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European Journal of Pharmacology 499 (2004) 257–263

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A role for L-glutamate ionotropic receptors in the development of rat neurogenic pulmonary edema[B]

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Received 11 March 2004; received in revised form 8 July 2004; accepted 23 July 2004

Abstract

The present study was undertaken to evaluate possible roles of L-glutamate ionotropic receptors in neurogenic pulmonary edema. Perfusion of L-glutamate into the fourth ventricles of rats increased nitric oxide (NO) signals in the efflux solution concentration-dependently, significantly reducing both the occurrence and severity of neurogenic pulmonary edema. This effect was completely reversed by prior intracisternal injection of an NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME), or an *N*-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine maleate (MK-801), and partially by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a 2-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA)/kainic acid receptor antagonist. Administration of MK-801 or CNQX alone, without L-glutamate, almost completely prevented neurogenic pulmonary edema development. These results suggest that endogenous L-glutamate may facilitate underlining disease process, whereas L-glutamate exogenously applied into the fourth ventricle may have an inhibitory action via release of NO, through ionotropic receptors.

Keywords: Neurogenic pulmonary edema; Nitric oxide; L-glutamate; Ionotropic receptor; MK-801; CNQX

1. Introduction

L-Glutamate, an excitatory neurotransmitter, plays an important role in the central nervous system (CNS) (Choi and Rothman, 1990; Bliss and Collingridge, 1993; Nakanishi and Masu, 1994). L-Glutamate receptors have been categorized into two distinct groups: ionotropic and metabotropic (Nakanishi, 1992; Hollmann and Heinemann, 1994; Pin and Duvoisin, 1995). The former can be further subdivided into *N*-methyl-D-aspartate (NMDA), 2-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA), and kainic acid receptors, all of which are coupled with cation-specific ion channels, causing neuronal depolarization (Nakanishi and Masu, 1994; Hollmann and Heinemann, 1994).

Recently, immunohistochemical studies have demonstrated that brain nitric oxide synthase (bNOS) and ionotropic glutamate receptors coexist within pre- and post-synaptic sites and in dendritic spines (Aoki et al., 1997), with a possible interaction. Garthwaite et al. (1989) and Donato di Paola et al. (1991) demonstrated that L-glutamate induces nitric oxide (NO) release from nerve terminals through action on the NMDA subtype receptor. In turn, NO stimulates the pre-synaptic terminal to release glutamate, activating positive feedback (Garthwaite, 1991; Vincent, 1994)). In contrast, in the post-synapse, NO inhibits the NMDA receptor-mediated current across the cell membrane through cGMP production, diminishing the intracellular Ca^{2+} concentration of cultured brain neurons (Lipton and Stamler, 1994).

[B] This study was supported, in part, by grants 09470325 and 12670097 from the Japanese Ministry of Education, Science, Sports, Culture and Technology, Japan.

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doi:10.1016/j.ejphar.2004.07.111

We previously demonstrated that NO in the CNS inhibits the development of fibrin-induced pulmonary edema, a model of neurogenic pulmonary edema (Hamdy et al., 2001) with marked pulmonary vascular congestion, protein-rich edema fluid, and intra-alveolar hemorrhage. Recently, unilateral left cervical vagotomy was found to increase the expression of bNOSmRNA in the ipsilateral nucleus tractus solitarii, strongly inhibiting neurogenic pulmonary edema formation (Feng et al., 2002). It is possible that interactions between an excitatory neurotransmitter such as L-glutamate, and an inhibitory agent such as NO may have different impacts on neurogenic pulmonary edema depending on the anatomical location of the excited neurons and the relative amounts of released neurotransmitters. It remains obscure, however, what neurotransmitters mediate neurogenic pulmonary edema development, and how they interact. The present study was undertaken to investigate the role of L-glutamate ionotropic receptors, with respect to interactions with NO.

