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Involvement of NMDA and non-NMDA receptors in transmission of spinal visceral nociception in cat[1]

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KEY WORDS *N*-methyl-*D*-aspartate receptors; quisqualic acid; kainic acid; 2-amino-5-phosphonovalerate; quinoxalines; spinal cord; viscera

ABSTRACT

AIM: To study the role of *N*-methyl-*D*-aspartic acid (NMDA) and non-NMDA receptors in processing nociceptive visceral information in the spinal cord. **METHODS**: The firing of spinal dorsal horn neurons to colorectal distension (3 – 15 kPa, 20 s) by inflation with air of latex balloon was recorded in 25 anesthetized cats. **RESULTS**: 1) According to the patterns of responses to colorectal distension, the neurons with increase and decrease in firing were classified as excitatory and inhibitory, respectively. The former consisted of 17 short-latency abrupt (SLA) neurons, 11 short-latency sustained (SLS) neurons, 9 long-latency (LL) neurons. The 15 inhibited (Inh) neurons were recorded. 2) Microelectrophoretic administration of NMDA, quisqualic acid (QA), and kainic acid (KA) activated 67.6 %, 78.4 %, and 59.5 % of the colorectal distension-excited neurons tested. Also, 60 %, 86.7 %, and 53.3 % of Inh neurons were activated by these 3 amino acids. 3) Colorectal distension-induced excitatory responses were reduced by 35 % ± 10 % and 65 % ± 14 % by a selective NMDA receptor antagonist *d*, *l*-2-amino-5-phosphonovalerate (APV) and a selective non-NMDA receptor antagonist 6, 7-dinitro-quinoxaline-2, 3-dione (DNQX), respectively. Such DNQX-induced inhibition was significantly more potent than that by APV ($P < 0.05$). Colorectal distension-induced inhibitory responses were partially relieved by 30 % – 50 % in 3/7 Inh neurons by DNQX, but not APV. **CONCLUSION**: Both NMDA and non-NMDA receptors are involved in transmission and/or modulation of spinal visceral nociceptive information and non-NMDA receptors may play more important role than NMDA receptors.

INTRODUCTION

Excitatory amino acids (EAA) receptors, NMDA, and non-NMDA, were involved in various kinds of spinal nociception[1-6]. Application of EAA receptor agonists elicited hyperalgesia and selective EAA receptor antagonists produced antinociception or analgesia[2,7,8]. NMDA receptors mediate excitatory transmission and are involved in "wind up" of nociceptive dorsal horn neurons[9,10]. The excessive excitation of NMDA receptors might be responsible for the generation of pain produced in rat models of tissue inflammation and nerve injury. Non-NMDA receptors might also participate in acute and inflammatory nociceptive transmission[8,11]. In a previous study[4], we found that NMDA and non-NMDA receptors were not only involved in nociception, but might preferentially mediate transmission of nociceptive information originating in skin and muscle, respectively. However, the contribution of NMDA and non-NMDA receptors in visceral nociception has not been evaluated. The skin, muscle, and visceral organs were sensitive to different kinds of stimuli, indicating that visceral and somatic sensation might occur through different mechanisms. The purpose of present study was to examine the role of NMDA and non-NMDA receptors in spinal visceral nociception.

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MATERIALS AND METHODS

Cats Experiments were performed on 21 adult cats of either sex weighing 2.0 – 3.5 kg (Shanghai Experimental Animal Center, Chinese Academy of Sciences, Certificate No 005) anesthetized with sodium pentobarbital (initial dose, 40 mg · kg^{-1}, ip). Catheters were inserted into radial vein, carotid artery and tracheal. The lumbar and sacral spinal cord were exposed and transected at L_{1-2} segments after pretreatment with 2 % procaine. Cats were artificially ventilated after neuromuscular paralysis with gallamine triethiodine (4 mg·kg^{-1}, iv) and end tidal CO_2 level was kept at 3.5 % – 5.0 %. A slow infusion pump continuously delivered both pentobarbitone (2 mg · kg^{-1}·h^{-1}) and gallamine (4 mg·kg^{-1}·h^{-1}). Blood pressure and body temperature were maintained at physiologic level.

