A gap junction blocker inhibits isolated whole bladder activity in normal rats and rats with partial bladder outlet obstruction

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ABSTRACT
We examined the effect of 18alpha-glycyrrhetinic acid (18alpha-GA), a gap junction blocker, or propiverine hydrochloride on the activity of isolated whole bladders obtained from intact rats and rats with partial bladder outlet obstruction (BOO). Thirty-two female Sprague-Dawley rats were divided into an intact group and a BOO group. The whole bladder was harvested from each rat and isovolumetric cystometry was performed in Krebs solution. Changes of bladder activity were recorded after addition of 18alpha-GA or propiverine hydrochloride to the perfusate. Propiverine hydrochloride inhibited the amplitude and duration of contraction in both intact and BOO groups. Propiverine hydrochloride also reduced the baseline bladder pressure in the BOO group, but not in the intact group. In contrast, 18alpha-GA inhibited the amplitude and duration of bladder contraction, and also reduced the baseline pressure, in both intact and BOO groups. BOO bladders showed inhibition of the amplitude and duration of bladder contraction at lower concentrations of 18alpha-GA than intact bladders. A gap junction blocker suppressed the in vitro activity of BOO bladders more effectively than that of intact bladders. Therefore, inhibition of intercellular communication in the bladder via gap junctions may be useful for treating detrusor overactivity, as well as propiverine hydrochloride.

Overactive bladder syndrome is a common urological problem in the elderly and muscarinic antagonists remain the first-line therapy for this condition (1). Bladder outlet obstruction (BOO) caused by benign prostatic hyperplasia in elderly men is associated with detrusor overactivity, which is also the most common cause of overactive bladder syndrome (11). Partial BOO is an established experimental model for producing detrusor overactivity in animals, which causes significant changes of bladder smooth muscle contraction (17). It has been reported that an increased intercellular communication via gap junctions in the bladder may play an important role in the onset of detrusor overactivity in both rats and humans (5, 10). Therefore, it is possible that administration of a gap junction blocker may be useful for the treatment of detrusor overactivity.

Gap junctions are intercellular channels that allow the passage of ions and small molecules (< 1.2 kDa) between adjacent cells (4). Connexin43 is one of the gap junction proteins, and it is the best-characterized connexin in rat and human bladder smooth muscle cells (5, 9, 14). Expression of Connexin43 increases after BOO in rats and is also increased in patients with detrusor overactivity (5, 9, 10). It has been reported that 18alpha-glycyrrhetinic acid acts as a blocker of gap junction communication (3). Because the most important gap junction protein in the
myocardium is also Connexin43 (8), it is impossible to administer 18alpha-glycyrrhetinic acid systemically to block intercellular communication in the bladder. Experiments on isolated whole bladders have shown that the bladder smooth muscle displays spontaneous activity in the absence of central nervous system input (6, 16). In the present study, we examined the influence of 18alpha-glycyrrhetinic acid on isolated whole bladders harvested from rats with or without partial BOO, and compared its effects with those of propiverine hydrochloride (a muscarinic antagonist).

MATERIALS AND METHODS

Animal model. A total of 32 female Sprague-Dawley rats weighing 250–300 g were divided into an intact group and a BOO group of 16 rats each. Connexin43 mRNA levels reach a peak at 2 weeks after partial BOO in rats (13), so we performed partial BOO at 2 weeks before these experiments. Rats from the BOO group were anesthetized with 2% halothane, and the bladder and proximal urethra were exposed through a lower abdominal incision. A polyethylene catheter (PE-50; outer diameter 0.965 mm) was placed under the urethra, and a 4–0 silk ligature was tied around both the urethra and cather to create partial BOO. After the ligature had been secured, the catheter was pulled out. Then the abdomen was sutured, and each rat was placed in a cage and treated with ampicillin (100 mg/day intramuscularly for 2 days) to prevent infection. The protocol of this study was approved by the Institutional Animal Care and Use Committee of the University of the Ryukyus.

