1] The present results indicate the following: (1) CI rats show altered urinary frequency, as evidenced by reduced pressure threshold for inducing bladder contractions, presence of NVCs, and increased number of voidings in the metabolic cage study compared with normal rats; (2) CI rats have lowered urethral basal tone, as evidenced by the reduced baseline pressure of UPP and no change after tramadol administration (i.e., no increase in residual urine volume) compared with normal rats; (3) tramadol could inhibit bladder afferents after CI, as evidenced by prolonged ICI after tramadol

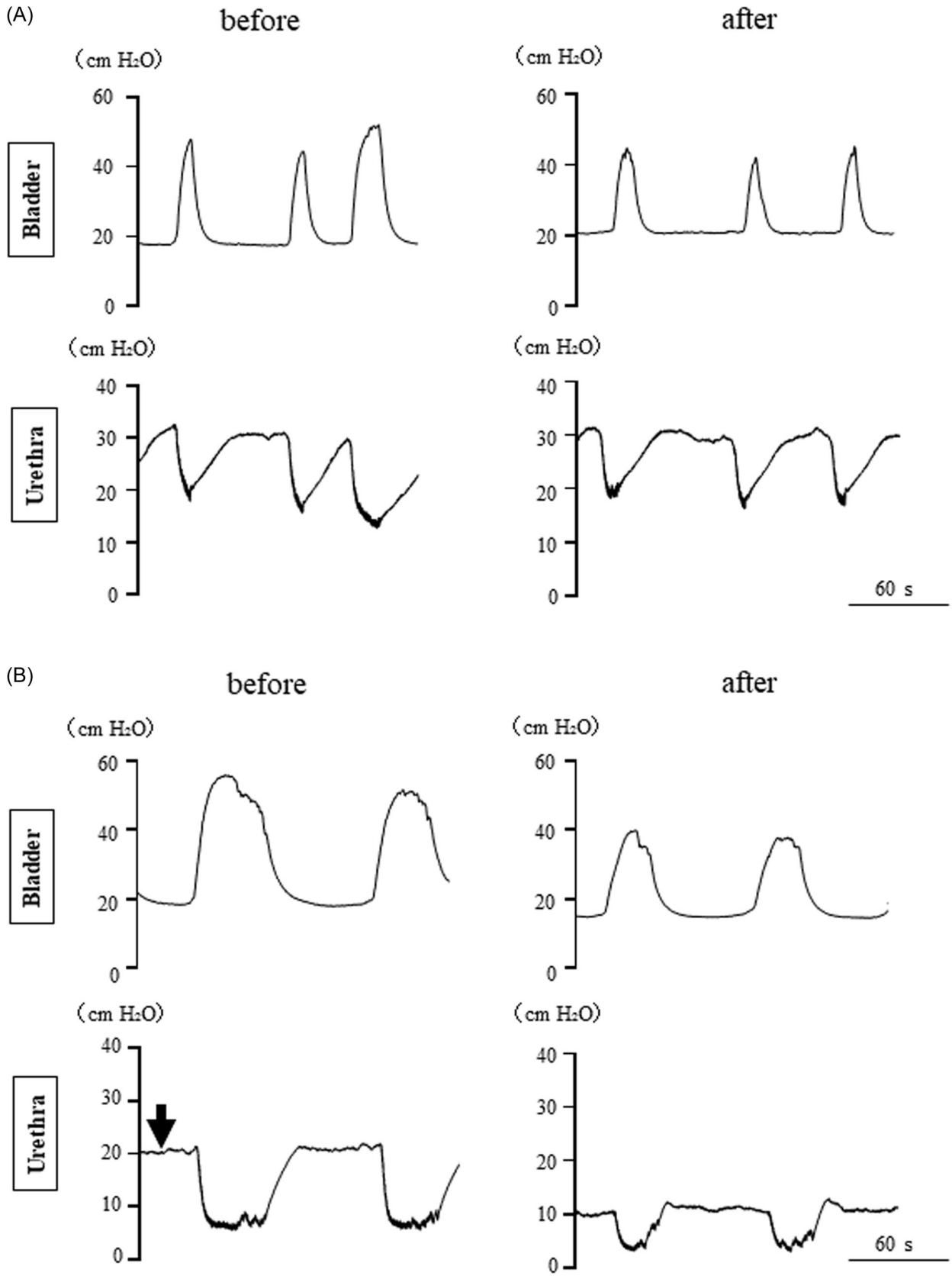

FIGURE 4 Simultaneous recordings of isovolumetric bladder contractions and UPP of a normal (A) and a CI (B) rat before and after intravenous tramadol (1 mg/kg). In a CI rat, baseline UPP (black arrow) was lower than that of a normal rat. After tramadol injection, the bladder activity and UPP did not change in normal and CI rats. CI, cerebral infarction; UPP, urethral perfusion pressure

