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Suppressive effect of spinal dorsal-horn neuronal activity by local spinal-cord cooling is reversed by naloxone in cats

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Abstract

Purpose. The purpose of this study was to assess the effect of local spinal cord cooling on spinal dorsal-horn neuronal activity, with special emphasis on the role of endogenous opioid.

Methods. Decerebrate, spinal-cord-transected cats ($n = 30$) were subjected to local spinal-cord irrigation, using 0.9 N saline solution (15°C; $n = 15$, and 35°C; $n = 15$) for 90 min. The extracellular, single-cell activity of spinal dorsal-horn neurons responding to noxious stimulation was recorded. Sixty-one minutes after induction of local spinal-cord irrigation, naloxone (0.1 mg·kg^{-1}) was administered intravenously. Local spinal-cord blood flow was measured using the hydrogen clearance technique.

Results. Local spinal cord cooling produced significant suppression of both spontaneous and evoked activity (33.1 ± 7.7% and 31.4 ± 5.5%, respectively; mean ± SE). Naloxone reversed this suppression immediately. Local spinal-cord blood flow was significantly reduced during spinal-cord cooling, but naloxone did not change local spinal-cord blood flow.

Conclusion. The results demonstrate that endogenous opioids may play an important role in dorsal-horn neuronal suppression induced by local spinal-cord cooling.

Key words Spinal cord · Dorsal-horn neuron · Hypothermia · Naloxone · Opioid

Introduction

The protective effects of hypothermia have been demonstrated in numerous experimental models of cerebral and spinal ischemia. In the clinical field, hypothermia has been employed in major vascular surgeries, such as thoracic aortic aneurysms. These observations also led to the employment of hypothermia in the form of local spinal-cord cooling [1–3]. In addition, the use of hypothermia on portions of the nervous system, such as subarachnoid irrigation with cold solution, has been reported to reduce pain in humans [4].

The purpose of this study was to assess the effect of local spinal-cord cooling on spinal dorsal-horn neuronal activity. We hypothesized that the antinociceptive effect of local spinal-cord cooling would involve the endogenous opioid system.

Materials and methods

Experimental procedure

After obtaining approval from the institutional Animal Ethics Committee, we prepared 30 cats of either sex, weighing 2.5 to 4.5 kg, with tracheostomy, cannulation of the common carotid artery for monitoring blood pressure, and cannulation of the external jugular vein for fluid (lactate Ringer's solution, 10 ml·kg^{-1}·h^{-1}) and drug administration under isoflurane (1%–2%), nitrous oxide (70%), and oxygen anesthesia. Muscle paralysis was maintained by pancuronium bromide (initial dosage of 0.5 mg·kg^{-1}, followed by intermittent dosage of 0.2 mg·kg^{-1} every 1 h) during mechanical ventilation. The head was restrained in a stereotactic apparatus and decerebration was performed by bilateral electrolytic lesions placed in the midbrain reticular formation. The vertebral column was immobilized with metal clamps, which held the spinal processes at T-7 and L-1. The pelvis was supported by stays at both sides. The spinal cords were transected at the T-11, T-12 level. Spinal cord transection allowed us to monitor drug effects on the dorsal horn of the spinal cord in the absence of descending supraspinal influences. Laminectomy was performed from the L2 through S1 vertebral level. The dura was opened, and the exposed lumbar spinal cord

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was then covered with normal saline solution at a temperature of 37°C. Anesthetics were then discontinued and the animals were ventilated with 100% oxygen. Arterial blood pressure, ECG, pulse rate, and endtidal CO2 were recorded continuously and kept within physiologically normal ranges. Body temperature was maintained at 36%–37°C (esophageal temperature) by use of a feedback-controlled heating plate (model ATB-1100; Nihon Kohden, Tokyo, Japan). Spinal cord temperature was monitored by a digital thermometer (model M405; Nihon Kohden) with a probe placed on the spinal cord surface.

