Enflurane Reduces the Excitation and Inhibition of Dorsal Horn WDR Neuronal Activity Induced by BK Injection in Spinal Cats

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The effects of enflurane (0.5%, 1.5% and 2.5%) on the excitation and inhibition of dorsal horn wide dynamic range (WDR) neuronal activity induced by bradykinin (BK) injection was studied in spinal cats. Extracellular activity was recorded in the dorsal horn from single WDR neurons responding to noxious and non-noxious stimuli applied to the cutaneous receptive fields on the left hind paw foot pads of decerebrate, spinal cord transected (L_{1-2}) cats. When 10 μg of BK was injected into the femoral artery ipsilateral to the recording site as the noxious test stimulus, 24 of 26 WDR neurons (92%) gave excitatory responses and 2 (8%) gave inhibitory resposes. On the other hand, when the injection of 10 μg of BK into the femoral artery contralateral to the recording site was used as the noxious test stimulus, 7 of 12 WDR neurons (58%) gave inhibitory responses, 3 (25%) gave excitatory responses, and 2 (17%) showed no response. The excitatory neuronal activity in WDR neurons was not depressed by 0.5% or 1.5% enflurane but was depressed significantly by 2.5%. However, the inhibitory neuronal activity in WDR neurons was significantly depressed by 0.5%, 1.5% and 2.5% enflurane. We have found that enflurane reduces the excitation as well as the inhibition of dorsal horn WDR neuronal activity induced by BK injection. These results suggest that the reduction of excitatory and inhibitory responses produced by noxious stimulation is likely to be the fundamental basis of the enflurane-induced anesthetic state in terms of WDR neurons. (Key words: enflurane, WDR neurons)

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We have reported the effects of enflurane and neuroleptanalgesia (NLA) on the wide dynamic range (WDR) neurons in the dorsal horn of spinal cats[1,2]. In our studies, the WDR neurons of the lumbar dorsal horn gave mainly either an excitatory or an inhibitory response to injection of bradykinin (BK) into the femoral artery ipsilateral to the recording site. It is considered a reasonable result that WDR neuronal activity was excited by noxious stimulation of their receptive field by BK. However, we were not able to understand the inhibitory effects produced by BK injection. Droperidol, which has neither analgesic nor anesthetic

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action, had little effect on the excitatory or inhibitory responses to BK injection. By contrast, enflurane, which has potent anesthetic action, and fentanyl, which has potent analgesic action, reduced the excitatory and inhibitory responses to BK. From the results of the above studies, there appears to be a relation between the pain mechanism and the inhibitory response to BK.

Le Bars et al.[3] reported an extensive study of noxious stimulation induced inhibition of intact rat dorsal horn cells (convergent neurons that can be excited by a convergent input from both sensitive mechanoreceptors and nociceptors) in many parts of the body including the contralateral foot (diffuse noxious inhibitory control: DNIC). In spinal rats[4], the inhibition was weaker. We have speculated that the inhibitory response to BK injection as the noxious test stimulus may be induced by DNIC and the propriospinal inhibitory mechanism. In other words, BK injection into the femoral artery ipsilateral to the recording site stimulates not only the receptive field of specific WDR neurons, but also areas outside of their excitatory receptive field.

In the present study, we compare the magnitude of responses induced by ipsilateral and contralateral injection of BK on dorsal horn WDR neurons of decerebrate, spinal cord-transected cats, and then investigated the effect of enflurane on these responses.

Methods

Thirty-two mongrel cats of either sex weighing 2.5 to 4.5 kg were used. Halothane, nitrous oxide, and oxygen anesthesia were used for tracheostomy, ligation of the right common carotid artery, and cannulation of the left internal carotid artery to monitor the arterial blood pressure and of the left external.juglar vein to use it for intravenous administration of fluids and drugs. The animals were maintained with a continuous drip infusion of pancronium bromide and were mechanically ventilated.

