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NMDA and non-NMDA receptors mediating nociceptive and non-nociceptive transmission in spinal cord of cat[1]

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ABSTRACT The effects of excitatory amino acid (EAA) receptor agonists and blockers on the nociceptive and non-nociceptive transmission in cat spinal dorsal horn neurons were studied. About 80% of neurons were facilitated by iontophoretic injection of EAA receptor agonists (10—100 nA), such as *N*-methyl-*D*-aspartate (NMDA), quisqualic acid (QA), kainic acid (KA), and *DL*-homocysteic acid (DLH). The nociceptive responses, irregular spontaneous discharges, and C afferent induced-response of dorsal horn neuron, were reduced by the different EAA receptor blockers (35 — 150 nA), such as *DL*-2-amino-5-phosphonovalerate (APV), ketamine, 6,7-dinitro-quinoxaline-2, 3-dion (DNQX), and kynurenic acid (Kyn). Ketamine and Kyn had different effects on the long-lasting response and the short-lasting response of neurons induced by stimulation of A afferent fiber of the tibial nerve. The long-lasting response in 8/12 neurons was reduced by Kyn, but not by ketamine, and the short-lasting response was reduced by neither Kyn nor ketamine. The DLH-induced excitations were reduced by DNQX and, to a less extent, by APV. The results suggest that both NMDA and non-NMDA receptors are involved in spinal nociception; the non-nociceptive information is in part mediated by non-NMDA receptors.

KEY WORDS nociceptors; spinal cord; amino acid receptors; *N*-methyl-*D*-aspartate; quisqualic acid; kainic acid; *DL*-homocysteic acid

In 1960 Curtis *et al* first reported that glutamate (Glu) and aspartate (Asp) excited spinal neurons, suggesting that these amino acids might act as excitatory transmitters in the central nervous system. In 1980's several lines of evidence have demonstrated that excitatory amino acids (EAA), Glu and Asp, may be involved in transmission of nociceptive information in the spinal cord[1—5] through interacting with EAA receptors. EAA receptors include at least two subtypes, NMDA receptors and non-NMDA receptors[6]. It is generally accepted that NMDA receptors contribute to the processing of peripheral nociceptive input in the spinal cord[2,4—6] despite some conflicting reports[8], while non-NMDA receptors are related to low threshold afferent[3]. However, whether non-NMDA receptors are involved in mediating nociceptive information is unclear. The present work was designed to re-examine the involvement of NMDA and non-NMDA receptors in nociceptive and non-nociceptive transmission.

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MATERIALS AND METHODS

Experiments were performed on 42 adults cats (♂/♀, weighing 2.5±*s* 0.5 kg, supplied by Experimental Animal Center of Shanghai Brain Research Institute, Chinese Academy of Sciences) anesthetized with sodium pentobarbital (40 mg · kg^{-1}, ip, initially). The spinal cord was transected at L_1-L_2 segments. Cats were artificially ventilated after neuromuscular paralysis with gallamine triethiodide (4 mg · kg^{-1}, iv) and end-tidal CO_2 level was kept at 3.5-5.0%. A slow infusion pump delivered continuously both pentobarbitone (2 mg · kg^{-1} · h^{-1}) and gallamine (4 mg · kg^{-1} · h^{-1}). Blood pressure and body temperature were maintained at physiological level. The tibial nerve was prepared for electric stimulation. The firing of single neuron in spinal segments of L_{6-7} was recorded extracellularly through single micropipette or the central barrel (filled with NaCl 4 mol · L^{-1}) of 7-barrel micropipettes. The data were collected with a computer bioelectric signal processing system through micro-electrode amplifier (MEZ-8201) and storage os-

ciloscope (VC-10). The drugs (ketamine: the First Pharmaceutic Factory of Shanghai; 6,7-dinitro-quinoxaline-2,3-dion (DNQX): Research Biochemicals Inc, kindly provided by Professor SHEN E; the others from Sigma) were administered by 3 methods:

(1) Iontophoresis. Within the outer barrels of 7-barrel micropipettes contained *N*-methyl-*D*-aspartate (NMDA) 50, quisqualic acid (QA) 5, kainic acid (KA) 5, *DL*-homocysteic acid (DLH) 200, *DL*-2-amino-5-phosphonovalerate (APV) 50, ketamine 50, Kynurenic acid (Kyn) 100, or DNQX 1 mmol · L^{-1}. Each barrel of the micropipettes was filled with NaCl 100 mmol · L^{-1} for current balance.