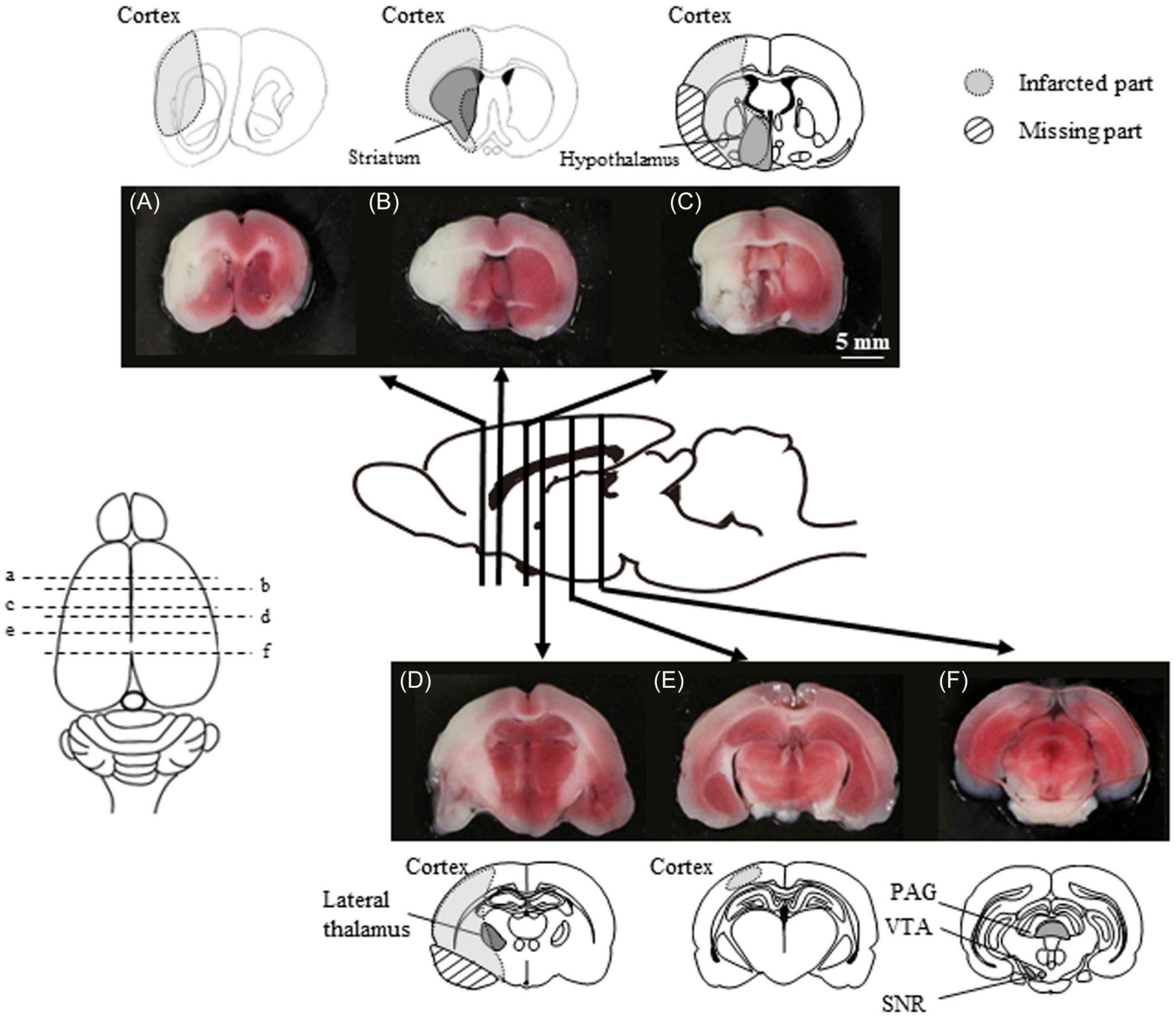

FIGURE 5 TTC staining in a CI rat. Coronal sections of brain spaced 2 mm (a–f) are shown. A large lesion of cortex (A–E) was infarcted. Infarction was involved in the striatum (B), hypothalamus (C), and lateral thalamus (D). Periaqueductal gray (PAG, F), ventral tegmental area (VTA), and substantia nigra (SNR) were not involved in the infarction. CI, cerebral infarction; TTC, 2,3,5-triphenyltetrazoliumchloride

