Original

Halothane Attenuates Excitation and Inhibition of Dorsal Horn Wide Dynamic Range Neuronal Activity Induced by Intraarterial Injection of Bradykinin in Spinal Cord-Transected Cats

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Abstract: We investigated the effects of various concentrations of halothane (0.2, 0.5 and 1.0%) on the excitation and inhibition of dorsal horn wide dynamic range (WDR) neuronal activity induced by bradykinin (BK) in spinal cord-transected cat. Extracellular activity was recorded from a single WDR neuron in the dorsal horn when noxious and non-noxious stimuli were applied to the cutaneous receptive fields on the left hind paw footpads of decerebrate, and spinal cord-transected (L_{1-2}) cats. Injection of 10 μg of BK (used as the noxious stimulus) into the femoral artery ipsilateral to the recording site resulted in excitatory response in 13 of 13 (100%) WDR neurons. On the other hand, when the same dose of BK was injected into the femoral artery contralateral to the recording site, 7 of 13 (54%) WDR neurons showed inhibitory responses and 6 showed no response. Halothane at 1.0% concentration, but not at lower concentrations (0.2 or 0.5%) significantly depressed the excitatory neuronal activity recorded in WDR neurons. Furthermore, halothane at 0.2, 0.5 and 1.0% significantly depressed the inhibitory neuronal activity in WDR neurons. Our results indicated that halothane reduces the excitation as well as inhibition of dorsal horn WDR neuronal activity induced by BK injection, suggesting that this anesthetic agent reduces the excitatory and inhibitory responses produced by noxious stimuli at spinal cord level.

Keywords: halothane, spinal cord
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Introduction

Spinal dorsal horn wide dynamic range (WDR) neurons can be excitated by low intensity innocuous, as well as high-intensity noxious stimuli applied to specific regions of the body (the excitatory receptive fields). These cells are known to be involved in the central processing of pain-related information. It is well known that anesthetic agents generally have depressant effects on the excitatory response of these spinal WDR neurons evoked by noxious stimulation[1-3].

WDR neurons are also known to possess widespread cutaneous inhibitory receptive fields[4-7] depending on the innocuous or nociceptive nature of the applied stimulus. However, the role of the abovementioned inhibitory mechanism has not been completely investigated. The effect of anesthetic agents on the inhibitory WDR neuronal activity by noxious stimulation is also not clearly understood.

With regard to the effects of halothane on spinal dorsal horn WDR neurons, previous studies reported that halothane depresses the excitation of WDR neurons induced by noxious stimulation[3,8-10]. This depressant effect is thought to be mediated at a spinal level, as demonstrated in experiments using systemically-administered halothane in spinal animals, in which the descending inhibitory control of spinal dorsal horn neurons was eliminated. Although several studies have examined the effect of halothane on spinal WDR neurons, to our knowledge, none examined the effects of halothane on the propriospinal inhibitory response of WDR neurons to noxious or non-noxious stimuli.

In the present study, we investigated the effects of halothane on WDR propriospinal excitatory and inhibitory mechanisms induced by bradykinin (BK) injection as a noxious test stimulus, in decerebrate and spinal cord-transected cats.

Materials and Methods

Twenty mongrel cats of either sex weighing 2.5 to 4.5 kg were used in our experiments. The experimental protocol was approved by the Institutional Animal Care and Use Committee. A detailed description of procedure has been reported previously[11]. Halothane, nitrous oxide and oxygen anesthesia were used for tracheostomy, ligation of the right common carotid artery, and cannulation of the left common carotid artery to monitor the arterial blood pressure and of the left

external jugular vein for intravenous administration of fluid and drugs. The left and right femoral arteries were also cannulated for administration of BK (10 $\mu g \cdot ml^{-1}$ in saline). A continuous drip infusion of pancronium bromide was used for muscle relaxation and the animal was mechanically ventilated throughout the experiment.