2. Materials and methods

2.1. Fibrin-induced pulmonary edema model

The protocol for the present animal study complied with the European Community guidelines, and was approved by the Aichi Medical University Ethics Committee. Wistar male rats, weighing 250–350 g, were anesthetized with an intraperitoneal injection of pentobarbital sodium (Abbott Laboratories North Chicago, IL, USA) at a dose of 35 mg/kg. Tracheal tubes were inserted after performing tracheotomy in the middle of cervical region. Catheters were inserted into the right femoral vein and artery for blood sampling and measurement of arterial blood pressure and heart rates (Multipurpose Polygram, W-1100, Nihon Kohden, Tokyo, Japan), respectively.

In all animals, the vagus nerves were severed, with removal of a 5-mm section in the bilateral mid-cervical region. Thereafter, the animals were fixed in a prone position with a stereotaxic instrument and the cisterna magna was accessed at the base of the dorsal side of the cranium using a needle (26 gauge, 10 mm long). To induce neurogenic pulmonary edema, all rats were consecutively treated with intracisternal injections of fibrinogen and thrombin, 0.075 ml each, at concentrations of 100 mg/ml and 200 units/ml, respectively (Ishikawa et al., 1988).

As previously reported, the severity of pulmonary edema was graded 0–3, with increasing severity. When edema fluid appeared in tracheal tubes within 10 min, it was collected in plastic tubes for later analysis and the grade of edema formation was denoted as grade 3. When edema fluid did not appear in the tracheal tubes within 10 min, chest walls were opened. Sometimes edema fluid spontaneously appeared in the tracheal tubes, and this was denoted as grade 2. However, in a few cases, edema fluid only appeared in the tracheal tubes when the lungs were gently compressed, and this was grade 1. When edema fluid did not appear even with such compression, the grade was 0. Finally, in all rats, lungs were dissected out, weighed, and dried at 80 °C overnight. The difference between wet and dry weights, relative to the dried lung weight, was used as the lung/water ratio. Lung/water ratios of grades 2 and 3 were different from those of grades 0 and 1, values being greater and less than 4.6, respectively (Ishikawa et al., 1988).

2.2. Materials and experimental protocol

All experimental drugs were purchased from Sigma, USA. All except 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, an antagonist of AMPA/kainic acid receptors) were dissolved in saline containing 0.02% $CaCl_2$. CNQX was dissolved with 0.1N NaOH/saline buffer and adjusted to pH 7.0. Animals were divided into nine groups of 8 each, and all underwent bilateral vagotomy. Amounts of 0.075 ml of saline or L-glutamate solution (3×10^{-6} M, L-Glu) were injected into the fourth ventricles of control and treatment groups, respectively. Pretreatments were performed with intra-cisternal injection of 0.075 ml 10^{-5} M of N^{ω}-nitro-L-arginine methyl ester (L-NAME, a non-specific antagonist of NOS), 10^{-5} M of N^{ω}-nitro-D-arginine methyl ester (D-NAME), 10^{-4} M of dizocilpine maleate (MK-801, an antagonist of NMDA receptor), or 10^{-5} M of CNQX 10 min before injection of L-glutamate. Thereafter, fibrinogen and thrombin were consecutively administered to cause neurogenic pulmonary edema. Furthermore, to investigate the role of endogenous L-glutamate, MK-801 or CNQX was pretreated into the fourth ventricle, before injecting fibrinogen and thrombin. Rats were thus divided into nine groups: control (saline), L-Glu, L-NAME, L-NAME+L-Glu, D-NAME+L-Glu, MK-801, CNQX, MK-801+L-Glu, and CNQX+L-Glu.

2.3. Measurement of protein concentrations in edema fluid and serum

Edema fluid and blood were collected in heparinized plastic tubes, centrifuged, and then 2% protease inhibitor (Protease Inhibitor Cocktail, Boehringer Mannheim, Germany) was added. The samples were stored at −30 °C until analyses of protein concentration were performed using a Biorad-protein-assay dye (Hercules, CA, USA) and a spectrometer (spectrometer, U-2000, Hitachi, Tokyo, Japan) with sampling of absorbance at 595 nm. The ratio of the protein concentration in edema fluid to that in serum was calculated as an index of vascular permeability.

