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

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INTRODUCTION

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH₂) 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 μ-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 (trigeminal nucleus caudalis) in anesthetizedrats. 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-Ala²,M-Ne-Phe³,Gly⁵-ol]-enkephalin, reduced the peak inhibitory effect of endomorphins on the NMDA- and natural stimulus-evoked responses. We suggest that endomorphins by acting at μ-opioid receptor selectively modulate noxious stimulus-evoked responses 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 ~40°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 μm, impedance: 4–6 MΩ, 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-Ala²,M-Ne-Phe³,Gly⁵-ol]-enkephalin...
(DAMGO, 10 mM in double-distilled water, pH 4.0); NMDA (50 mM in 150 mM NaCl, pH 8.0); α-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. A: endomorphin-2 and [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO) significantly reduced the N-methyl-D-aspartate (NMDA)-evoked responses [counts = spikes/bin (1 s)] of a wide dynamic range (WDR) neuron in the deeper dorsal horn of the medulla and naloxone antagonized the inhibitory effects of endomorphin-2 and DAMGO. B: endomorphin-1 significantly reduced the NMDA-evoked responses and naloxone antagonized the inhibitory effect of endomorphin-1 on the same neuron. Note the different scale range used for the x axis in A and B.](http://jn.physiology.org/content/357/1/351/F1.large.jpg)
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 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
The inhibitory effect on the noxious stimulus-evoked responses in the present study. These differences in the duration of the inhibitory effect could result from a number of factors including, different intracellular effector mechanisms or different mechanisms of action at a membrane level versus at an intracellular level and different firing patterns generated by NMDA and noxious stimuli. Further, endomorphin-2 produced a longer-lasting effect compared with that of endorphin-1. It is possible that both shared and separate intracellular effector mechanisms mediate effects of endomorphins. Although many subunits of the G protein, such as G11, G13 and G2, are involved in mediating the antinociceptive effects of both endorphin-1 and endomorphin-2, G12 is only involved in mediating the antinociceptive effect of endomorphin-2 (Sánchez-Blázquez et al. 1999). Further, endomorphin-2 appears to be more prevalent in the superficial laminae of the medullary dorsal horn than endorphin-1 (Martin-Schild et al. 1997, 1999; Wu et al. 1999). In contrast to our findings, behavioral observations indicate that the antinociceptive effect of endomorphin-2 is less potent than endorphin-1 administered intracerebrally (Przewlocka et al. 1999). The differences may arise from differential modulation of sensory versus motor circuits and different drug concentrations attained in behavioral and electrophysiological studies. Our observations that the inhibitory effect of endomorphins is modality-specific are consistent with the endomorphin-2 induced selective modulation of C-fiber-evoked responses reported in the spinal dorsal horn (Chapman et al. 1997).

Naloxone applied at a current that antagonized the effects of DAMGO, a selective μ-opioid receptor agonist, reduced the inhibitory effects of endomorphins, indicating that the inhibitory effect induced by endomorphins is mediated by μ-opioid 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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