administration compared with normal rats; and (4) there may be an injury of projection of the hypothalamus to sympathetic pathway after CI, as evidenced by ptosis, involvement of infarction of the hypothalamus revealed by TCC staining of brain, and decreased sympathetic activity after CI.

The bladder and urethra function reciprocally.[1] In the present study, tramadol (0.01–1 mg/kg) administration did not alter ICI and MVC; however, it augmented residual urine volume in normal rats in continuous cystometry. In contrast, while tramadol prolonged ICI without increased residual urine volume in CI rats. These different reactions can be explained by different urethral changes after tramadol treatment, prompting us to examine the simultaneous recordings of bladder and urethral activity in experiment 3. The present study showed that baseline UPP was 17% lower in CI rats than that in normal rats. Δ Baseline UPP ratio had a tendency to be decreased in CI rats compared with normal rats (-0.29 ± 0.15 vs. 0.00 ± 0.01, $p = 0.074$). In our previous study, microtip-induced urethral baseline pressure and tilt leak point pressure were 43% and 29% lower, respectively; in addition these did not increase after administration of duloxetine, a norepinephrine- and serotonin-reuptake inhibitor, in CI rats.[11] The results of our present and previous[11] studies suggest that

sympathetically innervated urethral basal tone may be impaired in CI rats. Microtip-induced urethral measurements can measure the pin-point area of the urethra, whereas UPP assesses the overall urethral resistance. Thus, further studies are necessary to evaluate the direct effect of CI-induced urethral activity before and after tramadol administration by microtip-induced urethral responses or leak point pressure.

Occlusion of the middle cerebral artery, which supplies the basal ganglia, induces CI in rats.[15,16] In the present study, CI rats showed TTC ischemic lesions in the right cerebral hemisphere, including the prefrontal cortex and hypothalamus. All CI rats exhibited drooping of the right upper eyelid. Horner's syndrome is a phenomenon characterized by ptosis, which arises from dysfunction of the oculosympathetic pathway innervating the smooth muscle of the eyelids, Müller's muscle.[17] The hypothalamus, located superior to the brain stem, is the highest center of the autonomic nervous system. Thus, in the present study, the hypothalamus and its projections from the brain stem to the sympathetic ganglion may be involved in the occurrence of ptosis after CI, as seen in cats.[17] Descending pathways through the raphe nuclei and locus coeruleus, which release norepinephrine and serotonin, respectively, project to Onuf's nucleus of the pudendal nerve innervating the external urethral sphincter.[1,18] Therefore, there may be an interaction between the hypothalamus, oculosympathetic pathway, raphe nucleus, and locus coeruleus (Figure 6). Alternatively, there may be a projection from the hypothalamus to the pontine micturition center, which is considered to directly project to the motor neurons in Onuf's nucleus.[1] Usually, patients after acute stroke are hypertensive as the body attempts to maintain blood circulation; this discrepancy in the present animal study can be explained by the distinct central lesions involved. In the present study, we confirmed no changes in sympathetic innervated baseline pressure of UPP in CI