2.4. Measurement of NO levels in the cerebrospinal fluid of rats perfused with L-glutamate solution into the fourth ventricle

Under deep anesthesia with intra-peritoneal injection of pentobarbital sodium (50 mg/kg, i.p.), rats were placed in a

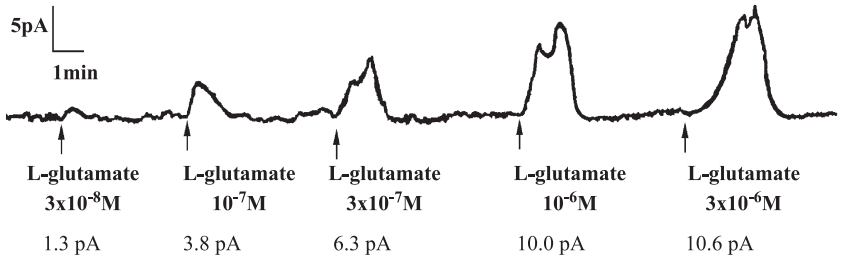

Fig. 1. Nitric oxide (NO) levels in effluent from the fourth ventricles of rats perfused with L-glutamate containing solution. Output current of an NO-selective electrode placed in the effluent from the fourth ventricle was recorded. Arrows indicate starting points of L-glutamate perfusion. After perfusion of artificial cerebrospinal fluid (ACSF) was started, the measured relative NO current was adjusted to 0 picoamperes for each animal. NO current clearly began increasing when L-glutamate reached the fourth ventricle, the peak levels being concentration-dependent as shown beneath the recording. The maximal response was obtained with 3×10^{-6} M of L-glutamate.

stereotaxic apparatus and the cisterna magna was exposed at the base of the dorsal side of the cranium. A double-lumen needle, consisting of an 18-gauge outer needle and a 29-gauge inner spinal needle, was inserted into the fourth ventricle, and the inner needle was attached to a micro-tube pump (Eyela MP-3, Rika-kikai, Tokyo). The flow-line was filled with artificial cerebrospinal fluid (ACSF: NaCl, 126 mM; $NaHCO_3$, 26.2 mM; NaH_2PO_4, 1.0 mM; KCl, 3.0 mM; $MgSO_4$, 1.5 mM; $CaCl_2$, 2.5 mM; D-glucose: 10 mM) at 37 °C and the speed of pumping through the inner needle was maintained at 500 μl/min. Outflow perfusion fluid, via the space between the inner and outer needles, was collected into plastic tubes (diameter 4 mm) in which two electrodes for NO monitoring were placed. NO currents were measured in accordance with the methods described by Ichimori et al. (1994). The NO-selective electrode (Nitric Oxide Monitor model NO-501; Medical, Tokyo) was a Pt/Ir alloy wire coated with a three-layer membrane consisting of KCl, an NO-selective nitrocellulose resin (pyroxyline lacquer), and a gas-permeable silicon membrane. The counter electrode was made of carbon fiber and was placed near the NO-selective electrode. Voltage from +0.4 to +0.8 V was applied to the working electrode to permit the electrochemical oxidation of NO and the picoampere-order redox current between the working and counter electrodes was detected with a current–voltage converter circuit in a high input impedance preamplifier. Currents indicating the level of NO in the ACSF effluent solution from the fourth ventricle were continuously recorded with a pen recorder (RJG-412; Nihon Kohden). When rats were perfused with ACSF alone, the measured relative NO current was adjusted to 0 pA for each animal. L-Glutamate was dissolved in ACSF at concentrations of 3×10^{-8}, 10^{-7}, 3×10^{-7}, 10^{-6} and 3×10^{-6} M and maintained at 37 °C until use.

2.5. Statistical analysis

Differences between means were examined for significance with analysis of variance, unless otherwise indicated in paired *t*-tests. Statistical significance was evaluated by Scheffe's method (Snedecor and Cochran, 1967), at a level of 0.05, with values expressed as means and standard errors. Differences in frequency mode for grades 0–3 were analyzed with the χ^2 or Fisher exact probability tests.

