Endomorphin-1 and Endomorphin-2 Modulate Responses of Trigeminal Neurons Evoked by N-Methyl-d-Aspartic Acid and Somatosensory Stimuli

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Endomorphin-1 and endomorphin-2 modulate responses of trigeminal neurons evoked by N-methyl-d-aspartic acid and somatosensory stimuli. J Neurophysiol 83: 3570–3574, 2000. The present study investigated the modulation of N-methyl-d-aspartate (NMDA)-evoked and peripheral cutaneous stimulus-evoked responses of trigeminal neurons by endomorphins, endogenous ligands for the \( \mu \)-opioid receptor. Effects of endomorphins, administered microiontophoretically, were tested on the responses of nociceptive neurons recorded in the superficial and deeper dorsal horn of the medulla (trigeminal nucleus caudalis) in anesthetized rats. Endomorphin-1 and endomorphin-2 predominantly reduced the NMDA-evoked responses, producing an inhibitory effect of 54.1 ± 2.96% (mean ± SE; \( n = 34 \), \( P < 0.001 \)) in 92% (34/37) of neurons and 63.6 ± 3.61% (\( n = 32 \), \( P < 0.001 \)) in 91% (32/35) of neurons, respectively. The inhibitory effect of endomorphins was modality specific; noxious stimulus-evoked responses were reduced more than nonnoxious stimulus-evoked responses. Naloxone applied at iontophoretic current that blocked the inhibitory effect of \([D-Ala^2, N-Me-Phe^4, Gly^5]-ol\]enkephalin, reduced the peak inhibitory effect of endomorphins on the NMDA- and natural stimulus-evoked responses. We suggest that endomorphins by acting at \( \mu \)-opioid receptor selectively modulate noxious stimulus-evoked responses in the medullary dorsal horn.

INTRODUCTION

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH\(_2\)) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH\(_2\)) isolated recently from the bovine (Zadina et al. 1997) and human (Hackler et al. 1997) brains have been suggested to be the endogenous ligands for the \( \mu \)-opioid receptor (Zadina et al. 1997). The presence of a dense aggregation of endomorphin-like immunoreactive elements in the superficial dorsal horns of the medulla and spinal cord indicates that endomorphins are likely to modulate nociceptive transmission (Martin-Schild et al. 1997, 1999; Wu et al. 1999). Behavioral evidence indicates that the peptides produce a potent and prolonged analgesia in mice (Zadina et al. 1997) and rats (Przewlocka et al. 1999).

We have been investigating the opioid-mediated modulation of somatosensory mechanisms in the medullary dorsal horn (trigeminal nucleus caudalis) (Mokha 1992; Wang et al. 1996, 1999; Zhang et al. 1996), a region considered important for the relay of somatosensory information originating from nociceptors, thermoreceptors, and mechanoreceptors in the orofacial region (reviewed in Light 1992; Sessle 1987). Glutamate, a putative excitatory neurotransmitter, is present in trigeminal primary afferent fibers (Clements et al. 1991; Watkins and Evans 1981; Wilcox 1993), and acts on N-methyl-d-aspartic acid (NMDA), non-NMDA ionotropic and metabotropic receptors. NMDA, non-NMDA ionotropic and metabotropic receptors are present in the medullary dorsal horn, especially in its superficial laminae (Kondo et al. 1995; Tallaksen-Greene et al. 1992). The NMDA receptor, in particular, has been shown repeatedly to be involved in mediating nociceptive neurotransmission and neural plasticity (hyperalgesia) in the spinal dorsal horn (reviewed in Wilcox 1993; Willis et al. 1996).

The functional significance of endomorphins in the trigeminal system remains unknown. Further, the role that endomorphins might play in modulating the NMDA-evoked and natural stimulus-evoked responses of nociceptive neurons has not been investigated previously in vivo studies. The present study was therefore designed to investigate the effects of endomorphins administered microiontophoretically on the NMDA-evoked and natural cutaneous stimulus-evoked responses of physiologically characterized nociceptive neurons in the medullary dorsal horn.