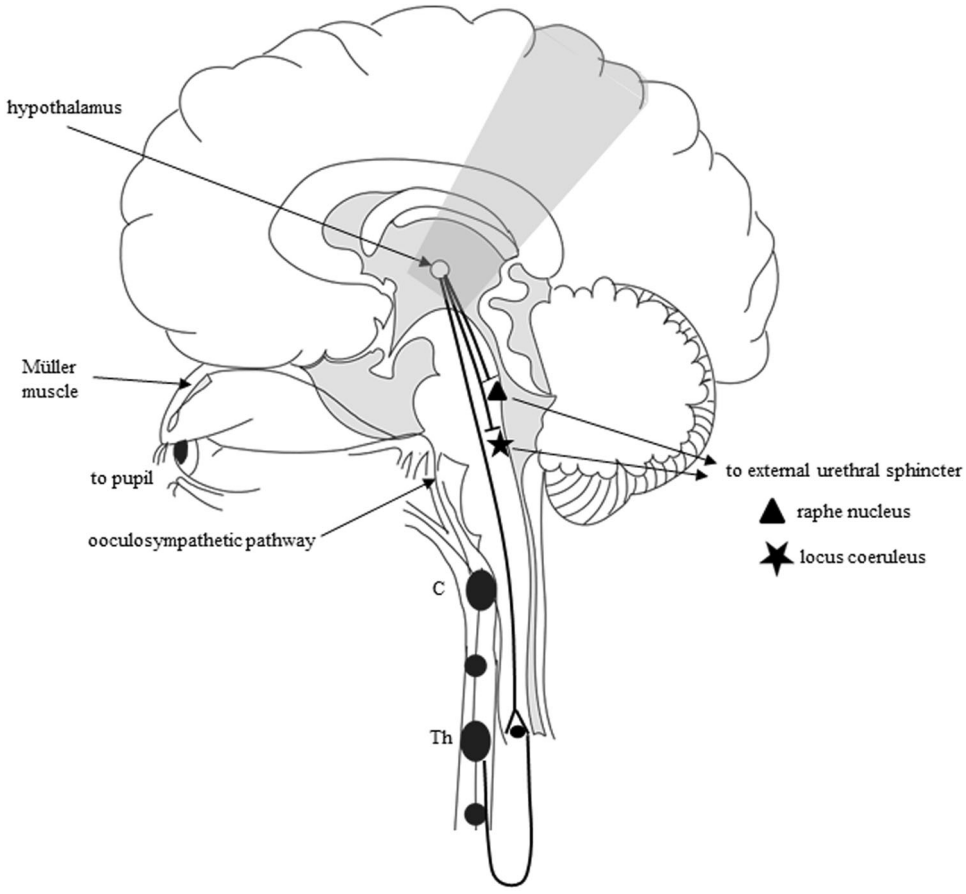

FIGURE 6 Hypothesis of impairment of oculosympathetic pathway and urethral continence reflex after CI. The oculosympathetic pathway, a projection from hypothalamus via thoracic (Th) and cervical (C) ganglion, innervates the smooth muscle of eyelids, Müller's muscle. After CI (gray shade), this pathway may be interrupted, thus inducing ptosis and Horner's syndrome. The hypothalamus is also considered to project to raphe nuclei and the locus coeruleus, releasing norepinephrine and serotonin, respectively, which terminates to Onuf's nucleus innervating the external urethral sphincter by the pudendal nerve (work as urethral continence reflex). CI, cerebral infarction

rats after tramadol, which has multiple pharmacological targets,[19] as well as norepinephrine and serotonin reuptake inhibition. Thus, further studies are necessary to examine the possibility of injured sympathetic activity related to urethral activity by selective stimulation of this pathway, by other drugs (e.g., duloxetine, phenylephrine), optogenetic, or chemogenetic methods. Overall, this is the first study to show a potential mechanism of impaired urethral continence reflex after CI.

Since the brain suppresses micturition,[1] voluntary control is disturbed after CI, resulting in an overactive bladder. In the present study, the prefrontal cortex, which inhibited bladder activity, was infarcted. The increased number of voiding episodes in the dark phase in the metabolic cage study, reduced pressure threshold for inducing bladder contractions, and occurrence of NVCs in CI rats suggest the presence of an overactive bladder. In the present study, pressure threshold did not change with prolonged ICI after tramadol administration in CI rats in the continuous cystometry (experiment 2), while it was decreased without prolonged ICI in simultaneous recording of isovolumetric bladder contractions and UPP (experiment 3). These different responses may be explained by different conditions of cystometry and changes, as evidenced by decreased bladder contraction only in isovolumetric conditions after tramadol administration, because urethral activity changes concomitant with bladder activity. Because a day-night difference in voiding behavior exists in humans and rodents,[20] the dark phase refers to the awake period in rats. On continuous cystometry, in normal rats, ICI was prolonged at a lower dose of tramadol (0.01 mg/kg), but returned to the control level at a higher dose (0.1–1 mg/kg). In the CI rats, ICI was prolonged after tramadol (0.01–1 mg/kg) administration. NVCs were also inhibited after tramadol. However, tramadol did not affect MVC in either the normal or CI rats. Changes in ICI and MVC seem to be related to the afferent and efferent activities of micturition reflex[1]; hence, tramadol may predominantly inhibit the afferent activity, especially in CI rats. These results are similar to those of a previous study on the inhibitory effect of tramadol on DO in CI rats.[14] In this study, urinary retention was observed only in normal rats after tramadol administration. Overall, tramadol predominantly inhibited bladder afferents by failing to enhance urethral activity in CI rats.

