Rociverine Citrate: A New Spasmolytic Agent, Potentially Useful in the Treatment of Urinary Bladder Hyperreflexia

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Abstract—Rociverine citrate was evaluated for its ability to affect the motility of rat urinary bladder, in vitro and in vivo, in comparison with flavoxate hydrochloride. Rociverine counteracted both methacholine- and high K+-induced tonic contractions of bladder strips. In anaesthetized rats, intravenous rociverine inhibited dose-dependently frequency and amplitude of the distension-induced rhythmic contractions (DIRCs) of urinary bladder and counteracted the topical high K+-induced pressure increase in the same organ. Orally administered rociverine produced a dose-related reversal of the reserpine-induced detrusor hyperreflexia in anaesthetized rats. In each of these experimental models rociverine was more effective than flavoxate. These results point to the usefulness of rociverine in the treatment of urinary bladder motility disorders.

In vitro studies indicated that rociverine antagonizes acetylcholine, K+, Ca2+ and transmural stimulation-induced contractions of rat urinary bladder (1, 2) as well as K+, histamine and eleidosin induced contractions of human ureteral strips (3). These findings suggest that rociverine might be of therapeutic usefulness in the treatment of urinary incontinence.

In view of the above we thought it worthwhile to investigate further the effects of rociverine on rat urinary bladder, by using an in vivo model mimicking cystometric findings of detrusor hyperreflexia in humans (4).

Materials and Methods

In vitro experiments: Male albino rats of the Sprague-Dawley strain, 320–380 g body weight, were killed by cervical dislocation. Longitudinal strips (from the dome to the urethra) of the urinary bladder were excised and mounted in a 20 ml organ bath containing warm (37°C) Krebs’ solution having the following composition (millimolar): NaCl, 118.0; KH2PO4, 1.18; NaHCO3, 25.0; KCl, 4.7; MgSO4·7H2O, 1.18; CaCl2, 2.52; and Glucose, 11.1, gassed with 95% O2 and 5% CO2. The preparations, under a resting tension of 1 g, were tied to a Basile isotonic transducer connected to a Basile 7060 recorder. After an equilibration period of 20 min, the preparations were exposed to methacholine (10−4 M). In other experiments, Krebs’ solution was replaced by a high K+ (54 mM) Krebs’ solution prepared by substituting equimolar amounts of NaCl with KCl. When tonic contraction produced by methacholine or KCl had reached a steady state, dose response curves to rociverine citrate and flavoxate HCl were obtained by exposing the preparations to increasing concentrations of the test substances, each concentration being added when the effects of the preceding one had reached a steady state. Spasmolytic activity of the test substances was calculated as percent inhibition of methacholine or K+-induced contractions.

Effect on distension-induced rhythmic contractions (DIRCs) of urinary bladder in anaesthetized rats: Rats were anaesthetized with intramuscular urethane (1.2 g/kg), and the left femoral vein was cannulated for administration of test substances. Through a small urethral incision, a polyethylene tubing (1 mm I.D., 1.5 mm O.D.) was inserted into the urinary bladder for recording intraluminal
pressure variations as described previously (5). The bladder was then emptied of urine and filled with 0.8-1.2 ml warm (37°C) saline. After a 15 min equilibration period, the test substances were injected in cumulative doses, each dose being administered when the main effects of the preceding one had subsided.

**Effect on topical high K⁺-induced pressure increase in urinary bladder of anaesthetized rats:** Surgical procedures and intraluminal pressure recording are similar to those described above, except that a 15 min equilibration period, the moistening solution was substituted with saline containing KCl (540 mM). This produced an increase of intraluminal pressure, which after a short-lived peak was stable for over 30 min. Topical NaCl (540 mM) was nearly ineffective, indicating that high K⁺-induced pressure increase was not ascribable to hyperosmolarity of the solution. When the pressure increase had reached a steady state, 0.5 ml/kg saline or test substances were injected intravenously in a cumulative manner, each dose being injected when the effects of the preceding one had reached a steady state.

**Effect on reserpine induced detrusor hyperreflexia in anaesthetized rats:** Surgical procedures and intraluminal pressure recording are similar to those described above, except that variations in intraluminal pressure were recorded in response to continuous infusion (2.8 ml/hr) of warm (37°C) saline (cystometrogram) for 30 min by means of a De Sage 131900 six-channel peristaltic pump connected to a polyethylene tubing inserted into the bladder. The volume of infused saline required to produce repetitive contractions of at least 4 mmHg in amplitude, which were followed by rhythmic contractions of increasing amplitude, was assumed to be an effective stimulus for triggering the micturition reflex in each animal and was chosen as end-point for testing the effects of the test substances. Intraperitoneal reserpine (5 mg/kg, 48 hr before induction of anaesthesia) significantly reduced the volume of saline required to elicit the micturition reflex (detrusor hyperreflexia) (4). Therefore, the effects of the test substances administered orally 60 min before cystometrogram were evaluated in terms of the ability to antagonize or reduce reserpine-induced detrusor hyperreflexia.