In vitro whole bladder preparation. In each rat from both groups, the bladder neck and both ureters were ligated and the bladder was removed. The isolated whole bladder was immobilized with pins in a recording chamber perfused with Krebs solution. The recording chamber had a central compartment serving as the organ bath, which was surrounded by an outer sealed compartment serving as the water jacket for regulation of bath temperature. The capacity of the recording chamber was 100 mL. Heated water was circulated through the outer jacket from an external, temperature-regulated fluid reservoir. A gravity perfusion system was used to superfuse the preparation with Krebs solution containing (mM): NaCl 113, KCl 4.7, CaCl2 2.5, MgSO4 1.2, NaHCO3 25, KH2PO4 1.2, and glucose 11.5. This solution was buffered to pH 7.4 and was equilibrated with 95% O2 and 5% CO2 prior to perfusion, while the solution in the recording chamber was also bubbled with the same gas mixture. The perfusion solution was preheated by passage through a heat exchanger before it entered the recording chamber and was maintained at 26 ± 0.2°C, which is the temperature at which relatively large bladder contractions can be induced stably (16). The organ bath was drained by gravity or suction, with the drainage rates being adjusted to match the fluid inflow rate.

Isovolumetric cystometry before and after exposure to 18alpha-glycyrrhetinic acid or propiverine hydrochloride. Pins were inserted into the urethra, with the anterior surface of the bladder dome facing upward. A 21-gauge needle was inserted into the bladder through the dome. The needle was connected to a polyethylene tube, which led to a saline infusion pump and a pressure transducer through a three-way stopcock. The bladder was slowly filled (0.05 mL/min) with physiological saline to a volume (1.0–3.0 mL) that elicited spontaneous contraction. The physiological saline contained 2% green food color dye (Kyoritsu Foods Co., Tokyo) to allow detection of fluid leakage around the needle or through the urethral orifice. Spontaneous isovolumetric bladder contractions were recorded on a rectilinear paper recorder. When bladder activity had been stable for at least 30 min, changes of bladder activity were recorded after addition of 18alpha-glycyrrhetinic acid (10−4–3 × 10−5 M; Sigma Chemical Co., St. Louis, MO) (3) or propiverine hydrochloride (10−7–10−3 M) to the perfusate. Before addition, 18alpha-glycyrrhetinic acid was dissolved in dimethyl sulfoxide (DMSO) and propiverine hydrochloride was dissolved in saline. The drug concentration was calculated by taking into account the total organ bath volume. Since 18alpha-glycyrrhetinic acid was dissolved in DMSO, the influence of this vehicle on spontaneous activity of the bladder preparation was also studied using bladders from intact rats and BOO rats (n = 2 each). Addition of DMSO to the perfusion fluid at the same volume (1.0 mL) used to dissolve 3 × 10−2 M 18alpha-glycyrrhetinic acid did not influence spontaneous contraction of either whole bladder preparation. The interval, amplitude, and duration of spontaneous bladder contractions were evaluated, as well as the baseline pressure of the bladder. These parameters were averaged for 30 min after application of drugs and then compared with control recordings obtained over a 30-min period before drug application.
**Statistical analysis.** Data are expressed as the mean ± standard error (SE). Statistical comparisons were performed by the paired or unpaired *t*-test, or by repeated measures analysis of variance (ANOVA), and *p* < 0.05 was considered to indicate statistical significance.

**RESULTS**

**Changes of activity in isolated normal and BOO whole bladders with or without application of 18alpha-glycyrrhetinic acid**

Using normal whole bladders harvested from intact rats (n = 7), isovolumetric cystometry showed that the interval (1.69 ± 0.19 min), amplitude (10.0 ± 2.0 cm H2O), and duration (1.62 ± 0.18 min) of bladder contraction, as well as the baseline bladder pressure (6.8 ± 0.5 cm H2O), were stable before drug infusion (control) (Figs. 1A, 3). The volume of fluid required to induce isovolumetric contractions was 1.0–1.2 mL. Application of 18alpha-glycyrrhetinic acid (10^-4 ~ 3 × 10^-2 M) did not change the interval between bladder contractions. However, 18alpha-glycyrrhetinic acid (10^-2 ~ 3 × 10^-2 M) caused a significant decrease of the amplitude (0.3 ± 0.3 cm H2O at 3 × 10^-2 M, *p* = 0.004) and the duration (0.22 ± 0.14 min at 3 × 10^-2 M, *p* < 0.001) of bladder contractions (Figs. 1A, 3A, 3B). In addition, 18alpha-glycyrrhetinic acid (10^-2 ~ 3 × 10^-2 M) significantly decreased the baseline bladder pressure (4.0 ± 0.8 cm H2O, *p* = 0.001) of bladder contractions, as well as the baseline pressure (9.3 ± 1.0 cm H2O, *p* = 0.007) of bladder contractions (Figs. 2A, 3A, 3B). However, propiverine hydrochloride (10^-4 ~ 10^-3 M) also did not change the interval between bladder contractions or the baseline pressure of the bladder (Fig. 4C).

