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Short communication

Interactions between NMDA and AMPA/kainate receptors in the control of micturition in the rat

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Abstract

In unanesthetized decerebrate rats, GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride), an AMPA/kainate receptor antagonist, and MK-801 (dizocilpine), an NMDA receptor antagonist, acted synergistically to depress the micturition reflex. MK-801 (1 mg/kg i.v.) and GYKI 52466 (4 mg/kg i.v.) administered separately had no or only a small depressant effect on reflex bladder contractions but markedly depressed external urethral sphincter activity. However, in MK-801-treated rats, GYKI 52466 decreased the amplitude, frequency and duration of reflex bladder contractions. These results suggest that both AMPA/kainate and NMDA glutamate receptors are important in the micturition reflex pathway and that these receptors may be activated in parallel at some site in the pathway so that excitatory transmission via only one receptor type is sufficient to mediate reflex activation of the bladder.

Keywords: GYKI 52466; MK-801; Cystometrogram; Urethral sphincter, external; Bladder reflex

1. Introduction

Previous pharmacological studies indicate that glutamatergic transmission has an important role in the reflex control of micturition in rats (Maggi et al., 1990; Yoshiyama et al., 1991, 1993a, b, 1994, 1995). However, the function of glutamate seems to vary under different experimental conditions. For example, in urethane-anesthetized rats, i.v. administration of a non-competitive (MK-801 (dizocilpine)) (Maggi et al., 1990; Yoshiyama et al., 1991, 1993a, 1994) or a competitive (LY274614) (Yoshiyama et al., 1993b) NMDA receptor antagonist as well as the administration of a non-competitive AMPA/kainate receptor antagonist (GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride)) (Yoshiyama et al., 1995) suppressed reflex bladder and external urethral sphincter activity induced by bladder distention. However, in unanesthetized decerebrate rats (Yoshiyama et al., 1994) or awake freely moving rats (Vera and Nadelhaft, 1991), the NMDA receptor antagonist (MK-801) had no effect or a slight facilitatory effect on bladder activity, but still suppressed external urethral sphincter activity. In unanesthetized decerebrate rats, GYKI 52466 also had only weak depressant effects on bladder reflexes but prominent effects on external urethral sphincter reflexes (Yoshiyama et al., 1995). These results indicate that urethane anesthesia facilitates or unmasks the depressant effects of glutamate receptor antagonists on the bladder reflex. This effect could be mediated by a direct depressant action of urethane on glutamate receptors (Daló and Larson, 1990; Gibbs et al., 1993). Since i.t. administration of glutamate antagonists suppresses bladder and sphincter reflexes in urethane-anesthetized rats, it seems likely that the interaction between urethane and glutamatergic transmission occurs at least in part at the level of the spinal cord.

To explain the prominent influence of urethane on the glutamatergic control of voiding function, we have speculated that in the spinal pathways controlling bladder activity, NMDA and AMPA/kainate receptors are activated in parallel during micturition and that transmission by either receptor is sufficient to initiate bladder contractions. Thus, pharmacological blockade of one receptor type does not produce a significant effect.

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On the other hand, after partial blockade of glutamate receptors with urethane, further blockade with either AMPA/kainate or NMDA receptor antagonists produces a prominent block of the micturition reflex.

In the present study, we have tested this hypothesis by examining the interaction between AMPA/kainate and NMDA receptor antagonists in unanesthetized decerebrate rats. The results indicate that doses of the antagonists which alone have weak depressant effects on reflex bladder activity, have prominent effects when administered in combination, supporting the view that excitatory transmission via both types of receptors is necessary during normal micturition.

2. Materials and methods

2.1. Animal preparation

Female Sprague-Dawley rats weighing 190–270 g (mean = 230 g, $n = 20$) were decerebrated under halothane anesthesia and then studied in the absence of anesthesia. Precollicular decerebrations were performed by ligating both carotid arteries and then removing the rostral part of the brain with a blunt spatula. Halothane was then discontinued. Cotton and Avitene (MedChem Products, Woburn, MA, USA) were placed in the intracranial cavity and covered with agar. Experiments were started 1 h after the decerebration. The trachea was cannulated with a polyethylene tube (PE-240) to facilitate respiration. Artificial respiration was used in some animals. A cannula (PE-50) was placed in the external jugular vein for i.v. drug administration in all animals, and in right carotid artery, in some animals, to monitor blood pressure.