**Materials:** The following substances were used: rociverine citrate (Laboratori Guidotti), flavoxate hydrochloride (Recordati), methacholine bromide (Sigma) and reserpine (Serva). The doses of rociverine citrate and flavoxate hydrochloride refer to the bases.

**Statistical analysis:** ED50s and 95% confidence limits were calculated by means of linear regression analysis of log dose-response curves. Some data were analyzed by means of Student’s t-test.

**Results**

**In vitro experiments:** Both rociverine citrate and flavoxate hydrochloride produced a concentration-dependent inhibition of methacholine-induced tonic contraction of bladder strips (Fig. 1); however, rociverine was eight times more potent than flavoxate, ED50s and 95% confidence limits being 1.6 (0.92-2.7) × 10⁻⁵ M and 1.3 (0.77-2.1) × 10⁻⁴ M, respectively. Rociverine inhibited also high K⁺-induced tonic contraction of bladder strips (Fig. 1), with an ED50 of 1.6 (1.1-2.5) × 10⁻⁴ M, while flavoxate produced a 27% inhibition at 10⁻⁴ M. Higher concentrations of flavoxate could not be used because precipitation occurred in the organ bath.

**Effect on distension-induced rhythmic contractions (DIRCs) of urinary bladder in anaesthetized rats:** Frequency and amplitude of DIRCs were not affected by 4 consecutive i.v. administrations of saline at 5 min intervals. Intravenous rociverine (0.62-5 mg/kg) produced a dose-dependent reduction of the frequency of DIRCs [ED50=2.0 (1.4-2.5) mg/kg] and of their amplitude [ED30=1.9 (1.5-3.0) mg/kg], as shown in Fig. 2. The duration of the effects of rociverine was also dose-dependent, but did not generally exceed 10 min even at the highest dose. Flavoxate (Fig. 2) was approximately two times less potent than rociverine in reducing the frequency of DIRCs [ED50=4.2 (3.0-5.5) mg/kg] and three times less potent in reducing their amplitude [ED30=5.5 (3.9-14.5) mg/kg].

**Effect on topical high K⁺-induced pressure
increase in urinary bladder of anaesthetized rats: Intravenous rociverine (1–10 mg/kg) produced a dose-dependent reduction (up to 58±5%, n=7, P<0.001) of K⁺-induced pressure increase in urinary bladder (Fig. 3). On the other hand intravenous flavoxate did not reduce this pressure increase even at the highest dose (Fig. 3).
Fig. 3. Representative tracings of the effects of vehicle, rociverine and flavoxate on the pressure increase in urinary bladder, evoked by topical high K+ in anaesthetized rats.

Fig. 4. Effects of oral rociverine (○) and flavoxate (●) on reserpine-induced detrusor hyperreflexia in anaesthetized rats. Each point is the mean±S.E. of at least six animals.

**Effect on reserpine-induced detrusor hyperreflexia in anaesthetized rats:** The volume of saline required to elicit micturition reflex in control animals was 0.59±0.09 ml (n=12), which is significantly higher than that (0.32±0.07 ml, n=12) required to elicit micturition in reserpine-pretreated animals. Orally administered rociverine produced a dose-related reversal of the reserpine-induced detrusor hyperreflexia (Fig. 4), with an ED50 of 13.2 (9.7–18.0) mg/kg, while flavoxate had no significant effect up to 50 mg/kg p.o.

**Discussion**

Spasmolytic drugs are widely used for the symptomatic treatment of urinary incontinence whose most common form, particularly in elderly people, is "detrusor instability" which, when dependent on neurologic disease, is termed "detrusor hyperreflexia" (6).