Using whole bladders from BOO rats (n = 7), isovolumetric cystometry showed that the interval (1.50 ± 0.23 min), amplitude (8.6 ± 1.9 cm H2O), and duration (1.27 ± 0.08 min) of bladder contraction, as well as the baseline pressure (9.3 ± 0.7 cm H2O), were stable before drug infusion (control) (Figs. 1B, 3). Although the volume of fluid required to induce isovolumetric contractions was increased to 2.0–3.0 mL, there were no differences between normal and BOO bladders with respect to the interval, amplitude, and duration of bladder contraction in the absence of drugs. However, the baseline pressure was significantly higher (*p* = 0.027) in BOO bladders compared with normal bladders (Fig. 3C). Application of 18alpha-glycyrrhetinic acid (10^-3 ~ 3 × 10^-2 M) also produced no change of the interval between bladder contractions. However, 18alpha-glycyrrhetinic acid (3 × 10^-4 ~ 3 × 10^-2 M) caused a significant decrease of the amplitude (0.8 ± 0.4 cm H2O at 3 × 10^-2 M, *p* = 0.004) and the duration (0.32 ± 0.15 min at 3 × 10^-2 M, *p* < 0.001) of bladder contractions (Figs. 1B, 3A, 3B). In addition, 18alpha-glycyrrhetinic acid (10^-2 ~ 3 × 10^-2 M) significantly decreased the baseline bladder pressure (6.8 ± 0.5 cm H2O, *p* = 0.012) (Fig. 3C). However, there were no differences (according to ANOVA) with respect to the effects of 18alpha-glycyrrhetinic acid on these cystometric parameters in normal and BOO bladders.

**Changes of activity in isolated normal and BOO whole bladders with or without application of propiverine hydrochloride**

In normal whole bladders harvested from intact rats (n = 7), isovolumetric cystometry showed that the interval (1.72 ± 0.16 min), amplitude (11.3 ± 2.4 cm H2O), and duration (1.42 ± 0.13 min) of bladder contractions, as well as the baseline pressure of the bladder (5.3 ± 1.0 cm H2O), were stable before drug infusion (control) (Figs. 2A, 4). The volume of fluid required to induce isovolumetric contractions was 1.0–1.3 mL. Propiverine hydrochloride (10^-4 ~ 10^-3 M) caused a significant decrease in the amplitude (1.0 ± 0.3 cm H2O at 10^-1 M, *p* = 0.007) and the duration (0.54 ± 0.04 min at 10^-1 M, *p* < 0.001) of bladder contractions (Figs. 2A, 4A, 4B). However, propiverine hydrochloride (10^-7 ~ 10^-1 M) did not alter the interval between contractions or the baseline pressure of the bladder (Fig. 4C).

Using whole bladders from BOO rats (n = 7), isovolumetric cystometry showed that the interval (1.56 ± 0.16 min), amplitude (10.8 ± 2.7 cm H2O), and duration (1.52 ± 0.25 min) of bladder contractions, as well as the baseline pressure (8.1 ± 0.6 cm H2O), were stable before drug infusion (control) (Figs. 2B, 4). The volume of fluid required to induce isovolumetric contractions was 1.9–3.0 mL. There were no differences between the normal and BOO bladders with respect to the control values of the interval, amplitude, and duration of bladder contractions. However, the control baseline pressure was significantly higher (*p* = 0.045) in BOO bladders compared with normal bladders (Fig. 4C). Application of propiverine hydrochloride (10^-7 ~ 10^-1 M) also did not alter the interval between contractions. However, propiverine hydrochloride (10^-5 ~ 10^-1 M) caused a significant decrease in the amplitude (0.8 ± 0.2 cm H2O at 10^-1 M, *p* = 0.010) and the duration (0.50 ± 0.03 min at 10^-1 M, *p* = 0.007) of bladder contractions (Figs. 2B, 4A, 4B). Propiverine hydrochloride (10^-6 ~ 10^-1 M) also significantly decreased the baseline pressure of the bladder (5.2 ± 0.6 cm H2O at 10^-1 M, *p* = 0.005) (Figs. 2B, 4C). There were no differences (according to ANOVA) in the effects of propiverine hydrochloride on these cystometric pa-
A. Intact