Measurement of dorsal-horn neuronal activity

A tungsten rod, platinum-sheathed microelectrode with an exposed tip of 1 to 2 μm was then inserted into the lumbar spinal cord near the L-7 root entry lateral zone, using a hydraulic microdrive. Single-unit activity from the recording microelectrode was amplified (MEG-1100; Nihon Kohden), filtered (0.3–5 kHz), and monitored with a dual-beam oscilloscope (VC-9; Nihon Kohden). Spikes were converted to pulses with a window discriminator (EN-601J; Nihon Kohden) and counted by a pulse counter (ET-612J; Nihon Kohden). When a single neuron was isolated, the cell type was identified by the spontaneous firing pattern, and by its response profile to peripheral stimuli (air puff, brush, and noxious pinch with forceps). Those neurons excited not only by noxious pinch but also responsive to non-noxious stimuli were classified as wide dynamic range (WDR) neurons and were used in this study. Units were observed for 30 min after isolation to obtain a stable firing pattern and to allow recovery from the effects of transient tissue distortion by the microelectrode. When recordings were started, at least 3 h had passed after endtidal isoflurane had disappeared. Noxiously evoked activity was produced by applying a pinching stimulus to a fixed site in the receptive field on the hind paw, using a quantitative pain-stimulating system (S-4896; Nihon Kohden), as described elsewhere [5]. Spikes were counted for 5 s after applying the pinching stimulus, and were converted to mean values (spikes/s).

Irrigating technique and drugs

An inflow stream of 0.9 N saline solution (15°C and 35°C, using an irrigating series pump [C-301; Sibata, Tokyo, Japan]) was applied at the level of S1, with the outflow catheter at L5 (Fig.1). After the irrigation with saline solution, spontaneous and noxiously evoked activities were measured every 15 min for 60 min. Sixty-one minutes after induction of local spinal-cord irrigation, 0.1 mg·kg^{-1} i.v. of naloxone was given, and then activity was observed for 25 min.

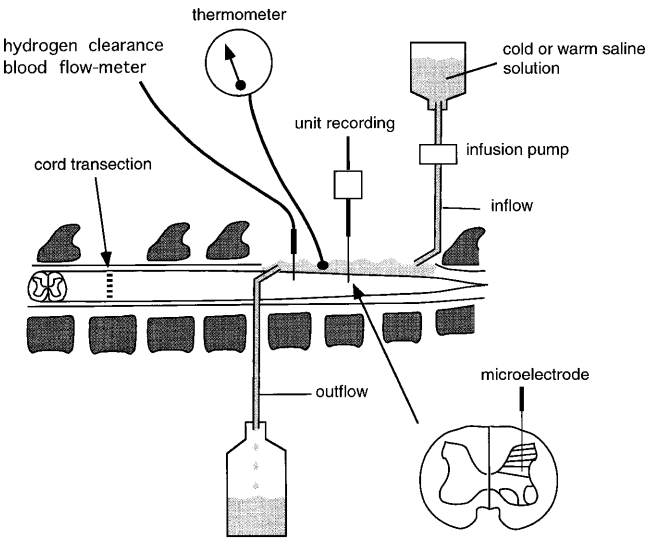

Fig. 1. Schematic drawing showing the local spinal-cord cooling, extracellular unit recording of spinal dorsal-horn neurons, and the local spinal-cord blood flow measurement

Blood flow measurement

Local spinal cord blood flow was measured by the hydrogen clearance technique, using Fick's principle, with the electrode inserted to the spinal cord at a level of L6.

Data analysis

Spinal dorsal-horn neuronal activity was transformed to relative values, and all results were expressed as means ± SEM. Comparisons between pre- and post-drug effects at each time were carried out by analysis of variance, and the significance of the difference between each time was calculated by Fisher's protected least significant difference. Differences were considered to be significant if P values were less than 0.05.

Results

There were no significant differences in esophageal temperature, arterial oxygen tension, carbon dioxide tension, pH, and mean arterial blood pressure between the control, cooling, and after-naloxone period in either of the groups (groups as stated below).