METHODS

Subjects, recording, and drug administration procedures

Techniques used for animal preparation, neuronal recording, and classification of trigeminal neurons have been described previously (Wang et al. 1996, 1999). Experiments were performed on 34 male Sprague-Dawley rats (body wt 240–350 g, Harlan Sprague Dawley, Indianapolis, IN) anesthetized with urethan (1.5 g/kg ip, initial dose). Subsequently, a smaller dose of urethan was given intravenously, if and when necessary, to maintain a stable level of anesthesia. The electrical activity of the heart and rectal core temperature were monitored continuously. The exposed surface of the medulla was covered with agar (4% agar in normal saline at \( \sim 40^\circ \)C) to improve the stability of recording from neurons in the superficial dorsal horn of the medulla. Extracellular single-unit recordings were made from nociceptive neurons using the central barrel of a seven-barrel micropipette (MS-7PB, tip diam 5–8 \( \mu \)m; impedance: 4–6 M\( \Omega \), Medical Systems, Harvard apparatus). The remaining barrels were filled with freshly made solutions of the following drugs: endomorphin-1 (10 mM in double-distilled water, pH 5.0); endomorphin-2 (10 mM in double-distilled water, pH 5.0); \([D-Ala^2, N-Me-Phe^4, Gly^5]-ol\]enkephalin made solutions of the following drugs: endomorphin-1 (10 mM in double-distilled water, pH 5.0); endomorphin-2 (10 mM in double-distilled water, pH 5.0); \([D-Ala^2, N-Me-Phe^4, Gly^5]-ol\]enkephalin...
(DAMGO, 10 mM in double-distilled water, pH 4.0); NMDA (50 mM in 150 mM NaCl, pH 8.0); γ-aminobutyric acid (GABA, 250 mM in 160 mM NaCl, pH 4.5); naloxone hydrochloride (10 mM in double-distilled water, pH 5.0); and 2 M NaCl for current balancing. All drugs, except NMDA, were ejected with positive current, whereas NMDA was ejected with negative current. Retaining currents of 5–10 nA were used routinely to prevent drug diffusion. Controls (pH and current) were performed as described previously (Zhang et al. 1996). All drugs, except endomorphin-1 and endomorphin-2, were obtained from Sigma Chemical (St Louis, MO). Endomorphin-1 and endomorphin-2 were purchased from Research Biochemicals International (Natick, MA).

Stimulation, data analyses, and histological procedures

Neurons were characterized as nociceptive specific (NS, responding only to noxious stimuli) and wide dynamic range (WDR, responding to noxious and nonnoxious stimuli). Noxious mechanical stimuli were applied briefly (3–5 s) at 3- to 5-min intervals with an arterial clip. Noxious thermal stimuli (58°C) were applied for 15 s starting from a baseline of 36°C (rise time: 10 s; 2°C/s) at 3- to 5-min intervals using a radiant heat stimulator (Beck et al. 1974). Brush stimuli were applied at 3- to 5-min intervals using a hand-held camel hair brush. Responses (total number of spikes/stimulus) evoked by at least three to five applications of natural stimuli or responses evoked by cyclical administration of NMDA over 5 min prior to the application of a test drug served as the control responses. In most cases, iontophoretic currents for NMDA were adjusted to generate peak firing rates of 40–60 Hz. Responses during iontophoresis of a test drug were compared with the control responses. The effect produced by the application of a test drug was defined as inhibitory or excitatory only when the NMDA-evoked responses differed from the mean of the control responses by ±2 SD in the same direction (facilitation or inhibition). The peak percentage change in the number of spikes was calculated by comparing the mean of the lowest number of spikes evoked by three to five applications of NMDA following applications of a test drug to the control responses. Group data are expressed as means ± SE. Statistical analysis was performed using the paired t-test and one-way ANOVA (followed by Student-Newman-Keuls test), and a probability level of <0.05 was considered significant. Some recording sites were marked and reconstructed as described previously (Wang et al. 1996, 1999).

RESULTS

Effects of microiontophoretically applied endomorphin-1 and endomorphin-2 were respectively tested on the NMDA-evoked responses of 37 neurons (8 NS, 29 WDR) and 35 neurons (4 NS, 31 WDR) in the medullary dorsal horn. Endomorphin-1 (10–70 nA) produced a peak inhibitory effect of 54.1 ± 2.96% (n = 34, P < 0.001) on the NMDA-evoked responses in 92% (34/37) of neurons (8 NS, 26 WDR, Fig. 1B). In general, the inhibitory effect was short-lasting (Fig. 1B). Facilitation was observed in three neurons (3 WDR). Similarly, endomorphin-2 (10–70 nA) produced a peak inhibitory effect of 63.6 ± 3.61% (n = 32, P < 0.001) on the NMDA-evoked responses in 91% (32/35) of neurons (4 NS, 28 WDR, Fig. 1A). The inhibitory effect was also short-lasting (Fig. 1A). Facilitation was observed in three neurons (3 WDR).

