Effects of LY274614, a competitive NMDA receptor antagonist, on the micturition reflex in the urethane-anaesthetized rat

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1 The effects of 3 competitive N-methyl-D-aspartate (NMDA) receptor antagonists, LY274614, LY233536 and LY235723, on the micturition reflex and external urethral sphincter EMG activity, were examined either under isovolumetric conditions or during continuous filling cystometry in urethane-anaesthetized (1.2 g kg⁻¹, s.c.) rats.

2 Intravenous administration of LY274614 (3–30 mg kg⁻¹) inhibited in a dose-dependent fashion both bladder and sphincter activity in the intact rats. In addition, the volume threshold for inducing micturition was increased and voided volume was decreased.

3 Intrathecal administration of LY274614 (0.06–30 µg) similarly inhibited bladder and sphincter activity during cystometry in intact rats.

4 In chronic spinal cord (T₆–T₈) transected rats LY274614 (0.1–30 mg kg⁻¹, i.v.) did not alter bladder activity under isovolumetric conditions but decreased the amplitude of micturition contractions and sphincter EMG activity during cystometry at a dose of 10–30 mg kg⁻¹.

5 The inhibitory effects of i.v. administration of LY274614, on bladder and sphincter activity induced by infusion of chemical irritant (0.1% acetic acid) or saline, were similar; except that a slightly larger dose was needed to inhibit sphincter activity during acetic acid infusion.

6 Peak amplitude of micturition contractions recovered to 50% of control 3 h following i.v. (30 mg kg⁻¹) or i.t. (6 µg) administration of LY274614.

7 Two other chemically related NMDA antagonists, LY233536 and LY235723 produced similar but less potent effects than LY274614 when given i.v.

8 These data indicate that glutamatergic transmitter mechanisms at the level of the spinal cord are important in modulating bladder activity in the intact animal, but that these mechanisms do not contribute to bladder reflexes in the chronic spinal rat. These mechanisms may, however, contribute to sphincter activity in both intact or chronic spinal rats.

Keywords: NMDA receptor antagonist; rat bladder; EMG; external urethral sphincter; glutamate; micturition reflex

Introduction

Reflex micturition is produced by a contraction of the smooth muscle of the urinary bladder and a reciprocal relaxation of bladder neck, urethra, and the external urethral sphincter. However, in rats (Kruse *et al.*, 1993) and to a lesser degree in dog (Nishizawa *et al.*, 1985) the striated muscle of the external urethral sphincter contracts intermittently in high (4–6 Hz) frequency bursts during voiding. This enhanced and pulsatile sphincter activity may aid in expulsion of urine in these animals, while continuous contraction of sphincter prevents urine release. In many species these responses are mediated by a spinobulbospinal reflex pathway that passes through relay centres in the lumbosacral spinal cord and the rostral brain stem (the pontine micturition centre) (de Groat *et al.*, 1992). The reflex pathway is activated by distension of the urinary bladder and subsequent firing in tension receptor afferent fibres that pass through the pelvic nerve to the lumbosacral spinal cord.

Pharmacological studies have implicated a number of neurotransmitters in the central nervous control of micturition (de Groat *et al.*, 1992). Among the putative excitatory transmitters, glutamic acid has attracted the most attention. Microinjection, of glutamic acid or related excitatory amino acids, into the pontine micturition centre or adjacent pontine area, facilitated micturition in the cat (Mallory *et al.*, 1991) and rat (Lumb & Morrison, 1987). Whereas, systemic administration of MK-801, an N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, inhibited bladder and sphincter reflexes in anaesthetized rats (Maggi *et al.*, 1990;

Yoshiyama *et al.*, 1991a,b; 1993). MK-801 also blocked the bladder contractions elicited by electrical stimulation of the pontine micturition centre (Suzuki *et al.*, 1991) and depressed the expression of the immediate early gene, *c-fos*, in spinal neurones induced by chemical irritation of the bladder mucosa (Birder & de Groat, 1992). Ligand binding studies have revealed that NMDA receptors are present at various locations along the micturition reflex pathway; including areas in proximity to the lumbosacral parasympathetic nucleus, the urethral sphincter motor nucleus and the pontine micturition centre (Jansen *et al.*, 1990; Shaw *et al.*, 1991).

The present study was undertaken to evaluate further the role of NMDA receptors in the micturition reflex pathway. We have examined the changes in bladder and sphincter activity induced by LY274614, a potent competitive NMDA receptor antagonist (Ornstein *et al.*, 1991), which enters the central nervous system following systemic administration. Two other competitive NMDA receptor antagonists (LY233536 and LY235723) were also examined in a few experiments. Unlike the non-competitive antagonist, MK-801, which blocks the cation channel of the NMDA receptor (Wong *et al.*, 1986), LY274614 acts at the glutamate binding site on the NMDA receptor (Ornstein *et al.*, 1991). In addition, LY274614 is likely to have a more selective action than MK-801 since, in high concentrations, MK-801 is known to block nicotinic acetylcholine receptor channels (Amador & Dani, 1991). The latter effect could alter ganglionic transmission in the peripheral autonomic pathways to the bladder, as well as transmission at the neuromuscular junction, in the striated sphincter muscle and thereby alter micturition reflexes by a peripheral non-glutamatergic mechanism. The

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present results with LY274614 confirm and extend the findings with MK-801; indicating that glutamatergic transmission at NMDA receptors has an important facilitatory influence on bladder and sphincter reflexes in the anaesthetized rat. This influence must occur, at least in part, at the level of the lumbosacral spinal cord, since intrathecally administered LY274614 mimicked the effect of systemic administration.

A preliminary account of this work has been presented in an abstract (Yoshiyama *et al.*, 1992).

Methods

Animal preparation

Experiments were performed on 79 female urethane-anaesthetized (1.2 g kg^{-1} , s.c.) Sprague-Dawley rats weighing 170 to 310 g (mean = 240 g). The trachea was cannulated with a polyethylene tube (PE-205) to facilitate respiration; and cannulae (PE-50) were placed in the external jugular vein or femoral vein for intravenous drug administration. A transurethral bladder catheter connected to a pressure transducer was used to record the bladder pressure isovolumetrically with the urethral outlet ligated or to record pressure during cystometry when the bladder was filled with a constant infusion of physiological saline or acetic acid (0.1%) and allowed to empty around the catheter. To evaluate voiding efficiency (% of bladder volume voided) saline was infused into the bladder (0.04 ml min^{-1}) until the peak of a voiding bladder contraction; then the infusion was stopped and the saline voided from the bladder was collected and measured to determine the volume voided. The bladder was then emptied, the residual volume measured and cystometrograms repeated. The procedure was repeated a minimum of three times for each drug dose and control. Continuous cystometry was performed by a constant infusion (0.21 ml min^{-1}) of saline into the bladder to elicit repetitive voidings, which allowed rapid collection of data for a large number of voiding cycles (Maggi *et al.*, 1986). PE-90 and PE-50 cannulae were used for isovolumetric recording and constant infusion cystometry (continuous and single), respectively. For isovolumetric recording, the ureters were tied distally, cut and the proximal ends cannulated (PE-10) and drained externally. The procedure prevented the bladder from filling with urine during the experiment.