A transurethral bladder catheter (PE-50 tubing) connected to a pressure transducer was used to record the bladder pressure during cystometry when the bladder was filled with a constant infusion of physiological saline (0.21 ml/min) and allowed to empty around the catheter. Continuous cystometrograms elicited repetitive voidings, which allowed rapid collection of data for a large number of voiding cycles.

In all experiments, fine (50 μm) wire EMG electrodes were placed percutaneously in the external urethral sphincter 5-10 mm lateral to the urethra, to record the electrical activity of the striated muscle. The EMG activity was amplified, passed through a rateme-ter and displayed on a chart recorder. The peak firing rate during each micturition contraction was measured in pulses/s.

2.2. Statistical analysis

All values are expressed as mean ± S.E.M. Repeated measures analysis of variance (ANOVA), Dunnett's test and Student's t-test were used when appropriate for statistical data analysis. For all statistical tests, $P < 0.05$ was considered significant.

2.3. Drugs

Drugs used include: halothane (Ayerst Lab., Philadelphia, PA, USA), sodium nitroprusside (Abbott Labs., North Chicago, IL, USA), MK-801 (dizocilpine, Merck, Sharp & Dohme Res. Labs., West Point, PA, USA) and GYKI 52466-HCl (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine hydrochloride, Institute for Drug Research, Budapest, Hungary). Sodium nitroprusside was diluted in 5% dextrose in water (pH 4.5). MK-801 was dissolved in physiological saline (pH 5.5). GYKI 52466-HCl was dissolved in sterile water (final pH 2.8). Drug doses were calculated for the base of each compound.

3. Results

3.1. Effect of MK-801 or vehicle (before GYKI 52466)

Four parameters of lower urinary tract activity were measured during continuous infusion (0.21 ml/min) cystometrograms. Control measurements before drugs were: (1) intravesical pressure during voiding (bladder contraction amplitude), 45 ± 5 cm H_2O, (2) contraction duration, 34 ± 2 s, (3) intercontraction interval, 92 ± 6 s, (4) external urethral sphincter EMG activity, 343 ± 23 pulses/s. There was no statistical difference in any parameters in different groups of animals prior to MK-801 or vehicle treatments. A 1 mg/kg i.v. dose of MK-801 was used based on previous experiments showing that this dose did not alter the amplitude of reflex bladder contraction in unanesthetized decerebrate rats although it did produce a significant depression (80%) of bladder contractions in urethane-anesthetized rats (Yoshiyama et al., 1994). In the present experiments, neither vehicle nor MK-801 altered the contraction amplitude or duration; however, MK-801 transiently depressed the peak amplitude of external urethral sphincter EMG activity by 28% and increased the intercontraction interval by 30%. Prior to GYKI 52466 administration, the external urethral sphincter EMG activity returned to control but the intercontraction interval was still increased.

3.2. Effects of GYKI 52466 after administration of vehicle

GYKI 52466 was tested, 90 min after the administration of MK-801 or the equivalent volume of vehicle (1 ml/kg of saline), in a single dose (4 mg/kg i.v.) that we showed in previous experiments to produce a signif-

icant depression (30%) of bladder contraction amplitude in urethane-anesthetized rats, but to have a minimal effect in unanesthetized decerebrate rats (Yoshiyama et al., 1995).

In rats pretreated with saline ($n = 8$), GYKI 52466 (4 mg/kg i.v.) elicited a rapid onset inhibition (75%) of sphincter activity, which persisted for 15 min (Figs. 1A and 2B) but produced only a small but significant suppression (25%) of bladder contraction amplitude (Figs. 1A and 2A). GYKI 52466 did not alter the duration of bladder contractions and intercontraction interval.

In 3 rats after complete recovery of bladder contraction amplitude and sphincter EMG activity, a second dose of GYKI 52466 (4 mg/kg i.v.) was given 75–120 min after the first dose of the drug, to test for reproducibility of the effect. The second dose produced a similar effect on bladder and sphincter activity.