3. Results

3.1. Effects of L-glutamate infused into the fourth ventricle on NO levels in the cerebrospinal perfusion fluid

Results for the output current of the NO-selective electrode in effluent ACSF from the fourth ventricle are shown in Fig. 1. NO current clearly began increasing when L-glutamate was infused into fourth ventricle, and the magnitude was dependent on the concentration applied.

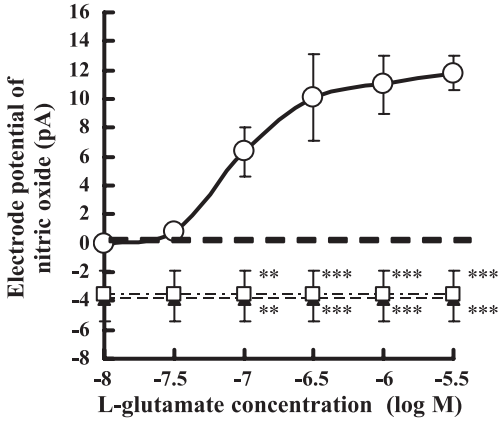

Fig. 2. Concentration–response curve for nitric oxide current induced by L-glutamate. L-Glutamate was infused into the fourth ventricle of rats via a double lumen catheter, and the amount of nitric oxide released was measured with a nitric oxide selective electrode. The concentrations of L-glutamate used were 10^{-8} M (−8), 3×10^{-8} M (−7.5), 10^{-7} M (−7), 3×10^{-7} M (−6.5), 10^{-6} M (−6), and 3×10^{-6} M (−5.5). The number of animals per group was five. Open circles, closed triangles, and open squares indicate data obtained without any other treatment, and after pretreatment with 10^{-4} M dizocilpine maleate (KM-801) or 10^{-5} M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), respectively. Asterisks, ** and ***, indicate statistical significance at levels of 0.01 and 0.001, respectively, compared to L-glutamate alone.

Table 1
Mean arterial pressure (mm Hg)

	Normal	After-vagotomy	L-NAME	D-NAME	MK-801	CNQX	L-Glu	After-fibrin
Control	118±6	122±5	–	–	–	–		180±5[a]
L-Glu	113±5	121±4	–	–	–	–	123±5	193±6[a]
L-NAME	111±6	114±5	138±5[b]	–	–	–	–	196±4[a]
L-NAME+L-Glu	115±2	120±3	140±5[b]	–	–	–	148±5[b]	195±7[a]
D-NAME+L-Glu	115±5	122±6	–	125±6	–	–	128±5	188±3[a]
MK-801	116±7	120±6	–	–	135±4[b]	–	–	199±5[a]
MK-801+L-Glu	114±4	118±3	–	–	133±3[b]	–	142±6[b]	195±7[a]
CNQX	110±5	115±4	–	–	–	127±5	–	207±4[a]
CNQX+L-Glu	107±5	111±4	–	–	–	116±3	126±5	194±3[a]

Control: without any drug treatment; L-Glu: pretreated with L-glutamate (L-Glu); L-NAME+L-Glu: pretreated with N^{ω}-nitro-L-arginine methyl ester (L-NAME) and L-glutamate; D-NAME+L-Glu: pretreated with N^{ω}-nitro-D-arginine methyl ester (D-NAME) and L-glutamate; MK-801: pretreated with dizocilpine maleate (MK-801); MK-801+L-Glu: pretreated with MK-801 and L-glutamate; CNQX: pretreated with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX); CNOX+L-Glu: pretreated with CNQX and L-glutamate.

[a] $P<0.001$, compared with values obtained after vagotomy.

[b] $P<0.01$, compared with values obtained after vagotomy.

The 50% effective concentration for L-glutamate was almost 10^{-7} M, as shown in Fig. 2. The increase in NO current induced with L-glutamate was completely inhibited by either 10^{-4} M MK-801 or 10^{-5} M CNQX (both $P<0.01$, or 0.001), continuously infused from 10 min before and during the infusion with L-glutamate. MK-801 or CNQX alone showed, non-significantly, a decrease in the basal NO current.