Eleven rats were spinalized under halothane anaesthesia. After a T₆–T₈ laminectomy, the dura and spinal cord were cut with scissors, and a sterile sponge (Gelfoam, The Upjohn

Company) was placed between the cut ends. The bladders of spinal rats were expressed manually two or three times daily, and perigenital stimulation with a cotton swab was performed to encourage reflex bladder emptying (Mallory *et al.*, 1989). The experiments on spinalized rats were performed 2 to 3 weeks postspinalization.

In 19 rats anaesthetized with urethane (1.2 g kg^{-1} , s.c.), an intrathecal (i.t.) catheter was inserted according to the technique of Yaksh & Rudy, 1976. The occipital crest of the skull was exposed and the atlanto-occipital membrane was incised at the midline using the tip of a 19 gauge needle as a cutting edge. A catheter (PE-10) was inserted through the slit and passed caudally to the L₆-level of the spinal cord. The volume of fluid within the catheter was kept constant at 6 μl . Cumulative dose-response curves were obtained by administering the drugs in 6 μl injections followed by 6 μl flush with artificial CSF at 15 min intervals. At the end of the experiment a laminectomy was performed to verify the location of the catheter tip.

In 23 experiments (5 intact, 5 chronic spinal, 8 intact with i.t. cannulae, and in 5 intact animals with acetic acid infusion

Figure 2 Log dose-response curves showing the effects of increasing doses of LY274614 (0.1 – 30 mg kg^{-1} , i.v.) on the amplitude of reflex bladder contractions under isovolumetric conditions in the urethane-anaesthetized intact (●) and chronic spinal (▲) rats. Abscissa scale: the cumulative dose of LY274614 (mg kg^{-1} , i.v.) plotted on a log scale. Ordinate scale: contraction amplitude as a % of control. Mean $\pm$ s.e.mean is plotted for each point. Data in the intact rat ($n = 5$) shows a dose-dependent significant decrease from control. Dose-response curve in the chronic spinal rat ($n = 5$) shows a small but significant increase at doses of 0.3 and 1 mg kg^{-1} . Individual doses are compared to control by paired t test (* $P < 0.05$, ** $P < 0.001$). As a comparison of dose-response curves between the intact and chronic spinal rats at each dose, significant differences were seen at 0.3 – 30 mg kg^{-1} (0.3 – 1 mg kg^{-1} , $P < 0.05$; 3 mg kg^{-1} , $P < 0.01$; 10 – 30 mg kg^{-1} , $P < 0.001$, unpaired t test).

Figure 1 The effects of increasing doses of LY274614 on rhythmic reflex bladder contractions under isovolumetric conditions in the urethane-anaesthetized intact (a) and chronic spinal rats (b). Doses represent total cumulative doses. Note that the amplitude of bladder contractions is reduced by increasing doses of LY274614 (3 – 10 mg kg^{-1} , i.v.) in the intact rat while a small increase is seen in the chronic spinal rat. The spinal cord was sectioned at the level of T₆–T₈, 3 weeks prior to the experiment.

instead of saline), fine (76 μm) wire EMG electrodes were placed percutaneously in the external urethral sphincter to record the electrical activity of the striated muscle. A 30 gauge needle with a hooked EMG electrode positioned at the tip was inserted into sphincter approximately 5–10 mm lateral to the urethra and then withdrawn leaving the EMG wires embedded in the muscle (Kruse *et al.*, 1990). The EMG activity obtained was passed through a ratemeter and recorded on chart recorder. The peak firing rate during each micturition contraction was measured.

Acetic acid (0.1%) infusion into the bladder

Acetic acid infused into the bladder was used as a model for bladder irritation (Birder & de Groat, 1992). In 12 experiments, 0.1% acetic acid (pH = 3.5) was infused into the bladder, after recording control bladder contractions induced by normal saline infusion. Control bladder activity during chemical irritation was analyzed 0.5–2.5 h after beginning acetic acid infusion, to allow time for the bladder contractions to stabilize.

Evaluation and statistical analysis

The effects of cumulative doses of LY274614, LY233536 or LY235723 injected at approximately 15 min intervals on the amplitude, duration and frequency of reflex bladder contractions were recorded under isovolumetric conditions with the urethra closed or with it open to allow the bladder to empty. The interval between two voiding cycles, termed the intercontraction interval (Maggi *et al.*, 1986), was also measured during continuous cystometry. During single cystometry, each dose of LY274614 was given 20 min prior to the first

test. Two to four records were obtained after each dose. The effects of the drug on volume threshold (V_T) to induce micturition and the volume of fluid released (voided volume; V_V) during each voiding reflex was measured. Based on this value, voiding efficiency (%) ($V_E = 100 V_V(V_T)^{-1}$) could be estimated. All values are expressed as mean $\pm$ s.e.mean. Repeated measures analysis of variance (ANOVA) and Student's *t* test were used when appropriate for statistical data analysis. For all statistical tests, $P < 0.05$ was considered significant. The ED_{50} values were calculated from the dose-response curves.

Drugs

Drugs used in these studies included urethane (ethyl carbamate, Sigma), halothane (Ayerst Lab. Inc.), LY274614 (($\pm$)-decahydro-6-(phosphonomethyl)-3-isoquinolinecarboxylic acid), LY233536 (($\pm$)-decahydro-6-(2H-tetrazol-5-ylmethyl)-3-isoquinolinecarboxylic acid) and LY235723 ([cis-(–)]-4-(2H-tetrazol-5-ylmethyl)-2-piperidinecarboxylic acid) (Lilly Res. Lab.). LY274614 was dissolved in a few drops of 1*N* sodium hydroxide and physiological saline for i.v. administration and in artificial CSF (Merlis, 1940; Feldberg & Fleischhauer, 1960) for i.t. injection. LY233536 and LY235723 were dissolved in physiological saline for i.v. administration. Drugs doses were calculated for the salts of each compound.