Control

18alpha-glycyrrhetinic acid

- $3 \times 10^{-4}$ M
- $3 \times 10^{-3}$ M
- $3 \times 10^{-2}$ M

B. BOO

Control

18alpha-glycyrrhetinic acid

- $3 \times 10^{-4}$ M
- $3 \times 10^{-3}$ M
- $3 \times 10^{-2}$ M

Fig. 1  Spontaneous contraction of an intact bladder (A) and a BOO bladder (B) before (control) and after application of 18alpha-glycyrrhetinic acid ($3 \times 10^{-4} - 3 \times 10^{-2}$ M). A) In the intact bladder, spontaneous contractions are similar between the control state and after $3 \times 10^{-4}$ of 18alpha-glycyrrhetinic acid, but contractions are almost abolished by $3 \times 10^{-2}$ M of the drug. B) In the BOO bladder, control spontaneous contractions are recorded with small waves. These contractions are gradually inhibited after application of 18alpha-glycyrrhetinic acid ($3 \times 10^{-3} - 3 \times 10^{-2}$ M) and are almost abolished at $3 \times 10^{-2}$ M.

A. Intact

Control

Propiverine Hydrochloride

- $10^{-5}$ M
- $10^{-3}$ M
- $10^{-1}$ M

B. BOO

Control

Propiverine Hydrochloride

- $10^{-5}$ M
- $10^{-3}$ M
- $10^{-1}$ M

Fig. 2  Spontaneous contraction of an in intact bladder (A) and a BOO bladder (B) before (control) and after application of propiverine hydrochloride ($10^{-5} - 10^{-1}$ M). A) In the intact bladder, spontaneous contractions are gradually inhibited after the application of propiverine hydrochloride ($10^{-5} - 10^{-1}$ M), but contractions with a small amplitude and duration remain. B) In the BOO bladder, spontaneous contractions are gradually inhibited by propiverine hydrochloride ($10^{-5} - 10^{-1}$ M) and are almost abolished at $10^{-1}$ M.
DISCUSSION

Spontaneous changes of intravesical pressure can be recorded from an isolated whole bladder in the absence of central nervous system input (6, 16). In the present study, whole bladders obtained from both intact and BOO rats also showed autonomous activity when maintained in Krebs solution at 26°C. Normally, the bladder is a sparsely innervated and electrically quiescent tissue in vivo, in which inter-

parameters in normal and BOO bladders.

Fig. 3  Effect of 18alpha-glycyrrhetinic acid (10^-4 – 3 x 10^-2 M) on the amplitude (A) and duration (B) of spontaneous bladder contractions, and on the baseline bladder pressure (C), compared with before administration (control) in intact and BOO bladders. A) In intact bladders, the amplitude of spontaneous contractions was concentration-dependently inhibited after application of 18alpha-glycyrrhetinic acid (10^-2 – 3 x 10^-2 M). In BOO bladders, the amplitude of spontaneous contractions was also concentration-dependently inhibited after application of 18alpha-glycyrrhetinic acid (3 x 10^-3 – 3 x 10^-2 M). B) In intact bladders, the duration of spontaneous contractions was concentration-dependently shortened after application of 18alpha-glycyrrhetinic acid (10^-2 – 3 x 10^-2 M). In BOO bladders, the duration of spontaneous contractions was also concentration-dependently shortened after application of 18alpha-glycyrrhetinic acid (3 x 10^-3 – 3 x 10^-2 M). C) In intact bladders, the baseline pressure was reduced after application of 18alpha-glycyrrhetinic acid (3 x 10^-3 – 3 x 10^-2 M). In BOO bladders, the baseline pressure was also reduced after application of 18alpha-glycyrrhetinic acid (10^-2 – 3 x 10^-2 M). Values are the mean ± SE. Significant differences from control (Cont) are indicated by: *p < 0.05, **p < 0.01, and ***p < 0.001.

Fig. 4  Effect of propiverine hydrochloride (10^-7 – 10^-1 M) on the amplitude (A) and duration (B) of spontaneous bladder contractions, and on the baseline bladder pressure (C), compared with before administration (control) in intact and BOO bladders. A) In intact bladders, the amplitude of spontaneous contractions was concentration-dependently inhibited after application of propiverine hydrochloride (10^-7 – 10^-1 M). In BOO bladders, the amplitude of spontaneous contractions was also inhibited after application of propiverine hydrochloride (10^-5 – 10^-1 M). B) In intact bladders, the duration of bladder contractions was concentration-dependently shortened after application of propiverine hydrochloride (10^-2 – 10^-1 M). In BOO bladders, the duration of spontaneous contractions was also concentration-dependently shortened after application of propiverine hydrochloride (10^-2 – 10^-1 M). C) In intact bladders, the baseline pressure did not change after application of propiverine hydrochloride (10^-7 – 10^-1 M). In BOO bladders, the baseline pressure was concentration-dependently reduced after application of propiverine hydrochloride (10^-6 – 10^-1 M). Values are the mean ± SE. Significant differences from control (Cont) are indicated by: *p < 0.05, **p < 0.01, and ***p < 0.001.
cellular signaling is highly dependent on gap junctional communication (4). Glycyrrhetinic acid does not inhibit gap junctional communication by blocking Connexin43 production (7). Instead, the inhibitory effect of glycyrrhetinic acid is mediated by either binding to sites in the plasma membrane or by altering the configuration of connexons within gap junction plaques (7). Accordingly, 18alpha-glycyrrhetinic acid probably inhibited the activity of bladder gap junctions containing Connexin43 in the present study.