Noxious visceral stimulation The distension control device was used to provide a distending stimulus previously described [12]. Colorectal distension was produced by inflation with air of 7- to 8-cm-long pressure-monitored flexible latex balloon inserted into the descending colon and rectum via anus. The balloon had a diameter (when inflated) greater than the distended colon so that recorded pressure was an accurate measure of colorectal distension. Intracolonic pressure was continuously monitored directly via an on-line pressure transducer. A colorectal distension (10.5 – 12.5 kPa pressures) was used as the search stimulus. Distending stimuli (3 – 15 kPa, 20 s) were administrated at 4-min intervals to provide stable neural responses to colorectal distension throughout the course of an experiment. According to the terminology of Ness and Gebhart's study[13], the neurons with increase and decrease in firing were classified as excitatory and inhibitory, respectively. The former included SLA neurons (short-latency abrupt increase infiring), SLS neurons (short-latency sustained increase), and LL neurons (long-latency increase). The later was Inh neurons with decrease in spontaneous firing.

Microelectophoresis and recording The central barrel (filled with NaCl 4 mol) of 7-barrel micropipettes were used for extracellular single-unit recording in the spinal S_{1-2} segment. The following solutions were contained within the outer barrels of 7-barrel micropipettes *N*-methyl-*D*-aspartic acid (NMDA, 50 mmol), quisqualic acid (QA, 5 mmol), kainic acid (KA, 5 mmol), *d*,*l*-2-amino-5-phosphono-valerate (APV, 50 mmol) and 6,7-dinitro-quinoxaline-2,3-dione (DNQX, 1 mmol), one barrel of the micro-pipettes was filled with NaCl 150 mmol for current balance.

The action potentials recorded with a glass microelectrode were amplified (MEZ-8201), displayed on a storage oscilloscope (VC10), and stored on a computer (IBM-PC-486). The depth of a recorded neuron was estimated by reading of the depth of micropipette tip indicated on the microdriver.

RESULTS

Fifty-two neurons of the dorsal horn responded to noxious colorectal distension (3 – 15 kPa, 20 s) were recorded from laminae Ⅰ – Ⅵ, mainly in laminae Ⅰ – Ⅳ, at S_{1-2}. More than 80 % of the neurons also received convergent somatic input from scrotal and pesianal skin, by responding to both noxious (squeeze, pinch) or non-noxious (brush) cutaneous stimuli. Spontaneous discharges (12 Hz ± 6 Hz) were recorded in 86.1 % of neurons (n = 52). The neurons responding to colorectal distension were classified as excitatory and inhibitory ones. The 17 SLA neurons were excited at short latency (< 1 s) in response to colorectal distension and, following termination of the distending stimuli, neural activity abruptly returned to the control levels in < 2 s. The 11 SLS neurons were also excited at short latency (< 1 s) in response to colorectal distension, but responses were sustained above base-line activity following termination of the distension stimulus. The 9 LL neurons were excited at long latency (5 – 15 s) by colorectal distension, and the responses were also sustained after termination of distending stimuli, returning to the control base-line about 30 s later. The 15 spontaneously active neurons (Inh) were inhibited by colorectal distension, and there was a slow return to the control level of spontaneous activity in 4 – 50 s. The thresholds for inhibition of most Inh neurons (n = 13) were more than 10.5 kPa (6 – 7.5 kPa in the other 2 neurons).

More than 50 % of both excited and inhibited neurons by noxious colorectal distension were activated by microelectrophoretic administration of NMDA, QA, and KA by 10 – 100 nA. There was no significant

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difference between SLA, SLS, and LL types of neurons. More Inh neurons were sensitive to QA than to NMDA and KA (χ^2-test, $P < 0.05$) (Tab 1). NMDA-, but not QA- or KA-, induced neural responses were reduced by ejection of APV (10 – 100 nA). DNQX (10 – 100 nA) inhibited QA- and KA-, but not NMDA-induced responses.