Results

The effects of LY274614 on bladder reflexes were studied in four different preparations: (1) animals with an intact

Figure 3 The effects of increasing doses of LY274614 on continuous filling (0.21 ml min⁻¹) cystometry and EMG of the external urethral sphincter muscle in urethane-anaesthetized intact (a) and chronic spinal (b) rats. Doses represent total cumulative doses. Note that the amplitude of micturition contractions and sphincter EMG activity are reduced by increasing doses of LY274614 (3–30 mg kg⁻¹, i.v.) both in the intact and chronic spinal rats. Some bladder and sphincter EMG activity in the chronic spinal rat remained even at the largest dose 30 mg kg⁻¹ while those in the intact rat are completely abolished at this dose. Data in the intact rat at 30 mg kg⁻¹ show a bladder inhibition with a higher baseline intravesical pressure. The reduction in amplitude of micturition contractions in the chronic spinal animal is probably due to inhibition of external urethral sphincter activity (see Discussion). EUS = external urethral sphincter.

neuraxis (intact) and (2) chronic spinal animals in which the i.v. route of administration was used, (3) intact animals in which drugs were administered intrathecally (i.t.) and (4) intact rats during bladder irritation produced by infusion of 0.1% acetic acid into the bladder. Six parameters of bladder activity were measured: peak amplitude of intravesical pressure, duration and frequency of bladder contractions, intercontraction interval (interval between voids during continuous cystometry), volume threshold for inducing micturition (V_T or bladder capacity) and voided volume (V_V). Based on V_T and V_V , voiding efficiency as percentage of bladder volume ($V_E = 100V_V (V_T)^{-1}$) was estimated. In some experiments the external urethral sphincter EMG activity was also recorded while recording micturition reflexes.

Intact rats

Bladder activity recorded under constant volume conditions ($n = 5$) consisted of rhythmic contractions occurring at peak amplitudes of 27–45 mmHg (mean = 33 mmHg), frequencies of 0.30–0.98 min^{-1} (mean = 0.69 min^{-1}) and durations of 32–43 s (mean = 38 s). The amplitude of bladder contractions was decreased or inhibited completely following the administration of increasing doses of LY274614 (Figure 1a). As shown in the dose-response curve in Figure 2, a significant decrease in amplitude of bladder contractions was seen at doses between 3–30 mg kg^{-1} with an ED_{50} of 2.3 mg kg^{-1} . LY274614 in doses of 0.1–30 mg kg^{-1} did not affect either frequency or duration of bladder contractions.

In 12 animals, urinary bladder contractions recorded during a continuous transurethral infusion (0.21 ml min^{-1}) of saline exhibited peak pressures ranging from 16–107 mmHg (mean = 50 mmHg), duration of 25–198 s (mean = 68 s) and intercontraction interval of 10–302 s (mean = 118 s). The amplitude of micturition contractions was reduced in a dose-dependent manner following the administration of increasing doses of LY274614 (3–30 mg kg^{-1} , i.v.; Figures 3a and 4a) with an ED_{50} of 12.2 mg kg^{-1} . Although changes in intercontraction interval and duration of micturition contractions were seen in some experiments, these changes were not consistent or statistically significant ($n = 12$). In 3 of 12 animals, 30 mg kg^{-1} or higher doses did not completely abolish the rhythmic bladder activity but these doses did reduce amplitude to 48% of control (range: 26–86% of control). In 5 of 9 animals following complete inhibition of rhythmic bladder

contractions, baseline bladder pressure was higher than control levels (range: 10–23 mmHg, above control baseline mean = 16 mmHg) (Figure 3a). Sphincter EMG recorded simultaneously with bladder reflex activity was also reduced in a dose-dependent fashion by LY274614 (0.1–30 mg kg^{-1} , $n = 5$) with an ED_{50} of 3.5 mg kg^{-1} (Figures 3a and 4b).

Figure 4 Log dose-response curves showing the effects of increasing doses of LY274614 (0.1–30 mg kg^{-1} , i.v.) on the amplitude of micturition contractions (a) and sphincter EMG (b), during continuous filling (0.21 ml min^{-1}) cystometry in the urethane-anaesthetized intact (●) and chronic spinal (▲) rats. Abscissa scale: the cumulative dose of LY274614 (mg kg^{-1} , i.v.) plotted on a log scale. Ordinate scale: contraction amplitude (a) or peak firing of EMG in spikes s^{-1} (b) as a % of control. Mean $\pm$ s.e.mean is plotted for each point. Dose-response curves for amplitude of micturition contractions or sphincter EMG both in the intact ($n = 12$, cystometrograms; $n = 5$, EMG) and chronic spinal ($n = 6$, cystometrograms; $n = 5$, EMG) rats show a significant decrease. Individual doses are compared to control by paired t test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). No significant difference between the pairs of dose-response curves was revealed by two-way repeated measures ANOVA in either (a) or (b).

Figure 5 The effects of increasing doses of LY274614 during single filling (0.04 ml min^{-1}) cystometry in the urethane-anaesthetized intact rat. Doses represent total cumulative doses. Note that the interval from the start of saline infusion to the point where micturition occurs was prolonged by increasing doses of LY274614 (3–30 mg kg^{-1} , i.v.). Amplitude of micturition contractions was also reduced by LY274614. M = micturition. V_T = volume threshold.

Figure 6 (a) The effect of LY274614 on the amplitude of micturition contraction during single filling (0.04 ml min^{-1}) cystometry in the urethane-anaesthetized intact rats ($n = 5$). LY274614 ($0.3\text{--}30 \text{ mg kg}^{-1}$, i.v.) decreases the amplitude in a dose-dependent manner similar to the result during continuous filling (0.21 ml min^{-1}) cystometry (Figure 4). There is no statistical difference at each dose between continuous and single cystometry. (b) LY274614 increases V_T (volume threshold) in a dose-dependent manner. (c) LY274614 decreases V_V (voided volume) in a dose-dependent manner. Mean $\pm$ s.e.mean is indicated for each column. Individual doses are compared to control by paired t test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

In 5 animals, urinary bladder activity was recorded during cystometry performed using a slower rate of transurethral infusion (0.04 ml min^{-1}) which more closely approximated the rate of urine formation (Sillen, 1980). The bladder was emptied at the end of each cystometrogram. The peak amplitudes of micturition contractions ranged from 26–40 mmHg (mean = 33 mmHg) and durations between 25–45 s (mean = 34 s). These parameters were not significantly different from the measurements obtained during continuous cystometry. LY274614 produced a dose-dependent decrease in the amplitude of micturition contractions (Figures 5 and 6a) similar to the effect during continuous cystometry (Figure 4a). Figure 5 shows that with increasing doses of LY274614 the volume of saline (bladder capacity or volume threshold) to produce a micturition contraction was increased 1.5 and 3 times control levels with 3 and 30 mg kg^{-1} of LY274614, respectively. In addition, as the bladder began to fill, small bladder contractions which did not produce a void were seen prior to