3.3. Effects of GYKI 52466 after administration of MK-801

In rats pretreated for 90 min with MK-801 (1 mg/kg, $n = 8$), a single dose of GYKI 52466 (4 mg/kg i.v.) elicited a rapid onset inhibition (98%) of sphincter activity, which persisted for 20 min (Figs. 1B and 2B) and produced a marked suppression (80%) of bladder contraction amplitude (Fig. 1B and Fig. 2A). Furthermore, GYKI 52466 significantly decreased the duration of bladder contractions and intercontraction interval.

In 2 experiments, a second dose of GYKI 52466 (4 mg/kg i.v.) administered 75 min after the first dose of the drug (when there was complete recovery from the effect of the first dose) produced similar depressant effects on both bladder contraction amplitude and sphincter EMG activity.

3.4. Influence of combined effects of GYKI 52466 and MK-801 on respiration and blood pressure

In all animals treated with MK-801 (1 mg/kg i.v.), strong respiratory inhibition lasting for 1.5–2 min was seen immediately after the administration of GYKI 52466 (4 mg/kg i.v.). To determine if the suppression of bladder activity by the combination of MK-801 and GYKI 52466 was indirect due to respiratory or cardiovascular effects, respiration was maintained artificially and blood pressure was monitored in 2 rats. In artificially respired animals, GYKI 52466 injected after MK-801 decreased mean arterial blood pressure by 38 ± 8 mm Hg and elicited a suppression of bladder and sphincter activity similar to that elicited in normally respiring animals. To determine whether a decrease of blood pressure could cause the depression of bladder and sphincter activity, sodium nitroprusside was infused intravenously (10 μg/kg/min for 10 min) in 2 rats, to mimic the decrease of blood pressure induced

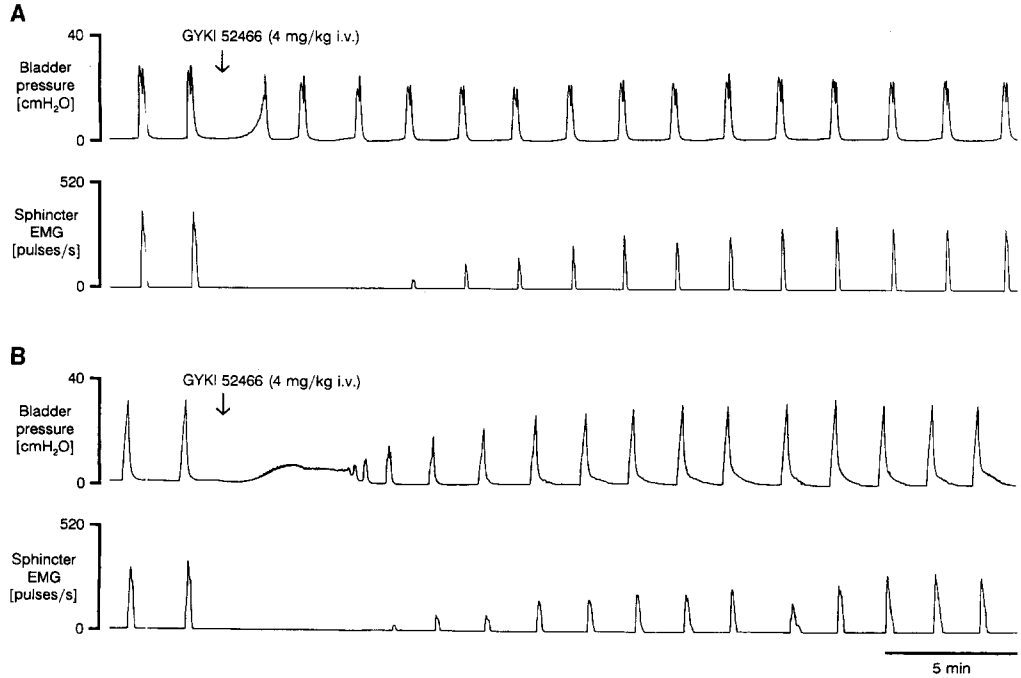

Fig. 1. The effects of GYKI 52466 (4 mg/kg i.v.) administered 90 min after either vehicle (A) or MK-801 (1 mg/kg i.v.) (B) on the reflex bladder contractions and EMG activity of the external urethral sphincter muscle during a continuous filling (0.21 ml/min) cystometrogram in unanesthetized decerebrate rat. Note that GYKI 52466 markedly reduced the amplitude of micturition contractions in the rats treated with MK-801 but had little effect in rats treated with vehicle. GYKI 52466 markedly depressed sphincter EMG activity in both animals.