Tab 1. Microelectrophoretic administration of NMDA, QA, and KA (10 – 100 nA) activates excited and inhibited spinal dorsal horn neurons by noxious colorectal distension.

Neuron	NMDA/%	QA/%	KA/%
Excited	67.6 (n = 18)	78.4 (n = 30)	59.5 (n = 28)
Inhibited	60 (n = 13)	86.7 (n = 15)	53.3 (n = 12)

The colorectal distension-induced nociceptive responses were reduced by (35 % ± 10 %) and (65 % ± 14 %) in 69.6 % and 78.3 % (n = 23) of the neurons by microelectrophoretic administration of APV and DNQX, respectively (Fig 1).

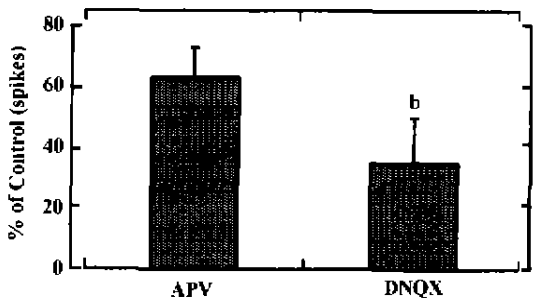

Fig 1. Reduction of the colorectal distension-induced nociceptive responses in 23 neurons by microelectrophoretic administration of selective NMDA receptor antagonist APV and selective non-NMDA receptor antagonist DNQX (10 – 100 nA).
$\bar{x} \pm s$. $^b P < 0.05$ *vs* corresponding APV.

There was no significant difference between the numbers of neurons inhibited by APV and DNQX (χ^2-test, $P > 0.05$); however, the inhibition in the neural response by DNQX was more potent than that by APV (t-test, $P < 0.05$). A representative example was illustrated in Fig 2.

We also found that the nociceptive response was markedly facilitated by QA, but not NMDA and/or KA, in 3 neurons (two SL-A and one SL-S neurons). The DNQX partially relieved the inhibitory effects or shortened the inhibitory time by (30 – 50) % in 3/7 Inh neurons, while the APV failed to relieve the colorectal distension-induced inhibition.

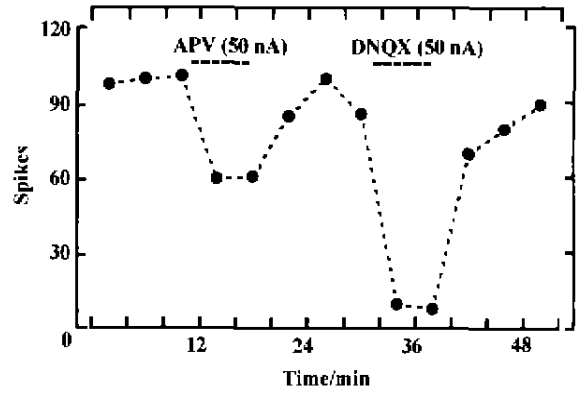

Fig 2. Inhibitory effects of APV and DNQX on colorectal distension-induced nociceptive responses in a dorsal horn neuron.

DISCUSSION

Visceral pain can be a very intense sensation, and is often associated with chronic disease states. However, the mechanisms underlying visceral pain are still poor understood. Since distension of hollow viscera is painful in humans, a constant-pressure distension of the descending colon and rectum was employed as a model for experimental visceral pain by Gebhart group[13-14]. With this method, spinal neurons responsive to colorectal distention have been characterized as conducting nociceptive information in the rat and cat.

