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# Substance P potentiates thermal hyperalgesia induced by intrathecal administration of *D*-serine in rats[1]

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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-chlorokynurenic acid 1 pmol, the selective antagonist for glycine regulatory site of NMDA receptor, or H-7 10 μmol, 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[1] and their receptors co-localize in some spinal dorsal horn neurons[2]. Previous electrophysiological results showed SP and SP receptor agonist potentiated *N*-methyl-*D*-aspartate (NMDA)-induced activity in dorsal horn neurons via calcium-dependent protein kinase C and A[3-5]. In the behavioral study, intrathecal co-administration of SP and NMDA produced more potent nociceptive responses than that of SP and NMDA given separately[6]. 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 ($Gly_{NMDAZ}$)[7,8]. There is considerable evidence for a regulatory role of $Gly_{NMDAZ}$ site in the spinal nociception[5,14]. HA-966, a $Gly_{NMDAZ}$ site antagonist, promoted SP receptor antagonist-induced antinociception in formalin pain model[9]. 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 $Gly_{NMDAZ}$ site antagonists[5]. 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 $Gly_{NMDAZ}$ site is functionally relevant to SP receptor-mediated regulation of nociception. To further open out the functional significance of $Gly_{NMDAZ}$ site, the present work was to examine the interaction of SP and *D*-serine, the endogenous ligand for $Gly_{NMDAZ}$[11], in the nociceptive behavior test of rats.

## MATERIALS AND METHODS

**Rats** Male Sprague-Dawley rats (Grade Ⅱ, Certificate № 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 ℃. 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 ℃ 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-isoquinolinesulfonyl)-2-methylpiperazine (H-7, 2 mol/L aqueous), and 7-chlorokynurenate (7-CK, 200 nmol/L in dimethyl sulfoxide). SP, *D*-serine, H-7, and 7-CK 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-CK + *D*-serine group and SP + H-7 + *D*-serine group, for rats in this two groups, 7-CK 1 pmol or H-7 10 μmol 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 $\bar{x} \pm s$. 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 × 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.** $\bar{x} \pm s$. $^bP < 0.05$ *vs* Pre-*D*-ser.

| *D*-Serine/nmol | Pre-*D*-ser/s | Post-*D*-ser/s | ΔTFL | Percent facilitation/% |
|---|---|---|---|---|
| 0.1 ($n = 5$) | 5.6 ± 0.2 | 5.7 ± 1.1 | −0.2 ± 1.1 | −2.6 ± 8.1 |
| 10 ($n = 6$) | 7.0 ± 1.8 | 5.7 ± 1.6 | −1.3 ± 0.6 | −18.9 ± 7.8 |
| 1000 ($n = 7$) | 6.6 ± 0.5 | 5.3 ± 0.8$^b$ | −1.2 ± 0.5 | −19.3 ± 8.6 |

Tab 2. Effects of different treatment on TFL after administration. $\bar{x} \pm s$. $^bP < 0.05$ *vs* Pre-*D*-ser.

| Group | Baseline/s | Post-pretreatment (or Pre-*D*-ser)/s | Post-*D*-ser/s | ΔTFL |
|---|---|---|---|---|
| Normal saline ($n = 8$) | | 7.0 ± 1.3 | 7.2 ± 1.2 | 0.2 ± 0.7 |
| *D*-Ser ($n = 6$) | | 7.0 ± 1.8 | 5.7 ± 1.6 | −1.3 ± 0.6 |
| *D*-Ser + SP ($n = 8$) | 7.1 ± 1.9 | 6.9 ± 1.6 | 4.6 ± 1.7$^b$ | −2.3 ± 1.8 |
| *D*-Ser + SP + 7-CK ($n = 8$) | 6.8 ± 1.0 | 6.7 ± 1.3 | 6.0 ± 1.4 | −0.8 ± 2.2 |
| *D*-Ser + SP + H-7 ($n = 8$) | 6.9 ± 1.2 | 6.4 ± 1.4 | 6.1 ± 1.2 | −0.3 ± 1.3 |

*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 ($P < 0.05$). ΔTFL 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 notably alter the baseline TFL at 3 min after injection (form baseline 7.1 s ± 1.9 s to 6.9 s ± 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 $NR_2$ subunits ($NR_{2A-D}$)[15]. $Gly_{NMDAZ}$ site locates in $NR_1$ 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[18], 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 alternation of it. This alternation 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.

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## P物质增强大鼠鞘内注射 *D*-丝氨酸诱发的热痛过敏[1]

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

**目的**: 研究脊髓伤害性信息传递中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**: 在浅麻大鼠, 采用行为学方法, 测定甩尾反射潜伏期(TFL)并结合鞘内给药途径观察药物作用. **结果**: 鞘内注射 *D*-serine 1000 nmol 后 1.5 分钟, TFL 明显缩短; 在注射 *D*-serine 10 nmol 前 6 分钟鞘内施加 SP 0.05 nmol, 明显增强 *D*-serine 10 nmol 引起的 TFL 缩短效应; 选择性 NMDA 受体甘氨酸位点拮抗剂 7-氯犬尿酸 1 pmol 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.

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