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# EFFECT OF MORPHINE ON THE RELEASE OF EXCITATORY AMINO ACIDS IN THE RAT HIND INSTEP: PAIN IS MODULATED BY THE INTERACTION BETWEEN THE PERIPHERAL OPIOID AND GLUTAMATE SYSTEMS

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**Abstract—Behavioral evidence supports a role for peripheral glutamate receptors in normal nociceptive transmission. In this study, we examined the release of the excitatory amino acids, glutamate and aspartate, in the s.c. perfusate of the rat hind instep by *in vivo* microdialysis. Antidromic stimulation of the sciatic nerve and noxious stimuli in the form of heat stimulation and local application of capsaicin cream (1%) to the instep caused an increase in excitatory amino acid release. This capsaicin-induced excitatory amino acid release was suppressed by pretreatment with capsaicin. Both systemic (10 mg/kg, i.v.) and local injections ($10^{-5}$ M in the perfusate) of morphine inhibited the increase in excitatory amino acid release evoked by local application of capsaicin cream to the instep. This inhibitory effect of morphine was antagonized by naloxone either given systemically (5 mg/kg, i.v.) or locally ($10^{-5}$ M).**

**These results suggest that excitatory amino acids are released from small diameter afferent fibers by heat stimulation in the periphery or local application of capsaicin cream, and that activation of opioid receptors, present on the peripheral endings of small-diameter afferent fibers, can regulate noxious stimulus-induced excitatory amino acid release.**


**Key words: excitatory amino acids, small-diameter afferent fiber, axon-reflex, morphine, capsaicin, opioid receptor.**

Glutamate is a major excitatory neurotransmitter in the CNS. Recently glutamate has been shown to have a role in transduction of sensory input at the periphery (Carlton, 2001). Electron microscope studies demonstrate that glutamate receptors (GluRs) are transported from the dorsal root ganglion (DRG) cell bodies into central and/or peripheral primary afferent terminals (Liu et al., 1994). In the glabrous and hairy skin of the rat (Carlton et al., 1995; Coggeshall and Carlton, 1998), and in hairy skin in the human (Kinkelin et al., 2000), *N*-methyl-D-aspartic acid (NMDA), $\alpha$-amino-3-hydroxy-5methyl-4-isoxazole propionic acid (AMPA) and kainate receptors are localized on unmyelinated axons at the dermal-epidermal junction. Approximately 20% of the fibers analyzed immunostained for one of the receptor subtypes. It is highly likely that two or more of the ionotropic GluRs are colocalized as Sato et al. (1993) report that virtually all DRG cells as well as their central (Westlund et al., 1989) and peripheral (Westlund et al., 1992) processes are positively labeled for the NMDA receptor (Battaglia and Rustioni, 1988; Keast and Stephensen, 2000). The labeled unmyelinated fibers are assumed to be sensory fibers and not sympathetic efferents that are also unmyelinated. Behavioral evidence supports a role for peripheral GluRs in normal nociceptive transmission. Intraplantar injection of L-glutamate into the hind paw evokes hyperalgesia in rats (Follenfant and Nakamura-Craig, 1992; Carlton et al., 1995). In addition, intraplantar injection of the specific GluR agonists NMDA, AMPA or kainate results in mechanical hyperalgesia and allodynia that can be blocked by appropriate antagonists (Zhou et al., 1996).

This behavioral and anatomical data led us to propose that tissue-damaging stimuli would release glutamate, leading to increased C fiber activation and thus to hyperalgesia. Omote et al. (1998) showed that s.c. administration of inflammatory substances such as formalin induced the release of peripheral excitatory amino acids (EAAs) (glutamate and aspartate) on the ipsilateral side. We previously reported that stimulation of sensory afferent fibers released substance P (SP) from the peripheral endings of small-diameter afferent fibers into the extracellular space (Yonehara et al., 1987), and that this stimulation-induced SP release was suppressed by pretreatment with opioids (Yonehara et al., 1988, 1992a, 1993). We suggested the existence of opioid receptors on the peripheral endings of primary afferent fibers that regulate SP release from the peripheral nerve endings into the extravascular space (Yonehara et al., 1992a). In addition, a recent immunohistochemical study suggests that $\mu$-opioid receptors exist on human cutaneous sensory nerve fibers, which contribute to the regulation of neurogenic inflammation and skin sensations such as pain and itch (Ständer et al., 2002).

The purpose of this study was to examine whether EAAs, glutamate and aspartate could be released peripherally following stimulation of sensory afferents, and then

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*Abbreviations:* AMPA, $\alpha$-amino-3-hydroxy-5methyl-4-isoxazole propionic acid; DRG, dorsal root ganglion; EAA, excitatory amino acid; EST, electrical stimulation; GluR, glutamate receptor; HPLC, high-performance liquid chromatography; HST, heat stimulation; MOR, morphine hydrochloride; NLX, naloxone hydrochloride; NMDA, *N*-methyl-D-aspartic acid; SP, substance P.

