Synergistic effects of loxoprofen and glycine on the micturition reflex in conscious rats

Yumiko FUKIYA, Masaru YOSHIZUMI, Mikako SAITO, Kazumasa MATSUMOTO-MIYAI, Toshie NIMURA, and Masahito KAWATANI
Department of Neurophysiology, Akita University Graduate School of Medicine 1-1-1 Hondo, Akita, Akita 010-8543, Japan
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ABSTRACT
We examined the inhibitory effects of loxoprofen, a cyclooxygenase inhibitor, and glycine, a major inhibitory neurotransmitter, on the micturition reflex in conscious rats and hypothesized that these drugs would interact synergistically to inhibit micturition. Voiding behaviors were assessed using a metabolic cage. Oral loxoprofen decreased the urinary frequency, and only a high dose (10 mg/kg) significantly reduced the voided volume. With cystometry, intravenous loxoprofen (0.1–3 mg/kg) and glycine (30 and 100 mg/kg) prolonged the intercontraction intervals (ICI) in a dose-dependent manner, but did not change the maximum voiding pressure (MVP) in conscious rats. The combination of loxoprofen (3 mg/kg) and glycine (100 mg/kg) strongly prolonged the ICI more than with either drug alone. The lowest dose of loxoprofen (0.1 mg/kg) and glycine (30 mg/kg) did not affect either the ICI or the MVP, but their combination resulted in a significant increase in the ICI. These results suggest that the combined administration of loxoprofen and glycine produced a synergistic inhibitory effect on the micturition reflex.

Bladder outlet obstruction secondary to benign prostatic hyperplasia (BPH) induces various bladder dysfunctions. Nocturia in patients with BPH is one of the most bothersome symptoms of the disease. The presence of nocturia disrupts sleep, leading to daytime somnolence, depressive symptoms, cognitive dysfunction, and reduction in quality of life (10). Although nocturia in patients with BPH is commonly treated with α, blockers and/or 5α-reductase inhibitors, using these drugs to reduce nocturia is not effective in many cases.

Clinical reports have been published indicating that loxoprofen, a non-steroidal anti-inflammatory drug (NSAID), reduces not only pain and inflammation but also nocturia in patients with BPH (6, 22). Other NSAIDs such as indomethacin also reduce the frequency of voiding and decrease the urine volume in patients with enuresis (1). NSAIDs including loxoprofen prevent prostaglandin (PG) synthesis by inhibiting the cyclooxygenase pathway. PGs, in particular PGE₂, play an important role in regulating bladder function, and intravesical infusion of PGE₂ stimulates the micturition reflex by activating capsacin-sensitive afferent nerves, leading to bladder overactivity in rats and humans (13, 14, 24). In addition, the urinary PGE₂ level has been reported to increase in patients with lower urinary tract obstruction (4). Previous studies have shown that NSAIDs decrease urinary frequency and increase bladder capacity in both normal and cystitis rats under urethane anesthesia (26, 27). We also reported that loxoprofen inhibits enhancement of the micturition reflex after acetic acid-induced inflammation in conscious rats (24). However, the inhibitory effect of loxoprofen on the micturition reflex in normal, conscious states has not been fully examined. In addition, long-term use of NSAIDs is not recommended.
for this purpose in the clinic, because they have a risk of causing side effects such as gastric damage (23). We therefore focused on low-dose combination therapy for the purpose of reducing their toxicity in normal, conscious rats.

Glycine, a major inhibitory neurotransmitter, plays a role in the control of spinal nociceptive processing (29) and bladder function in both physiological and pathological conditions (16, 19). Previous studies have suggested that spinal and serum glycine levels in rats after partial bladder outlet obstruction (17) and in humans with BPH (19) decrease, and thereby may partly cause detrusor overactivity. In rats under urethane anesthesia, systemic or intrathecal administration of glycine inhibits the micturition reflex in both intact and spinal cord-injured rats. This effect relies on inhibitory mechanisms that modulate the spinobulbospinal and spinal micturition reflex pathways at the level of the lumbosacral cord (16). Although intrathecal injection of a glutamate receptor antagonist decreases glutamate and glycine levels in the lumbosacral cord in urethane-anesthetized rats (7), different effects of glutamate receptor antagonists on the micturition reflex between conscious and urethane-anesthetized rats have been reported (28). Thus, a study on unanesthetized animals is needed.