3.2. Cardiovascular responses

Intracisternal injections of fibrinogen and thrombin caused significant increase in mean systemic arterial blood pressure ($P<0.001$) and heart rate ($P<0.001$) as shown in Tables 1 and 2. Injections of L-NAME and MK-801 into the fourth ventricle significantly increased the mean arterial pressure and heart rate, compared to the values obtained before the injection ($P<0.01$). Injection of CNQX into the fourth ventricle significantly increased only the heart rate ($P<0.01$).

3.3. Effects of exogenous L-glutamate on the development of fibrin-induced pulmonary edema

3.3.1. Effects of L-NAME on fibrin-induced pulmonary edema development in the presence of L-glutamate

In the control group, fibrin-induced pulmonary edema was induced in all eight rats (one at grade 2 and seven at grade 3), while the incidence declined to 14.3% (one at grade 3) in the L-Glu alone group ($P<0.01$) (Table 3). Compared to the L-Glu group, pre-injection of L-NAME into the fourth ventricle significantly increased the incidence of pulmonary edema in the presence of L-glutamate to 100% (eight at grade 3), whereas administration of D-NAME failed to exert this effect (one at grade 2 and one at grade 3, 25%). Treatment with L-NAME alone into the fourth ventricle caused a pulmonary edema rate of 100% (eight all at grade 3) with a 5.69±0.25 (n=8) lung/water ratio. The lung/water ratio obtained in the L-Glu group was 3.89±0.26, significantly less than that obtained with the control group, 5.29±0.17 and L-NAME+L-Glu group,

Table 2
Mean heart rate (beats/min)

	Normal	After-vagotomy	L-NAME	D-NAME	MK-801	CNQX	L-Glu	After-fibrin
Control	397±14	406±13	–	–	–	–	–	478±17[a]
L-Glu	393±6	397±8	–	–	–	–	416±8	494±8[a]
L-NAME	391±6	398±8	430±11[b]	–	–	–	–	487±8[a]
L-NAME+L-Glu	395±10	401±12	424±9[b]	–	–	–	432±8	493±8[a]
D-NAME+L-Glu	395±5	399±7	–	409±5	–	–	426±8	496±9[a]
MK-801	396±11	402±10	–	–	428±10[b]	–	–	499±8[a]
MK-801+L-Glu	399±8	401±8	–	–	422±8[b]	–	439±7	501±7[a]
CNQX	398±10	402±6	–	–	–	437±11[b]	–	496±19[a]
CNQX+L-Glu	398±6	406±13	–	–	–	411±13	430±11	488±11[a]

Control: without any drug treatment; L-Glu: pretreated with L-glutamate (L-Glu); L-NAME+L-Glu: pretreated with N^{ω}-nitro-L-arginine methyl ester (L-NAME) and L-glutamate; D-NAME+L-Glu: pretreated with N^{ω}-nitro-D-arginine methyl ester (D-NAME) and L-glutamate; MK-801: pretreated with dizocilpine maleate (MK-801); MK-801+L-Glu: pretreated with MK-801 and L-glutamate; CNQX: pretreated with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX); CNOX+L-Glu: pretreated with CNQX and L-glutamate.

[a] $P<0.001$, compared with values obtained after vagotomy.

[b] $P<0.01$, compared with values obtained after vagotomy.

Table 3
Incidence, grades and lung/water ratios for fibrin-induced pulmonary edema, in animals pretreated with L-glutamate, L-NAME or D-NAME

	Incidence				Lung/water ratio
	Grade				
	0	1	2	3	
Control	0	0	1	7	5.29±0.17
L-Glu	6	1	0	1[a]	3.89±0.26[b]
L-NAME	0	0	0	8	5.69±0.25
L-NAME+L-Glu	0	0	0	8	5.03±0.22
D-NAME+L-Glu	5	1	1	1[a]	4.14±0.22[b]

Control: without any drug treatment; L-Glu: pretreated with L-glutamate (L-Glu); L-NAME+L-Glu: pretreated with N^{ω}-nitro-L-arginine methyl ester (L-NAME) and L-glutamate; D-NAME+L-Glu: pretreated with N^{ω}-nitro-D-arginine methyl ester (D-NAME) and L-glutamate.