After fixation to a stereotaxic apparatus, a lumber laminectomy was performed. The dura was removed to expose the spinal cord, and the cord was bathed with 36°C liquid paraffin in order to control the temperature. Carotid artery pressure was recorded continuously on a polygraph and most of the values of the systolic pressure were above 100 mmHg. The data were neglected when the systolic pressure was below 100 mmHg. Ventilation was controlled to keep the end expiratory CO_2 concentration at about 4%. Rectal temperature was maintained in the range of 36°C to 37°C, and if necessary, the body was warmed with infrared rays. Lactated Ringer solution was administered at the rate of 10 $ml \cdot kg^{-1} \cdot hr^{-1}$ through the intravenous catheter. The left and right femoral arteries were cannulated to provide a route for administration of BK, 10 $\mu g \cdot ml^{-1}$ in saline. Decerebration was performed at the intracollicular level of the midbrain and the spinal cord was transected at level L_{1-2}. When the surgical procedure was finished, anesthesia was discontinued and the animal were ventilated on 100% oxygen. Two hours later, the responses of WDR neurons were determined by extracellular recording from the left side of the spinal cord. Only cells with excitatory receptive fields in the left hindlimb were included in this study. The WDR neurons were identified by the evoked reponse to peripheral stimuli of several types: (1) air puff, (2) light touch, (3) light forceps pinch, and (4) strong forceps squeeze. Neuronal activity was expressed as the number of impulses per 5 second and recorded on the polygraph. The protocol of enflurane administration is shown in figure 1. The changes induced by enflurane were expresed as percentage change of the control values, and the differences between the control values and the post-administration values of enflurane were analyzed statistically using the paired t-test.

Results

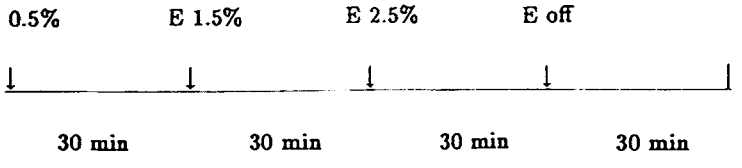
All values were expressed as mean ± SE. Data were obtained from 32 cats.

Spontaneous activity group

Administration of 0.5% enflurane (E) in 100% oxygen for 30 min significantly depressed spontaneous activity and 1.5% and

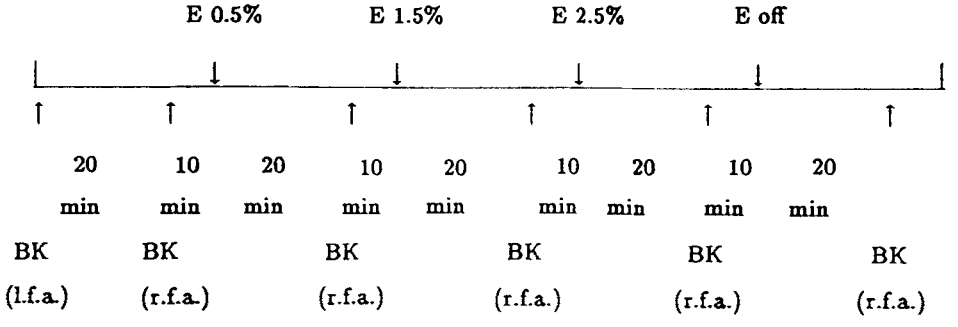
[Spontaneous activity group] n = 6

Enflurane (E) concentration

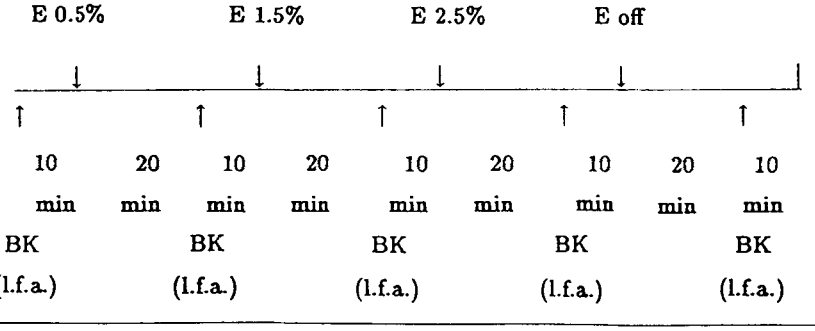


[Evoked activity group]