(2) Intravenous injection (iv). Ketamine 5 and 10 mg · kg^{-1} iv.

(3) Topical administration on the area of spinal cord recorded. A small chamber was made with agar for topical ketamine (50 mmol · L^{-1}, 10 μl) and Kyn (20 mmol · L^{-1}, 10 μl).

The locations of neurons were examined by ejection of 2% potamine sky blue or by reading of the depth of micropipette tip indicated on the microdriver.

RESULTS

Sixty neurons were recorded in laminae I - VI of the dorsal horn. These neurons were classified into 3 types: lower threshold neurons (LT, $n=21$) responded to Aβ afferent; wide dynamic range neurons (WDR, $n=34$) responded to A and C afferents; high threshold neurons (HT, $n=5$) responded to Aδ and C afferents.

EAAs-induced responses Firing of 48 (80%) neurons was increased following iontophoretic application of selective NMDA and non-NMDA receptors agonists (10—100 nA), ie, NMDA ($n=6$), QA ($n=25$), KA ($n=32$). NMDA-, but not QA- or KA-, induced excitations were antagonized by iontophoretic injection of selective NMDA receptor blockers, APV and ketamine (10—100 nA), but not by the selective non-NMDA receptor blocker DNQX. DNQX (10—100 nA, $n=4$) preferentially blocked QA- and KA-, but not NMDA-, induced excitations. The broad spectrum EAA receptor blocker Kyn (10—100 nA) inhibited all the EAA-induced excitations (Fig 1 A—C). DLH-induced excitations were all reduced by APV, ketamine, DNQX, and Kyn. Reduction of DLH-induced excitation by DNQX was much powerful than that by APV in 4 neurons tested (Fig 2).

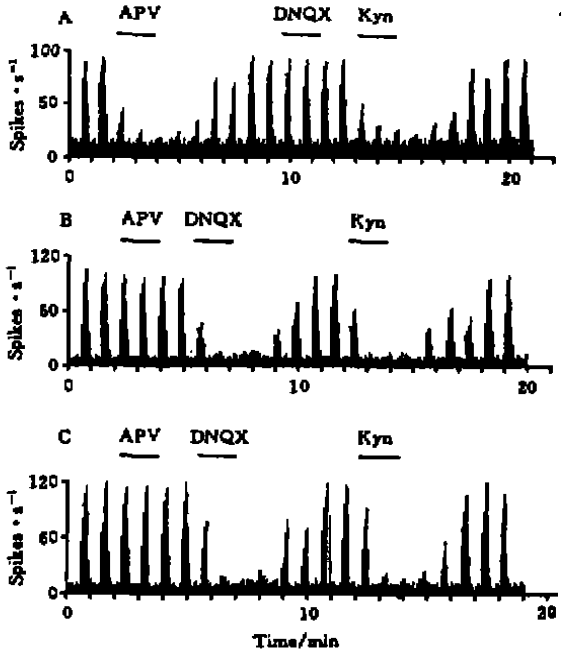

Fig 1. Effects of EAA receptor blockers on EAA-induced excitations of spinal dorsal horn neurons—firing rate of neurons in response to electrophoretic injection of excitatory amino acids NMDA (40 nA, A), QA (40 nA, B), and KA (30 nA, C). APV (40 nA) selectively reduced the excitations of NMDA, but not those of QA and KA. DNQX (40 nA) selecitively reduced the excitations of QA and KA, but not those of NMDA. Kynurenate (Kyn, 40 nA) reduced the excitations of all 3 agonists.

Nociceptive response Neurons responded to electric stimulation of tibial C fiber were mainly recorded in laminae IV - V. As shown in Tab 1, in most neurons the nociceptive responses were reduced about 30—60% by iv or topical ketamine, topical Kyn and iontophoretic injection of APV, ketamine, DNQX, or Kyn (Fig 3), while in some neurons the

Tab 1 . Effects of EAA receptors blockers on the C responses induced by stimulation of tibial nerve and the irregular spontaneous activities of cats spinal dorsal horn neurons. * Number of neurons, T: Total; I: Inhibition; E: Excitation; N: No effect.