We therefore examined whether intravenously administered loxoprofen and glycine inhibit the micturition reflex in normal, conscious rats. We also tested whether either drug potentiates the other when administered intravenously.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats, weighing 200–250 g (10–12-week-old), were used in this study. Animals were housed under a 12-h light-dark cycle with food and water ad libitum. All experiments were approved by the Animal Research Committee of Akita University and followed the American Physiological Society guidelines for animal research.

Metabolic cage studies. Rats were acclimatized in individual metabolic cages with a 12-h light-dark cycle for 3 days before this test. After acclimatization, each rat was given vehicle (0.5% gum tragacanth solution) by gavage on days 1 and 2 and immediately placed in the metabolic cage. Baseline urine outputs were recorded. Then, vehicle or loxoprofen dissolved in vehicle was administered orally by gavage on day 3, and urine was immediately collected. During the experiment, rats received an oral dose of 4 mL/rat of test drugs and were allowed free access to food and water. Because urinary frequency and voided volume were greater during the dark cycle than the light cycle, evaluation of the urinary inhibitory effect of loxoprofen was performed during the dark cycle. Therefore, micturition parameters were measured continuously after the evening administration and were assessed after 2, 4, and 6 h. Urine output was monitored every 60 s by recording the weight of urine voided in a container that rested on a digital scale below each cage. The scales were connected to a personal computer using a data acquisition program.

Cystometric studies. Rats were anesthetized with 2% sevoflurane (sevofrane; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan), and a midline abdominal incision was made to expose the bladder. A PE-50 polyethylene tube (Cray Adams, Parsippany, NJ, USA) with a fire-flared tip was implanted into the bladder dome for bladder filling and pressure recording. A PE-10 tube was inserted into the right jugular vein for intravenous drug administration. After surgery, rats were placed in a Ballman restraining cage (Natsume, Tokyo, Japan) and were allowed to recover from anesthesia for 1 h. Physiological saline was infused at room temperature into the bladder at a rate of 2.4 mL/h. Intravesical pressure was recorded on an AP601 polygraph (Nihon Kohden, Tokyo, Japan) and digitized with a converter for recording on a PowerLab system (ADInstruments Pty., Ltd., NSW, Australia). During the course of saline infusion, before drug administration, three voiding cycles were recorded as control values, and each parameter was averaged. Loxoprofen sodium (gift from Daiichi Sankyo Healthcare Co., Ltd., Tokyo, Japan) and glycine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in saline, and the pH was adjusted to 6.4–7.4 just before use.

Statistical analyses. The data are presented as the mean ± SE. The statistical significance of differences among groups for metabolic cage experiments was determined using two-way analysis of variance (ANOVA), and other data were analyzed using one-way ANOVA. P < 0.05 was considered significant.

RESULTS

Effect of oral administration of loxoprofen on voiding behavior

Oral administration of loxoprofen (3 and 10 mg/kg)
significantly decreased the urinary frequency at 6 h during the dark period compared with the vehicle-administered group ($P < 0.05$; Fig. 1A). Only a high dose of loxoprofen (10 mg/kg) significantly decreased the voided volume at 4 and 6 h during the dark period compared with the vehicle-administered group ($P < 0.05$ and $P < 0.01$, respectively; Fig. 1B).

