Naloxone and Flumazenil Fail to Antagonize the Isoflurane-Induced Suppression of Dorsal Horn Neurons in Cats

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Effects of naloxone and flumazenil on isoflurane activities were examined on dorsal horn neurons in cats. Isoflurane suppressed bradykinin-induced nociceptive responses in transected feline spinal cords. The bradykinin-induced neuronal firing rates were significantly suppressed by 60.0%, 35.3% and 32.2% at 10, 20 and 30 min after isoflurane administration, respectively. The 32.2% suppression on bradykinin-induced neuronal responses at 30 min after isoflurane administration was not reversed 5 min after administration of naloxone (36.4% suppression). The suppressive effects of isoflurane were not reversed by naloxone (0.2 $mg \cdot kg^{-1}$, i.v.). Similarly, the benzodiazepine antagonist, flumazenil (0.2 $mg \cdot kg^{-1}$, i.v.), did not affect the suppressive effects of isoflurane. Failure of naloxone and flumazenil to reverse the suppressive effects of isoflurane suggests that isoflurane interacts with neither opioid nor benzodiazepine receptors in producing its suppressive action on nociceptive responses in dorsal horn neurons of the feline spinal cord. (Key words: isoflurane, spinal dorsal horn neuron, nociceptive response, naloxone, flumazenil)

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Interactions of volatile anesthetics with either the opioidergic or benzodiazepine systems are currently of considerable interest. Several studies have shown that opioidergic[1–3] or benzodiazepine[4–7] systems partially mediate the action of volatile anesthetic agents. However, other investigators have been unable to find any interactions of volatile anesthetics with either the opioidergic[8–12] or benzodiazepine[13–15] systems.

The dorsal horn of the spinal cord is thought to be an important site for drugs to mediate the transmission of noxious inputs. Suppressive effects on the nociceptive stimuli with volatile anesthetics may be, at least in part, due to the direct action on the spinal dorsal horn[16–18]. Halothane (0.5 and 1.0%) and enflurane (1.5–2.5%) suppressed nociceptive responses of lamina V-type neurons in the spinal cord[17–19]. The purpose of this study was to determine whether naloxone and flumazenil might reverse the sup-

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pressive effects of volatile anesthetics on nociceptive responses of the spinal dorsal horn neurons.

Methods

The protocol was approved by the Institutional Animal Care Committee. A total of 21 cats (2.4–3.2 kg) were used in the experiments. Details of the experimental method have been previously described[19]. Briefly, surgical procedures were carried out under enflurane-nitrous oxide and oxygen anesthesia. Following tracheostomy, cannulations of the internal jugular vein for drug administration and carotid artery for continuous blood pressure monitoring were performed. Nociceptive responses of spinal dorsal horn neurons were induced by intraarterial bradykinin injections. For the intraarterial bradykinin injection, a cannula was introduced retrograde into the bifurcation site of bilateral femoral arteries. The animals were then placed in a stereotaxic apparatus and decerebrated at the midbrain reticular formation. An initial laminectomy extending from L4 to L6 was performed to render the insertion of a recording microelectrode. Another laminectomy was performed at the T12 level for transection of the spinal cord. After these surgical procedures, anesthesia was discontinued. Animals were immobilized by pancuronium bromide and artificially ventilated with oxygen. The Pa_{CO_2} and systolic blood pressure were maintained at 35–40 mmHg and more than 100 mmHg, respectively. The body temperature was maintained consistently by the use of a rectal probe connected to a servocontrolled heating pad adjusted at 37–38°C. As a noxious stimulus, bradykinin was injected via the cannula inserted into the femoral artery. Bradykinin was dissolved in physiological saline (100 $\mu g \cdot ml^{-1}$), and 0.1 ml of the solution was injected as a single bolus administration within 1–2 sec and injected at 10–12 min intervals. Only dorsal horn neurons that responded to bradykinin injections were selected for this study. Extracellular recordings from lamina V-type cells (1500–2500 μm deep from the dorsum of the spinal dorsal horn) were traced via a tungsten microelectrode (tip diameter, 3–4 μm). Single unit activity from the recording tungsten microelectrode was amplified (MZ-4, Nihon Kohden, Japan), monitored with a dual-beam oscilloscope (EN-601J, Nihon Kohden) and the action potentials counted (ET-612J, Nihon Kohden) with a discriminator (EN-601J, Nihon Kohden). The number of unitary spikes for each neuron per 60 sec before and after each bradykinin injection was counted. The pre-bradykinin recording was referred 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 to 3 recordings of the spontaneous and bradykinin-induced neuronal activities, 1.0% isoflurane was inhaled for 40 min and the neuronal responses were observed. With regards to the concentration of isoflurane, 1.0% was far greater than that required to suppress the nociceptive stimuli in the spinal cord. Naloxone (0.2 $mg \cdot kg^{-1}$) and flumazenil (0.2 $mg \cdot kg^{-1}$) were administered intravenously after isoflurane administration to study the pharmacological aspects of isoflurane-induced suppression on spinal dorsal horn neurons. Results are expressed as mean ± S.E.M. Statistical significance was verified with Wilcoxon signed rank test. Differences were considered significant at $P < 0.05$.

