Indirect and Direct Suppressive Actions of Morphine on Dorsal Horn Neurons in Rabbits

Takahiko OKUDA, Kazuyuki MIYAMOTO and Keita SUEKANE
Department of Anesthesiology, Kinki University School of Medicine, Osaka 589, Japan
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Abstract—The analgesic mechanism of morphine on the spinal nociceptive transmission was compared in rabbits with the intact and cold-blocked states of the spinal cord. The degree of the suppressive effect of morphine (2 mg/kg) on the bradykinin-induced activity was significantly greater in the intact than in the cold-blocked states. Morphine (4 mg/kg) suppressed the nociceptive responses to similar levels in both states. These results suggest that in a small dose, the indirect suppressive action is more important than the direct action. In a larger dose, the suppressive action is probably exerted primarily by the direct spinal action.

It is generally accepted that morphine interacts with opiate receptors which are distributed in the brain and spinal cord and exerts its suppressive effects on the lamina V type cells of the spinal dorsal horn. There are two different suppressive mechanisms that act on this neuron. One is the indirect suppressive effect by activation of a descending inhibitory system originating in the brain stem. This mechanism was clarified by experiments in which morphine was micro-injected into the cerebral ventricles or certain brain stem areas such as the periaqueductal grey (PAG), nucleus raphe magnus (NRM), nucleus reticularis paragigantocellularis (NRPG) and nucleus reticularis gigantocellularis (NRGC) (1), and the electrical stimulation of such a site (2). Other supporting evidence includes the finding that the suppressive effect of systemically administered morphine is reduced by spinal transection (3–5) and by lesions of the dorsolateral funiculus (6). The other suppressive mechanism is the direct effect at the spinal level which has been demonstrated by systemically administered morphine in spinal animals (7), by iontophoresis in the spinal cord (8, 9) by intrathecal administration (10) and by spinally administered morphine (11). Clinically, many reports have shown that intrathecal and epidural morphine produce intensive, prolonged and segmental analgesic action.

In the present experiments, in order to identify the primary site of the total suppressive effect of morphine on the spinal cord, we compared the effect of morphine in spinal cord nociceptive transmission on the identical lamina V type cells in the intact and temporarily spinalized states of the spinal cord. Temporary spinalization was obtained by a reversible cold block of the upper part of the spinal cord.

Twenty-nine male rabbits weighing 1.6–2.8 kg were used. Surgical preparation was carried out under halothane-nitrous oxide, oxygen anesthesia. Following tracheostomy, cannulation of an internal jugular vein for drug administration and a carotid artery for continuous blood pressure recording were performed. For intra-arterial bradykinin injections, a cannula was retrogradely introduced into the femoral artery according to the method described by Satoh et al. (3). A laminectomy was performed at L4 through L6 to insert the micro-electrode and another laminectomy was performed at the Th12 level to practice a reversible spinalization by cooling the spinal cord. The dura was incised, and 37°C physiologic saline was dripped on the spinal cord intermittently. After the surgical procedures, anesthesia was discontinued. Animals were immobilized by pancuronium bromide and artificially venti-
Lidocaine was applied to all wound edges through the experiments. As a noxious stimulus, the injection of bradykinin through the canula of the femoral artery was applied. Bradykinin was dissolved in saline (10 μg/ml), and 0.1 ml of the solution was rapidly injected at intervals of no less than 10 min. The dorsal horn neurons responding to the bradykinin injection were chosen for this study.

Extracellular recordings from lamina V type cells of the spinal dorsal horn were obtained using tungsten microelectrodes. The number of single unit spikes of each neuron was counted for 60 sec. before and after each bradykinin injection, the former is referred to as the spontaneous activity reading. The bradykinin-induced activity was determined by subtracting the spontaneous activity reading from the numbers of unit discharges after bradykinin injection. Following 2 to 3 recordings of the control values in the spontaneous and bradykinin-induced activity, the cold block was performed, and the degree of the tonic descending inhibition was compared for each neuron in the intact spinal cord and cold blocked states. Temporary spinalization was made, with slight modification, according to the method described by Kawajiri and Satoh (12). Following the control cold block study, 2 and 4 mg/kg of morphine were slowly administered systemically, and the effects of morphine were examined on the spontaneous and bradykinin-induced activity. In each experiment, the level of the spontaneous activity and the magnitude of the bradykinin-induced activity were determined in the presence and absence of the tonic descending inhibition both before and after morphine administration.