Cold saline group (15°C; n *= 15)*

The surface cord temperature the during cooling period was 21.7 ± 0.2°C. Figure 2*A* shows an example of the activity of the spinal dorsal-horn neurons before cool-

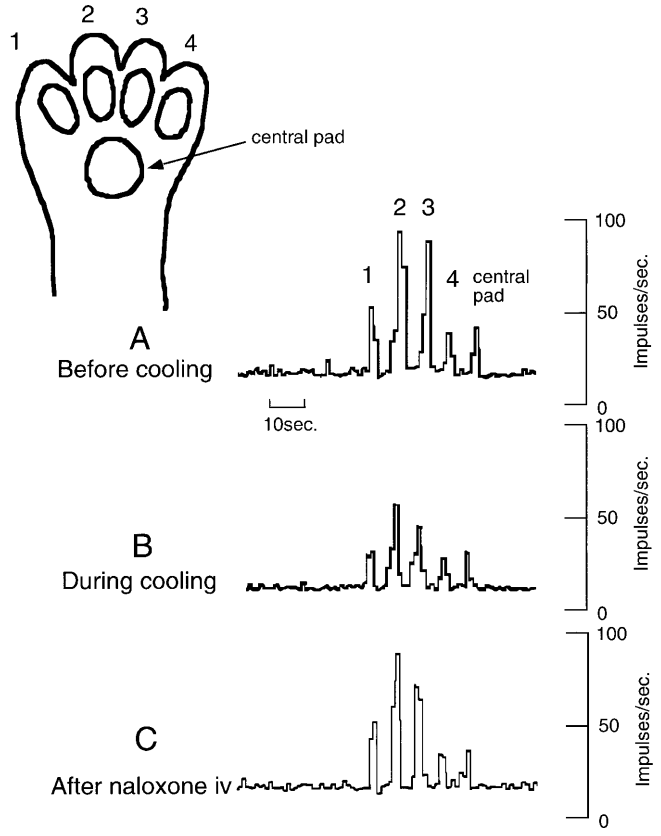

Fig. 2. An example of the response profile of noxiously evoked activity of the spinal dorsal-horn neuron. *A* The evoked activity of a single dorsal-horn neuron before cooling. *B* During cooling, spontaneous and evoked activities were suppressed. *C* After naloxone (0.1 mg·kg^{-1}) i.v., the activity returned to the control level

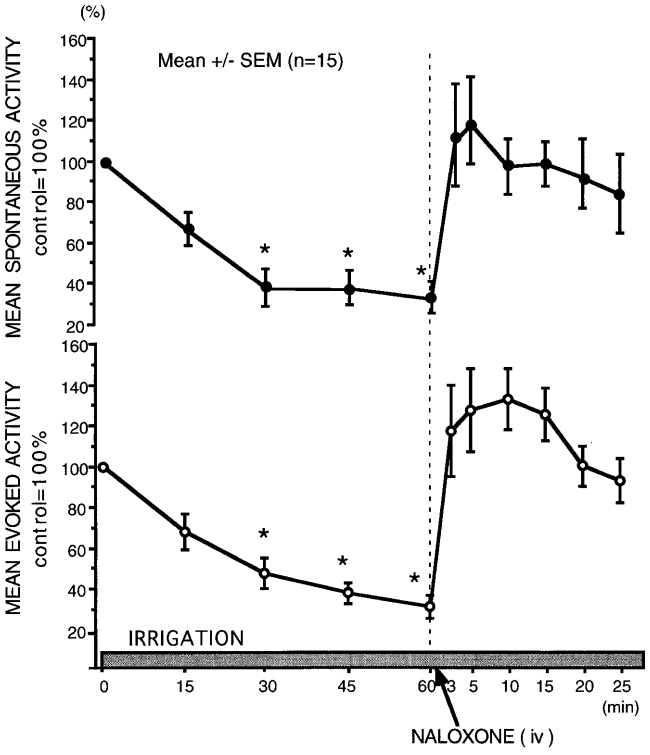

Fig. 3. The effects of cold saline irrigation on the mean spontaneous and evoked activities of the spinal dorsal-horn neurons (expressed as percentages of the control values along the time course). Cold saline irrigation (15°C) produced distinct suppression of the activity of spinal dorsal-horn neurons (*$P < 0.05$ versus the control values). Naloxone (0.1 mg·kg^{-1}, i.v.) reversed the suppressive effect of local spinal-cord cooling immediately. Fifteen cats were used