[a] $P<0.01$, compared with the control value, determined by Fisher exact probability test.

[b] $P<0.001$, compared with the control value.

5.03±0.22 (both $P<0.001$). In the D-NAME+L-Glu group, the ratio was 4.14±0.22, no different from that obtained for the L-Glu group (Table 3).

A higher concentration of L-glutamate, 10^{-3} M, resulted in a 25% (two at grade 2, one at grade 1, and five at grade 0) incidence, and a 4.04±9.14 lung/water ratio.

3.3.2. Effects of MK-801 and CNQX on fibrin-induced pulmonary edema development in the presence of pretreatment with L-glutamate

In order to evaluate the role of ionotropic receptors activated by 3×10^{-6} M of exogenously administered L-glutamate, MK-801, an antagonist of NMDA receptor subtype, or CNQX, a non-NMDA (AMPA/kainic acid) receptor antagonist, were injected into fourth ventricle before the L-glutamate treatment. Compared to the L-Glu group, addition of MK-801 and L-glutamate significantly increased the incidence to 87.5% (two at grade 2 and five at grade 3) ($P<0.01$), and the lung/water ratio to 5.25±0.25 ($P<0.001$). CNQX plus L-glutamate failed to increase the incidence (three at grade 3, 37.5%), the lung/water ratio was increased to 5.24±0.31 ($P<0.001$), as compared with the L-Glu group (Table 4). In the CNQX+L-Glu group, the lung/water ratio for grade 0/1 was 4.77±0.52, significantly greater than those obtained in the L-Glu and MK-801+L-Glu groups, 3.66±0.14 and 3.84±0.16, respectively ($P<0.001$) (Fig. 3).

3.4. Effects of MK-801 and CNQX on fibrin-induced pulmonary edema development in the absence of pretreatment with L-glutamate

Pretreatment of MK-801 or CNQX alone, without L-glutamate, was performed to evaluate the effects of endogenous L-glutamate on the incidence and severity of fibrin-induced pulmonary edema. As shown in Table 5, compared to the control group, pretreatment with MK-801 or CNQX alone almost completely prevented the occurrence

Table 4
Incidence, grades, and lung/water ratios for fibrin-induced pulmonary edema in rats pretreated with MK-801 or CNQX

	Incidence				Lung/water ratio
	Grade				
	0	1	2	3	
L-Glu	6	1	0	1	3.89±0.26
MK-801+L-Glu	0	1	2	5[a]	5.25±0.25[b]
CNQX+L-Glu	2	3	0	3	5.24±0.31[b]

L-Glu: pretreated with L-glutamate (L-Glu); MK-801+L-Glu: pretreated with dizocilpine maleate (MK-801) and L-glutamate; CNOX+L-Glu: pretreated with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and L-glutamate.

[a] $P<0.01$, compared with the L-Glu group, determined by Fisher exact probability test.

[b] $P<0.001$, compared with the L-Glu group.

of fibrin-induced pulmonary edema. In the MK-801 group, the edema positive case was 0 for grades 2 and 3, and no edema fluid was collected ($P<0.001$). In the CNQX group, only one at grade 3 was obtained ($P<0.001$). Both agents significantly attenuated the lung/water ratio to 3.85±0.06 for MK-801 and 4.0±0.21 for CNQX ($P<0.001$).

3.5. Edema fluid to serum protein concentration ratios

The protein edema fluid/serum concentration ratio in the control group was 0.75±0.02, whereas the value in the L-Glu group was 0.74±0.05. The ratios obtained with L-NAME+L-Glu, D-NAME+L-Glu, MK-801+L-Glu, CNQX+L-Glu, and CNQX were 0.79±0.01 (n=8), 0.75±0.02 (n=2), 0.78±0.02 (n=7), 0.74±0.03 (n=3), and

