Substance P potentiates thermal hyperalgesia induced by intrathecal administration of D-serine in rats

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KEY WORDS N-methyl-D-aspartate receptors; glycine; substance P; protein kinases; spinal cord; hyperalgesia

ABSTRACT

AIM: To investigate the functional interaction between substance P (SP) and D-serine, agonist for glycine regulatory site of N-methyl-D-aspartate (NMDA) receptor, in processing spinal nociception. METHODS: Behavior studies, by testing tail-flick latency (TFL) combined with intrathecal application of drugs, were conducted in lightly anesthetized rats. RESULTS: Decrease in TFL was observed 1.5 min after intrathecal injection of D-serine 1000 nmol. Following pretreatment with SP 0.05 nmol 6 min prior to injection of D-serine 10 nmol, D-serine-induced decrease in TFL was greatly enhanced. The potentiation was blocked by co-administration of 7-chlorokynurenine acid 1 pmol, the selective antagonist for glycine regulatory site of NMDA receptor, or H-7 10 \(\mu\)mol, the PKC non-selective inhibitor, with SP 0.05 nmol. CONCLUSION: SP potentiates the D-serine-induced thermal hyperalgesia, glycine regulatory site of NMDA receptor and intracellular protein kinase system may participate in the interaction of SP and NMDA receptor in the spinal cord.

INTRODUCTION

Substance P (SP) and glutamate (Glu) co-exist in small dorsal root ganglion (DRG) neurons\(^{11}\) and their receptors co-localize in some spinal dorsal horn neurons\(^{2}\). Previous electrophysiological results showed SP and receptor agonist potentiates N-methyl-D-aspartate (NMDA)-induced activity in dorsal horn neurons via calcium-dependent protein kinase C and A\(^{13,15}\). In the behavioral study, intrathecal co-administration of SP and NMDA produced more potent nociceptive responses than that of SP and NMDA given separately\(^{16}\). An interaction between SP and NMDA receptor in the spinal cord may play a prominent role in central sensitization. NMDA receptor-channel complex possesses a variety of regulatory sites such as the polyamine site, phencyclidine (PCP) site, phosphorylation site, Zn\(^{2+}\) sites, Mg\(^{2+}\) sites, and glycine site (Gly\(_{NMDA}\))\(^{1,8}\). There is considerable evidence for a regulatory role of Gly\(_{NMDA}\) site in the spinal nociception\(^{1,14}\). HA-966, a Gly\(_{NMDA}\) site antagonist, promoted SP receptor antagonist-induced antinociception in formalin pain model\(^{19}\). Moreover, iontophoretic application of SP receptor agonist greatly facilitated NMDA receptor agonist 1-aminocyclobutane-cis-1, 3-dicarboxylic acid (ACBD)-produced increase in the firing rate of spinal dorsal horn neurons, which was prevented in the presence of Gly\(_{NMDA}\) site antagonists\(^{3}\). Our recent study has shown that SP enhanced NMDA/glycine-induced increase in [Ca\(^{2+}\)]\(_i\) in the spinal dorsal horn neurons\(^{10}\). Taken together, it is suggested that the status of the Gly\(_{NMDA}\) site is functionally relevant to SP receptor-mediated regulation of nociception. To further open out the functional significance of Gly\(_{NMDA}\) site, the present work was to examine the interaction of SP and D-serine, the endogenous ligand for Gly\(_{NMDA}\)\(^{11}\), in the nociceptive behavior test of rats.

MATERIALS AND METHODS

Rats Male Sprague-Dawley rats (Grade II, Certificate No 003, weighing 220–250 g) were obtained from Shanghai Experimental Animal Center, Chinese Academy of Sciences. Animals were caged individually with food and water available. The room temperature was controlled at 22 – 25 °C. All experimental protocols

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followed the guidelines of the International Association for the Study of Pain (IASP) concerning the use of laboratory animals.

