J Anesth (1994) 8:49–51

Journal of
Anesthesia

Suppressive action of enflurane on dorsal horn neurons in rabbits

TAKAHIKO OKUDA, KATSUTOSHI WAKITA, NORIO TSUCHIYA, KAZUHIKO TANAKA, and KEITA SUEKANE

Department of Anesthesiology, Kinki University School of Medicine, 377-2 Oonohigashi, Osakasayama-shi, Osaka-fu, 589 Japan

Abstract: The neurophysiologic mechanism of the suppressive action of enflurane on spinal nociceptive transmission was examined in rabbits with intact and with transected spinal cords. Enflurane suppressed nociceptive responses in both intact and transected spinal cord groups. The suppressive effects of enflurane were significantly greater in the intact group than in the transected group. The suppressive effects of enflurane were not reversed by the addition of 0.2 $mg \cdot kg^{-1}$ of naloxone. These results suggest that enflurane suppresses nociceptive responses by activating descending inhibitory systems and directly suppressing activity at the spinal level. This suppressive action of enflurane does not interact with the opioid receptor.

Key words: Enflurane, Spinal dorsal horn neuron, Nociceptive response

Introduction

Volatile anesthetics have been shown to suppress the activity of dorsal horn neurons in the spinal cord [1–3]. However, the relative role of direct (spinal) and indirect (supraspinal) action of enflurane has not been reported. In addition, it is controversial whether the suppressive effects of enflurane partially regulate the opioid system. The dorsal horn of the spinal cord is thought to have an important role in modulating afferent noxious input. In the present experiments, the effects of enflurane on neuronal responses of lamina V cells of the spinal dorsal horn, which are concerned with the integration of noxious stimuli, in animals with intact spinal cords and those with transected spinal cords were compared. Furthermore, naloxone was administered systemically to determine whether the suppressive action of enflurane is reliably reversed by opioid antagonists.

Address correspondence to: T. Okuda
Received for publication on June 9, 1992; accepted on March 2, 1993

Methods

Experiments were performed on 24 Japanese white rabbits (1.8–2.6 kg, Hokuetsu, Settsu City). Details of the experimental method have been previously described [4]. Surgical preparation was carried out under enflurane-nitrous oxide and 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 injection, a cannula was retrogradely inserted into the femoral artery. The animals were then placed in a stereotaxic apparatus and decerebrated at the superior colliculus in the midbrain reticular formation as previously described, to later achieve an unanesthetized and decerebrated experimental preparation [5]. A laminectomy was performed at L4 through L6 to insert a microelectrode, and another laminectomy was performed at the T12 level to transect the spinal cord. After the surgical procedures, anesthesia was discontinued. The animals were immobilized by pancuronium bromide and artificially ventilated with oxygen. The $PaCO_2$ and systolic blood pressure were maintained at 35–40 mmHg and at more than 100 mmHg, respectively. The body temperature was kept constant by the use of a rectal probe, connected to a servocontrolled heating pad adjusted to 37°–38°C. As a noxious stimulus, an injection of bradykinin through the cannula of the femoral artery was applied. Bradykinin was dissolved in saline (10 μg/ml), and 0.1 ml of solution was rapidly injected at 10- to 12-min intervals. Only dorsal horn neurons that responded to bradykinin injections were chosen for this study. Extracellular recordings from lamina V cells 1500–2500 μm from the dorsum of the spinal dorsal horn were obtained using tungsten microelectrodes. The number of unitary spikes for each neuron per 60 s before and after each bradykinin injection was counted; the former is referred to as the spontaneous neuronal activity. The

bradykinin-induced activity was then determined by subtracting the spontaneous reading from the number of unit discharges immediately after bradykinin injection, and this corrected bradykinin-induced neuronal count was taken as the control of nociceptive response before drug treatments. After repeating 2–3 recordings of the spontaneous and bradykinin-induced neuronal activities, before the anesthetic administration, enflurane 1.0% was administered for 40 min and neuronal responses were observed. Naloxone 0.2 mg·kg^{-1} was administered intravenously to examine the reversal of the effect of enflurane. Naloxone 0.02 and 0.04 mg·kg^{-1} is adequate for the reversal of the effect of morphine 1 and 2 mg·kg^{-1}, respectively, on the spinal cord neurons [6]. The dose of naloxone 0.2 mg·kg^{-1} administered is far in excess of that needed to antagonize opioid receptor-mediated responses. All variables were analyzed by the Mann-Whitney U-test for inter-group comparisons or the Wilcoxon signed rank test for intra-group comparisons. Differences were considered significant at $P < 0.05$. Results are expressed as mean ± SEM.Second Reading of the Married Women's Property Bill

Results

The bradykinin-induced neuronal firing rates (control) were 942.5 ± 125.9 and 1747.1 ± 247.6 spikes/min in the intact and transected groups, respectively (Table 1). After administration of enflurane, the bradykinin-induced neuronal firing rates were significantly suppressed by 61.4%, 71.6%, and 69.8% at 10, 20, and 30 min after administration of enflurane in the intact group, respectively. Similarly, the bradykinin-induced neuronal firing rates were significantly suppressed by 18.4%, 29.6%, and 36.3% at 10, 20, and 30 min in the transected group, respectively, although the degree of suppression was significantly greater in the intact group. Naloxone 0.2 mg·kg^{-1} i.v. did not reverse the suppressive effect of enflurane on bradykinin-induced activity in either group (Fig. 1).