Various neurotransmitters regulate the micturition reflex in the CNS. Glutamic acid is a major excitatory neurotransmitter that facilitates bladder function, whereas glycine, γ-amino butyric acid, and opioids inhibit this function.[8,9] It has been reported that reduced bladder capacity was inhibited by dizocilpine, a glutamate N-methyl-D-aspartate (NMDA) receptor antagonist in CI rats.[10] Glutamatergic activation through α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors triggers the urethral closure reflex during sneezing at the spinal level.[21] These results suggest that glutamate stimulates both bladder and urethral functions. In our previous study, we reported that duloxetine, an inhibitor of norepinephrine- and serotonin-reuptake,[22] and tramadol enhanced the sneeze-induced urethral continence reflex in normal rats.[13] In the present study, residual urine was increased after tramadol administration in normal rats. Thus, the activation of norepinephrine, serotonin, and μ-opioid receptor stimulation in the spinal cord can work as storage (i.e., inhibition of the bladder and stimulation of the urethra). The urethral basal tone was lowered after CI, and it did not change after tramadol administration in the present study, suggesting that CI impair the noradrenergic, serotonergic, or opioid-related urethral activity.

The main lower urinary tract symptoms after CI are wet or dry bladder overactivity.[4] In our recent human studies, chronic stroke patients reported bladder-related urgency incontinence in 9.8% and urethra-related SUI in 17.6%.[6] Thus, CI patients further show SUI twice as often as overactive bladder symptoms. Our present and previous studies[11] with lower urethral basal tone in CI rats may support these clinical observations. Current functional imaging studies, such as functional magnetic resonance imaging, can help to understand that the frontal lobe, superior frontal gyrus, insula, and periaqueductal gray are involved in exaggerated brain activity and lead to overactive bladder.[23,24] Therefore, it can be speculated that CI induces not only overactive bladder but also SUI, which can account for mixed incontinence in CI patients. The clinical efficacy of tramadol for SUI could be reduced in patients with CI. This pharmacologic agent is useful for the treatment of overactive bladder. Tramadol is a less addictive type analgesics, but transported in the brain, and its concentration is five times high than plasma.[14] Therefore, it is important to use the minimal dose for reducing side effects, such as nausea, seizures, and slow breathing. Our dose is compatible with human use.

This study had some limitations. First, we used female rats after 2–3 days of CI and did not identify any differences in sex hormones. Second, cystometry was performed under urethane anesthesia, which may have affected the bladder and urethral activities. Third, the bladder and urethral activities were recorded separately by ligating the bladder neck. Fourth, there may be species differences in the neuroanatomical role of sympathetic activity. Despite these limitations, this study can support the clinical findings after stroke, which shows that urethral activity is weak and tramadol or duloxetine fails to enhance urethral activity. Future studies are required to identify distinct neural

networks between the PNS and CNS, which control the urethral continence reflex.

5 | CONCLUSIONS

This study provides evidence that CI rats have impaired bladder and urethral functions. After tramadol administration, μ-opioid receptor stimulation, and inhibition of norepinephrine and serotonin reuptake, ICI was prolonged in continuous cystometry, whereas the reduced baseline UPP did not change in CI rats. Infarction of the prefrontal cortex and hypothalamus by TCC staining of the brain was shown with ptosis and a decrease in blood pressure. This supports our initial hypothesis that CI-induced ischemic changes in the brain and its projection to the spinal sympathetic pathway may be correlated with impairment of the urethral activity. Further, tramadol may be effective for the treatment of overactive bladder after CI.

AUTHOR CONTRIBUTIONS

Satoko Nagamine: investigation, data curation, and original draft writing. **Tadanobu Chuyo Kamijo**: investigation, data curation, and original draft writing and reviewing. **Asuka Ashikari**: data curation and original draft writing and reviewing. **Minoru Miyazato**: conceptualization, methodology, supervision, and original draft writing, reviewing and editing.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data of the present study are available from the corresponding author on the request.

ETHICS STATEMENT

Experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of the Ryukyus (protocol no. A2019234) in compliance with ARRIVE guideline (https://arriveguidelines.org/).

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