Figure 7 The depressant effect of increasing doses of LY274614 ($0.3\text{--}30 \text{ mg kg}^{-1}$, i.v.) on the efficiency of voiding during single filling (0.04 ml min^{-1}) cystometry in the urethane-anaesthetized intact rats ($n = 5$). Voiding efficiency (V_E , %) is defined as $100 V_V (V_T)^{-1}$. Abscissa scale: the dose of LY274614 (mg kg^{-1} , i.v.). Ordinate scale: voiding efficiency (%). Mean $\pm$ s.e.mean is plotted for each column. Individual doses are compared to control by paired t test (* $P < 0.05$, ** $P < 0.01$). C = control.

micturition (Figure 5). Control V_T and V_V were $0.36\text{--}0.60 \text{ ml}$ (mean = 0.41 ml) and $0.13\text{--}0.53 \text{ ml}$ (mean = 0.31 ml), respectively, with a V_E of 74%. Increasing doses of LY274614 increased V_T (Figure 6b) and reduced V_V (Figure 6c). V_E was reduced to 11% of control at the maximal dose of LY274614 (Figure 7).

Chronic spinal rats

In 5 chronic spinal animals, bladder activity recorded under constant volume conditions consisted of rhythmic contractions occurring at peak amplitudes of 10–23 mmHg (mean = 16 mmHg), frequencies of $0.71\text{--}1.14 \text{ min}^{-1}$ (mean = 0.96 min^{-1}) and durations of 33–41 s (mean = 37 s). As shown in Figure 1b, LY274614, at doses of 0.3 and 1 mg kg^{-1} in this preparation slightly but significantly increased the amplitude of bladder contractions (Figure 2), while the effects of larger doses were not significantly different from control. Doses of LY274614 ($0.1\text{--}30 \text{ mg kg}^{-1}$ i.v.) had no effect on frequency or duration of bladder contractions.

In 6 chronic spinal animals, micturition contractions recorded during a continuous transurethral infusion (0.21 ml min^{-1}) of saline exhibited peak pressures ranging from 10–42 mmHg (mean = 18 mmHg), durations of 24–46 s (mean = 31 s) and intercontraction intervals of 8–42 s (mean = 18 s). Increasing doses of LY274614 ($0.1\text{--}30 \text{ mg kg}^{-1}$, i.v.) reduced the amplitude of the micturition contractions (Figure 3b). A significant decrease was seen at doses of 10 and 30 mg kg^{-1} of LY274614 (Figure 4a) with an ED_{50} of 18.5 mg kg^{-1} . Durations and intercontraction intervals were not affected by LY274614. Following the 30 mg kg^{-1} dose of LY274614 bladder activity was depressed for the remainder of the experiment (2–8 h). In 5 animals in which sphincter EMG was recorded during cystometry, LY274614 significantly decreased sphincter activity with an ED_{50} of 4.7 mg kg^{-1} (Figures 3b and 4b).

Intact rats: i.t. administration of LY274614

In 13 animals with an intrathecal catheter, voiding reflexes induced by a continuous infusion of saline (0.21 ml min^{-1}) had peak intravesical pressures of 21–127 mmHg (mean = 58 mmHg), intercontraction intervals of 25–317 s (mean = 106 s) and durations of 22–287 s (mean = 92 s). An i.t. administration of LY274614 ($0.06\text{--}30 \mu\text{g}$) produced a

dose-related reduction in micturition contraction amplitude (ED_{50} of $0.11 \mu\text{g}$) (Figures 8 and 9a). In 7 animals (greater than 50% of animals tested) bladder activity was abolished at a dose of $12 \mu\text{g}$ of LY274614. In 9 animals after complete inhibition, the baseline bladder pressure markedly increased (20 mmHg; range: 15–27 mmHg) and saline constantly leaked from the external urethral orifice (overflow incontinence). In 7 animals, bladder activity was measured 5–18 h following complete inhibition with doses ranging from 12–72 μg . During this period, 5 of 7 animals showed a partial recovery of bladder activity (35–141% of beginning control, mean = 71%). In 4 of these animals, a second dose of LY274614 also abolished the bladder activity. The effects of LY274614 on duration and intercontraction interval were quite variable and although a few doses showed a statistically significant decrease, the effects were not dose-dependent. Sphincter EMG recorded simultaneously with bladder reflex activity was also reduced in a dose-dependent fashion by LY274614 (0.06 – $30 \mu\text{g}$, $n = 8$) (Figures 8 and 9b). A statistically significant reduction in the EMG activity was noted at a dose of $0.12 \mu\text{g}$ (64.0% of control) with an ED_{50} of $0.16 \mu\text{g}$.

Time course of LY274614 effects on amplitude of micturition contractions measured during continuous cystometry

The duration of bladder inhibition produced by LY274614 was studied following both i.v. and i.t. administration of the drug. During continuous cystometry, a single dose (30 mg kg^{-1} , i.v., $n = 5$ or $6 \mu\text{g}$ i.t., $n = 5$) of LY274614 was given to each animal and time course of recovery recorded for up to 10 h following drug administration. Figure 10 shows the time course (up to 6 h) of recovery. Some recovery was seen at 2.5 to 3 h following LY274614. In 2 animals following i.v. and in 3 animals following i.t. administration, the amplitude of the micturition contractions had not recovered to control levels for up to 10 h following administration.

Influence of bladder irritation with acetic acid (0.1%) on the effects of LY274614 (i.v.)

Since drugs that suppress the micturition reflex might be used clinically to diminish bladder hyperactivity induced by neurological disorders, a series of experiments was conducted to examine the effect of LY274614 on bladder hyperactivity induced by chemical irritation. In 12 animals, urinary bladder activity was recorded during a continuous transurethral infusion (0.21 ml min^{-1}). Control micturition contractions induced by saline infusion occurred at peak amplitudes of 21–78 mmHg, intercontraction intervals of 35–271 s and durations of 27–86 s (Table 1). Following a control period of 30 min with saline infusion, the infusion solution was swit-

Figure 9 Log dose-response curves showing the effects of increasing doses of LY274614 (i.t.) on the amplitude of micturition contractions (a) and sphincter EMG (b) during continuous filling (0.21 ml min^{-1}) cystometry in the urethane-anaesthetized intact rats ($n = 13$, cystometrogams; $n = 6$, sphincter EMG). Abscissa scale: the cumulative dose of LY274614 (μg) plotted on a log scale. Ordinate scale: contraction of micturition amplitude (a) or peak firing of EMG in spikes s^{-1} (b) as a % control. Mean $\pm$ s.e.mean is plotted for each point. Individual doses are compared to control by paired t test (α : $P < 0.05$, β : $P < 0.01$, γ : $P < 0.001$).