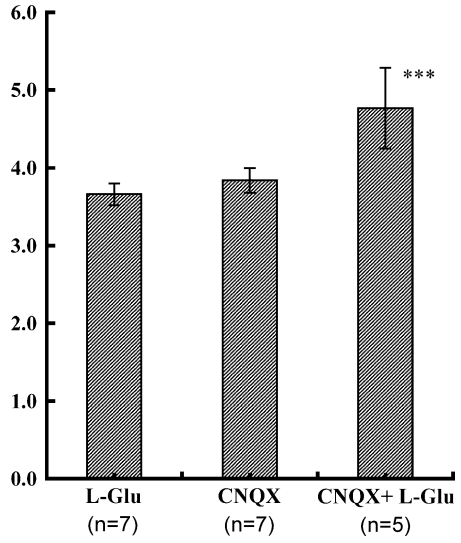

Fig. 3. Lung/water ratios in grade 0/1 lungs of edema-negative rats. Lung/water ratios in the grade 0/1 lungs were calculated by dividing the difference between the wet and dry lung weights (lung/water content) with the dry lung weight. L-Glu: pretreated with L-glutamate; CNQX: pretreated with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX); CNOX+L-Glu: pretreated with CNQX and L-glutamate; n: animal numbers. The symbol, ***, indicates statistical significance at $P<0.001$, compared with the L-Glu group.

Table 5
Incidence, grades, lung/water ratios for fibrin-induced pulmonary edema in rats pretreated with MK-801 or CNQX alone

	Incidence				Lung/water ratio
	Grade				
	0	1	2	3	
Control	0	0	1	7	5.29±0.17
MK-801	8	0	0	0[a]	3.85±0.06[b]
CNQX	6	1	0	1[a]	4.00±0.21[b]

Control: without any drug treatment; MK-801: pretreated with dizocilpine maleate (MK-801); CNQX: pretreated with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX).

[a] $P<0.001$, compared with the control group, determined by Fisher exact probability test.

[b] $P<0.001$, compared with the control group.

0.77 (n=1), respectively. No significant differences among the values were apparent.

4. Discussion

The results obtained in the present study showed MK-801, an NMDA receptor antagonist, and CNQX, an AMPA/kainic acid receptor antagonist, to clearly diminish the incidence of fibrin-induced pulmonary edema in animals not stimulated with glutamate, suggesting that ionotropic receptors mediate the neurogenic pulmonary edema development. Administration of 3×10^{-6} M L-glutamate into the fourth ventricle abolished fibrin-induced pulmonary edema, but this was paradoxically completely reversed by MK-801, and to a lesser extent by CNQX. These controversial results indicate that L-glutamate exerts two kinds of influence, excitatory and inhibitory, depending on whether endogenously or exogenously applied. As a possible explanation, when exogenous L-glutamate diffuses from the surface of brain or spinal cord, reuptake to neurons may make a concentration gradient, resulting in an insufficient concentration at specific sites to stimulate neurons for edema development. However, since 10^{-3} M L-glutamate, i.e., at a much higher concentration, even elicited a similar influence on the edema development as that obtained at 3×10^{-6} M, it is not probable that exogenous L-glutamate may only activate inhibitory neurons near the surface. It cannot be precluded that such inhibitory cells are located distant from the stimulatory neurons, which may be activated by endogenous L-glutamate.

The increase in electrode current signal for NO in the cerebrospinal perfusion fluid during the administration of L-glutamate, which was blocked by L-NAME, but not by D-NAME, suggested that NO may mediate the inhibitory action of exogenous L-glutamate on the neurogenic pulmonary edema development. Furthermore, MK-801 diminished the release of NO induced by L-glutamate infusion, consistent with the results obtained by Garthwaite et al. (1989) who demonstrated stimulation of NMDA receptors to mediate activation of NO synthesis from arginine in rat brain slices. Therefore, it is likely that exogenous L-glutamate, injected into the fourth ventricle, prevents neurogenic pulmonary edema development by releasing NO through ionotropic, mainly NMDA, receptors.