After fixation to a stereotaxic apparatus, a lumbar laminectomy was performed. The dura was removed to expose the spinal cord, and the cord was bathed with 36°C liquid paraffin to control the temperature and prevent tissue dryness. Carotid artery pressure was recorded continuously on a polygraph paper and systolic blood pressure was maintained above 100 mmHg. Data were excluded from analysis when systolic blood pressure was below 100 mmHg. Ventilation was controlled to keep the expiratory CO_2 concentration at about 4%. Rectal temperature was maintained at 36 to 37°C, and when necessary, the body was warmed with an infrared lamp. Lactated Ringer solution was administered at a rate of 10 $ml \cdot kg^{-1} \cdot hr^{-1}$ through the intravenous catheter. Decerebration was performed at the intracollicular level of the midbrain and the spinal cord was transected at level $L_{1\text{-}2}$. Following completion of the surgical procedure, anesthesia was discontinued and the animals were ventilated using 100% oxygen.

Two hours later, the response of WDR neurons was determined by extracellular recording from the left side of the spinal cord. Cells with excitatory receptive field in the left hindlimb were identified and selected for experiment. The WDR neurons were identified by the evoked response to various peripheral stimuli including: air puff, light touch, light forceps pinch, and strong forceps squeeze. Neuronal activity was expressed as the number of impulses per 5 second and recorded on the polygraph. The protocol of halothane inhalation is shown in Fig. 1. Changes in the evoked response induced by halothane inhalation were expressed as percentage of the control values, and differences between control and post-inhalation values were analyzed statistically using the Student's paired *t*-test. All data were expressed as mean standard error of the mean (SEM). A *P* value less than 0.05 denoted the presence of a statistically significant difference.

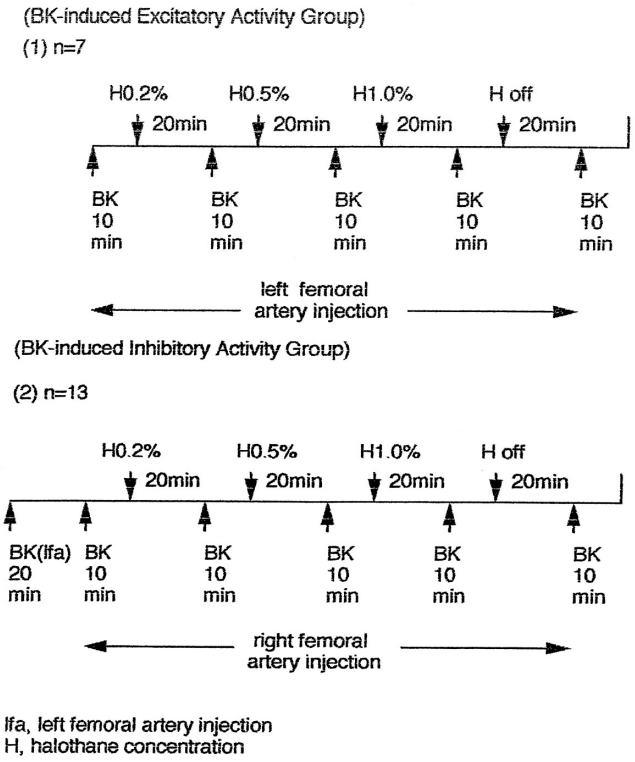

Fig. 1. The protocol used for halothane inhalation.

Results

Innocuous cutaneous stimuli (light touch) were routinely tested and found to have no inhibitory effects on both hindlimbs. When 10 μg of BK (used as the noxious stimulus) was injected into the femoral artery ipsilateral to the recording sites, all (100%) WDR neurons exhibited excitatory responses. Halothane at 1.0%, but not at 0.2% or 0.5% significantly depressed the excitatory neuronal activity in WDR neurons (Fig. 2). When 10 μg of BK was injected into the femoral artery ipsilateral to the recording sites, 13 of 13 (100%) WDR neurons showed excitatory responses. Twenty minutes later, after contralateral BK injection, 7 of 13 (54%) WDR neurons showed inhibitory responses, and 6 (46%) showed no response. Fig. 3 shows that BK injection into the contralateral (right) femoral artery resulted in a significant reduction of discharge at one-minute period. These inhibitory neuronal activities in WDR neurons were significantly depressed by 0.2%, 0.5% and 1.0% halothane (Fig. 4).