Microiontophoretic applications of endomorphin-1 and endomorphin-2 were tested respectively on the natural stimulus-evoked responses in 16 neurons (7 NS, 9 WDR) and 17 neurons (5 NS, 12 WDR). Endomorphins primarily reduced the natural-stimulus-evoked responses of nociceptive neurons. The inhibitory effect on a nociceptive-specific neuron located in the superficial dorsal horn is illustrated in Fig. 2. The inhibitory effects of endomorphins were more prolonged on noxious stimulus-evoked responses than NMDA-evoked responses, particularly the inhibition evoked by endomorphin-2 (Fig. 2, A and B). Endomorphin-1 produced a peak inhibitory effect of

![Fig. 1](http://jn.physiology.org/doi/abs/10.1152/jn.1997.277.7.3571)
55.6 ± 6.87% (n = 9, 2 NS, 7 WDR, Fig. 3A) and 74.8 ± 7.80% (n = 6, 4 NS, 2 WDR, Fig. 3A) on the pinch- and heat-evoked responses, respectively. In contrast, endomorphin-1 produced only a small reduction of 11.3 ± 6.01% (n = 6, 6 WDR, Fig. 3A) on the brush-evoked responses. Similarly, endomorphin-2 produced a peak inhibitory effect of 57.8 ± 5.7% (n = 10, 2 NS, 10 WDR, Fig. 3B) and 86.8 ± 6.61% (n = 4, 3 NS, 1 WDR, Fig. 3B) on the pinch- and heat-evoked responses, respectively. The brush-evoked responses were reduced only 7.1 ± 6.6% (n = 10, 10 WDR, Fig. 3B).

Naloxone applied iontophoretically at currents that blocked the effects of DAMGO, a μ-opioid receptor agonist, blocked or reduced the inhibitory effects of endomorphin-1 and endomorphin-2 by 70.39% (n = 9, P < 0.01, Fig. 1A) and 65.96% (n = 10, P < 0.01, Fig. 1B) on the NMDA-evoked responses, respectively. The antinociceptive effects of endomorphins on the noxious stimulus-evoked responses were also reduced by iontophoretic application of naloxone (Fig. 2). However naloxone applied at identical parameters (current × time) did not alter the inhibitory effects of GABA on the same neurons.

**DISCUSSION**

This is the first in vivo electrophysiological study that examined the effects of endomorphins on the NMDA-evoked and natural-stimulus-evoked responses of nociceptive neurons. The present results indicate that endomorphins primarily produced inhibitory effects on the NMDA-evoked responses and the noxious natural-stimulus-evoked responses through a naloxone-sensitive opioid receptor in the medullary dorsal horn. Our observations are consistent with the behavioral observations demonstrating an analgesic effect of endomorphins administered intracerebroventricularly (Zadina et al. 1997) or intrathecally in mice (Stone et al. 1997; Zadina et al. 1997) and rats (Przewlocka et al. 1999), and with the electrophysiological observations demonstrating the inhibitory effects of endomorphins on the C-fiber-evoked responses of spinal dorsal horn neurons in the rat (Chapman et al. 1997). The inhibitory effect of endomorphins is presumably mediated by postsynaptic inhibitory mechanisms since endomorphins have been shown to hyperpolarize substantia gelatinosa neurons (Wu et al. 1999). However, presynaptic mechanisms may also play a role since endomorphins have been shown to decrease peripheral stimulus-evoked excitatory postsynaptic potentials (Wu et al. 1999) and inhibit high-threshold Ca\(^{2+}\) channel currents (Higashida et al. 1998).

The time course of the inhibitory effect of endomorphins on the NMDA-evoked responses in the present study is shorter as compared with the long-lasting effects observed in some behavioral (more than an hour) (Przewlocka et al. 1999; Zadina et al. 1997) and electrophysiological (Chapman et al. 1997) studies. However, endomorphins did produce a longer-lasting
and endomorphin-2, Gi2 is only involved in mediating the antinociceptive effects of both endomorphin-1 and endomorphin-2 subunits of the Gi protein, such as Gi1, Gi3 and Gz, are involved in mediating the antinociceptive effects of endomorphins. Although many intracellular effector mechanisms mediate effects of endomorphins, it is possible that both shared and separate intracellular effector mechanisms or different intracellular effector mechanisms or different firing patterns generated by NMDA receptor activation. Therefore the predominantly inhibitory modulation of the NMDA-evoked and noxious-stimulus-evoked responses of nociceptive neurons suggests that endomorphins are involved in producing an antinociceptive effect at the level of the medullary dorsal horn by acting at the μ-opioid receptor.

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