Experimental Neurogenic Bladder in Rats and Effect of Robaveron, a Biological Prepared from Swine Prostate, on It

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Abstract—An experimental neurogenic bladder was induced in rats by an intraspinal injection of 10% phenol-glycerin solution. The functional and biochemical changes in the bladder were studied in vivo and ex vivo, and the effect of Robaveron, a biological prepared from swine prostate, on these changes were examined. The forced voiding pressure in the control rats which had been caused by the neurogenic bladder was reduced to 1/4–1/5 that of the intact animals. Robaveron recovered a decrease in the pressure, and the effect of 150–250 mg/kg, p.o., corresponded nearly to that of 40 mg/kg, i.m. In the ex vivo study with the strips of isolated neurogenic bladder muscle, the time required to cause 50% of the maximum contraction by transmural electric stimulation prolonged to about four times that of the intact one. Such a prolongation was recovered by Robaveron in a dose-dependent fashion, and the effect of Robaveron given p.o. corresponded to about 1/6 that of the drug given i.m. No change in Ca2+-influx, was found, although Ca2+-efflux was inhibited in the strip of neurogenic bladder muscle. Such an inhibition was significantly relieved in that of animals treated with Robaveron. In the neurogenic bladder rat, the ratio of bladder weight to body weight increased, and the activities of total ChE and AChE in the bladder muscle decreased, on which Robaveron did not show any influence. Phasic and tonic contractions in the strips of intact rat bladder and guinea pig ileum were inhibited by La3+, particularly phasic response in both the strips. Robaveron recovered the inhibition in a dose-dependent fashion. The present study suggests that the urinary dysfunction in neurogenic bladder may be caused not only by nervous disorders but also by changes in the bladder muscle itself. Robaveron was found to be effective for most of such changes. These findings may support the clinical efficacy of Robaveron for improving the attenuated bladder function.

Neurogenic bladder is clinically defined as a dysfunction of the bladder caused by injuries of the central nervous system including the brain and spinal cord, e.g., by workmen's or traffic accident and by peripheral nervous injuries seen in the cases of sequelae after operations for uterine and/or rectal cancer and diabetes mellitus. This is one of the irremediable diseases because the etiology and the symptoms are very complicated, and complete restoration of injured nerves can not usually be expected. Although there are some basic studies on the neurogenic bladder, most of them are based on electrophysiological approaches and cystometrograms (1–6).

On the pharmacological study of the neurogenic bladder, only a few reports can be found on the effects of Robaveron, a prostatic extract, (7–10) and a derivative of prostaglandins (11) on the cystometrogram and electromyogram. The therapeutic effect
of Robaveron has been already proven with clinical signs such as reinforcement of vesical contraction, reduction of residual urine and improvement of subjective symptoms, although the reports concerning the study on changes in the muscle of neurogenic bladder are few. In the present study, we prepared an experimental model of neurogenic bladder in rats by damage of their spinal cord, examined changes in the bladder muscle and observed the effect of Robaveron given i.m. and p.o. on the dysfunction of the bladder.

Materials and Methods

Animals: Female Wistar rats (Nihon Rat) weighing 250–350 g were exclusively used for the present study from the results of preliminary experiments for preparing the model of neurogenic bladder; the operated animals showed hematuria for about 3 days, which was followed by coagulation of blood in the bladder and urinary tract, resulting in urinary retention in most of the animals, particularly in male rats with a longer urinary tract. Each group included 14 rats, and 2 to 4 animals were housed in a cage (30×45×15 cm size), floored with sterilized sawdust, at room conditions of 23±2 °C and 55±5% humidity during the experimental period.

Drugs: Robaveron (injection and powder) was provided by Robapharm, Ltd, Basel, Switzerland. It is a biological prepared from swine prostate that contains numerous bioactive substances such as amino acids and nucleic acids. The other agents including ethyleneglycol-bis-(‘aminoethyl-ether)-N,N’-tetraacetic acid (EGTA, Sigma), lanthanum chloride (LaCl₃, Nakarai Chemicals), ethopropazine hydrochloride (lysivane, Sigma), 5,5’-dithio-bis-2-nitrobenzoic acid (DTNB, Wako Chemicals), acetylthiocholine iodide (AThCh, Sigma) and tetrodotoxin (TTX, Sigma) were purchased from the companies indicated in parentheses, respectively. All drugs except for Robaveron injection were dissolved in water before use.

