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The effects of substance P analogues on the scratching, biting and licking response induced by intrathecal injection of N-methyl-D-aspartate in mice

[1]Tsukasa Sakurada, Yoichi Manome, Koichi Tan-No, Shinobu Sakurada & Kensuke Kisara

Department of Pharmacology, Tohoku College of Pharmacy, 4-4-1 Komatsushima, Sendai 981, Aoba-ku, Japan

1 Intrathecal (i.t.) administration of N-methyl-D-aspartate (NMDA) elicited a dose-dependent behavioural response consisting of licking, biting and scratching in mice.

2 Repeated i.t. injections of 0.4 nmol NMDA, at 5 min intervals, resulted in the rapid development of desensitization to this NMDA-induced behavioural phenomenon.

3 The NMDA-induced response was dose-dependently inhibited by the simultaneous injection of a selective NMDA-receptor antagonist, D-2-amino-5-phosphonovaleric acid.

4 The substance P (SP) analogues [D-Pro^2, D-Trp^{7,9}] SP and [D-Arg^1, D-Trp^{7,9}, Leu^{11}] SP (spantide) inhibited NMDA-induced behavioural responses in a dose-dependent manner. However, [D-Phe^7, D-His^9] SP (6–11), a SP analogue selective for neurokinin$_1$ (NK_1) receptors, failed to inhibit NMDA-induced responses even at a dose of 4.0 nmol.

5 These results indicate that NMDA-induced behavioural responses are mainly mediated through NMDA receptors without affecting NK_1 receptors in the spinal cord.

Introduction

In recent years, much attention has been directed toward the study of the pharmacological and physiological effects of excitatory amino acids (EAAs) in the mammalian central nervous system (CNS). The EAAs are considered to function as neurotransmitters in the CNS. It is most likely that glutamate is one of the EAAs under discussion. Receptors for EAAs have recently been classified into three different types, N-methyl-D-aspartate (NMDA), quisqualate and kainic acid receptors, based on their sensitivity to these three agonists (Davies *et al.*, 1980; McLennan, 1981; Watkins & Evans, 1981). These three receptors are mostly characterized by using pharmacological treatments (Davies *et al.*, 1980; Watkins & Evans, 1981) and are ubiquitously but unevenly distributed in the mammalian CNS (Cotman *et al.*, 1987).

Several lines of evidence indicate that EAAs may be involved in the neurotransmission of painful stimuli in the spinal cord (Anis *et al.*, 1983; Aanonsen & Wilcox, 1987). This suggestion is supported by the fact that NMDA can induce biting and scratching behaviour resembling that of substance P (SP) when administered intrathecally (i.t.) in the behavioural assay (Aanonsen & Wilcox, 1986). The behavioural effects indicative of activation of sensory pathways are brought about by i.t. injections of not only EAAs, but also tachykinins (Hylden & Wilcox, 1981; 1983; Vaught *et al.*, 1984), somatostatin (Seybold *et al.*, 1982), bombesin (O'Donohue *et al.*, 1984), 5-hydroxytryptamine (5-HT) (Fasmer & Post, 1983), and muscarinic agonists (Raffa *et al.*, 1987).

The main purpose of the present investigation was to examine the effects of SP analogues on NMDA-induced behavioural responses in order to see the interaction with SP receptors in the mouse spinal cord.

Methods

Male ddY-strain mice weighing between 22–26 g were used for all experiments. Mice were housed in colony cages with food and water continuously available. Testing took place during the light period of a 12–12 h light-dark cycle. All i.t. injections were adapted from the method of Hylden & Wilcox (1980). The lumbar puncture was made in unanaesthetized mice at the L5 and L6 intervertebral space with a 28 gauge needle on a 50 μl Hamilton syringe. NMDA was dissolved in sterile artificial cerebrospinal fluid containing (g 1000 ml^{-1}): NaCl 7.4, KCl 0.19, $MgCl_2$ 0.19 and $CaCl_2$ 0.14 and administered in a total volume of 5 μl, with the exception of repeatedly injected NMDA. For repeated injections of NMDA, 0.4 nmol/5 μl was delivered twice at 5 min or 15 min intervals. Before the i.t. injection, the mice were adapted for 1 h to an individual plastic cage (22.0 × 15.0 × 12.5 cm) which served as the observation chamber. Immediately after the i.t. injection, the mice were placed in the transparent cage and the accumulated response time of scratching, biting, forepaw and hindpaw licking was measured for 5 min. The inhibitory actions of SP analogues and D-(−)-2-amino-5-phosphonovalerate (D-APV), an NMDA antagonist, were determined by co-administration of NMDA (0.4 nmol).