**Effect of intravenous injection of loxoprofen alone or glycine alone on the micturition reflex**

During cystometrogram with intravesical saline infusion, intravenous injection of saline (vehicle) did not elicit detectable changes compared with the control. Intravenously injected loxoprofen (0.1–3 mg/kg) increased the intercontraction intervals (ICI) in a dose-dependent manner but did not alter the maximum voiding pressure (MVP) of bladder contractions in conscious rats (Fig. 2). Loxoprofen showed a significant increase in the ICI at doses of 1 mg/kg (34.7 ± 6.8% increase from control value, $P < 0.05$) and 3 mg/kg (50.9 ± 7.5% increase, $P < 0.01$) compared with vehicle (Fig. 2A). Similarly, intravenous injection of glycine (30 and 100 mg/kg) dose-dependently increased the ICI without affecting the MVP (Fig. 2). Glycine produced a significant increase in the ICI at a dose of 100 mg/kg (24.3 ± 4.8% increase, $P < 0.05$) compared with vehicle (Fig. 2A).

**Combination effect of loxoprofen and glycine on the micturition reflex**

We then examined a combination of loxoprofen and glycine on bladder contractions in conscious rats. Based on our results of the effects of individual drugs on ICI, we selected a maximum and an ineffective dose of each drug for the combination studies. Administration of loxoprofen (3 mg/kg) alone or glycine (100 mg/kg) alone produced a significant ($P < 0.05$) increase in the ICI but not the MVP, and

![Fig. 1](image1.png)

**Fig. 1** Changes in frequency of voiding (A) and voided volume (B) in rats after oral (p.o.) vehicle (VEH, n = 8) or loxoprofen (LOX; 3 and 10 mg/kg, n = 10). Data are presented over time as the change in the post-test minus baseline in each group. *$P < 0.05$, **$P < 0.01$ vs. VEH.

![Fig. 2](image2.png)

**Fig. 2** Effects of loxoprofen and glycine on the intercontraction interval (ICI; A) and the maximum voiding pressure (MVP; B) of bladder contractions in conscious rats. Rats were given intravenous (i.v.) vehicle (VEH), loxoprofen (0.1–3 mg/kg), or glycine (30 and 100 mg/kg). Data are presented as the percentage of control. Value in each column represents the number of animals. *$P < 0.05$, **$P < 0.01$ vs. VEH.
this combination of the two drugs also drastically and significantly ($P < 0.01$) increased the ICI only (Figs. 3 and 4). Significant differences were found between the ICI values of each drug (loxoprofen $50.9 \pm 7.5\%$; glycine $24.3 \pm 4.8\%$ increase) and the combination of both drugs ($98.5 \pm 11.4\%$ increase, $P < 0.01$; Fig. 4A). Individual administration of the lowest dose of loxoprofen (0.1 mg/kg) or glycine (30 mg/kg) did not affect either the ICI or the MVP, but their combination produced a significant ($P < 0.05$) increase in the ICI compared with vehicle (Figs. 5 and 6). Significant differences were found between the ICI values of each drug (loxoprofen $10.1 \pm 4.8\%$; glycine $11.2 \pm 3.5\%$ increase) and the combination of both drugs ($34.3 \pm 6.2\%$ increase, $P < 0.05$; Fig. 6A).

**DISCUSSION**

Loxoprofen is a well-known short-acting NSAID that inhibits PG synthesis via inhibition of cyclooxygenase. PGs have various effects on the kidney, urinary bladder, urethra, and sympathetic nervous system (11), and loxoprofen may act on these sites as PG synthesis inhibitors. Several authors have reported that loxoprofen is an effective and useful treatment for patients with nocturia. The main mechanism of this effect in patients with nocturia is considered to be a reduction in nocturnal urine volume (5, 22). Here, we examined voiding behavior in conscious rats and demonstrated that a high dose of loxoprofen reduced urine volume without increasing the voided volume, suggesting that loxoprofen reduces urine production in the kidney, similar to reports in patients with nocturia. However, in the current study, oral administration of loxoprofen at a dose less than 10 mg/kg showed a reduction in the

**Fig. 3** Effects of individual or combined use of loxoprofen and glycine on continuous infusion cytometrograms in conscious rats. Rats were given intravenous (i.v.) loxoprofen only (3 mg/kg; A), glycine only (100 mg/kg; B), or a combination of both drugs (C). Arrows indicate the time of drug administration.