Induction of neurogenic bladder: A method for inducing experimental neurogenic bladder by laminectomy of the fifth lumbar vertebra in rabbits has been already reported by Nakaarai et al. (7). However, as it is difficult to apply the method to a small animal such as a rat, we applied the method of nervous block with an injection of phenol-glycerin solution, generally employed in clinical fields, to rats for preparing the model. By this method, the nerve of cauda equina is incidentally damaged due to insertion of the needle. Rats anesthetized with a mixture of ether-alcohol (4:1) whose backs had been shaved were disinfected with 10% hibitane in alcohol solution. The back skin was incised and muscles around the spine were operated on to disclose the fifth lumbar vertebra (Fig. 1). A part of the spine was then transsected with a pair of bone forceps. From the transsected site, 0.05 ml of 10% phenol-glycerin solution was injected with a 24 G needle into the lower part of the spinal cord. Convulsions of the hind legs and a tail were seen during the injection. Crystalline procaine penicillin G in oil (0.5 ml, 150,000 units, Meiji Seika) was dropped into the operated site before suturing the muscles and skin. To protect against infection of the
bladder and urinary tract, the animals were given intraperitoneally the same dose of the antibiotics for 3 days. This operation procedure from incision to suturing could be completed within 10 min, and most of animals subjected to this method developed a typical neurogenic bladder. Immediately after the operation, paralysis in the hind legs was observed, and this was followed by urinary dysfunction accompanied with urinary incontinence and residual urine from about 2 days after the operation. The drug (Robaveron) was given p.o. in doses of 100 to 250 mg/kg or i.m. in a dose of 40 mg/kg daily from the day of operation. The concentration of Robaveron was adjusted with saline to make the volume of administration 0.02 ml/100 g body weight. For the control and intact animals, the same dose of saline was given. As the operated animals suffered from severe urinary retention from 2 days after the operation, they were subjected to forced urination by fingers.

**Forced voiding pressure:** From the 3rd day when hematuria disappeared to the 14th day, to measure the pressure which provokes voiding, the lower abdominal part of the rat was covered with a cotton-bagged balloon connected to a water-manometer and gradually pressed by fingers. Thus, the pressure which causes voiding was determined.

**Contractile response to transmural electric stimulation:** On the 14th day, after the measurement of forced voiding pressure, the animal was sacrificed and the bladder was isolated. The organ was cut longitudinally into 4 strips so that the trigone of the bladder was included in each strip. The strip was suspended in an organ bath filled with Tyrode’s solution (10 ml, 37°C) aerated with a gas mixture of 95% O₂ and 5% CO₂, in which a pair of Ag-AgCl electrodes (5 mm x 3 cm size, 3 mm interval) was fixed to stimulate the strip. After the strip was allowed to stand for about one hour, electric stimulation was performed, and the contraction was recorded on a recticorder (RJG4024, Nihon Kohden) under loading with 1 g of resting tension. Stimulation was carried out transmurally by a rectangular-pulse stimulation (MSE-3, Nihon Kohden) with a constant condition of 30 V, 0.5 msec pulse-duration and 5 Hz for 40 sec. The contraction evoked by the electric stimulation was confirmed to be completely blocked by the treatment with 10⁻⁶ M TTX. The time required to cause 50% of the maximum contraction, “t”, was calculated from the response curve.

**Examination of Ca²⁺-dynamics:** The Ca²⁺-efflux and-influx in neurogenic bladder was examined according to the method of Shibata et al. (12). On the 14th day after the operation, the bladder was isolated and cut longitudinally between both the ureters into 2 strips. The strip was suspended in an organ bath filled with Tyrode’s solution (10 ml, 37°C) aerated with a gas mixture of 95% O₂ and 5% CO₂, and the movement was recorded on a recticorder (RJG4024, Nihon Kohden) with an isotonic transducer (TD-112S, Nihon Kohden) under loading with 1 g of resting tension. After the strip was allowed to stand for about one hour, 100 mM KCl was added to the organ bath. The strip was washed twice at intervals of 20 min with Ca²⁺-free Tyrode’s solution containing 2 mM EGTA. The bath fluid was replaced with Ca²⁺-free Tyrode’s solution. After 10 min, the strip was allowed to depolarize with 100 mM KCl. Then, the time required to cause 50% of the maximum contraction of the strip by 1.8 mM CaCl₂ was determined from the recorded curves. Ca²⁺-influx was thus examined. After the maximum contraction was caused, the time required to relax the contraction to 50% level was also determined by adding 2 mM EGTA. Ca²⁺-efflux was thus examined.