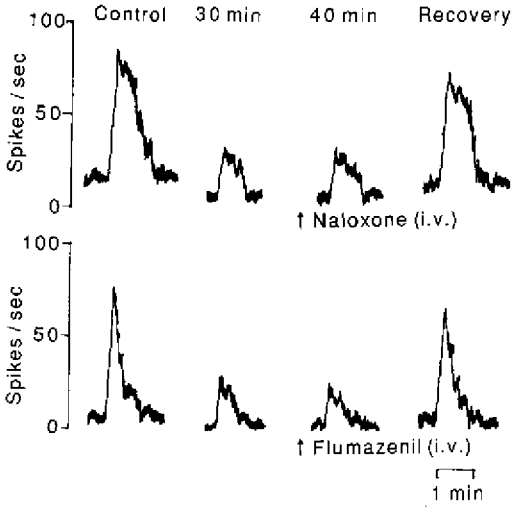

Fig. 1. Effects of naloxone and flumazenil on isoflurane-elicited suppression of bradykinin-induced activity of neuron of the spinal dorsal horn. Neither naloxone (0.2 $mg \cdot kg^{-1}$) nor flumazenil (0.2 $mg \cdot kg^{-1}$) reversed the suppressive effects of isoflurane.

Results

Figure 1 demonstrates the effects of naloxone and flumazenil on isoflurane-elicited suppression of bradykinin-induced activity of the spinal dorsal horn neurons. Table 1 summarizes the effects of naloxone and flumazenil on isoflurane-elicited suppressions of bradykinin-induced activities of the spinal dorsal horn neurons. A total of 7 spinal lamina V-type neurons were studied with the use of naloxone on the suppressive effects of isoflurane. The bradykinin-induced neuronal firing rate was 978.6 ± 158.2 (control) spikes/min. These bradykinin-induced neuronal firing rates were significantly suppressed by 60.0%, 35.3% and 32.2% at 10, 20 and 30 min after isoflurane administration, respectively. Naloxone (0.2 $mg \cdot kg^{-1}$, IV) did not affect the suppressive effects of isoflurane. The bradykinin-induced neuronal responses (32.2% suppression at 30 min after isoflurane) was not reversed comparing with the 36.4% suppression at 5 min after administration of naloxone. Similarly, a total of 7 spinal neurons were studied with the use of the flumazenil on the suppressive effects of isoflurane. The bradykinin-induced neuronal firing rate was 1025.3 ± 161.2 (control) spikes/min. These bradykinin-induced neuronal firing rates were significantly suppressed by 54.5%, 32.0% and 29.2% at 10, 20 and 30 min after isoflurane administration, respectively. Flumazenil (0.2 $mg \cdot kg^{-1}$, IV) did not affect the suppressive effects of isoflurane. The bradykinin-induced neuronal responses (29.2% suppression at 30 min after isoflurane) was not reversed comparing with the 33.6% suppression recorded at 5 min after administration of naloxone.