Discussion

We observed in the present study that halothane has suppressive effects on the excitatory response of spinal WDR neuronal activity induced by BK injection ipsilateral to the recording sites. Previous studies reported that halothane at 0.5, 1.0 and 1.5% concentrations suppressed the spontaneous activity of neurons in lamina V of the dorsal horn as well as the activity (excitation) of

lamina V neurons induced by noxious mechanical and noxious heat stimuli[3,9]. Our results, using noxious chemical stimuli, confirmed the findings of the above studies and demonstrated that halothane suppressed the excitatory neuronal activity of spinal WDR neurons induced by noxious mechanical stimulation. These electrophysiological studies may correspond to our behavioral studies, which indicated that halothane blocks the tail flick response in a dose-dependent manner in rats[12]. The present results may explain that this anesthetic agent has a direct analgesic action at the spinal level, because spinal cord transection severs pathways to and from the supraspinal structure. Therefore, in conjunction with our behavioral studies, it is likely that this direct action is not associated with the hypotensive action of halothane[9].

In addition to our previous reports[11,13-15], Fig. 3 of the present study shows that BK injection into the contralateral femoral artery induced a clear inhibition of spinal WDR neuronal activity in spinal cord-transected cats. In contrast, injection of BK into the ipsilateral femoral artery resulted in an excitatory response. Although this excitatory system may play an important role in pain processing to the upper brain, the role of this propriospinal inhibitory system could not be rationally explained.

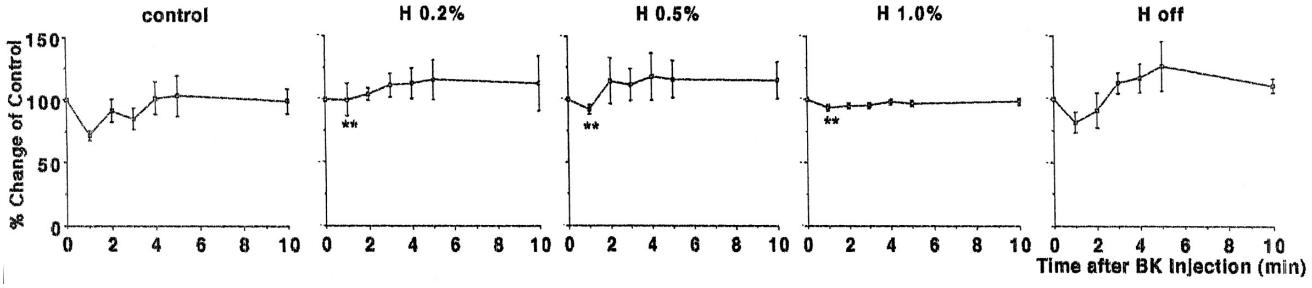

Fig. 2. Effects of halothane on excitation of dorsal horn WDR neurons induced by ipsilateral injection of BK into the recording site. Data from 7 neurons in each group. *P< 0.05 compared with the control.

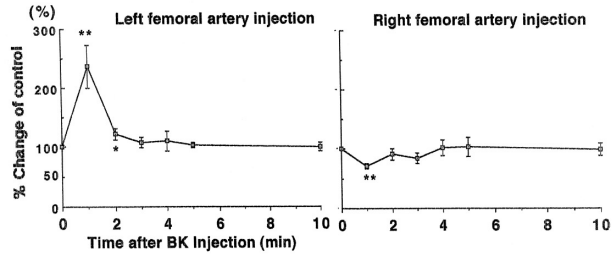

Fig. 3. Comparison of effects of ipsilateral and contralateral BK injection. Time-course of the effects of BK injection on seven WDR neurons that were inhibited by contralateral BK injection (right) and ipsilateral BK injection (left). **P< 0.01, *P<0.05 compared with the respective neuronal activity before BK injection.