**Ratio of bladder weight to body weight and ChE activity in neurogenic bladder:** On the 14th day after the operation, the bladder was isolated and fat and connective tissues surrounding the bladder were thoroughly removed in a Petri dish cooled with ice. The bladder was then blotted with filter paper and weighed to determine the ratio of bladder weight to body weight. After that, a part of the bladder was used for preparing crude enzyme solution. That is, 1.0 ml of 0.1 M phosphate buffer (pH 8.0) was added to 10 mg of the bladder tissue and the mixture was homogenized by an Ultraturrax T18.
The homogenate thus obtained was centrifuged at 0°C, 12,000 G for 20 min. The supernatant was used as the enzyme solution for determining activities of total cholinesterase (ChE) and acetylcholinesterase (AChE) according to the method of Goto et al. (13) using the Ellman reaction (14). The supernatant in a volume of 0.4 ml each was put into 2 tubes. To one of the tubes, 2.4 ml of 0.1 M phosphate buffer (pH 8.0) and 0.1 ml of 0.01 M DTNB were added (A). To the other tube, 2.35 ml of 0.1 M phosphate buffer, 0.05 ml of 8.52 x 10^{-4} M lysivane and 0.1 ml of 0.01 M DTNB were added (B). After preincubation at 25°C for 5 min, 0.1 ml of 0.015 N AThCh was added to each tube as a substrate. Absorbances of both A and B were measured at 412 nm using water as a blank, and protein content in the supernatant was determined by the Lowry method. The activity of total ChE was calculated from the absorbance of A, and the activity of AChE was calculated by subtracting the absorbance of B from that of A. The unit of enzyme activity was expressed as O.D./min/mg protein.

Phasic and tonic contractions inhibited by La^{3+}: In order to examine the effects of Robaveron on the phasic and tonic contractions inhibited by La^{3+}, the strips of intact rat bladder and intact guinea pig longitudinal ileal muscle were employed. A rat bladder was isolated to prepare two strips cut longitudinally between both the ureters, while a guinea pig ileum was isolated to prepare the strip of longitudinal ileal muscle according to Weiss and Goodman (15). Each strip was suspended in an organ bath filled with the solution prepared by Hurwitz et al. (16) (125 mM Na⁺, 2.7 mM K⁺, 1.8 mM Ca²⁺, 23.8 mM Tris, 153.3 mM Cl⁻, 11 mM glucose, pH 7.5) aerated with 100% O₂ and loaded with 1 g of resting tension. Contraction was recorded on a recticorder (RJG4024, Nihon Kohden) with an isotonic transducer (TD-112S, Nihon Kohden). After the strip was allowed to stand for about one hour, 40 mM KCl was repeatedly added until a constant contraction had been gained. The lanthanum ion was then added to the bath (2 x 10^{-6}–10^{-5} M for the ileal strip and 5 x 10^{-5} M for the bladder strip as final concentrations) 3 min prior to 40 mM KCl. To examine the effect of Robaveron, the drug was pretreated 5 min prior to La^{3+}. Phasic and tonic contractions evoked by KCl could be clearly observed in the ileal strips, but the contractions in most of the bladder strips were very vague. Therefore, only strips that responded well were selected and used for the experiments.

Statistical analysis: Data were statistically evaluated using the Student’s t-test. They were considered significant when P was equal to or less than 0.05.

Results

Influence of Robaveron of forced voiding pressure in neurogenic bladder of rats: The forced voiding pressure was daily determined from the 3rd to the 14th day after the spinal operation. As shown in Fig. 2, the forced voiding pressure in rats of the control group was approximately 20 cmH₂O which corresponded to 1/4 to 1/5 that of intact animals (data not shown). Such a reduced pressure in the control group scarcely recovered through the experimental period. On the other hand, the groups treated with Robaveron showed a gradual increase in the forced voiding pressure and the treatment of Robaveron 150–250 mg/kg, p.o., exerted an increasing activity on the reduced pressure to the same extent as that for the dosage of 40 mg/kg, i.m.