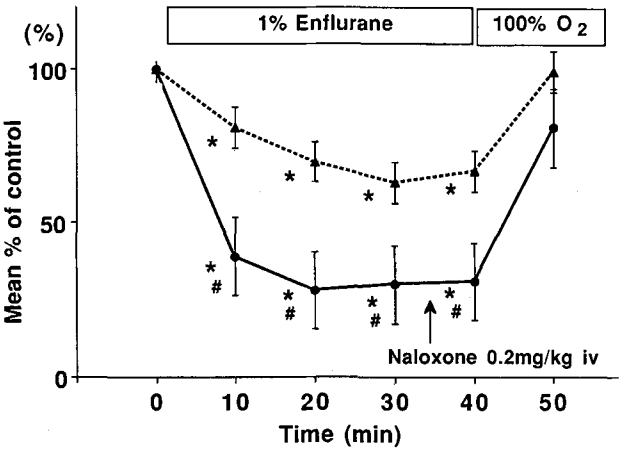

Fig. 1. Effects of 1% enflurane administration on bradykinin-induced activity on spinal dorsal horn neurons in intact and transected states. Naloxone 0.2 mg·kg^{-1} was systemically administered at the time indicated by *arrows*. Changes induced by enflurane are expressed as a percentage of the control values. Enflurane suppressed the bradykinin-induced activity in the intact and transected groups. The degree of suppression was significantly greater in the intact group. Naloxone did not reverse the suppressive effect of enflurane on bradykinin-induced activity. *, Significantly different from the values of control groups ($P < 0.05$); #, Significantly different from the values of transected groups ($P < 0.05$); ●, intact spinal; ▲, transected spinal

Discussion

This study showed that enflurane suppresses the response of nociceptive neurons in the presence and absence of tonic descending inhibitory systems. The descending inhibitory systems from the brain stem play an important role in the suppressive action of enflurane on lamina V cells, but it is now evident that enflurane acts directly at the spinal level. These results suggest that enflurane suppresses nociceptive responses by activating descending inhibitory systems and by directly suppressing action at the spinal level. We have previ-

Table 1. Effects of 1% enflurane and reversal effects of naloxone on the bradykinin-induced activity of the spinal dorsal horn neurons in rabbits

		Time after 1% enflurane administration				
	Control	10 min	20 min	30 min	naloxone i.v.	Recovery[b]
Intact spinal[a] (spikes/minute)	942.5 ± 125.9(8)	373.0 ± 82.9(8)*	267.7 ± 45.6(8)*	284.6 ± 53.9(8)*	295.9 ± 54.9(7)*	708.6± 102.0(7)
Transected spinal[a] (spikes/minute)	1747.1 ± 247.6(9)	1375.2 ± 176.3(9)*	1294.3 ± 245.4(9)*	1129.9 ± 189.1(8)*	1218.6 ± 218.2(7)*	1750.1 ± 291.2(7)

* $P < 0.05$ *vs* control values.
[a] Values are mean ± SEM(n).
[b] 10 min after termination of enflurane.

ously reported that, at a low concentration of 50% nitrous oxide, indirect suppressive action plays a more important role than direct action, and at a higher concentration of 75% nitrous oxide, direct action becomes predominant [7]. It is possible that the suppressive effect of enflurane thus resembles nitrous oxide in its ability to suppress nociceptive neurons.

Several groups have investigated the interaction between volatile anesthetics and opioid systems [8–10]. The anesthetic effect of enflurane and halothane are partially mediated through opioid receptors; naloxone administration increases the number of animals responding to stimulus and alters the depth of inhalational anesthesia [8]. A previous in vivo study has shown that halothane interacts with opioid receptors in the rat brain membrane [9]. Furthermore, cardiovascular changes induced by volatile anesthetics are antagonized by naloxone [10]. However, other studies have failed to confirm the interaction between volatile anesthetics and endogenous opioid systems. Beside indicating no effects on the suppression of volatile anesthetics on tail flick ED_{50} and halothane requirement (MAC) [8], naloxone fails to reverse the righting response in rats [12]. Moreover, naloxone neither reverses halothane anesthesia in dogs [13] nor alters the MAC in rats [14]. With regard to sympathetic nerve activity, sympathetic inhibition induced by halothane is mediated in part by the activation of opioid receptors, whereas halothane analgesia is not mediated by the opioid mechanism [15]. In this study, naloxone did not antagonize the suppressive effect of enflurane. This result does not support the previous reports suggesting that endogenous opioid systems play a role in the suppressive effect of enflurane on the spinal cord.

In conclusion, it was shown that enflurane suppressed nociceptive responses by activating descending inhibitory systems and directly suppressing the spinal cord. This suppressive action of enflurane did not interact with the opioid receptor.

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