ched to 0.1% acetic acid, which had little effect on the amplitude (32–69 mmHg) or duration (25–56 s) of micturition contractions but significantly decreased intercontraction interval (9–83 s) (Table 1). In these animals the amplitude of micturition contractions ($n = 12$) and sphincter EMG activity ($n = 5$) were reduced following the administration of increasing doses of LY274614 (0.1 – 30 mg kg^{-1} i.v.; Figure 11). The ED_{50} s for these effects were 24.3 mg kg^{-1} and 16.6 mg kg^{-1} , respectively. Although reduction in amplitude of micturition contractions seemed to require a larger dose of LY274614 in the acetic acid-infused bladder (Figure 11a), this effect was

Figure 8 The effects of increasing doses of LY274614 administered by i.t. injection on continuous filling (0.21 ml min^{-1}) cystometry and sphincter EMG in the urethane-anaesthetized intact rat. Doses represent total cumulative doses. Drug injections were made subdurally at the L_6 – S_1 level of spinal cord. Note the decrease in the amplitude of the micturition contractions and the sphincter EMG activity following LY274614 (0.12 – $18 \mu\text{g}$). EUS = external urethral sphincter.

Table 1 The effects of acetic acid (0.1%) continuous filling infusion (0.21 ml min⁻¹) to the bladder

Continuous filling cystometrogram	Amplitude (mmHg)	Intercontraction interval* (s)	Duration (s)
Saline	53 ± 5	95 ± 20	41 ± 6
Acetic acid	52 ± 3	27 ± 6	40 ± 3

*Significant difference between 'saline' and 'acetic acid' ($P < 0.01$, paired t test). Only the intercontraction interval was changed by acetic acid continuous infusion to the bladder but not the amplitude and duration of micturition contractions. All values are expressed as mean $\pm$ s.e.mean ($n = 12$).

not statistically significant (two-way repeated measures ANOVA) from saline infusion. Reduction in sphincter EMG did, however, require a larger dose of LY274614 in the acetic acid infused bladder as compared to saline-infused preparations (Figure 11b). A significant reduction in sphincter EMG was only detected at a dose of 30 mg kg⁻¹ (23.2% of control); and a significant difference from the control experiment with saline infusion occurred at 10 mg kg⁻¹ (two-way repeated measures ANOVA followed by unpaired t test, $P < 0.05$). A significant decrease in duration of micturition contractions was seen at 10 and 30 mg kg⁻¹ for acetic acid-infused bladder.

Effects of LY233536 and LY235723 on continuous filling cystometry

Two other competitive NMDA receptor antagonists were studied on micturition reflexes. LY233536 is related chemically to LY274614, while LY235723 is chemically distinct. Both compounds (1–100 mg kg⁻¹, i.v. of LY233536, $n = 5$ or LY235723, $n = 6$) decreased the amplitude of micturition contractions in a dose-dependent manner when studied with continuous cystometry. Micturition contractions were completely inhibited by LY233536 or LY235723 at 100 mg kg⁻¹ with ED₅₀s of 18 or 20 mg kg⁻¹, respectively. A significant decrease in amplitude occurred at doses of 10 mg kg⁻¹ and larger of LY235723, and 30 mg kg⁻¹ and larger of LY233536. These drugs did not change durations or intercontraction intervals with the exception of an increase of these intervals by LY235723 at doses of 1 mg kg⁻¹ ($P < 0.05$, paired t test) and 3 mg kg⁻¹ ($P < 0.01$, paired t test).

Discussion

The results of the present study indicate that LY274614, LY233536 and LY235723, competitive NMDA receptor antagonists that enter the central nervous system (CNS) following systemic administration, can inhibit bladder and external urethral sphincter reflexes in the urethane-anesthetized rat. The site of these inhibitory effects is, at least in part, at the level of the lumbosacral spinal cord and dependent on an intact descending pathway from supraspinal sites. These data support the conclusions that emerged from previous studies with the non-competitive NMDA receptor antagonist, MK-801, that CNS glutamatergic mechanisms involving NMDA receptors are important in modulating bladder and sphincter activities.

Although the block of the micturition reflex produced by MK-801 is at the cationic channel associated with the NMDA receptor complex (Foster & Fagg, 1987) while LY274614, LY233536 and LY235723 block at the recognition site for NMDA, the effects of the drugs are qualitatively similar in most respects. Both LY274614 and MK-801 inhibit bladder and sphincter reflexes when administered either intra-

Figure 10 Graph showing the time course of the effect of LY274614 (30 mg kg⁻¹ i.v., ●, $n = 6$ and 6 μ g i.t., ▲, $n = 6$) on the amplitude of micturition contractions during continuous filling (0.21 ml min⁻¹) cystometry in the urethane-anesthetized intact rats. Abscissa scale: time scale (h) since LY274614 was administered. Ordinate scale: contraction amplitude as a % of control. Mean $\pm$ s.e.mean is plotted for each point. Individual points are compared to control by paired t test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). A comparison between the i.v. and i.t. administration at each period, showed no significant difference up to 6 h. Note that some recovery from LY274614 was seen 2.5 to 3 h after injection but complete recovery was not seen in all animals even 6 h after LY274614.

Figure 11 Log dose-response curves showing the effects of increasing doses of LY274614 (i.v.) on the amplitude of micturition contraction (a) and sphincter EMG (b) during continuous filling (0.21 ml min⁻¹) cystometry with either saline (●) or 0.1% acetic acid (○) in intact rats. Abscissa scale: the cumulative dose of LY274614 (mg kg⁻¹, i.v.) plotted on a log scale. Ordinate scale: contraction amplitude (a) or ratemeter output of EMG in spikes s⁻¹ as a % control (b). Mean $\pm$ s.e.mean is plotted for each point. Dose-response curves both with saline ($n = 12$, cystometrograms; $n = 5$, sphincter EMG) and acetic acid ($n = 12$, cystometrograms; $n = 5$, sphincter EMG) infusion show a statistically significant decrease in amplitude and sphincter EMG peak firing. Individual doses are compared to control by paired t test (* $P < 0.01$, ** $P < 0.001$). There is no significant difference between pairs of dose-response curves with saline or acetic acid infusion in (a) although a significant difference is seen in (b) at a dose of 10 mg kg⁻¹ (two-way repeated measured ANOVA followed by unpaired t test, $P < 0.05$).

venously (i.v.) or intrathecally (i.t.). MK-801 is however more potent (ED₅₀ = 0.36 mg kg⁻¹) than LY274614 (ED₅₀ = 12.2 mg kg⁻¹) on i.v. administration but LY274614 is more potent on i.t. administration (ED₅₀ of MK-801 = 17 μ g; of LY274614 = 0.11 μ g) (Yoshiyama *et al.*, 1993). LY274614 has a slightly

longer time for onset than MK-801 (4–6 min compared to 1–2 min). These differences are consistent with the poorer penetration of LY274614 across the blood-brain barrier and the greater affinity of LY274614 for its binding to the NMDA receptor complex (personal communications with Drs Ornstein & Schoepp), in comparison to MK-801. Similar differences in effective doses of MK-801 and LY274614 were seen by other investigators examining the effects of these NMDA antagonists on pain pathways (Elliott *et al.*, 1991) and on neurotoxic effects of excitatory amino acids and amphetamine (Fuller *et al.*, 1992).