Influence of Robaveron on the time required to cause maximum contraction of neurogenic bladder muscle by transmural electric stimulation: The height of maximum contraction was almost the same extent in any group, but the time required to cause the maximum contraction retarded in the neurogenic bladder muscle. The time required to cause 50% of the maximum contraction which had been expressed as “t” was about 3.2 sec in the intact group, while it was significantly retarded to about 12 sec in the control group (P<0.01). Such a retardation was recovered by the treatment of Robaveron in a dose-dependent fashion (Fig. 3). The oral treatment with approx. 210 mg/kg of Robaveron was presumed to exert a recovery of the retardation to the same extent as that
seen in the case of 40 mg/kg, i.m. This indicates that by the oral dose needs to be 5–6 fold higher than the dose by injection to obtain the same potency.

Influence of Robaveron on the Ca\(^{2+}\)-dynamics in neurogenic bladder muscle: As
shown in Table 1, Ca\(^{2+}\)-efflux from the tissue cells would be inhibited in the strip of neurogenic bladder muscle because a slight contraction was observed in the neurogenic bladder muscle but not in the intact one by the depolarization with KCl. The contraction was slightly relieved in the groups treated with Robaveron, p.o. and i.m. No difference was observed in the height of contraction and the time required to cause 50% of maximum contraction, "t", evoked by Ca\(^{2+}\) among all the groups. However, the time required to relax to 50% of the Ca\(^{2+}\)-induced maximum contraction by the addition of EGTA was 44.1±4.24 min in the control group and significantly retarded in comparison with 12.7±0.61 min of the intact group. Such a retardation recovered in the groups treated with Robaveron, particularly in the groups treated with 65 mg/kg, p.o., and 40 mg/kg, i.m., although not in a dose-dependent fashion.

**Influences of Robaveron on bladder weight and cholinesterase activity:** The ratio of bladder weight to body weight and cholinesterase activity in each group are shown in Table 2. The ratio of bladder weight to body weight was 0.08±0.005% in the control group and significantly increased in comparison with 0.03±0.001% of the intact group (P<0.01). No significant change was observed between the Robaveron-treated

| Table 1. Influence of Robaveron on Ca\(^{2+}\)-efflux and influx in the strip of neurogenic bladder muscle |
|-----------------------------------------------------|
| **Drugs** | **% of contraction by K\(^+\)** | **% of contraction by Ca\(^{2+}\)** | **50%-contraction time by Ca\(^{2+}\)** (min) | **50%-relaxation time by EGTA** (min) |
|----------|-------------------------------|-------------------------------|---------------------------------|---------------------------------|
| Intact  | 0  | 116.4±3.09                   | 1.6±0.11                       | 12.7±0.61                       |
| Control | 7.4±0.53 | 128.1±6.48                  | 3.4±0.35                       | 44.1±4.24*                     |
| Robaveron (mg/kg) | | | | |
| 65 p.o. | 4.9±1.48 | 140.1±11.69                  | 2.8±0.32                       | 30.5±4.33†                     |
| 125 p.o. | 4.8±1.49 | 136.9±6.91                   | 3.1±0.27                       | 33.6±3.94                      |
| 250 p.o. | 5.3±1.39 | 131.3±3.54                   | 3.5±0.31                       | 40.5±4.15                      |
| 40 i.m. | 5.3±1.63 | 130.8±4.87                   | 3.4±0.27                       | 32.1±2.93†                     |

The time required to cause 50% of the maximum contraction of the strip evoked by 1.8 mM CaCl\(_2\) (50%-contraction time) and the time required to relax to 50% of the Ca\(^{2+}\)-induced maximum contraction by EGTA (50%-relaxation time) were determination from the recorded curves. The contraction induced under Tyrode’s solution with 100 mM in each preparation was taken as 100%. The strip was washed twice at intervals of 20 minutes with Ca-free Tyrode’s solution containing 2 mM EGTA. The bath fluid was replaced with Ca-free Tyrode’s solution. After the 10 minutes, the strip was allowed to depolarize with 100 mM KCl. Each value represents the mean±S.E. of 9–11 experiments. *: significantly different from the intact control at P<0.01, †: significantly different from the control at P<0.05.

| Table 2. Influences of Robaveron on the ratio of bladder weight to body weight and activities of total ChE and AChE |
|-----------------------------------------------------|
| **Drugs** | **Bladder weight/body weight** | **Total ChE activity O.D./min/mg protein ×10\(^{-5}\)** | **AChE activity O.D./min/mg protein ×10\(^{-5}\)** |
|----------|-----------------------------|------------------------|------------------------|
| Intact  | 0.03±0.001                  | 4.45±0.599             | 1.73±0.013 (9)        |
| Control | 0.08±0.005*                  | 2.03±0.436*            | 0.92±0.083* (9)       |
| Robaveron (mg/kg) | | | | |
| 32 p.o. | 0.09±0.009                  | 2.19±0.506             | 0.72±0.117 (9)        |
| 64 p.o. | 0.09±0.012                  | 1.75±0.237             | 0.77±0.091 (10)       |
| 128 p.o. | 0.08±0.010             | 1.94±0.382             | 0.81±0.090 (10)       |
| 20 i.m. | 0.08±0.007                  | 2.69±0.531             | 1.09±0.258 (11)       |