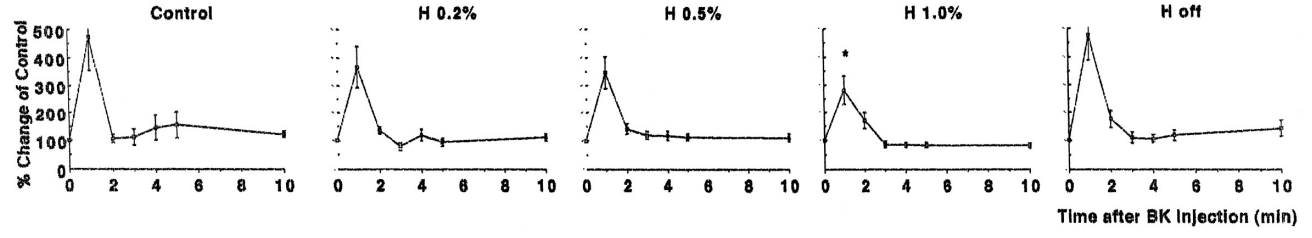

Fig. 4. Effects of halothane on inhibition of dorsal horn WDR neuron activity induced by contralateral injection of BK into the recording site. (n= 7) **P<0.01 compared with the control.

The inhibitory effects of other anesthetic agents have also been examined. For example, thiamylal[13,14], enflurane[15], and ketamine[11] reduce BK-induced excitation and inhibition of dorsal horn WDR neuron activity in spinal cord-transected cat. Thus, the effects of halothane are similar to the above agents. It has been reported also that deep halothane anesthesia depresses or abolishes the inhibition of spinal dorsal horn WDR neuron activity evoked by noxious mechanical stimuli in spinal cord-intact rats[16]. These studies, however, could not clarify whether the inhibitory phenomenon was due to the descending inhibitory mechanism, or propriospinal mechanism. The neurochemical mechanism of halothane-induced depression of spinal nociceptive neurons in the spinal transected animal is not clear. Naloxone fails to antagonize halothane-induced depression of nociceptor-driven spinal cord response in cats[17]. However, halothane may affect other neurotransmitter systems in the central nervous system including interaction with gamma-aminobutyric acid (GABA) receptors[18] and serotonin receptors[19].

In conclusion, we have demonstrated in the present study that halothane reduces the excitation as well as inhibition of dorsal horn WDR neurons induced by BK injection in the propriospinal system.

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脊髄切断ネコにおける脊髄後角第五層型細胞活動のブラディキニンによる興奮性および抑制性活動のハロタンによる抑制

水元　裕，長坂　浩*

除脳、L_{1-2} 間で脊髄切断した脊髄ネコを用いて左足先付近に興奮性受容野をもつ脊髄後角第五層型細胞活動（WDR 細胞活動）を細胞外微小電極法により導出し、0.2％，0.5％，1.0％ハロタンの WDR 細胞活動に及ぼす影響を観察した。痛み刺激としてブラディキニン（BK）を用い、左足先を灌流する左大腿動脈投与及び反対側の右大腿動脈投与とした。BK の左右大腿動脈投与を比較した症例では，左大腿動脈投与で、13 例中 13 例 BK 投与により興奮性反応を示し、右大腿動脈投与では、13 例中 7 例で、BK 投与により抑制性反応を示した。BK 投与による興奮性反応 に対して 1.0％ハロタンで有意の減弱を、BK 投与による抑制性反応に対して 0.2％，0.5％，1.0％ハロタンで有意の減弱を示した。この興奮性および抑制性反応のハロタンによる減弱は、ハロタン麻酔の脊髄レベルにおける作用機序の一つとして推測される。

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