Drugs administered were NMDA (Nakarai Tesque, Inc., Kyoto, Japan), D-APV (Cambridge Research Biochemicals, Cambridge, England), [D-Pro^2, D-Trp^{7,9}] SP (DPDT-SP) and [Arg^1, D-Trp^{7,9}, Leu^{11}] SP (spantide) (Peptide Institute, Inc., Osaka, Japan). [D-Phe^7, D-His^9] SP (6–11) was synthesized by solid phase peptide methodology (Sakurada *et al.*, 1989).

Statistical analysis of the results included determination of ED_{50} values by the method of Litchfield & Wilcoxon (1949) and Dunnett's test for multiple comparisons after analysis of variance (ANOVA). All values are expressed as the mean ± s.e.mean.

Results

Behavioural response induced by intrathecally administered N-methyl-D-aspartate

Intrathecal administration of NMDA resulted in a characteristic behavioural response consisting of vigorous biting, licking and scratching, which peaked at 0–5 min and had disappeared at 15 min post-injection. As seen in Figure 1, a dose-dependent increase in the total time of biting, licking and scratching was observed following i.t. administration of

[1] Author for correspondence.

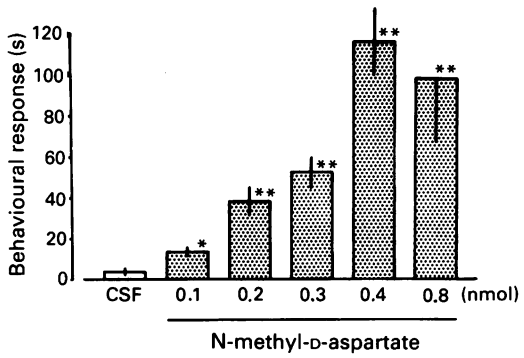

Figure 1 The effect of varying doses (0.1–0.8 nmol per mouse) of N-methyl-D-aspartate (NMDA) administered i.t. in the mouse. The duration of scratching, biting and licking induced by NMDA was determined over a 5 min period starting immediately after injection. * $P < 0.05$, ** $P < 0.01$ when compared with CSF-controls.

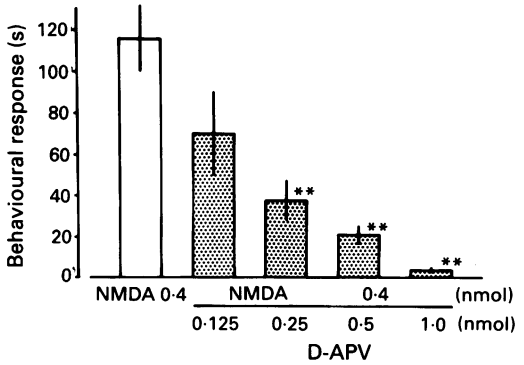

Figure 3 Effect of D-(−)-2-amino-5-phosphonovalerate (D-APV) on N-methyl-D-aspartate (NMDA)-induced scratching, biting and licking response in mice. D-APV was co-administered i.t. with NMDA in a 5 μl volume. The duration of scratching, biting and licking induced by NMDA was determined over a 5 min period starting immediately after injection. ** $P < 0.01$ when compared to NMDA (0.4 nmol) alone.

NMDA (0.1–0.8 nmol). The highest dose (0.8 nmol) of NMDA tested produced seizures in 80% of mice. NMDA-induced seizures were seen immediately after administration and subsided in 2–3 min. In further experiments, 0.4 nmol of NMDA was therefore used in combination with D-APV and SP analogues to test their antagonistic actions. Intrathecal injection of artificial CSF (5 μl) had no apparent effect on the behaviour of animals.

Mice were injected i.t. with 0.4 nmol of NMDA at 5 or 15 min intervals twice to assess the reproducibility of scratching, biting and licking response produced by NMDA. Desensitization to the behavioural response produced by i.t. administration of NMDA (0.4 nmol) was observed when administered at 5 min intervals (Figure 2); injection of 0.4 nmol of NMDA resulted in a significant decrease in the response to a second injection at 5 min intervals.

Effects of D-(−)-2-amino-5-phosphonovalerate and substance P analogues on N-methyl-D-aspartate-induced behavioural response

The selective NMDA receptor antagonist, D-APV (0.125–1.0 nmol), co-administered i.t. with NMDA (0.4 nmol) caused a dose-dependent inhibition of the NMDA-induced response (Figure 3). The ID_{50} for D-APV was 0.195 (0.126–0.303) nmol.

Interaction of putative tachykinin antagonists with the NMDA receptor agonist was investigated. When co-administered i.t. with DPDT-SP (1.0–4.0 nmol), the NMDA-induced response was reduced dose-dependently (Figure 4a).