**Drugs and reagents** SP was stored frozen below -20 °C in double distilled water at 10 mmol/L. The other drugs and their concentrations for store as follows: D-serine (0.01 - 200 mmol/L aqueous), 1-(5-isouquinolinesulfonfyl)-2-methylpiperazine (H-7, 2 mol/L aqueous), and 7-chlorokynurenic acid (7-CR, 200 mmol/L in dimethyl sulfoxide). SP, D-serine, H-7, and 7-CR were all purchased from Sigma (Chemical Co, St Louis, MO, USA).

**Intrathecal injection** Animals were anesthetized with sodium pentobarbital (40 mg/kg, ip), and then an intrathecal (it) polyethylene (PE-10) cannula was implanted surgically into the subarachnoid space of lumbar spinal cord according to the classic procedure\(^{(12)}\). The animals were allowed a 4-d recovery from surgery. Only those animals with no sign of neurological impairment were used in the experiments.

**Behavioral testing** The nociceptive tail-flick reflex test was performed in animals lightly anesthetized with chloral hydrate (50 mg/kg) and sodium pentobarbital (28 mg/kg). The animals were placed on a glass plate, and a high-intensity light beam was focused on the ventral skin at least 3 cm from the end of the tail. The tail-flick latency (TFL) was measured from the time triggering the light until the rat withdrew its tail from the heat source. Intensity was set to such a level that baseline TFL was typically 6 - 7 s. Drugs were administered through the PE-10 cannula (5 μL followed by 10 μL saline). The animals that showed unstable TFL were discarded.

**Experimental protocol** In each experiment, baseline TFL was recorded three times at intervals of at least 5 min. Post-drug TFL was measured 1.5, 3, and 6 min after its administration, respectively.

According to its administration of different drugs, rats were divided into following groups. Normal saline group; D-serine group, D-serine 0.1 nmol, 10 nmol, or 1000 nmol was given alone; SP + D-serine group, rats were intrathecally pretreated with SP 0.05 nmol 6 min prior to injection of D-serine 10 nmol; SP + 7-CR + D-serine group and SP + H-7 + D-serine group, for rats in this two groups, 7-CR 1 pmol or H-7 10 pmol was co-administered with SP 0.05 nmol 6 min prior to D-serine 10 nmol injection.

**Data and statistics** All data are presented as \(x \pm s\). Comparisons were made by two-tailed Student's \(t\) test. Baseline TFL was evaluated as mean of three consecutive TFL values. Changes in TFL were expressed as ΔTFL or percentage of facilitation. ΔTFL = Post-drug TFL - Pre-drug TFL. Percentage of facilitation = ΔTFL/pre-drug TFL \times 100 \%. Increasing negative values indicate increasing magnitudes of thermal hyperalgesia. \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Acute thermal hyperalgesia induced by D-serine** Intrathecal administration of D-serine (5 μL, 1000 nmol, 10 nmol, and 0.1 nmol, respectively) decreased baseline TFL elicited by noxious thermal stimuli to some extent, indicating that acute thermal hyperalgesia occurred. D-serine at the dose of 10 nmol produced decrease in TFL from baseline 7.0 s ± 1.8 s to 5.7 s ± 1.6 s at 1.5 min and recovered to 6.5 s ± 3.1 s at 3 min after its administration, though both showed no statistical significance when compared with baseline, while D-serine at the dose of 1000 nmol produced great decrease in TFL at 1.5 min from baseline 6.6 s ± 0.5 s to 5.3 s ± 0.8 s (\(P < 0.05\)) and restored to 5.9 s ± 1.2 s at 3 min after its administration (\(P > 0.05\) vs baseline) (Tab.1). Therefore we chose the dose of 10 nmol and the time point of 1.5 min to observe the effect of SP on the thermal hyperalgesia induced by D-serine. The administration of 5 μL NS had no effect on the baseline of TFL (Tab.2).