Thus it has been reported that L-glutamate, released from pre-synaptic nerves, may activate post-synaptic NMDA receptors, causing membrane depolarization and allowing Ca^{2+} to enter the cell, finally activating bNOS which produces NO and L-citrulline stoichiometrically from L-arginine (Bredt and Snyder, 1990; Bredt et al., 1992). On the other hand, Lawrence and Jarrott (1993) and Jones et al. (1995) have demonstrated that NO enhances release of excitatory amino acids such as L-aspartate and/or L-glutamate via cGMP-dependent processes at the pre-synaptic terminals. It is very conceivable that NMDA receptors may activate NOS, enhancing release of NO, which in turn would be expected to stimulate the release of pre-synaptic glutamate in a positive feedback loop (Garthwaite, 1991; Vincent, 1994). Recently, Moreira et al. (2004) demonstrated that glutamate ionotropic receptors have an important function in an aversive reaction caused by NO donors injected into the dorsolateral periaquedutal gray area of rats. NO may mediate physiological responses mediated by ionotropic glutamate receptors, through bNOS activation and increase in cGMP. Such a glutamate ionotropic receptor–NOS–NO system appear to be clearly involved in neurogenic pulmonary edema development, because L-NAME injected into the fourth ventricle completely enhanced fibrin-induced pulmonary edema in vagus nerve intact rats (Hamdy et al., 2001).

Recently, a controversial action of the NMDA receptor antagonist, MK-801, has been reported regarding neuronal cell death/loss caused by injecting high doses of L-glutamate. Schori et al. (2002) showed that the treatment with MK-801 enhanced the L-glutamate-induced retinal ganglion cell death/loss, but reduced it in mice devoid of T cell-dependent endogenous protection. The protective effect of L-glutamate on cell death/loss might be mediated by an immune-related mechanism via neuronal or non-neuronal NMDA receptors. High doses of L-glutamate may stimulate non-neuronal cells via AMPA/kainic acid receptor subtypes, finally activating such cells as resident microglia, invading macrophages, and T cells (Rimaniol et al., 2000), because NMDA-induced neuronal cell death/loss differs from that due to L-glutamate.

In this context, the unexpected action of MK-801 observed in the present study raises an interesting possible explanation for paradoxical effects of L-glutamate on neurogenic pulmonary edema development. The authors propose that endogenous L-glutamate, through ionotropic receptors, may affect neuronal cells more greatly than non-neuronal elements such as microglia. Both MK-801 and CNQX inhibited the fibrin-induced pulmonary edema, indicating an interaction between NMDA and AMPA/kainic acid receptors: the two types of glutamate ionotropic receptors may interact to trigger edema development, but each alone may be without effect. The

exogenously applied L-glutamate may diffuse and reach non-neuronal cells, and stimulate NMDA and AMPA/kainic acid receptors simultaneously, so that they cooperate to activate NOS and increase NO release.

The lung/water ratio of grade 0/1 in the CNQX+L-Glu group was greater than those obtained in the MK-801+L-Glu and control groups (Table 5), suggesting that AMPA/kainic acid receptors in neuronal and/or non-neuronal cells may have a major role in promoting neurogenic pulmonary edema incidence without affecting the lung vascular permeability, whereas NMDA receptors may enhance neurogenic pulmonary edema severity, the latter possibly associated with so-called permeability nerves in part, as shown by Malik (1985). Different functions among ionotropic receptors have been reported: Protective effects of AMPA/kainic acid receptor antagonists on cultured neurons under ischemia was lacking, or weaker than that afforded by NMDA receptor antagonists (Koh and Choi, 1991; Koretz et al., 1994). Furthermore, among NMDA, AMPA or kainic acid receptor agonists, only the latter have been shown to induce a release of L-glutamate from cultured neurons, and not the NMDA or AMPA forms (Kimura et al., 1998). It is still obscure why NMDA, but not AMPA/kainic acid receptors, mediate increase in vascular permeability in the lungs.

We conclude that under physiological conditions, endogenous L-glutamate in the medulla oblongata exerts a promoting effect on neurogenic pulmonary edema development through NMDA and AMPA/kainic acid receptors and their interaction. In contrast, high concentrations of L-glutamate injected into the fourth ventricle inhibit the disease process via activation of ionotropic receptors on non-neuronal cells and increasing NO release. It is probable that NMDA receptors mediate both the incidence and severity of neurogenic pulmonary edema, whereas AMPA/kainic acid receptors impact only on the incidence.

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