(1) n = 12



(2) n = 14



l.f.a., left femoral artery: r.f.a., right femoral artery

Fig. 1. The protocol of drug administration

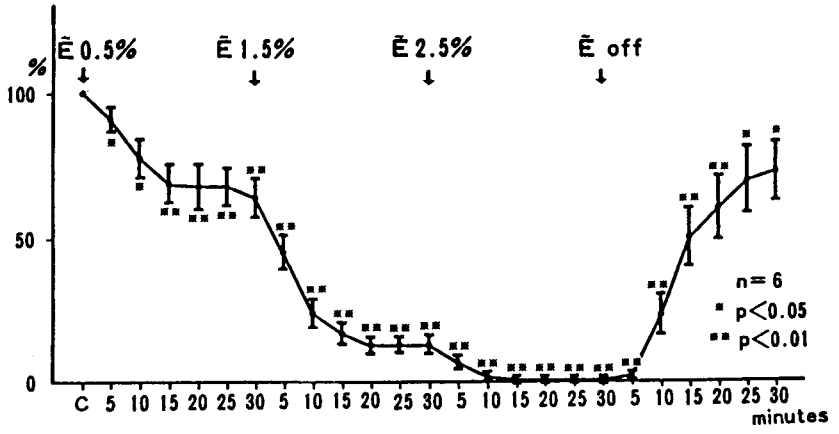


Fig. 2. Dose-response curves of the effects of various concentrations of enflurane on spontaneously firing single-unit activity of dorsal horn WDR neurons. $*P < 0.05$; $**P < 0.01$ compared with control

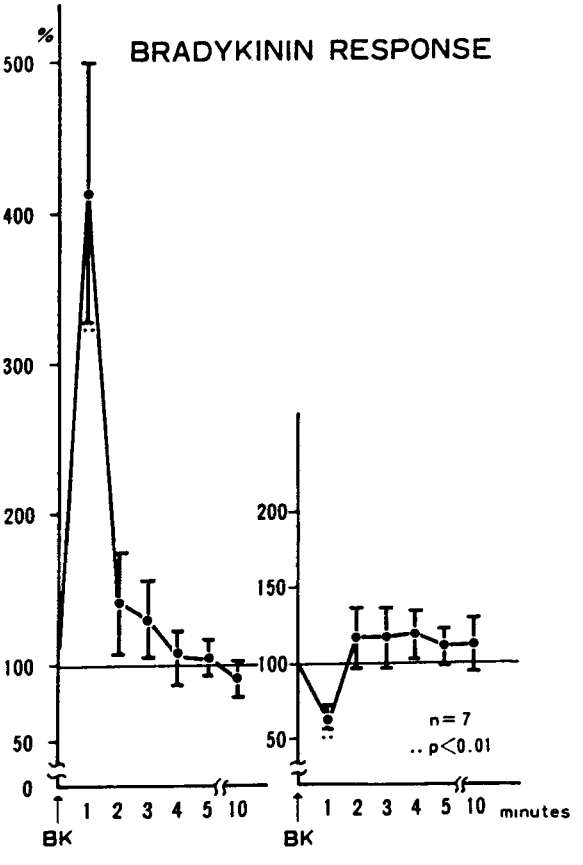


Fig. 3. Comparison of effect of ipsilateral and contralateral BK injection on the magnitude of the spinal WDR neuron response. The left side of the graph indcates the excitation of the WDR neuronal activity by the ipsilateral BK injection. The right side indicates the inhibitory response on WDR neuronal activity by the contralateral BK injection. $**P < 0.01$ compared with control.