One other qualitative difference between these two drugs was the elevated bladder tone following complete inhibition of bladder contractions with LY274614, seen when bladder reflexes were studied during continuous cystometry. The baseline bladder pressure was high (average 20 mmHg) with LY274614 (i.v. and i.t.), but this was rarely seen with MK-801. Overflow incontinence occurred with both drugs following inhibition of bladder contractions but only with LY274614 was an elevated resting pressure observed. The magnitude of the pressure recorded in the bladder during continuous infusion cystometry depends on two factors, the contractile force of the bladder and the pressure at which the striated and smooth muscle sphincters relax (outlet resistance). Since both LY274614 and MK-801 inhibit micturition contractions, the increase in baseline pressure seen following LY274614 is probably due to some residual urethral resistance reducing flow through the urethra. The exact mechanism of this high resting tone is not known but may represent some differential action of the two drugs on either urethral smooth or striated muscle sphincter activity. Although the mechanism of this elevated bladder pressure was not investigated in the present study one might speculate from *in vitro* data, which shows that MK-801 blocks nicotinic channels in cultured striated muscle cells (Amador & Dani, 1991), that MK-801 may produce a more effective relaxation of external urethral sphincter than LY274614. An alternative explanation would be that MK-801 is more effective in producing relaxation of the smooth muscle of the urethra.

The site of action of LY274614 within the CNS appears to be similar to that proposed for MK-801 (Yoshiyama *et al.*, 1993). Since an i.t. administration of LY274614 or MK-801 at the level of L₆–S₁ spinal cord inhibited bladder and sphincter activity, a spinal site of action is likely. However, the absence of an effect of LY274614 or MK-801 (Yoshiyama *et al.*, 1993) on bladder contractions in chronic spinal animals studied under isovolumetric conditions would suggest that an intact descending pathway from the pontine micturition centre is necessary for the action of the drugs. However, a decrease in sphincter activity with LY274614 or MK-801 was seen both in animals with an intact spinal cord and in chronic spinal cord transected animals; suggesting that NMDA receptor mechanisms are important for both the spinal and supraspinal control of external urethral sphincter activity. It should be noted, however, that LY274614 did partially reduce the amplitude of the micturition contractions in chronic spinal animals when using continuous cystometry with the urethral outlet open. This effect is probably produced by decreased outlet resistance rather than a decrease in bladder contractility since with continuous cystometry, bladder pressure begins to decrease as soon as the urethral outlet relaxes and voiding begins. Furthermore, LY274614, even in very large doses (> 30 mg kg⁻¹), produced no decrease in reflex bladder contractions in a group of animals studied with closed bladder outlets (ligated) and at constant bladder volume. In these animals, changes in outlet resistance could not modify bladder pressure since a ligature separates the bladder from the urethra. It is, therefore, concluded that reflex bladder contractions are not reduced by LY274614 in chronic spinal animals.

The lack of effect on bladder contractions in chronic spinal animals with both LY274614 and MK-801 would suggest that neural elements in the spinal bladder reflex pathway including bladder afferent neurones, interneurones and preganglionic

efferent neurones do not utilize a glutamatergic NMDA receptor mechanism. On the other hand i.t. administration of very small doses of LY274614 to animals with an intact spinal cord inhibited bladder activity, indicating that the descending limb of the spinobulbospinal micturition reflex pathway (de Groat *et al.*, 1992) may utilize glutamate as a transmitter and must be intact for LY274614 to be effective. However, since the drugs do depress sphincter activity in chronic spinal animals, NMDA receptor-related glutamatergic mechanisms must be involved in the spinal reflex pathway controlling sphincter function. In intact animals, the sphincter activity is suppressed at a lower dose (ED₅₀ = 3.5 mg kg⁻¹) than bladder activity (ED₅₀ = 12.2 mg kg⁻¹), which suggests that the drug affects bladder and sphincter by different mechanisms. NMDA receptors in the spinal cord are prominent in the vicinity of the motoneurones which control sphincter, and around the central canal and in the intermediolateral gray (Jansen *et al.*, 1990; Shaw *et al.*, 1991). The latter regions of the cord contain bladder preganglionic neurones or interneurones which are involved in lower urinary tract function (de Groat *et al.*, 1992), and thus are likely to be one site of action of the NMDA antagonists.

Although the present study suggests a spinal site of action for LY274614, an effect on supraspinal pathways is also possible. A supraspinal site of action is supported by receptor binding studies which show that NMDA receptors are present in the brainstem near the pontine micturition centre (Monaghan & Cotman, 1985). Further support for a supraspinal site of action is provided by studies from this and other laboratories which examined the effects of neuromodulators at the level of the pontine micturition centre (Lumb & Morrison, 1987; Willette *et al.*, 1988; Mallory *et al.*, 1991). In these studies glutamate was an effective agent in modulating micturition reflexes producing a reduction in V_T when injected directly into the pontine micturition centre. LY274614, much like MK-801 (Yoshiyama *et al.*, 1993), increased the V_T and decreased the V_V when given i.v. and tested using cystometry. The ED₅₀ for LY274614 in these experiments is about 30 times that for MK-801: suggesting that LY274614 is less potent than MK-801.

The duration of action of LY274614 following either i.v. or i.t. was long, requiring approximately 4 h for a 50% recovery. These results are consistent with those reported by other laboratories which have evaluated different pharmacological effects of LY274614 (Schoepp *et al.*, 1991; Fuller *et al.*, 1992). We therefore were able to use, in the majority of our studies, cumulative doses of LY274614 given at 15 min intervals with complete dose-response curves usually requiring less than 90 min. This approach could be used since there was no evidence for rapid tolerance development to repeated i.v. administration of LY274614. Following partial or total recovery from LY274614, a second dose of the drug produced the same magnitude of response as seen with the first dose.