**Potentiation of D-serine-induced thermal**

### Tab 1. Effects of different doses of D-serine on TFL at 1.5 min after administration. \(x \pm s\). \(^{(P < 0.05 vs Pre-D-ser.})\)

<table>
<thead>
<tr>
<th>D-Serine/μmol</th>
<th>Pre-D-ser/s</th>
<th>Post-D-ser/s</th>
<th>ΔTFL</th>
<th>Percent facilitation/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 (n = 5)</td>
<td>5.6 ± 0.2</td>
<td>5.7 ± 1.1</td>
<td>-0.2 ± 1.1</td>
<td>-2.6 ± 8.1</td>
</tr>
<tr>
<td>10 (n = 6)</td>
<td>7.0 ± 1.8</td>
<td>5.7 ± 1.6</td>
<td>-1.3 ± 0.6</td>
<td>-18.9 ± 7.8</td>
</tr>
<tr>
<td>1000 (n = 7)</td>
<td>6.6 ± 0.5</td>
<td>5.3 ± 0.96</td>
<td>-1.2 ± 0.5</td>
<td>-19.3 ± 8.6</td>
</tr>
</tbody>
</table>
Tab 2. Effects of different treatment on TFL after administration. $x \pm s$. $^{a} P < 0.05$ vs Pre-D-ser.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline/s</th>
<th>Post-treatment (or Pre-D-ser)/s</th>
<th>Post-D-ser/s</th>
<th>$\Delta$TFL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline ($n = 8$)</td>
<td>7.0 ± 1.3</td>
<td>7.2 ± 1.2</td>
<td>0.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>D-Ser ($n = 6$)</td>
<td>7.0 ± 1.8</td>
<td>5.7 ± 1.6</td>
<td>-1.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>D-Ser + SP ($n = 8$)</td>
<td>7.1 ± 1.9</td>
<td>6.9 ± 1.6</td>
<td>4.6 ± 1.7$^{a}$</td>
<td>-2.3 ± 1.8</td>
</tr>
<tr>
<td>D-Ser + SP + 7-CK ($n = 8$)</td>
<td>6.8 ± 1.0</td>
<td>6.7 ± 1.3</td>
<td>6.0 ± 1.4</td>
<td>-0.8 ± 2.2</td>
</tr>
<tr>
<td>D-Ser + SP + H-7 ($n = 8$)</td>
<td>6.9 ± 1.2</td>
<td>6.4 ± 1.4</td>
<td>6.1 ± 1.2</td>
<td>-0.3 ± 1.3</td>
</tr>
</tbody>
</table>

$D$-Ser: D-serine 10 nmol; SP: 0.05 nmol; 7-CK: 1 pmol; H-7: 10 μmol.

**Hyperalgesia by SP** In D-serine group, D-serine alone at the dose of 10 nmol produced a decrease in TFL, but without statistical significance when compared with baseline ($P > 0.05$). However, in SP + D-serine group, when SP at the dose of 0.05 nmol was intrathecally applied 6 min prior to administration of D-serine 10 nmol, TFL was further shortened. TFL at 1.5 min post-D-serine was greatly less than that of baseline in this group ($P < 0.05$). $\Delta$TFL was -1.3 s ± 0.6 s and -2.3 s ± 1.8 s in D-serine and SP + D-serine group, respectively. Mean percentage of facilitation was -18.9 % ± 7.8 % and -31.8 % ± 22.9 % in D-serine and SP + D-serine group, respectively, indicating that SP potentiated the D-serine-induced responses (Tab 2). We chose the dose of SP 0.05 nmol due to the fact that, at this dose, SP alone did not alter the baseline TFL at 3 min after injection (form baseline 7.1 s ± 1.9 s to 6.9 ± 1.6 s, $n = 8$, $P > 0.05$).