**Fig. 4** Graphs showing the effects of individual or combined use of loxoprofen and/or glycine on the intercontraction interval (ICI; A) and the maximum voiding pressure (MVP; B) of bladder contractions in conscious rats. Rats were given intravenous (i.v.) vehicle (VEH), loxoprofen only (LOX; 3 mg/kg), glycine only (GLY; 100 mg/kg), or a combination of both drugs. Data are presented as the percentage of control. Value in each column represents the number of animals. *$P < 0.05$, **$P < 0.01$ vs. VEH. ##$P < 0.01$ vs. LOX and GLY alone.
frequency of voiding without a significant change in
the urine volume. Therefore, additional mechanisms
other than suppression of urine production appear to
contribute to the efficacy of loxoprofen. Possible
mechanisms for other effects of loxoprofen include
a reduction in detrusor smooth muscle tone and an
increase in urethral tone, as well as suppression of
the afferent nerve from the urinary bladder. Previous
studies have suggested that an important physiologic
role of PGs on bladder function may be sensitization
of the sensory nerves (2) and that PGs may di-
rectly affect bladder activity by affecting the smooth
muscle or indirectly by affecting neurotransmission
as neurotransmitters/modulators (3, 15, 20). Indeed,
some studies have reported the clinical efficacy of
NSAIDs on detrusor overactivity (8, 9, 21). In addi-
tion, Araki et al. (5) suggested that loxoprofen af-
ffects urinary sensation in the central nervous system
by suppressing afferent and/or efferent nerve path-
ways. In the cystometric study, we showed that in-
travenous loxoprofen less than 10 mg/kg prolonged
the bladder contraction interval without producing
pressure changes in normal, conscious rats. Changes
in the contraction interval and pressure are thought
to be due to alterations in afferent and efferent ac-
tivity in the micturition reflex pathway, respectively
(25). Although further study is required to determine
whether loxoprofen affects the micturition reflex
when administered intrathecally, the efficacy of
loxoprofen may depend on urinary sensation via
suppression of afferent nerve pathways. However,
because the current study tested rats acutely im-
planted with a cannula, urinary PGs, which are in-
creased by mucosa injury, may have enhanced the
micturition reflex by activating afferent nerves.

In conscious rats, we showed that intravenous
glycine prolonged the bladder contraction interval,
but did not alter the voiding pressure, consistent with previous observations in urethane-anesthetized rats (18). However, the threshold dose of glycine on the micturition reflex was lower in urethane-anesthetized rats than in conscious rats, and this difference may have been due to the depth of anesthesia. A previous study demonstrated that intrathecal injection of glycine prolongs the interval and decreases the amplitude of bladder contraction in normal rats, suggesting that a spinal glycinergic mechanism inhibits the spinal afferent and efferent limbs of the spinobulbospinal micturition reflex pathway (16). Miyazato et al. (18) demonstrated that the inhibitory effects of intravenous glycine on the micturition reflex are abolished by intrathecal strychnine, a selective glycine receptor antagonist, and that isotonic bladder contractions due to intravesical infusion of 1% glycine are not different from those using saline. These results suggest that intravenous injection of glycine inhibits the afferent limbs of the micturition reflex pathway in the lumbosacral cord after crossing the blood-brain barrier.

We also demonstrated a relatively strong synergy between intravenous loxoprofen and glycine in rats. In a clinical study, Shin et al. (23) reported that side effects were noted when loxoprofen was administered for longer than 6 months. Although all of these complications were mild and did not require stopping the administration of loxoprofen, the long-term use of loxoprofen in older people may involve relatively high risks of side effects such as gastric discomfort and renal failure. The current study in rats showed that the combination of a low dose of loxoprofen and glycine acted synergistically to inhibit bladder contraction without affecting the voiding pressure. Although further study is needed to determine the inhibitory mechanism of this combination, the addition of glycine may lower the effective dose of loxoprofen and provides some guidance on whether this combination may produce side effects in humans. In clinical trials, oral glycine reduces negative symptoms of schizophrenia and the efficacy of olanzapine and risperidone augment us-
tive dose of loxoprofen and provides some guidance on whether this combination should be performed in patients with nocturia.

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