ing. Figure 2*B* demonstrates that, during local spinal-cord cooling, the activity of the spinal dorsal-horn neurons was suppressed. Figure 2*C* shows that, after naloxone i.v., the activity of the spinal dorsal-horn neurons returned to the control level. The effects of local spinal-cord cooling on the spontaneous and evoked activities of the spinal dorsal horn are summarized in Fig. 3. At 30, 45, and 60 min after induction of local spinal-cord cooling, spinal dorsal-horn neuronal activity was significantly lower than the control values ($P < 0.005$). Naloxone (0.1 mg·kg^{-1} i.v.) produced a significant reversal of the local spinal-cord cooling-induced suppression of spinal dorsal-horn neuronal activity ($P < 0.005$). Local spinal-cord blood flow was significantly reduced during local spinal-cord cooling, but naloxone did not change the local spinal-cord blood flow (Fig. 4).

Warm saline group (35°C)

The surface cord temperature during irrigation was 35.3 ± 0.3°C. No significant changes in the spontaneous and

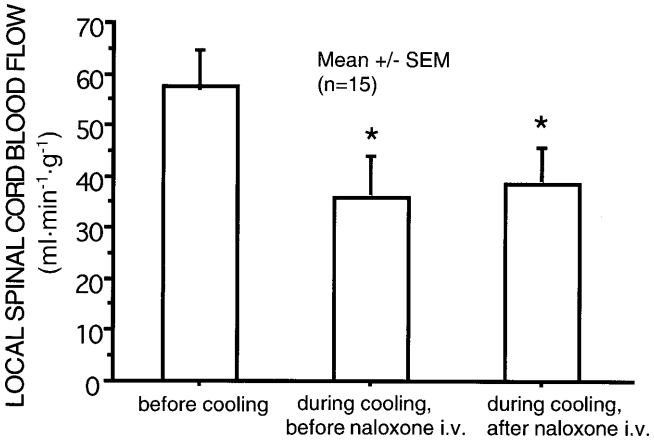

Fig. 4. Local spinal-cord blood flow was significantly reduced during local spinal-cord cooling (*$P < 0.05$ versus before cooling). However, there were no significant differences between the before- and after-naloxone (0.1 mg·kg^{-1}, i.v.) periods

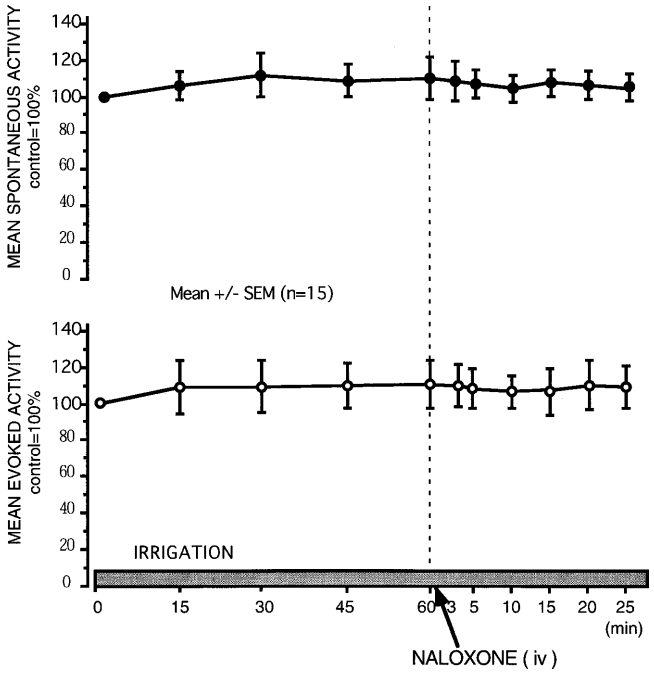

Fig. 5. The effects of warmed saline irrigation on the mean spontaneous and evoked activities of the spinal dorsal-horn neurons (expressed as percentages of the control values along the time course). Warmed saline irrigation produced no significant changes in the activity of spinal dorsal-horn neurons. Naloxone (0.1 mg·kg^{-1}, i.v.) did not affect the activity of spinal dorsal-horn neurons