**Blockade of SP-induced potentiation by H-7 or 7-CK** When 7-CK 1 pmol, selective glycine site antagonist, was co-administered with SP 0.05 nmol, SP-induced potentiation of D-serine action was completely prevented. Similarly, co-administration of H-7 10 μmol, non-selective PKC inhibitor, with SP 0.05 nmol also completely blocked SP-induced potentiation (Tab 2). Neither 7-CK 1 pmol nor H-7 10 μmol co-administration with SP greatly altered the baseline TFL at 3 min after injection.

**DISCUSSION**

D-serine has been presumed to be an endogenous ligand for the Gly$_{NMDAZ}$ site, as localization of D-serine and its biosynthetic enzyme approximate the distribution of NMDA receptors more closely than glycine$^{[11]}$. Occupation of Gly$_{NMDAZ}$ site by its agonist is an absolute requirement for NMDA receptor activation$^{[13]}$. The result that D-serine facilitated spinal thermal nociception was in agreement with the previous report$^{[14]}$. Our data showed that SP potentiated D-serine-induced acute thermal hyperalgesia, and this potentiation was blocked by 7-CK, selective antagonist for Gly$_{NMDAZ}$ site. It provided the behavioral evidence for involvement of the interaction of SP receptor and Gly$_{NMDAZ}$ site in mediating the spinal transmission of nociceptive information.

NMDA receptor-channel complex is composed of an NR1 subunit and at least one of the NR2 subunits (NR2A-D)$^{[15]}$. Gly$_{NMDAZ}$ site locates in NR1 subunit, which possesses several amino acid residues that could be phosphorylated by PKC$^{[16]}$. SP receptor belongs to G-protein-coupled receptor superfamily that is linked with phospholipase C$^{[17]}$. Activity of SP receptor increases production of IP$_3$ and DAG$^{[16]}$, which in turn activate PKC. It is, therefore, reasonable to assume that PKC may have a link role between SP and NMDA receptors. Activation and translocation of PKC triggered by SP may produce phosphorylation of NMDA receptor-channel complex including Gly$_{NMDAZ}$ site resulting in allosteric alteration of it. This alteration will increase in the affinity of the Gly$_{NMDAZ}$ site for D-serine. The present finding that H-7 blocked SP-induced potentiation of thermal hyperalgesia by D-serine supports this proposal. Since H-7 is a non-selective PKC inhibitor, a role of PKA could not be excluded out. However, our recent observation showed that the selective PKC inhibitor chelerythrine blocked both SP-induced increases in inward current and $[Ca^{2+}]_{i}$ by NMDA/glycine in the spinal dorsal horn neurons strengthened the view of PKC participation. Thus, modulation of Gly$_{NMDAZ}$ site by PKC mediates the interaction of SP and NMDA receptor and hence contributes to the transmission of nociceptive information in the spinal cord.
REFERENCES


P物质增强大鼠脑内注射D-丝氨酸诱发的热痛过敏

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关键词 N-甲基-D-天冬氨酸受体; 甘氨酸; P物质; 蛋白激酶C; 脊髓; 痛觉过敏

目的: 研究脊髓性糖信息传递中P物质(SP)与N-甲基-D-天冬氨酸(NMDA)受体甘氨酸位点激动剂D-丝氨酸(D-serine)之间的功能联系。方法: 在浅麻醉大鼠, 采用行为学方法, 测定尾反射潜伏期(TTL)并结合脊内给药途径观察药物作用。结果: 麻内注射D-serine 1000 mmol 毫升后1.5 分钟, TTL 显著缩短; 在注射D-serine 10 mmol 前6 分钟脊内施加SP 0.05 mmol, 显著增强D-serine 10 mmol 引起的TTL 缩短效应; 选择性NMDA受体甘氨酸位点拮抗剂7-氯-D-天冬氨酸1 mmol 及非选择性PKC抑制剂H-7 10 μmol 均可阻断这种增强作用。结论: SP可使D-丝氨酸诱发的热痛过敏明显加强, NMDA受体甘氨酸位点及胞内蛋白激酶系统参与了脊髓SP与NMDA受体的相互作用。