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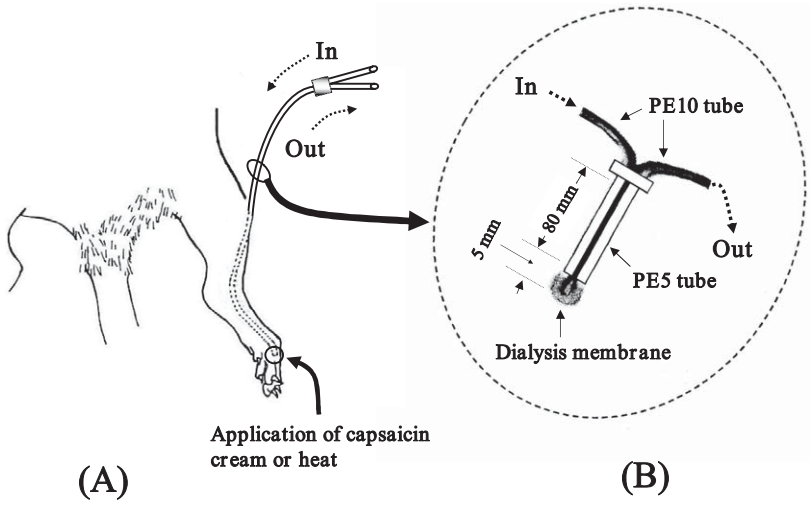


**Fig. 1.** (A, B) A schematic of the single loop catheter introduced into the s.c. space of a rat hind instep and the experimental arrangement for collection of samples, respectively.

whether the effects of this stimulation-induced EAA were modulated by treatment with a $\mu$-opioid receptor agonist.

## EXPERIMENTAL PROCEDURES

All surgical and experimental procedures for animals were reviewed and approved by the Osaka University Graduate School of Dentistry Intramural Animal Care and Use Committee and conformed to the guidelines of the International Association for the Study of Pain (Zimmermann 1983).

### Release of EAAs into the s.c. space

Male Sprague–Dawley rats (approx. 200–300 g body weight) were anesthetized with urethane (1 g/kg i.p.). A single loop catheter whose tip was covered with a 5000 molecular weight dialysis membrane (Fig. 1A; MS 0045, PSS® Select, Jacksonville, FL, USA) was introduced in to the s.c. space of the instep using a 2.2 mm outer diameter polyethylene tube as a guide (Fig. 1B). Ringer's solution was perfused at 15 $\mu$l/min through this catheter with a micro syringe pump (EP-60, Eicom, Kyoto, Japan) and perfusate was collected into the tubes placed in an ice bath at intervals of 20 min. The samples were kept at −80 °C until analysis.

### Electrical stimulation (EST)

The sciatic nerves were placed on bipolar enamel-insulated stimulating electrodes under liquid paraffin. Antidromic stimulation of the nerves for 20 min with a 10 V 2 Hz square wave, with a duration of 1 ms, 1 h or more after the beginning of perfusion produced, in addition to the rapidly conducting volley derived from A$\beta$, a complex series of waves containing conduction velocities ranging from less than 2 M/s to approx. 20 M/s (Yonehara et al., 1992a).

### Heat stimulation (HST)

The threshold temperature for heat-induced plasma extravasation is about 45 °C, which is similar to the threshold for excitation of cutaneous polymodal nociceptors (Fleischer et al., 1983) and a temperature of 47–48 °C induces a marked increase in release of SP and the formation of thermal edema (Yonehara et al., 1987). In this study, the hind instep in the immediate vicinity of the tip of the single loop catheter was stimulated for a period of 20 min using a probe with a 10 mm×10 mm square sensor area which was connected to a thermo-stimulator (UDH-104, Unique Medical Co., Ltd., Tokyo) Allowing the temperature to be adjusted to 50 °C.

**Table 1.** The basal value was taken as the average EAA concentration in two 20-min fractions collected before stimulation (100%)

| | Glutamate (*n*) (%) | Aspartate (*n*) (%) |
|---|---|---|
| EST | 493±28 (8)[a] | 201±29 (8)[a] |
| HST | 356±11 (8)[a] | 265±11 (8)[a] |
| Capsaicin cream | 442±30 (11)[a] | 233±20 (11)[a] |
| +Saline (i.v.) | 540±38 (10)[a] | 187±12 (10)[a] |
| +Capsaicin pretreatment | 199±11 (10)[b] | 144±20 (10)[b] |
| +Morphine (i.v.) | 121±16 (8)[b] | 77±8 (8)[b] |
| +Morphine+naloxone (i.v.) | 363±54 (12)[c] | 275±25 (12)[c] |
| +Saline (local peripheral administration) | 622±38 (6)[a] | 184±21 (6)[a] |
| +Morphine (local peripheral administration) | 136±12 (8)[d] | 79±8 (8)[d] |
| +Morphine+naloxone (local peripheral administration) | 319±22 (8)[e] | 212±12 (8)[e] |

The average EAA concentration in two 20-min fractions collected during/after stimulation were expressed as percentages of the control value before stimulation. EST, antidromic stimulation of the sciatic nerves; HST, thermal stimulation of the rat hind instep on the perfusion side; capsaicin cream, topical application of about 100 mg 1% capsaicin cream to the hind instep on the perfusion side. Values represent the mean±S.E.M., and the difference of the means was analyzed with the Student's *t*-test.

[a] Significant difference at $P<0.05$ between the basal values and those during/after stimulation.

[b] Significant difference at $P<0.05$ between saline (i.v.) and capsaicin pretreatment, or morphine (i.v.) in capsaicin cream-treated group.

[c] Significant difference at $P<0.05$ between morphine (i.v.) and morphine+naloxone (i.v.) in capsaicin cream-treated group.

[d] Significant difference at $P<0.05$ between saline (local peripheral administration) and morphine (local peripheral administration) in capsaicin cream-treated group.

[e] Significant difference at $P<0.05$ between morphine (local peripheral administration) and morphine+naloxone (local peripheral administration) in capsaicin cream-treated group.