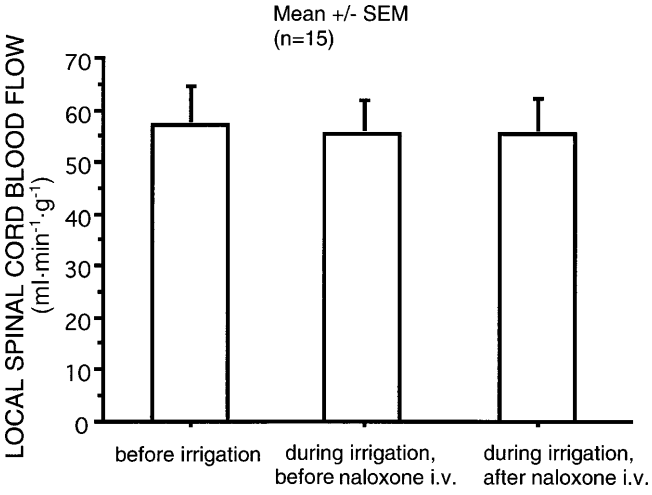

Fig. 6. Local spinal-cord blood flow in warmed saline-irrigation group. Local spinal-cord blood flow did not significantly change during the before-irrigation, during-irrigation, and after-naloxone (0.1 mg·kg^{-1}, i.v.) periods

evoked activities of the spinal dorsal-horn neurons were detected during the irrigation or after naloxone (0.1 mg·kg^{-1} i.v.) (Fig 5). No significant changes in local spinal-cord blood flow were detected during the irrigation or after naloxone (0.1 mg·kg^{-1} i.v.) (Fig. 6).

Discussion

The present study investigated the possibility of activation of the endogenous opioid system by local spinal-cord cooling in cats. Local spinal-cord cooling was able to produce a stable and reversible change in spinal-cord temperature without altering general body temperature.

The effects of hypothermia on the central nervous system have been studied for many years, with efforts mainly focused on protection against the ischemic state [6,7]. However, the spinal-cord temperature of 21°C–22°C in our study is not sufficient for the blockade of neuronal conduction. Furthermore, the spinal-cord blood flow did not increase after the administration of naloxone in the hypothermic condition while the spontaneous and evoked activities increased. The uncoupling between neuronal activities and blood flow after the administration of naloxone in the hypothermic condition indicates that nonspecific suppressive effects of cooling played no role in suppressing the spontaneous and pinching-evoked neuronal activities.

The role of endogenous opioids in stress-induced antinociception has been widely reported [8,9]. Pain research has increasingly focused on the role of the spinal cord in the modulation and processing of nociceptive inputs. Some of this attention has focused on the possible role of opioids in modulating nociceptive inputs from primary afferent fibers in the dorsal horn of the cord [10]. The dorsal horn of the spinal cord has been recognized as an important site for modifying the transmission of noxious inputs. WDR neurons in the spinal dorsal horn are thought to be responsible for relaying afferent pain information to the higher center [11]. It is generally agreed that opioids suppress the noxiously evoked activity of WDR neurons. In the present study, naloxone reversed the suppressive effect of local spinal-cord cooling on the spinal dorsal-horn neuronal activity without changing the local spinal-cord blood flow. On the other hand, naloxone had no effect on the spinal dorsal-horn neuronal activity or on the local spinal-cord blood flow in the warmed saline irrigation group. These results provide evidence that local spinal-cord cooling may induce the release of endogenous opioid which acts directly at opioid receptors within the spinal cord.

In conclusion, spinal dorsal-horn neuronal suppression induced by local spinal-cord cooling may involve the activation of endogenous opioid systems.

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