The effects of LY274614 on bladder hyperactivity due to bladder irritation was also examined in the present study. Bladder irritation was produced by substituting 0.1% acetic acid for normal saline during a continuous cystometry. In the irritated bladder model, LY274614 reduced the amplitude of micturition contractions and sphincter EMG activity with the dose-response curve for sphincter EMG showing a small shift to the right. The typical effect of LY274614 in reducing bladder and sphincter activity occurred at a slightly higher dose for sphincter EMG activity when the bladder was irritated with acetic acid. The major effect produced by acetic acid was to decrease the intercontraction interval (increase in frequency), and this effect of irritation was not reduced by LY274614. This latter result would be in agreement with our conclusions that neither LY274614 nor MK-801 (Yoshiyama *et al.*, 1993) have any effect on the afferent limb of the micturition reflex or on nociceptive afferents that modulate the reflex. These findings are also consistent with the effects of MK-801 on increased expression of the immediate early gene, *c-fos*, in spinal neurones induced by stimulation of bladder afferents. MK-801

in doses that blocked the micturition reflex did not alter *c-fos* expression elicited by non-noxious distension of the bladder of the rat (Birder & de Groat, 1993). Large doses of MK-801 did reduce by 50% the increased *c-fos* expression induced by chemical irritation of the bladder (Birder & de Groat, 1992). However, this effect was remarkably reduced in spinal transected animals, suggesting that the effect of MK-801 was dependent upon the integrity of supraspinal pathways and was not mediated by direct actions on spinal nociceptive mechanisms.

Although the pharmacology and possible sites of action of LY274614 were the primary focus of this study, two additional competitive NMDA receptor antagonists were examined for their effects on micturition reflexes. LY233536 is chemically similar to LY274614 except that the phosphonic acid moiety is replaced with a tetrazole group, while LY235723 is the (–)-isomer of LY233053, a 4-tetrazolylalkyl substituted piperidine-2-carboxylic acids with competitive NMDA antagonist activity (Schoepp *et al.*, 1990; Ornstein *et al.*, 1991; 1992a,b; Zimmerman *et al.*, 1992). Both LY233536 and LY235723 produced effects, qualitatively similar to those of LY274614 and MK-801, but were less potent in reducing micturition bladder contractions. The reduced potency of these compounds is in agreement with lower NMDA receptor affinity *in vitro* and lower *in vivo* and *in vitro* antagonist activity reported by others (Ornstein *et al.*, 1991). The fact that a variety of compounds which possess a high affinity for NMDA receptors *in vitro*

produce profound effects on micturition reflexes, provides a strong support for the idea that glutamatergic mechanisms are important in modulating reflex micturition.

In summary, these experiments suggest that glutamic acid is an important neurotransmitter in the micturition reflex pathway of the rat. This substance acts via NMDA receptors in the lumbosacral spinal cord when descending pathways from the brainstem to the cord are intact. On the other hand, in chronic paraplegic rats when the descending pathways are eliminated, NMDA receptor mediated glutamatergic transmission is not essential for the generation of spinal micturition reflexes. The inhibitory effects of LY274614 on external urethral sphincter, however, still occurred after chronic spinalization; indicating that glutamate is a transmitter in the spinal reflex pathways controlling the urethral sphincter. LY274614 is a long acting NMDA antagonist and may be potentially useful in the treatment of neurogenic bladder hyperactivity. It has the advantage over MK-801 of having fewer phencyclidine-like side effects (Rasmussen, 1991; Rasmussen *et al.*, 1991).

This work was supported by a research grant from Eli Lilly and Company and NIH Grants DK37241 (W.D.), DK42369 (W.D.) and NS21137 (J.R.R.). We would like to acknowledge Dr P.L. Ornstein and Dr D.D. Schoepp of the CNS Research Division of Lilly Research Laboratories (Eli Lilly and Company) for their helpful discussions throughout this study and during preparation of this manuscript.

References

- AMADOR, M. & DANI, J.A. (1991). MK-801 inhibition of nicotinic acetylcholine receptor channels. *Synapse*, **7**, 207–215.
- BIRDER, L.A. & DE GROAT, W.C. (1992). The effect of glutamate antagonists on *c-fos* expression induced in spinal neurons by irritation of the lower urinary tract. *Brain Res.*, **580**, 115–120.
- BIRDER, L.A. & DE GROAT, W.C. (1993). Induction of *c-fos* gene expression in spinal neurons of the rat by nociceptive and non-nociceptive stimulation of the lower urinary tract. *Am. J. Physiol.*, (in press).
- DE GROAT, W.C., BOOTH, A.M. & YOSHIMURA, N. (1993). Neurophysiology of micturition and its modification in animal models of human disease. Chapter 8. In *The Autonomic Nervous System*, Vol. 3: *Nervous Control of the Urogenital System*. ed. Maggi, C.A. pp. 227–290. London: Harwood Academic Publishers.
- ELLIOTT, K.J., CERBONE, D.J., FOLEY, K.M. & INTURRISI, C.E. (1991). NMDA receptor antagonists are antinociceptive in the mouse formalin model during acute and chronic administration. *Soc. Neurosci. Abstr.*, **17**, 588.
- FELDBERG, W. & FLEISCHHAUER, K. (1990). Penetration of bromophenol blue from the perfused cerebral ventricles into the brain tissue. *J. Physiol.*, **150**, 451–462.
- FOSTER, A.C. & FAGG, G.E. (1987). Taking apart NMDA receptors. *Nature*, **329**, 395–396.
- FULLER, R.W., HEMRICK-LUECKE, S.K. & ORNSTEIN, P.L. (1992). Protection against amphetamine-induced neurotoxicity toward striatal dopamine neurons in rodents by LY274614, an excitatory amino acid antagonist. *Neuropharmacol.*, **31**, 1027–1032.
- JANSEN, K.L.R., FAULL, R.L.M., DRAGUNOW, M. & WALDVOGEL, H. (1990). Autoradiographic localization of NMDA, quisqualate kainic acid receptors in human spinal cord. *Neurosci. Lett.*, **108**, 53–57.
- KRUSE, M.N., BELTON, A.L. & DE GROAT, W.C. (1993). Changes in bladder and external urethral sphincter function following spinal cord injury in the rat. *Am. J. Physiol.*, (in press).
- KRUSE, M.N., NOTO, H., ROPPOLO, J.R. & DE GROAT, W.C. (1990). Pontine control of the urinary bladder and external urethral sphincter in the rat. *Brain Res.*, **532**, 182–190.
- LUMB, B.M. & MORRISON, J.F.B. (1987). An excitatory influence of dorsolateral pontine structures on urinary bladder motility in the rat. *Brain Res.*, **435**, 363–366.
- MAGGI, C.A., GIULIANI, S., GIACHETTI, A. & MELI, A. (1990). The effect of MK-801 on the micturition reflex in anesthetized rats. *Eur. J. Pharmacol.*, **181**, 105–109.
- MAGGI, C.A., SANTICIOLI, P. & MELI, A. (1986). The nonstop trans-vesical cystometrogram in urethane-anesthetized rats. *J. Pharmacol. Methods*, **15**, 157–167.
- MALLORY, B.S., ROPPOLO, J.R. & DE GROAT, W.C. (1991). Pharmacological modulation of the pontine micturition center. *Brain Res.*, **546**, 310–320.
- MALLORY, B., STEERS, W.D. & DE GROAT, W.C. (1989). Electrophysiological study of micturition reflexes in rats. *Am. J. Physiol.*, **257**, R410–R421.
- MERLIS, J.K. (1940). The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. *Am. J. Physiol.*, **131**, 67–72.
- MONAGHAN, D.T. & COTMAN, C.W. (1985). Distribution of *N*-methyl-D-aspartate-sensitive L-[³H]glutamate-binding sites in rat brain. *J. Neurosci.*, **5**, 2909–2919.
- NISHIZAWA, O., FUKUDA, T., MATSUZAKI, A., MORIYA, I., HARADA, T. & TSUCHIDA, S. (1985). Role of the sympathetic nerve in bladder and urethral sphincter function during the micturition cycle in the dog evaluated by pressure flow EMG study. *J. Urol.*, **134**, 1259–1261.
- ORNSTEIN, P.L., ARNOLD, M.B., AUGENSTEIN, N.K., LEANDER, J.D., LODGE, D. & SCHOEPP, D.D. (1991). Characterization of (–)-LY235959, (–)-LY202157 and (–)-LY235723 as the active isomers of the racemic competitive NMDA antagonists LY274614, LY233536 and LY233053, respectively. *Soc. Neurosci. Abstr.*, **17**, 392.
- ORNSTEIN, P.L., SCHOEPP, D.D., ARNOLD, M.B., AUGENSTEIN, N.K., LODGE, D., MILLAR, J.D., CHAMBERS, J., CAMPBELL, J., PASCHAL, J.W., ZIMMERMAN, D.M. & LEANDER, J.D. (1992a). 6-Substituted decahydroisoquinoline-3-carboxylic acids as potent and selective conformationally constrained NMDA receptor antagonists. *J. Med. Chem.*, **35**, 3547–3560.
- ORNSTEIN, P.L., SCHOEPP, D.D., ARNOLD, M.B., JONES, N.D., DEETER, J.B., LODGE, D. & LEANDER, J.D. (1992b). NMDA antagonist activity of (±)-(2SR,4RS)-4-(1H-tetrazol-5-ylmethyl) piperidine-2-carboxylic acid residues with the (–)-2R,4S-isomers. *J. Med. Chem.*, **35**, 3111–3115.
- RASMUSSEN, K. (1991). Afferent effects on locus coeruleus in opiate withdrawal. *Prog. Brain Res.*, **88**, 207–216.
- RASMUSSEN, K., FULLER, R.W., STOCKTON, M.E., PERRY, K.W., SWINFOR, R.M. & ORNSTEIN, P.L. (1991). NMDA receptor antagonists suppress behaviors but not norepinephrine turnover or locus coeruleus unit activity induced by opiate withdrawal. *Eur. J. Pharmacol.*, **197**, 9–16.