It has been shown that in urethane-anaesthetized rats, distension induced rhythmic contractions (DIRCs) of urinary bladder are due to rhythmic activation of a supraspinal vesico-vesical reflex similar to that which subserves bladder voiding in normal adult animals (7–9). On the other hand, micturition following transurethral infusion depends upon activation of a spinal vesico-vesical reflex which under normal conditions is suppressed by a supraspinal descending inhibitory pathway (10). The threshold for the spinal reflex is under sympathetic inhibitory control which possibly occurs at various sites (4) and a picture of detrusor hyperreflexia can be obtained by either chemical (reserpine, 6-OH dopamine) or surgical sympathectomy (9, 11). Therefore, the ability of rociverine to revert the reserpine-induced detrusor hyperreflexia, presumably due to its antimuscarinic and musculotropic activity (1, 2, 12, 13), supports clinical observations indicating that this drug is effective in increasing bladder capacity in patients suffering from urinary bladder spasm of varying etiology (14, 15) and in reducing markedly the frequency of micturition in elderly patients affected by nycturia (16).

It should be noted that intravenous doses of rociverine required to inhibit transiently (10 min or less) DIRCs were not very far from those which, one hour after oral administration, reverted the reserpine-induced...
detrusor hyperreflexia. Since DIRCs represent a rhythmic activation of a reflex similar to that producing bladder voiding in normal animals (9, 11), these findings suggest that at doses effective in treating bladder hypermotility, rociverine may have little or no effect on normal voiding function.

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References

1 Schiantarelli, P. and Murmann, W.: Antispasmodic activity of rociverine on the smooth musculature of the urinary tract: comparative in vitro study. Arzneimittelforsch. 30, 1102-1109 (1980)

2 Downie, J.W. and McGuire, R.P.: Antagonism of calcium-induced contraction in potassium-depolarized rabbit detrusor muscle strips by dicyclomine hydrochloride and rociverine. Can. J. Physiol. Pharmacol. 59, 853-856 (1981)

3 Potenzoni, D., Zappia, L., Sacchini, P. and Bezzi, E.: Effects of rociverine on the human ureter; in vivo and in vitro experimental study. Pharmacol. Res. Commun. 16, 765-774 (1984)

4 Maggi, C.A. and Meli, A.: Reserpine-induced detrusor hyperreflexia: an in vivo model for studying smooth muscle relaxants at urinary bladder level. J. Pharmacol. Meth. 10, 79-91 (1983)

5 Maggi, C.A., Grimaldi, G. and Meli, A.: The effects of nifedipine and verapamil on spontaneous and carbachol stimulated contractions of rat urinary bladder in vivo. Arch. Int. Pharmacodyn. Ther. 257, 288-294 (1984)

6 Stanton, S.L.: Classification of incontinence. In Clinical Gynecological Urology. Edited by Stanton, S.L., p. 169-192. Mosby Co., St. Louis (1984)

7 Maggi, C.A., Furio, M., Santicioli, P. and Meli, A.: Intracisternal glycine activates the micturition reflex in urethane anaesthetized rats. J. Pharm. Pharmacol. 37, 517-520 (1985)

8 Maggi, C.A., Santicioli, P. and Meli, A.: The non-stop transvesical cystometrogram in urethane anaesthetized rats: a simple procedure for quantitative studies on the various phases of urinary bladder voiding cycles. J. Pharmacol. Meth. 15, 157-167 (1986)

9 Maggi, C.A., Santicioli, P., Furio, M. and Meli, A.: Dual effects of clonidine on micturition reflex in urethane anaesthetized rats. J. Pharmacol. Exp. Ther. 235, 528-536 (1985)

10 Maggi, C.A., Santicioli, P. and Meli, A.: Somato-vesical and vesico-vesical excitatory reflexes in urethane-anaesthetized rats. Brain Res. (1986) (in press)

11 Maggi, C.A., Santicioli, P. and Meli, A.: Sympathetic inhibition of reflex activation of bladder motility during filling at a physiological-like rate in urethane anaesthetized rats. Neuourol. Urodynamics 4, 37-45 (1985)

12 Toson, G., Schiantarelli, P. and Murmann, W.: Rociverine, a new antispasmodic agent with balanced neurotropic and myotropic activity. Arzneimittelforsch. 28, 1130-1142 (1978)

13 D’Agostino, G., Zonta, F., Santagostino Barbone, M.G., Grana, E., Bruni, P. and Subissi, A.: Mechanism of smooth muscle relaxation by rociverine. Arzneimittelforsch. 34, 584-589 (1984)

14 Manganelli, A.: Cystometric evaluation of the activity of a new spasmytic agent: rociverine. Farmaco [Prat.] 34, 384-392 (1979)

15 Laudi, M. and Fontana, G.: Clinical evaluation of a new synthetic spasmytic (rociverine) in the urological field. Farmaco [Prat.] 34, 563-566 (1979)

16 Dacco’, L. and Intrieri, L.: Rociverine treatment for nycturia of the elderly. Clin. Ther. 8, 170-174 (1986)