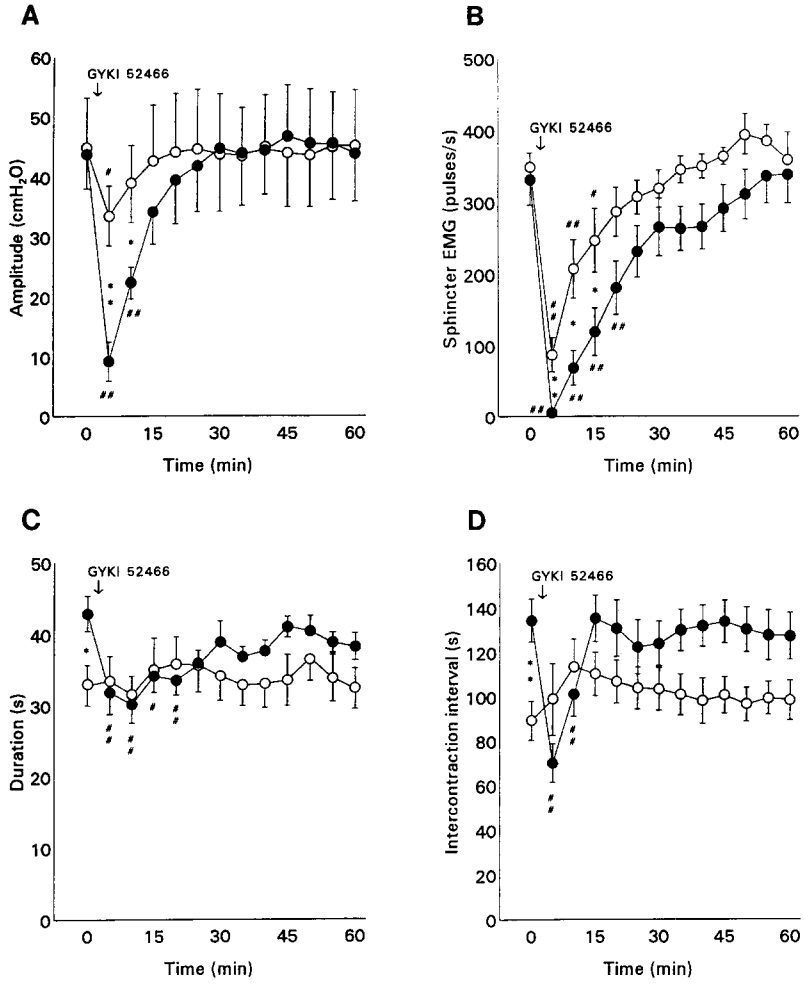

Fig. 2. Graphs showing the time course of the effects of GYKI 52466 (4 mg/kg i.v.) on the reflex bladder contractions and sphincter EMG activity during continuous filling (0.21 ml/min) cystometrograms, in vehicle (○, $n = 8$) or MK-801 (●, $n = 8$) treated rats. Abscissa: time in min, after injection of GYKI 52466. Ordinates: bladder contraction amplitude (mm Hg) (A), sphincter EMG (pulses/s) (B), bladder contraction duration (s) (C) and intercontraction interval (s) (D). Mean ± S.E.M. is plotted for each point. A comparison between both graphs at each period was evaluated by unpaired t-test (* $P < 0.05$, ** $P < 0.01$) following two-way repeated measures ANOVA. The values of each graph at every 5 min were compared to control (before GYKI 52466, at 0 min), by Dunnett's test ($^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$) following repeated measures ANOVA.

by the combination of MK-801 and GYKI 52466. Sodium nitroprusside decreased mean arterial blood pressure by 40 ± 2 mm Hg but had no or little effect on bladder and external urethral sphincter EMG activity.

4. Discussion

The present study revealed that in the unanesthetized decerebrate rat, MK-801, an NMDA receptor antagonist, enhanced the depression of reflex bladder activity elicited by GYKI 52466, an AMPA/kainate receptor antagonist. This finding raises the possibility that AMPA/kainate and NMDA excitatory synaptic mechanisms interact or converge at some site in the central reflex pathways controlling bladder function.