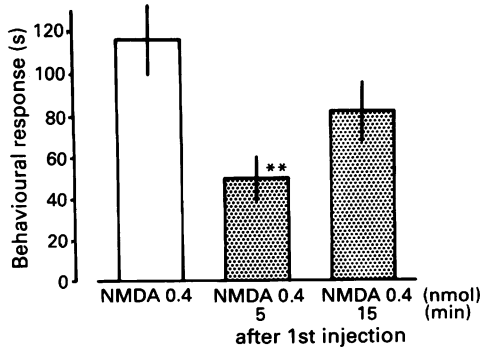

Figure 2 Comparison of the scratching, biting and licking response induced in the mouse by two i.t. injections of N-methyl-D-aspartate (NMDA) 5 or 15 min apart. The duration of scratching, biting and licking induced by NMDA was determined over a 5 min period starting immediately after injection. ** $P < 0.01$ when compared to a single injection of NMDA.

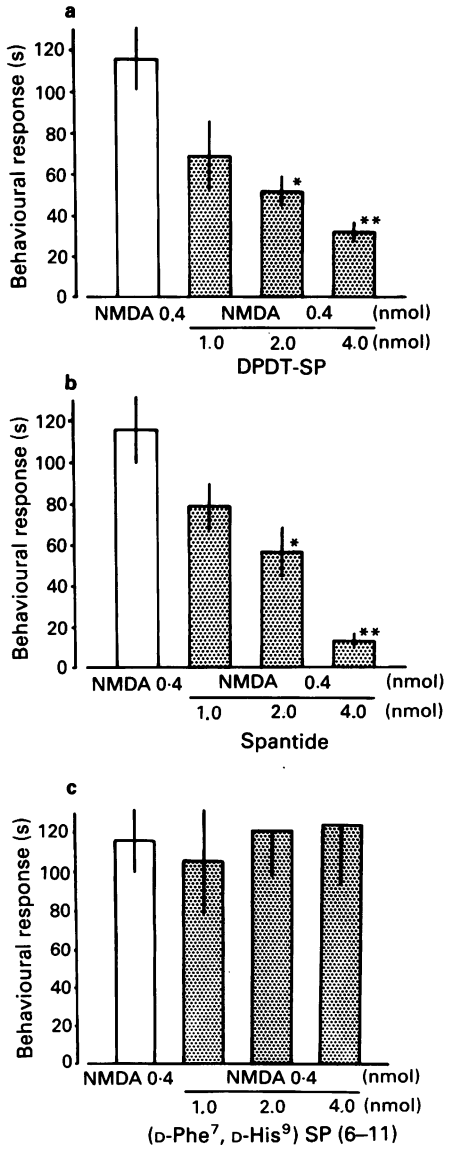

Figure 4 Effect of [D-Pro^2, D-Trp^{7,9}] substance P (DPDT-SP) (a), spantide (b) and (D-Phe^7, D-His^9)-SP (6–11) (c) on N-methyl-D-aspartate (NMDA)-induced scratching, biting and licking response in mice. The duration of scratching, biting and licking induced by NMDA was determined over a 5 min period starting immediately after injection. * $P < 0.05$, ** $P < 0.01$ when compared to NMDA (0.4 nmol) alone.

Spantide, a putative tachykinin antagonist, yielded results similar to those observed for DPDT-SP (Figure 4b). The ID_{50} for DPDT-SP and spantide was 1.66 (0.66–3.88) nmol and 1.70 (1.03–2.81) nmol, respectively. In order to characterize more fully tachykinin receptors, a shortened analogue of SP, (D-Phe^7, D-His^9) SP (6–11) with greater selectivity for spinal NK_1 receptors was used. However, (D-Phe^7, D-His) SP (6–11), in doses ranging from 1.0–4.0 nmol did not antagonize NMDA-induced behavioural response (Figure 4c).

Discussion

The principal finding of the present experiments is that these NMDA-induced behavioural responses are not mediated through spinal NK_1 receptors in mice. Furthermore, desensitization occurs to the behavioural response induced by repeatedly administered NMDA, suggesting that NMDA may act on the NMDA receptors directly in the spinal cord.

The present experiments showed that NMDA injected i.t. produced a dose-dependent behavioural response consisting of biting, licking and scratching. This NMDA-induced behaviour was dose-dependently blocked by co-administration of the NMDA antagonist, D-APV. These data are consistent with the hypothesis that NMDA may have a nociceptive action in the spinal cord (Aanonsen & Wilcox, 1986). It was previously reported that SP and NMDA are able to elicit a behavioural syndrome indicative of nociceptive behavioural response such as biting, licking and scratching (Hylden & Wilcox, 1981; Aanansen & Wilcox, 1986; Sakurada *et al.*, 1987). Both substances can also produce hyperalgesia as measured by the tail-flick and pressure tests in rats or mice (Yasphal *et al.*, 1982; Moochhala & Sawynok, 1984; Matsumura *et al.*, 1985b; Aanonsen & Wilcox, 1986). Desensitization to the actions of i.t. administered SP in eliciting scratching and biting response has been found in mice (Larson, 1988). Similarly, apparent desensitization has been reported to occur to the hyperalgesic effect of i.t. administered SP in rats in the tail-flick assay (Moochhala & Sawynok, 1984). In the present experiments, a similar phenomenon was observed with repeated i.t. injections of NMDA. Taking these results into consideration, i.t. administered NMDA is involved in the transmission of nociceptive information by acting on the spinal NMDA receptors.