Each value represents the mean±S.E. of 9–11 experiments. *: significantly different from the intact at P<0.01.
groups and the control one. The activity of total ChE was 4.45±0.599 unit in the intact group and 2.03±0.436 unit in the control group, which indicates a significant decrease in the latter (P<0.01). On the other hand, the activity of AChE was 1.73±0.113 unit in the intact group and 0.92±0.083 unit in the control group, which also indicates a significant decrease in the latter (P<0.01). Administration of Robaveron did not show any significant effect on decreases in the activities of both enzymes.

Influence of Robaveron on La³⁺-induced inhibition of phasic and tonic contractions:
The effect of Robaveron on La³⁺-induced inhibition of phasic and tonic contractions which had been provoked by 40 mM KCl was examined in the strips of intact rat bladder and intact guinea pig longitudinal ileal muscle. As can be seen in Fig. 4, 5×10⁻⁵ M La³⁺ showed 16.6–37.5% inhibition of the phasic contraction and little inhibition of the tonic contraction in the bladder. On the other hand, as shown in Fig. 5, in the ileal muscle, 2×10⁻⁶–10⁻⁶ M La³⁺ revealed 22–45% inhibition and 15–17% inhibition, respectively. Thus, the inhibitory effect of La³⁺ was rather dominant on the phasic contraction in both the strips of bladder and ileal muscle, and the latter strip possessed better reproducibility with smaller fluctuations than the former. The inhibitory effect of La³⁺ on the bladder was reduced by 10⁻⁶–10⁻³ g/ml Robaveron in the phasic contraction, and that on the ileal muscle was also reduced nearly dose-dependently by 5×10⁻⁶–10⁻⁴ g/ml of the drug even in the tonic contraction. The inhibition induced by 2×10⁻⁶–5×10⁻⁶ M La³⁺ in the phasic contraction of the ileal muscle was recovered nearly completely by 5×10⁻⁵ g/ml Robaveron, and that induced by 10⁻⁵ M La³⁺ was recovered approximately 90% by the same dose of the drug. Such a recovery was also observed in the tonic contraction of the ileal muscle.

Discussion
In the present study, we have produced an experimental neurogenic bladder in rats which was accompanied with paralysis of the hind legs, urinary incontinence, residual urine, atony of the bladder-neck, retardation of contraction, abnormality of Ca²⁺-dynamics, decrease in cholinesterase activities and increase in the ratio of bladder weight to body weight. The results suggest that the urinary dysfunction in neurogenic bladder may be caused not only by disorders of innervation on the urinary function but also by changes in the bladder muscle itself. Symptoms such as urinary incontinence and residual urine which have been observed in this model presented here are similar to those seen in the case of humans. There was no difference in the height of contraction between the neurogenic bladder muscle and intact bladder muscle by the transmural stimulation, though the time, "t", required to cause 50% of the maximum contraction was distinctly retarded in the neurogenic bladder muscle. This retardation would be caused by the inhibition of Ca²⁺-influx or release of the stored Ca²⁺. From the result of the experiment with the strip of neurogenic bladder muscle in Ca²⁺-free medium, it was considered that the Ca²⁺-influx via the potential channel had been unchanged at least. Therefore, it was conclusively considered that the Ca²⁺-dynamics involved in the phasic response had been disordered.
Our results also suggest that neurogenic bladder would be concerned with not only changes in the ACh-receptor due to injuries of the nervous system but also a decrease in the AChE activity, though it is clinically said that neurogenic bladder shifts to cholinergic hypersensitivity. Such a decrease in the AChE activity was also reported by Katsumi et al. (17) using an experimentally caused neurogenic bladder, and they suggested that the decrease in this enzyme activity displays certain roles in the causation of the neurogenic bladder.

