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SPECIAL REPORT

Differential modulation of κ and μ opioid antinociception by the glycine/NMDA receptor agonist D-serine

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D-Serine, a selective agonist for the strychnine-insensitive glycine allosteric site associated with the NMDA receptor-ion channel complex, was found to modulate differentially the antinociception produced by κ and μ-opioid receptor agonists in the rat formalin test. D-Serine (100 μg, i.c.v.) attenuated the antinociception produced by the selective κ-opioid agonist, enadoline (0.003–0.1 mg kg^{-1}, s.c.) against the tonic, but not acute, phase of the formalin response. Conversely, D-serine potentiated the antinociception produced by morphine (0.3–10 mg kg^{-1}, s.c.) against both the acute and tonic phases. These results demonstrate an important interaction between the opioid and NMDA/glycine systems in the control of nociceptive information possibly at different levels of the neuraxis.

Keywords: Enadoline; morphine; rat formalin test; inflammatory pain

Introduction The excitatory amino acids (EAA) glutamate and aspartate play a major role in the mediation of formalin-induced nociception through an interaction with both non-NMDA (acute phase) and NMDA/glycine (tonic phase) types of receptors (Haley *et al.*, 1990). κ and μ opioid agonists exhibit potent antinociceptive properties against such prolonged tonic/chronic forms of noxious stimulation, particularly when there is an associated inflammatory component (Wheeler-Aceto & Cowan, 1991). κ- and μ-agonists have been shown to inhibit glutaminergic transmission through a predominantly pre- and postsynaptic mechanism of action, respectively, at both a spinal and supraspinal level (Allerton *et al.*, 1988; Lambert *et al.*, 1991; Pinnock, 1992). It is possible that such a modulatory influence may contribute towards the antinociceptive potency of the opioids against formalin-induced nociception. We have previously demonstrated that activation of the glycine-NMDA receptor indirectly modulates the anticonvulsant action of the κ-receptor selective agonist CI-977 (enadoline; Singh *et al.*, 1990). In the present study we have therefore investigated the potential interaction between the opioid system mediating antinociception and the glycine/NMDA receptor complex in the formalin model of acute and prolonged noxious stimulation.

Methods Male Wistar rats (60–90 g) were administered with 50 μl of formalin (5% formaldehyde) into the plantar surface (i.pl.) of the left hindpaw and the time (s) each animal spent licking and biting the injured paw recorded in 5 min bins for a total of 60 min. Cumulative data (mean ± s.e.mean) were then recorded for the early, acute phase (0–10 min) and the late, tonic phase (10–45 min). Enadoline/saline or morphine/saline were administered, respectively, as a 15 and 30 min subcutaneous pretreatment prior to formalin in groups of 6–10 animals. The intracerebroventricular (i.c.v.) injections were performed by exposing the skull of the rats under isoflurane anaesthesia. D-serine (100 μg)/saline was administered, 30 min prior to formalin, by direct injection (5 μl) into the 4th ventricle at lambda with a 5 mm long, 27 guage needle attached to a 10 μl Hamilton syringe and the wound sealed with epoxy resin. Similar injections of Evans Blue dye demonstrated a uniform distribution of injectate throughout the ventricular system within 10–15 min post-administration.

Statistical analyses of the data were carried out using a Mann-whitney U-test. At each dose-level of the opioid agonist, the group that had received D-serine as a pretreatment was compared with the group given the opioid drug plus saline (i.c.v.). A probability level of $P<0.05$ was regarded as significant. ED_{50} (dose producing a 50% reduction in the licking/biting response of control animals) values from graphs of the cumulative data were determined by GRAPHPAD and presented as the mean with 95% confidence limits.

Drugs used were enadoline (CI-977, ((5**R**)-(5α,7α,8β)-N-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide monohydrochloride (Parke-Davis Neuroscience Research Centre, Cambridge), morphine sulphate (Savory & Moore, Cambridge), D-serine (Sigma) and isoflurane (Abbot Laboratories, Kent).

Results In control animals receiving saline pretreatments (i.c.v. and s.c.), the formalin-induced acute and tonic phases of licking and biting lasted for 93 ± 10 and 337 ± 22 s ($n = 12$), respectively.

Enadoline (0.003–0.1 mg kg^{-1}, s.c.) caused a dose-dependent inhibition of both the acute and tonic phases of the formalin response with ED_{50} values of 0.009 (0.008–0.010) mg kg^{-1} and 0.007 (0.004–0.013) mg kg^{-1}, respectively (Figure 1a and b). Similarly, morphine caused a dose-dependent inhibition of both acute and tonic phases with ED_{50} values of 2.9 (1.3–6.1) mg kg^{-1} and 3.0 (2.2–4.0) mg kg^{-1}, respectively (Figure 1a and b).