The characteristic behavioural response produced by i.t. administered SP was inhibited by a number of SP analogues and opioid peptides (Hylden & Wilcox, 1983; Post & Folkers, 1985; Takahashi *et al.*, 1987; Sakurada *et al.*, 1987; 1988; 1989) when co-administered with SP. Of these SP analogues, DPDT-SP and spantide have often been used as pharmacological and physiological tools to study the participation of SP, though the specificity of SP analogues as antagonists has been discussed (Salt *et al.*, 1982; Stoppinin *et al.*, 1983; Wiesenfeld-Hallin *et al.*, 1987). DPDT-SP has been found to block the 5-hydroxytryptamine induced behavioural syndrome which is similar to that seen after injection of SP (Fasmer & Post, 1983; Vaught & Scott, 1988). Subsequently, we have reported that co-administered spantide resulted in a reduction of the behavioural response produced by not only SP but also by eledoisin, neurokinin A and somatostatin (Sakurada *et al.*, 1989). These results suggest that the two SP analogues do not have a selective action on the NK_1 receptor, at least, in the mouse spinal cord. In addition to the antagonistic effect at tachykinin receptors, neurotoxic actions of DPDT-SP have been reported: (1) I.t. administered DPDT-SP produced long-lasting hindlimb paralysis (Åkerman *et al.*, 1982; Rodgriguez *et al.*, 1983; Matsumura *et al.*, 1985a). (2) Microinjected DPDT-SP into the ventral tegmentum area led to wide-spread neuronal necrosis (Hökfelt *et al.*, 1981). (3). I.t. administered DPDT-SP produced a profound reduction in glutamate, glycine, γ-aminobutyric acid and aspartate (Sakurada *et al.*, 1990) as well as SP (Matsumura *et al.*, 1985a) in the spinal cord of paralysed rats. However, there were no significant changes in the level of amino acids and SP in non-paralysed rats. These alterations may be attributed to the neurotoxic action on the spinal cord (Post & Paulsson, 1985).

In the present study, i.t. co-administration of DPDT-SP produced a dose-dependent inhibition of NMDA-induced behavioural responses. However, this result is not consistent with the previous report that NMDA-induced behaviour was not significantly changed by co-administration of DPDT-SP (DeLander & Wahl, 1989). The difference in the results may be due to the difference in the dose of DPDT-SP used, and of the procedure for behavioural observation. However, the inhibitory effects of DPDT-SP and spantide on the NMDA-induced behavioural response is not due to their selectivity for NMDA receptors, as mentioned above. It is possible that NMDA-induced behaviour is mediated through NK_1 receptors in the spinal cord, since the behavioural response produced by tachykinins has been shown to be mediated mainly by NK_1 receptors (Sakurada *et al.*, 1989). The NMDA-induced behavioural response was also inhibited by co-administration of spantide, a putative tachykinin antagonist. Recently, we have found that a novel peptide (D-Phe^7, D-His^9) SP (6–11) is a selective NK_1 receptor antagonist (Sakurada *et al.*, 1989). SP-induced behavioural response was decreased to less than 20% by 2.0 nmol of this antagonist with an ED_{50} of 0.47 (0.23–0.95) nmol. Thus, this has a relatively low ED_{50} as compared to the ED_{50} values of DPDT-SP and spantide which were 1.88 and 1.00 nmol, respectively, when assayed against SP-induced behavioural responses. It is therefore probable that (D-Phe^7, D-His^9) SP (6–11) is a highly selective and potent NK_1 receptor antagonist in the spinal cord as compared to DPDT-SP and spantide. Moreover, this SP analogue is able to inhibit the SP-induced response without affecting the behavioural responses produced by NK_2 agonists such as neurokinin A and eledoisin, somatostatin and bombesin (Sakurada *et al.*, 1989). The results presented in this paper with the NK_1 receptor antagonist show that it did not antagonize the NMDA-induced behavioural response. Therefore, it seems evident that i.t. administered NMDA acts independently of SP receptors in the mouse spinal cord.

Our investigations have demonstrated the capacity for DPDT-SP and spantide to inhibit NMDA-induced responses. The finding that a selective antagonist for spinal NK_1 receptors, (D-Phe^7, D-His^9) SP (6–11) failed to antagonize NMDA-induced action reveals that i.t. administered NMDA does not interact with the NK_1 receptor to induce scratching, biting and licking responses.

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