Previous studies revealed that MK-801 inhibited bladder activity in urethane-anesthetized (intact and decerebrate) rats (Maggi et al., 1990; Yoshiyama et al., 1991, 1993a) but had no effect or a slight facilitatory effect in unanesthetized decerebrate (Yoshiyama et al., 1994) and awake freely moving (Vera and Nadelhaft, 1991) rats. Similarly, GYKI 52466 prominently decreased the amplitude of bladder contractions in urethane-anesthetized (intact and decerebrate) rats but had little effect on bladder activity in unanesthetized decerebrate rats (Yoshiyama et al., 1995).

Thus, the unmasking of GYKI 52466 inhibition after pretreatment with MK-801 is similar to the emergence of this inhibitory effect in urethane-anesthetized rats. Because urethane is known to suppress both NMDA and AMPA receptor-mediated responses (Daló and

Larson, 1990; Gibbs et al., 1993), it is possible that urethane and MK-801 produce their effects via a similar mechanism, i.e., a suppression of glutamatergic transmission. Because urethane also unmasks the depressant effect of MK-801 on bladder reflexes (Yoshiyama et al., 1994), administration of either one of the glutamate receptor antagonists in the presence of urethane seems to be equivalent to administering the two antagonists in combination in the absence of urethane. Based on these findings, it seems reasonable to conclude that glutamatergic synaptic transmission is essential for the initiation of reflex bladder activity and that the transmission can be mediated by either NMDA or AMPA/kainate receptors. Under normal conditions, block of one type of receptor produces minimal effects on micturition. However, in the presence of urethane, which is known to suppress the micturition reflex (Maggi and Meli, 1986), and which presumably reduces glutamatergic transmission (Daló and Larson, 1990; Gibbs et al., 1993), block of either receptor significantly reduces reflex bladder activity.

One site at which glutamate antagonists act to suppress bladder reflexes must be in the spinal cord because both NMDA and AMPA/kainate glutamate receptors can mediate excitatory transmission at synapses on parasympathetic preganglionic neurons (Araki and De Groat, 1994). Short duration and long duration excitatory postsynaptic currents in the preganglionic neurons, which were evoked by the stimulation of single interneurons at L_6-S_1 spinal cord level of neonatal rats, were blocked, respectively, by CNQX, an AMPA/kainate receptor antagonist, and by 2-amino-5-phosphonovaleric acid (APV), an NMDA receptor antagonist. In addition, the i.t. administration of NMDA receptor antagonists inhibited bladder activity in urethane-anesthetized rats (Yoshiyama et al., 1993a, b), implying the existence of spinal NMDA receptors. The i.v. administration of MK-801 or GYKI 52466 also blocked bladder contractions elicited by electrical stimulation of the pontine micturition center in urethane-anesthetized rats (Matsumoto et al., 1994). The latter findings indicate that AMPA/kainate and NMDA glutamatergic excitatory mechanisms are involved in the descending limb of the spinobulbospinal micturition reflex.

It is clear that glutamatergic mechanisms in bladder and sphincter pathways are markedly different because bladder activity was maintained even during the marked suppression of sphincter EMG activity produced by either MK-801 or GYKI 52466 in unanesthetized decerebrate rats (Yoshiyama et al., 1994, 1995). Thus, both AMPA/kainate and NMDA transmitter mechanisms are essential for the maintenance of sphincter reflexes, whereas only one type of transmitter mechanism is necessary to initiate the bladder reflex. These data suggest that AMPA/kainate and NMDA receptor mechanisms are linked serially in the reflex pathway to the sphincter; whereas these mechanisms may occur in parallel at the same synapse in the pathway to the bladder. A synergistic interaction between NMDA and AMPA/kainate receptor antagonists has also been noted in other systems in the spinal cord (Honoré et al., 1988) and brain (Löscher et al., 1993), using in vitro as well as in vivo preparations (Foutz et al., 1994).

In conclusion, the present study suggests that glutamic acid has multiple and complex transmitter functions in the reflex pathways controlling the lower urinary tract. Further investigation is necessary to determine the site and mechanism of interaction between NMDA and AMPA/kainate receptors in these pathways.

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