Drugs		C response				Spontaneous activity			
		T*	I*	E*	N*	T*	I*	E*	N*
Ketamine, iv,	5	28	10	5	13	36	21	6	9
(mg · kg^{-1})	10	24	17	2	5	36	27	3	6
Ketamine	(topical)	23	14	2	7				
Kyn	(topical)	18	11	2	5				
Ketamine+Kyn	(topical)	14	10	2	2				
APV	(40—150 nA)	12	8	1	3	21	12	2	7
Ketamine	(40—150 nA)	18	12	3	3	39	24	4	11
DNQX	(40—150 nA)	8	5	0	3	8	5	0	3
Kyn	(35—150 nA)	16	12	2	2	33	20	6	7

nociceptive responses were facilitated.

Low threshold response The A fiber excited-responses exhibited 2 different patterns: short-lasting response (a few spikes lasting 10—30 ms with a latency of 2—3 ms) and long-lasting response (some dozen of spikes lasting 500—1000 ms with a latency of 2—50 ms). The short-lasting responses in 20 neurons tested were not reduced by iv ketamine, 5 or 10 mg · kg^{-1}, topical ketamine and Kyn, and iontophoretic injection of APV, ketamine, or Kyn. In contrast, the long-lasting responses of most neurons (8/12) were reduced by 55± 16% following topical Kyn, but not ketamine.

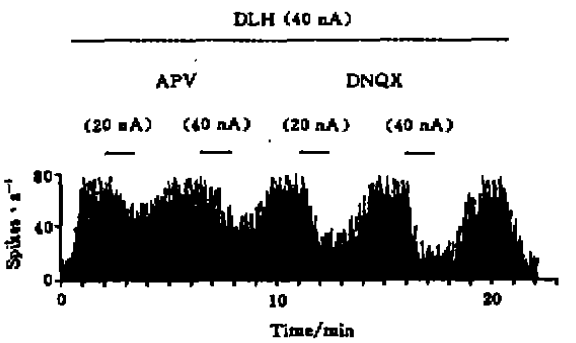

Fig 2. Reductions of DLH-excitation by APV and DNQX. The DLH-induced excitations were reduced more by iontophoretic ejection of DNQX (selective non-NMDA receptor blocker) than by APV (selective NMDA receptor blocker).

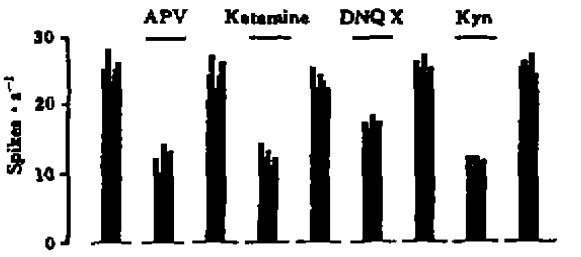

Fig 3. Effects of EAA receptor blockers on C response of a spinal dorsal horn neuron excited by stimulation of C afferent fiber of tibial nerve. The C responses were reduced by iontophoretic injection (40 nA), ketamine, DNQX, or kynurenate (Kyn).

Spontaneous discharges Spontaneous discharges in 64 neurons were recorded in laminae I-VI. The 2 patterns of spontaneous discharges were found: the regular activities with rhythmic discharges at 20—50 Hz, and the irregular spontaneous ones mainly in laminae I and V with burst of spikes at 4—12 Hz. The irregular discharges, but not the regular discharges, were inhibited by iontophoretic injection of APV, ketamine, Kyn or DNQX, and iv ketamine 5 or 10 mg · kg^{-1}

in most neurons (37/52) (Tab 1). In some neurons (6/37), the irregular discharges were almost abolished by the EAA receptor blocker. The regular discharges of neurons (n = 12) tested were not affected by the EAA receptor blocker.

DISCUSSION

It has been considered that non-NMDA and NMDA receptors mediate spinal non-nociceptive and nociceptive transmission, respectively[2,4,5,7]. In previous study, we found that non-NMDA receptors are involved in mediating transmission of muscular nociceptive information, while cutaneous nociceptive information was mediated by NMDA receptors in the spinal cord[7], suggesting involvement of both non-NMDA and NMDA receptors in spinal nociception. The present findings that both non-NMDA and NMDA receptors agonists or blockers excited or inhibited nociceptive neurons further support above notion. Consistent with our result, (R,S)-a-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), a non-NMDA receptor agonist, facilitated the nociceptive response in rat[4].