2.5% E also depressed spontaneous activity in a dose-related manner. At 30 min after termination of enflurane administration, the extent of the depression was 73.1 ± 10.3% of the control (fig. 2). (n = 6)

Evoked activity group

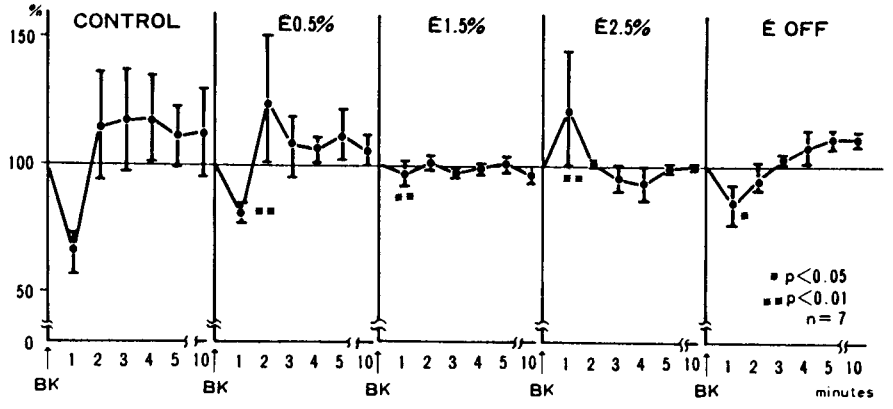


Fig. 4. The effects of enflurane on the inhibition of dorsal horn WDR neuronal activity induced by injection of BK contralaterally to the recording site. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with control

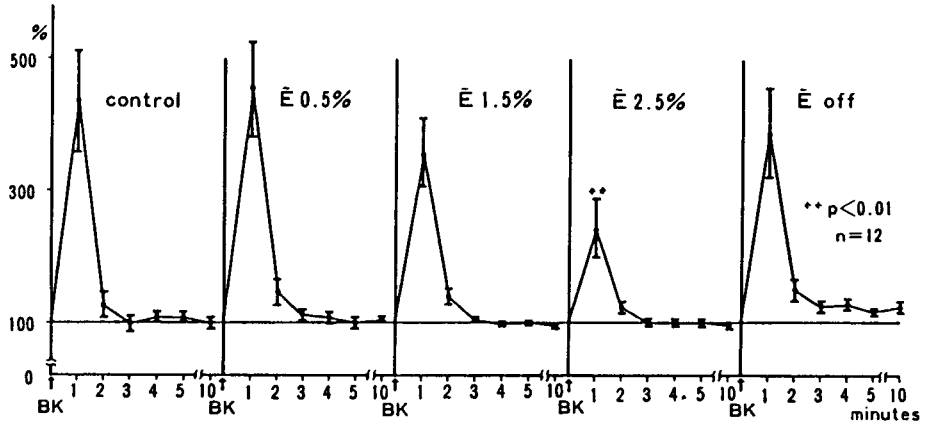


Fig. 5. The effects of enflurane on the excitation of dorsal horn WDR neuronal activity induced by injection ipsilaterally to the recording site. $^{**}P < 0.01$ compared with control

Innocuous cutaneous stimuli (light touch) were routinely tested but never produced inhibition in either hindlimb.

[I] The magnitude of the responses induced by ipsilateral (left femoral artery) and contralateral (right femoral artery) injection of BK was compared (12 cats). Following ipsilateral BK injection, the activity of WDR neurons was excited in all 12 cats. Twenty minutes later, following contralateral BK injection, the activity of the WDR neurons was inhibited in 7 of the 12 cats, slightly excited in 3, and was not affected in 2 animals. The inhibitory neuronal activity in 7 WDR neurons was significantly depressed by 0.5%, 1.5% and 2.5% enflurane and in 2 of 7, WDR neuronal activity was excited by 2.5% enflurane. At 20 min after termination of administration of 2.5% enflurane, inhibitory neuronal activity in the 7 WDR neurons appeared again (figs. 3,4).