Many clinical investigations have revealed that the injection of Robaveron, a biological prepared from swine prostate, is effective for the therapy of the urinary dysfunction accompanying cord bladder (18, 19), brain bladder (20, 21) and neurogenic bladder due to diabetes mellitus (22, 23) or post-operation of uterine cancer (24, 25) as well as the urinary disturbance in benign prostatic hypertrophy (26, 27). The clinical efficacy of the drug was proved by a double blind test (28). In addition, the effectiveness on an experimental neurogenic bladder in rabbits has been already clarified (7–10). However, there is no report on the mode of action and on the effectiveness by the oral administration of the drug. Only a report by Koda et al. (29) has suggested that the oral administration of a prostatic extract prepared by their own method exhibits influences on the metabolism in rat prostatic tissue. Since the patients suffering from such diseases require the medication for a fairly long period, the oral application of drugs would be desired.

In the present study, we prepared a reproducible model of neurogenic bladder in

| $La^{3+}$ (M) | Robaveron (g/ml) | % of contraction |
|--------------|-----------------|-----------------|
| 0            |                 | 100 (%)         |
| $2 \times 10^{-6}$ |                 |                 |
| 0            |                 |                 |
| $5 \times 10^{-6}$ |                 |                 |
| 0            |                 |                 |
| $10^{-5}$    |                 |                 |
| $5 \times 10^{-5}$ |                 |                 |
| 0            |                 |                 |
| $10^{-4}$    |                 |                 |
| $5 \times 10^{-5}$ |                 |                 |
| 0            |                 |                 |
| $10^{-5}$    |                 |                 |
| $5 \times 10^{-5}$ |                 |                 |
| 0            |                 |                 |

* , †: significantly different from the control at $P<0.05$ and $P<0.01$, respectively.

Fig. 5. Influence of Robaveron on the $La^{3+}$-induced inhibition of phasic (■) and tonic (□) contractions in the strip of intact guinea pig longitudinal ileal muscle. Robaveron was pretreated 5 min prior to $2 \times 10^{-6}–10^{-6}$ M $La^{3+}$. The phasic and tonic contractions induced by 100 mM KCl in each preparation were taken as 100%, respectively. Each column represents the mean±S.E. of 8 experiment.
rats and examined the influence of Robaveron given by two different routes, i.m. and p.o., on this model. The forced voiding pressure in the neurogenic bladder decreased to 1/4 to 1/5 that of the intact animals. For this decrease, the administration of Robaveron by either route displayed an improvement, and the potency in the oral route nearly corresponded to 1/6-1/4 that of the intramuscular administration. On the other hand, a retardation in the time, “t”, required to cause 50% that of the maximum contraction of the isolated neurogenic bladder muscle which had been stimulated transmurally was recovered by Robaveron given by either route. Many investigators, e.g., Weiss et al. (15), Hurwitz et al. (16) and Magaribuchi et al. (30) have reported that La3+ has influences on the Ca2+ channel, particularly on the phasic contraction. Since Robaveron recovered an inhibition of phasic contraction by La3+ in both strips of rat bladder muscle and guinea pig ileum, even that of the tonic contraction in the ileal muscle, the drug appears to improve the urinary dysfunction seen in the neurogenic bladder through an improvement of the attenuated phasic contraction; namely, the drug would be expected to reinforce the strength of micturition due to intensification of the detrusor muscle related to the phasic contraction. Robaveron, however, did not show any influence on decreases in the total ChE and AChE activities resulting from neurogenic bladder. As to the clinical effect of Robaveron, Nakaarai (10) suggested that it would act directly on the detrusor muscle. Our results presented here may support their report. The relaxation of Ca2+-induced contraction by EGTA was dramatically inhibited in the neurogenic bladder muscle. Such an inhibition was recovered by the treatment with Robaveron, which suggests that the drug improves the Ca2+-efflux. Therefore, Robaveron would normalize the Ca2+-efflux after the contraction of the bladder and contribute to the improvement of urinary dysfunction by enhancing the relaxation. As our model represents a flaccid type of bladder, further studies will be required on the other type of bladder disturbance. The increasing effect of Robaveron on the forced voiding pressure will be useful for the improvement of urinary incontinence. A question inevitably arises that Robaveron would be rather disadvantageous for the bladder when its neck is under a tonic condition as seen in the case of the reflex type of bladder. However, Hojo et al. (31) have reported that Robaveron shows desirable effects on the attenuated bladder function but practically no effect on the normal or hypertonic bladder. Therefore, our finding that Robaveron recovers a decrease in the forced voiding pressure in the experimentally-caused neurogenic bladder may support the clinical efficacy of Robaveron. It also suggests that the drug would be effective even when given orally.

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