Discussion

It is controversial whether volatile anesthetics mediate the opioid systems. Several groups have investigated the interaction between volatile anesthetics and opioid systems[1-3]. The anesthetic action of enflurane and halothane is partially attributed to activation of the endogenous opioid systems because naloxone increases the number of animals responding to nociceptive stimulus and alters the depth of inhalational anesthesia[1]. A previous *in vivo* study has shown that halothane interacts with opioid receptors in rat brain membrane[2]. Furthermore, cardiovascular changes induced by volatile anesthetics are antagonized by naloxone[3]. 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 the tail flick ED_{50} and MAC[8], naloxone fails to reverse the anesthetic-elicited righting response in rats[9]. Moreover, this opioid antagonist neither reverses halothane anesthesia in dogs[10] nor al-

Table 1. Effect of naloxone or flumazenil on isoflurane-elicited suppression of bradykinin-induced activities of the spinal dorsal horn neurons in cats

Time (min) after 1% isoflurane administration					
Control	10 min	20 min	30 min	naloxone or flumazenil	Recovery
				(naloxone i.v.)	
978.6±158.2	587.2±131.5 (60.0)*	345.7±89.7 (35.3)*	314.9±90.2 (32.2)*	356.2±78.6 (36.4)*	792.7±123.1 (81.0)
				(flumazenil i.v.)	
1025.3±161.2	558.8±124.4 (54.5)*	328.1±79.6 (32.0)*	299.3±70.8 (29.2)*	344.6±89.6 (33.6)*	762.0±161.5 (74.3)

(spikes/min)

Values are mean ± SEM (n) (%): change in percentage referse to control values

*$P < 0.05$ vs. control values.

Recovery: 10 min after termination of isoflurane

ters halothane requirement (MAC) in rats[11]. With regards to sympathetic nerve activities, sympathetic inhibition induced by halothane is mediated in part by the activation of opioid receptors, whereas halothane analgesia is not mediated via the opioid mechanism[12]. In this study, naloxone did not antagonize the suppressive effects of isoflurane. This result does not support other findings that suggest the role played by endogenous opioid systems in the mechanism of isoflurane analgesia. Particular attention has been focused on the interaction between volatile anesthetics and benzodiazepine receptors in recent years. Volatile anesthetics are known to alter central nervous system (CNS) activity mediated by gamma aminobutyric acid (GABA). Volatile anesthetics effectively increase the GABA concentration within synapses by inhibiting metabolic breakdown of GABA, leading to an anesthetic state as a result of accumulation of this inhibitory neurotransmitter in the synaptic cleft[20]. Benzodiazepines influence the CNS by facilitating its interaction of GABA because benzodiazepine receptors are linked to GABA receptors via a chloride coupling unit. If such benzodiazepine-GABA receptor complex systems were responsible for the effect of volatile anesthetics, effects of volatile anesthetics may in part be mediated at the level of the benzodiazepine receptors. It remains to be proved whether flumazenil (a specific benzodiazepine antagonist that reverses the effect of benzodiazepine) can antagonize the action induced by volatile anesthetics. If flumazenil did antagonize the action of volatile anesthetics, the latter might directly or indirectly activate the benzodiazepine receptors. Beside partially manifesting reversal effects of isoflurane-elicited depressions on cerebral metabolic rate of oxygen ($CMRO_2$) and EEG in dogs[4], flumazenil antagonizes CNS depression caused by halothane, but not at concentrations sufficient to produce anesthesia[5]. Furthermore, midazolam, a hydrophilic benzodiazepine, decreases MAC requirements of halothane and enflurane in humans[6] and dogs[7] in a dose-related manner. However, flumazenil does not manifest any effects on the MAC of volatile anesthetics in dogs[14] and rats[14]. In addition, volatile anesthetics exert no direct action on the benzodiazepine receptors either *in vivo*

or *in vitro*[15]. These studies confirm that volatile anesthetics do not interact with benzodiazepine receptors, directly or indirectly, to produce the anesthetic action. In this study, flumazenil did not affect the suppressive effects of isoflurane, suggesting that isoflurane does not interact with the benzodiazepine receptors to produce its suppressive action. It is believed that volatile anesthetics act nonspecifically on the lipid membranes in the CNS independent of the direct anesthetic effects on specific receptors. However, some of the effects of volatile anesthetics are antagonized by either naloxone or flumazenil. This is probably attributed to either direct binding of the drugs to the specific receptors or via indirect mechanisms such as an alteration of the binding sites and/or release action of neurotransmitters by the drugs.

In conclusion, neither naloxone nor flumazenil indicated reversal effects on isoflurane-elicited suppressions on bradykinin-induced nociceptive neuronal responses in the feline spinal cord. As such, participation of opioid and benzodiazepine receptors in the mechanism of analgesia for isoflurane in the CNS may be discounted.

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