[II] The activity of WDR neurons was excited in 12 cats and inhibited in 2 cats by the administration of BK into the left femoral artery ipsilateral to the recording site (figs. 5–7). Excitatory neuronal activity in 12 WDR neurons was not depressed by 0.5% or 1.5% enflurane but was de-

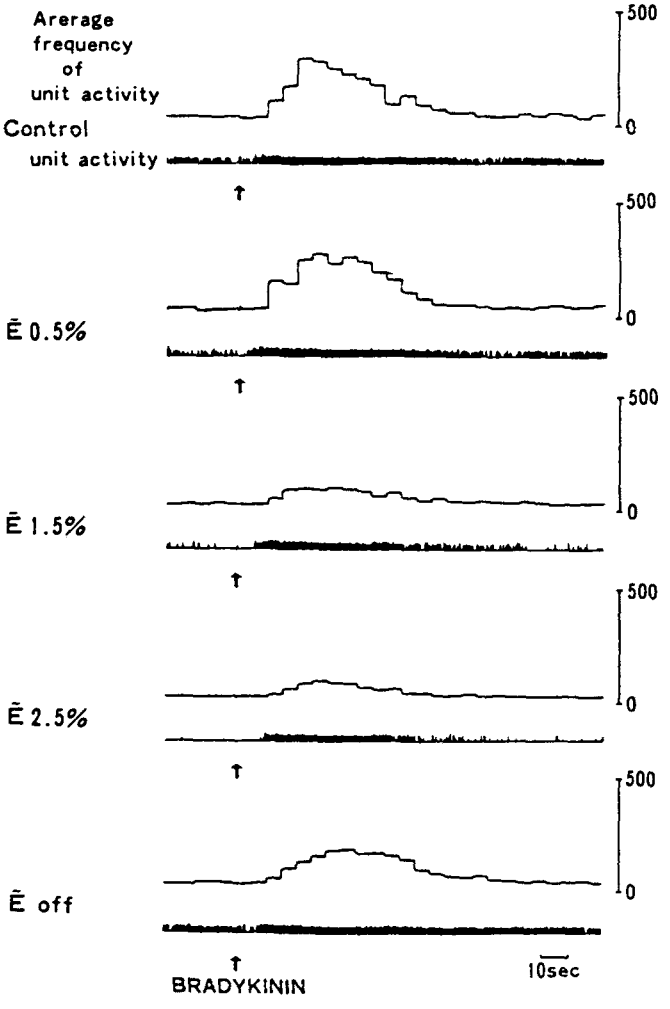


Fig. 6. Typical result of the experiment shown in Figure 5.

pressed significantly by 2.5%. At 20 min after termination of administration of 2.5% enflurane, excitatory neuronal activity in 12 WDR neurons had recovered to the control level. Inhibitory neuronal activity in 2 WDR neurons was depressed by 0.5%, 1.5% and 2.5% enflurane.

Discussion

Clinically, nitrous oxide, morphine and pentazocine, which possess potent analgesic action, do not always induce unconciousness and attenuate pain sensation in small doses. It is harder for them to alter the heart rate, blood pressure, respiratory rate and tidal volume responses to a given noxious stimulus than is the case with halothane and barbiturates. In regard to the neurophysiological basis of the analgesic action on spinal cord dorsal horn cells, it has been reported that the above mentioned agents selectively depress the spontaneous activity of lamina V cells in spinal cats[5–7]. By contrast, halothane and barbiturates, which possess both a weak analgesic action and a potent anesthetic action, induced unconciousness and attenuated pain sensation but also tended to alter the patients physical responses (heart rate, blood pressure, respiratory rate) to a given noxious stimulus more easily than do morphine and pentazocine. In regard to the neurophysiological basis of the anesthetic action on spinal cord dorsal horn cells, it has been reported that agents with such action have a lamina-