It merits our attention that the irregular spontaneous discharges of spinal dorsal horn neurons in laminae I and V were reduced by non-NMDA and NMDA receptors blockers. The reduction of irregular spontaneous discharge may be related to spinal antinociception. Chang reported that irregular spontaneous discharges in parafascicularis neurons of thalamus were abolished by iv morphine and markedly reduced by procaine infusion of the wound, suggesting that these discharges were attributed to the tissue injuries by surgery[10].

DL-homocysteic acid (DLH) is a common tool to excite central neurons in electrophysiological study. But its potency of affinity to subtypes of EAA receptors is still unclear. In the present experiments, the effects of non-NMDA and NMDA receptors blockers on DLH-induced excitation were compared. Non-NMDA and NMDA receptors blockers reduced DLH-induced excitations by 85% and 46%, respectively, indicating that DLH mainly acts on non-NMDA receptors, at least, in the spinal cord.

In agreement with previous studies that the monosynaptic excitation of the low threshold neuron in the spinal cord is readily attenuated by non-NMDA receptors blockers[3,11], long-lasting response of low threshold neurons related to non-nociceptive information was reduced by the broad spectrum EAA receptors blocker kynurenic acid (Kyn), but not by the selective NMDA receptors blocker ketamine, suggesting that non-NMDA receptor, not NMDA receptor, may be involved in mediating the low threshold long-lasting response. The neurons recorded in present study were mainly located in laminae IV-V where large myelinated afferent fibres are terminated. So they may receive A fibre afferents monosynaptically.

The present studies suggest that bpth NMDA and non-NMDA receptors are involved in spinal nociception; the non-nociceptive information is in part mediated by non-NMDA receptors. DLH is a broad spectrum EAA receptors agonist, but it mainly acts on non-NMDA receptors.

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NMDA 和非 NMDA 受体介导猫脊髓伤害性和非伤害性信息传递

R 965.2

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摘要 用多管微电极离子电泳技术研究猫脊髓背角神经元活动. 80%的神经元被兴奋性氨基酸 NMDA、使君子氨酸、卡因酸或 *DL*-高磺丙氨酸（DLH）激活. NMDA 受体拮抗剂 APV 和氯胺酮，非 NMDA 受体拮抗剂 DNQX 以及广谱拮抗剂 4-羟基喹啉酸(Kyn)均抑制神经元的伤害性反应. Kyn 抑制非伤害性反应大大强于氯胺酮. NMDA 和非 NMDA 受体均参与介导脊髓伤害性反应，非伤害性信息主要由非 NMDA 受体介导.

关键词 伤害性感受器；脊髓；氨基酸受体；*N*-甲基-*D*-门冬氨酸；使君子氨酸；卡因酸；*DL*-高磺丙氨酸

高磺丙氨酸

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Midazolam pharmacokinetics and electroencephalographic changes in eight Chinese men[1]

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ABSTRACT Eight Chinese healthy male volunteers aged 27±*s* 4 a were injected iv midazolam (Mid) 15 mg. Blood samples were collected at 0, 2, 5, 7, 10, 20, 30, 45, 60, 90, 120, 180, and 240 min. A HPLC method was established for determining the Mid concentrations in serum. The concentration-time data was fitted with biexponential curve. Pharmacokinetic parameters were: $T_{\frac{1}{2}\alpha}$=6.8±2.5 min, $T_{\frac{1}{2}\beta}$=118±27 min, V_c=25±7 L, Cl=393±79 ml·min^{-1}, V_{ss}=59±13 L, $AUC_{0-\infty}$=39.6±8.6 g·min·L^{-1}. The electroencephalogram (EEG) showed a decrease in α activity and an increase in β activity. The EEG pattern reverted toward baseline after 2—3 h.

Pharmacokinetic and EEG findings suggest that Mid is a preferable anesthesia inducing agent.

KEY WORDS midazolam; pharmacokinetics; high pressure liquid chromatography; electroencephalography

Midazolam (Mid) is used in anesthesia for premedication, induction of anesthesia, and intraoperative hypnosis[1,2]. But there has

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