Student's paired and unpaired t tests were used for statistical analysis. The changes induced by morphine were expressed by percent changes of controls because the level of the spontaneous activity and magnitude of the bradykinin-induced activity differed markedly before and during the cold block. Percent changes were calculated and the data were compared in the presence and absence of the tonic descending inhibition both before and after morphine administration. The difference was considered significant at P<0.05. The results were expressed as the mean±S.E.

The effects of 2 and 4 mg/kg of morphine on the activity of an identical lamina V type cell of the spinal dorsal horn are shown in Fig. 1. The effects of the spontaneous and bradykinin-induced activity of morphine in both intact and cold-blocked states are summarized in Fig. 2. Morphine (2 mg/kg) did not suppress the spontaneous activity in either the intact or the cold blocked states significantly, although there was a tendency for it to be suppressed when compared to the control. Morphine (4 mg/kg) significantly suppressed the spontaneous activity by 52±28% and 40±21% of the control in the intact and cold blocked states (P<0.05). On the other hand, morphine had a greater suppressive effect on the bradykinin-induced activity than the responses of the spontaneous activity in both states. Morphine (2 mg/kg) suppressed the nociceptive responses by 65±13% and 28±12% of the control in the intact and cold blocked states, respectively (P<0.05), while 4 mg/kg of morphine suppressed these responses by 67±17% and 66±15%, respectively (P<0.01). A number of observations have demonstrated that the suppressive action of morphine may be dependent on the direct suppression at the spinal level and the indirect action exerted by activation of the descending inhibitory systems through mediating opiate receptors. Particularly, in most of these experiments, the effects of morphine on nociceptive neuronal responses in intact or decerebrate and spinal preparations were examined on respectively different neurons. On the other hand, we compared those of morphine in intact and spinalized states on the same neurons. In the present experiments we used the cold block technique with the advantage that one can then compare the response of the same neurons in the intact spinal cord and cold blocked state.

When morphine is administered systemically, it was demonstrated that morphine has a greater suppressive effect on the nociceptive responses than on spontaneous activity responses. These results indicate that morphine acts preferentially on the
Fig. 1. The changes produced by cold block of the spinal cord before and after systemically administered 2 mg/kg (A) and 4 mg/kg (B) of morphine on the activity of the lamina V type cells of the spinal dorsal horn. Cold blocked states were obtained by cooling the cord at the Th12 levels.
nociceptive responses rather than on the spontaneous activity responses. Furthermore, morphine has been shown to suppress the response of nociceptive neurons in the presence and absence of the tonic descending inhibitory systems. The degree of the suppressive effect produced by 2 mg/kg of morphine on the bradykinin-induced activity was significantly greater in the intact states than in the cold blocked states. On the other hand, 4 mg/kg of morphine suppressed the nociceptive responses to similar levels in both states. These results suggest that the indirect suppressive action produced by 2 mg/kg of morphine may be more important for the production of analgesia than the direct action at the spinal level. In a larger dose, 4 mg/kg of morphine, the suppressive effect is probably exerted primarily by direct spinal action, and there is little possibility of suppression of the nociceptive response by activation of the descending inhibitory system, at least in the cold blocked states.

The question of which is the primary site of narcotic action at the spinal or supraspinal site has not been resolved sufficiently. To resolve this question, the spinal transection or the cold block and the lesion of the dorsolateral funiculus have been carried out. Satoh et al. (3), Takagi et al. (4) and Hanaoka et al. (5) compared the effect of morphine on spinal cord nociceptive neurons in rabbits and cats in the intact spinal cord and transected spinal cord. They suggested that the suppressive action of morphine, in small doses (0.3–2.0 mg/kg, i.v.), acted on spinal cord neurons significantly via the descending inhibitory system, while in large doses, morphine acts directly at the spinal level. Our results are basically in agreement with their results.

Many contradictory results on the analgesic systems of morphine exist (13, 14). These conflicting conclusions may result from differences in the method of analyzing the data, the type of noxious stimulation, the species used and other experimental conditions used. Furthermore, the type of noxious stimulation is important in the design and evaluation of experimental
analgesic action, because each acts on different neuronal mechanisms (15) and produces different results (16).

The present studies using the cold block technique to compare the effect of morphine on the same spinal dorsal horn neurons in the intact and cold blocked states provide direct evidence that morphine suppresses the responses of nociceptive neurons by activation of the descending inhibitory systems and by direct suppressive action at the spinal level. In small doses, the indirect suppressive action may be more important for the production of analgesia than the direct suppressive action at the spinal level. On the other hand, in larger doses, the direct suppressive action becomes predominant.

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References
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