The present study shows that most spinal dorsal horn neurons in response to noxious colorectal distension were excited by NMDA and non-NMDA agonists, and that the colorectal distension-induced nociceptive responses in the same neurons were facilitated and reduced by the EAA agonists and antagonists, respectively. These results provide evidence that the EAA, glutamate and/or aspartate, may be contributed to the transmission of visceral afferent information. In the previous studies, we demonstrated the involvement of NMDA and non-NMDA receptors in mediating transmission of nociceptive cutaneous and muscular information[5].

Taken together with the present results, we do believe an important and universal role of NMDA and non-NMDA receptors in processing spinal pain messages originating from various tissues, including skin, muscle, and viscera.

It was noted that our results here showed that there was a profound difference in the degree of inhibition between the selective NMDA receptor antagonist APV and the selective non-NMDA receptor antagonist DNQX on the nociceptive visceral responses of dorsal horn neurons, ie, the nociceptive responses were reduced more by DNQX and, to a lesser extend, by APV in most neurons tested. This finding indicates that non-NMDA receptors may play more important roles in mediating spinal visceral nociception than NMDA receptors. This is consistent with the previous work in our laboratory[4] and Sluka *et al*[8] that shows nociceptive inputs from deep tissues, muscle, and knee, are mainly mediated by non-NMDA receptors.

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NMDA 和非 NMDA 受体参与介导猫脊髓内脏伤害性信息传递[1]

R338.3
R338.21

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NMDA

关键词 *N*-甲基-*D*-天冬氨酸受体；使君子氨酸；卡英酸；2-氨基-5-膦酰基戊酸盐；喹噁啉类；脊髓；内脏传入

目的：研究 NMDA（*N*-methyl-*D*-aspartic acid）和非 NMDA 受体在介导脊髓内脏痛传入中的作用. **方法**：气球膨胀(3－15 kPa、20 s)麻醉猫结－直肠诱发脊髓背角痛敏神经元发放. **结果**：1）扩张结－直肠引起神经元发放增加的为兴奋性型：17 个 SLA 型(短潜伏期突然增加)；11 个 SLS 型(短潜伏期渐增)；9 个 LL 型(长潜伏期). 15 个神经元属于抑制性的 Inh 型. 2）67.6 %，78.4 %和59.5 %的膨胀肠诱发兴奋的神经元，分别被微电泳 NMDA、使君子酸(QA)和海人藻酸(KA)激活；60 %，86.7 %和53.3 %的 Inh 神经元也分别被 3 个酸激活. 3）微电泳 NMDA 受体拮抗剂 *d*,*l*-2-amino-5-phosphonovalerate（APV）和非 NMDA 受体拮抗剂 6,7-dinitro-quinoxaline-2,3-dione（DNQX），分别使兴奋性反应减少 35 % ± 10 %和65 % ± 14 %，DNQX 明显强于 APV（$P < 0.05$）. DNQX 使 3/7 个 Inh 神经元抑制翻转 30% － 50 %，而 APV 无效. **结论**：NMDA 和非 NMDA 受体均参与介导脊髓内脏伤害性信息传递，而非 NMDA 受体的作用更强.

（责任编辑 李 颖）

全国心血管药理学术会议通知

中国药理学会心血管药理专业委员会决定，由心血管药理专业委员会主办，第三军医大学大坪医院野战外科研究所承办于 1999 年 11 月初在重庆市召开全国心血管药理学术会议，并向全国征文.

征文内容：(1) 抗休克药物基础与临床. (2) 心血管离子通道药理学及临床应用. (3) 心血管神经体液调控及临床意义. 要求邮寄 600－800 字未发表过的论文摘要一份.

会议期间将邀请知名学者作专题报告并评选四十岁以下青年优秀论文，参加评选者邮寄全文及摘要各两份.

截稿日期 1999 年 6 月 30 日，稿件邮寄 400042 重庆市第三军医大学大坪医院野战外科研究所二室胡德耀研究员、肖 南副研究员收. 电话：023-6875-7522 或 023-6875-7432.

会议热烈欢迎药理、有关临床及药剂工作人员参加.

中国药理学会心血管药理专业委员会
第三军医大学大坪医院野战外科研究所