- SCHOEPP, D.D., ORNSTEIN, P.L., LEANDER, J.D., LODGE, D., SALHOFF, C.R., ZEMAN, S. & ZIMMERMAN, D.M. (1990). Pharmacological characterization of LY233053: a structurally novel tetrazole-substituted competitive N-methyl-D-aspartic acid antagonist with a short duration of action. *J. Pharmacol. Exp. Ther.*, **255**, 1301–1308.
- SCHOEPP, D.D., ORNSTEIN, P.L., SALHOFF, C.R. & LEANDER, J.D. (1991). Neuroprotectant effects of LY274614, a structurally novel systemically active competitive NMDA receptor antagonist. *J. Neural. Transm. (Gen. Sect.)*, **85**, 131–143.
- SHAW, P.J., INCE, P.G., JOHNSON, M., PERRY, E.K. & CANDY, J. (1991). The quantitative autoradiographic distribution of [³H]MK-801 binding sites in the normal human spinal cord. *Brain Res.*, **539**, 164–168.
- SILLEN, U. (1980). Central neurotransmitter mechanisms involved in the control of the urinary bladder function. *Scand. J. Urol. Nephrol. Suppl.*, **58**, 1–45.
- SUZUKI, T., BIRDER, L.A., YOSHIYAMA, M., ROPPOLO, J.R. & DE GROAT, W.C. (1991). Electrophysiological studies of the effects of glutamate antagonists (MK801 and CNQX) on the micturition reflex in the rat. *Soc. Neurosci. Abstr.*, **17**, 1002.
- WILLETTE, R.N., MORRISON, S., SAPRU, H.N. & REISS, D.J. (1988). Stimulation of opiate receptors in the dorsal pontine tegmentum inhibits reflex contraction of the urinary bladder. *J. Pharmacol. Exp. Ther.*, **244**, 403–409.
- WONG, E.H.F., KEMP, J.A., PRIESTLEY, T., KNIGHT, A.R., WOODRUFF, G.N. & IVERSEN, L.L. (1986). The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 7104–7108.
- YAKSH, T.L. & RUDY, T.A. (1976). Chronic catheterization of the spinal subarachnoid space. *Physiol. Behav.*, **17**, 1031–1036.
- YOSHIYAMA, M., ROPPOLO, J.R. & DE GROAT, W.C. (1991a). The effects of glutamate receptor antagonists on the micturition reflex in the rat. *Soc. Neurosci. Abstr.*, **17**, 1001.
- YOSHIYAMA, M., ROPPOLO, J.R. & DE GROAT, W.C. (1993). Effects of MK-801 on the micturition reflex in the rat – possible sites of action. *J. Pharmacol. Exp. Ther.*, **265**, 844–850.
- YOSHIYAMA, M., ROPPOLO, J.R., RIHMLAND, J., BLASTOS, B. & DE GROAT, W.C. (1991b). The effects of MK-801, an NMDA receptor antagonist, on the micturition reflex in the rat. *Neurosci. Lett.*, **126**, 141–144.
- YOSHIYAMA, M., ROPPOLO, J.R., THOR, K.B. & DE GROAT, W.C. (1992). The effects of LY-274614, a competitive NMDA receptor antagonist, on the micturition reflex in the rat. *Soc. Neurosci. Abstr.*, **18**, 126.
- ZIMMERMAN, D.M., SCHOEPP, D.D., LEANDER, J.D. & ORNSTEIN, P.L. (1992). The discovery and characterization of the competitive NMDA antagonists LY274614, LY233536 and LY233053. *Mol. Neuropharmacol.*, **2**, 77–81.

(Received February 12, 1993

Revised April 4, 1993

Accepted April 20, 1993)