D-Serine (100 μg, i.c.v.), administered 15 min before enadoline (0.01–0.1 mg kg^{-1}, s.c.), caused a significant inhibition of the antinociceptive response to enadoline, at 0.01 and 0.03 mg kg^{-1}, against the tonic phase of the formalin nociceptive response (Figure 1b) with a rightward shift in the control dose-response curve and an increase in the ED_{50} to 0.044 (0.033–0.060) mg kg^{-1}. D-Serine pretreatment had no significant effect on the enadoline-mediated inhibition of the acute phase at any dose (Figure 1a) as shown by the ED_{50} of 0.010 (0.009–0.011) mg kg^{-1}. Conversely, when administered immediately before morphine (0.3–10 mg kg^{-1}, s.c.), D-serine (100 μg, i.c.v.) caused a significant potentiation of the morphine antinociceptive effect, at 1 and 3 mg kg^{-1} against the

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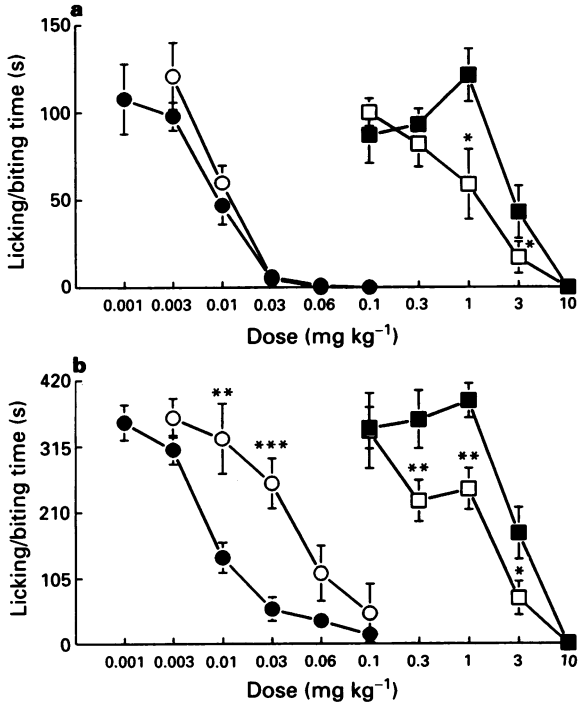

Figure 1 Dose-dependent (mg kg^{-1}, s.c.) effect of enadoline (●, ○) and morphine (■, □) on the (a) acute and (b) tonic phases of the formalin nociceptive response in the absence (solid symbols) and presence (open symbols) of D-serine (100 μg, i.c.v.). Significant differences between D-serine and the respective saline pretreated groups are indicated by asterisks ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, Mann-Whitney U-test).

acute phase (Figure 1a) and at 0.3, 1 and 3 mg kg^{-1} against the tonic phase (Figure 1b) of the formalin nociceptive response. A leftward shift was observed in the morphine dose-response curve with a decrease in the ED_{50} to 1.2 (0.5–2.6) and 1.3 (0.4–4.1) mg kg^{-1} for the acute and tonic phases, respectively.

D-Serine (100 μg, i.c.v.) had no significant effect on either the acute or tonic phase of the formalin-induced licking/biting response with values of 62 ± 18 s and 336 ± 53 s ($n = 6$), respectively. In saline (i.c.v.) pretreated control animals the respective values were 83 ± 8 s and 318 ± 22 s ($n = 8$).

Discussion The present study has demonstrated that opioid receptor mediated antinociception in the formalin model can be differentially modulated by the glycine-NMDA agonist D-serine. The most likely site for this interaction is in the spinal cord where κ and μ receptors are widely distributed throughout the superficial layers (laminae I and II) and lamina V of the dorsal horn, regions associated with nociceptive input and transmission (Stevens *et al.*, 1991).

The D-serine-mediated inhibition of tonic but not acute antinociception produced by the κ-selective agonist, enadoline (Hunter *et al.*, 1990), is consistent with the ability of enadoline to cause presynaptic inhibition of EAA release (Lambert *et al.*, 1991; Pinnock, 1992). Thus, since the glycine-NMDA receptor is located on the dorsal horn cells, D-serine would not be able to influence a presynaptic action of enadoline on the terminals of the primary afferents to reduce EAA release and, consequently, the EAA-evoked monosynaptic response (acute phase). In contrast, D-serine was able to reverse the inhibitory effect of enadoline on the subsequent polysynaptic response to formalin which has been associated with the sensitization and plasticity of dorsal horn neurones, particularly transmission neurones located in the deeper laminae (Haley *et al.*, 1990). This could be explained if the κ receptors involved in the modulation of the tonic phase were predominantly located on the terminals of spinal interneurons, *e.g.* immediately presynaptic to the transmission cells, especially those located in the deeper laminae of the dorsal horn. Of course, such an explanation cannot exclude the possibility that the antinociceptive effect of enadoline on the tonic phase may be mediated in part through activation of supraspinal κ receptors.

In contrast to the glycine-NMDA influence on the κ-opioid system, D-serine potentiated morphine-induced attenuation of *both* acute and tonic phases which would suggest a predominantly postsynaptic site of interaction, possibly on the spinal dorsal horn cells. The paradoxical effect of the glycine-NMDA agonist might therefore reflect modulation of distinct interneuronal pathways by the μ and κ opioid systems in the spinal cord. Alternatively, however, it may reflect possible differences in the anatomical localization of the interaction with, for example, a predominant involvement of supraspinal sites in the D-serine modulation of the morphine response. Thus, morphine antinociception has been shown previously to be potentiated by NMDA agonists injected into the periaqueductal grey (Jacquet, 1988) and nucleus raphe magnus (Van Praag & Frenk, 1990).

In conclusion, the present study has shown that an interaction appears to exist between two major systems in the control of nociceptive information, particularly following a prolonged noxious stimulus, that may have important consequences for the treatment of chronic pain.

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