We found that administration of propiverine hydrochloride decreased the amplitude and shortened the duration of spontaneous contraction in both intact and BOO bladders. Propiverine hydrochloride is a muscarinic antagonist (2) and its blocking effect on calcium channels is similar to that of nifedipine (18). Therefore, propiverine hydrochloride may have blocked both muscarinic receptors and calcium channels, resulting in a decrease of the amplitude and duration of bladder contractions. We also found that propiverine hydrochloride decreased the baseline pressure of BOO bladders, but not intact bladders. The urothelium of the bladders contributes to nonneuronal release of acetylcholine, especially in elderly patients with an overactive bladder (19). Therefore, propiverine hydrochloride might have blocked nonneuronal acetylcholine release from the urothelium in BOO bladders, resulting in a decrease of baseline pressure only in such bladders. However, this drug did not change the interval between bladder contractions in either intact or BOO bladders. The spontaneous electrical and mechanical activity of gastrointestinal smooth muscle is generated by specialized pacemaker cells, which are known as the interstitial cells of Cajal (15). Recently, cells resembling the interstitial cells of Cajal have been found at the edges of smooth muscle bundles in the guinea pig bladder (12), suggesting that pacemaker cells may also trigger spontaneous bladder contractions. Therefore, propiverine hydrochloride might not have influenced pacemaker cell activity, resulting in no change of the interval between bladder contractions.

Application of 18alpha-glycyrrhetinic acid decreased the amplitude and shortened the duration of spontaneous bladder contractions, but did not change the interval between contractions in both intact and BOO bladders, as was the case for propiverine hydrochloride. While, 18alpha-glycyrrhetinic acid decreased the baseline pressure both intact and BOO bladders. ANOVA showed that there were no differences between intact and BOO bladders with regard to the effects of 18alpha-glycyrrhetinic acid on the amplitude and duration of bladder contractions, as well as on the baseline bladder pressure. However, inhibition of the amplitude and duration of contraction in BOO bladders, but not the baseline pressure, occurred at lower concentrations of 18alpha-glycyrrhetinic acid ($3 \times 10^{-2} - 3 \times 10^{-3} \text{M}$) than were required for intact bladders ($10^{-2} - 3 \times 10^{-3} \text{M}$). Connexin43 transcript levels have been shown to increase by approximately four-fold after acute BOO (7–9 hours) (9) and show a 15-fold increase from 3 days to 6 weeks afterwards (5). In our previous study, the expression of Connexin43 mRNA increased five-fold to reach a peak at 2 weeks after partial BOO (13). Therefore, the inhibitory effect of 18alpha-glycyrrhetinic acid (but not propiverine hydrochloride) on bladder activity was somewhat superior in BOO bladders compared with intact bladders, suggesting that 18alpha-glycyrrhetinic acid was able to block the increased number of gap junctions in BOO bladders.

Clinically, detrusor overactivity is common in patients with benign prostatic hyperplasia and outflow obstruction (11), and Connexin43 expression is increased in patients with neurogenic detrusor overactivity (10). Our present findings suggest that bladder activity is more effectively inhibited by a gap junction blocker after BOO has increased the number of gap junctions. Connexin43, which is a target of 18alpha-glycyrrhetinic, is abundantly expressed in cardiac myocytes (8), so it is impossible to use this drug systemically for the treatment of detrusor overactivity. Therefore, a more bladder-selective gap junction blocker will need to be developed in the future.

In summary, exposure of 18alpha-glycyrrhetinic acid decreased the amplitude and duration of spontaneous bladder contractions, and also reduced the baseline pressure in both intact and BOO bladders. Therefore, inhibition of intercellular communication in the bladder via gap junctions may be a potential new method for treating detrusor overactivity.

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REFERENCES