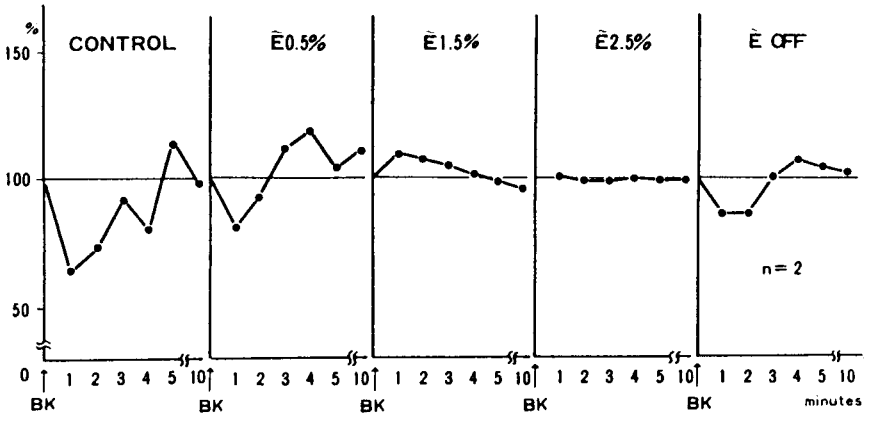


Fig. 7. The effects of enflurane on the inhibition of dorsal horn WDR neuronal activity induced by BK injection ipsilaterally to the recording site.

non-specific depressive action in the spinal cord dorsal horn[8,9]. In the present study, only WDR neurons were observed, except for lamina type IV and VI cells. Although the anesthetic action of enflurane is strong, its analgesic action is weak. We suggest that enflurane, as well as halothane and barbiturates, reduces the spontaneous activity in cells of lamina IV, V, and VI in spinal cats.

Inhibition by noxious test stimuli to a wide area of skin outside the excitatory receptive field in WDR neurons has been reported by not only Le Bars et al.[3] but also many other investigators. The noxious stimuli that inhibit WDR neurons may be (a) a chemical stimulus such as BK injection[10], (b) a mechanical stimulus such as a paw pinch[11,17], (c) a thermal stimulus[4,11,12], or (d) a stimulus that produces visceral pain (increase in pressure in the gall bladder, increase in pressure in the rectum)[13,14]. The inhibition is produced regardless of the kind of noxious stimulus.

The inhibition of primate spinothalamic tract (STT) cells described by Willis's group[15] can be compared to the DNIC mechanism in the rat described Le Bars's group[3]. However, there are several differences between the inhibition described for STT cells and DNIC.

(1) DNIC primarily affects dorsal horn cells classified as convergent neurons, whereas the inhibition described for STT cells has some effect on high threshold cells as well. (2) When the spinal cord is transected, there may still be inhibition in the rat spinal cord of the type seen in the monkey, but the long-lasting form of inhibition disappears. Willis has explained that species differences, as well as differences in anesthetic, can account for these different results in monkeys and rats.

Enflurane at 0.5% reduces the inhibition of dorsal horn. WDR neuronal activity induced by BK injection into the femoral artery contralateral to the recording site. At first, Le Bars et al. did not find a propriospinal inhibitory mechanism in 0.5% halothane-anesthetized rats following cervical section of the spinal cord[3]. However, in the spinal preparation, they found such a mechanism by using unanesthetized rats[4]. In comparison with that in the intact animals, this inhibition is weak. Therefore we assumed that 0.5% halothane might depress the activity of dorsal horn convergent neurons by a propriospinal mechanism triggered by noxious input as well as by enflurane.

Le Bars et al.[16] have proposed that the pain signalling message sent to higher centers by dorsal horn WDR neurons might be the difference between the excitatory activity of the segmental neuronal pool evoked by noxious stimuli and the DNIC-mediated

silence of the remaining neuronal population. In other words, DNIC could improve the spatial and intensive contrast in the response to noxious stimulation.

In this study, we have found that enflurane may depress this system of dorsal horn WDR neurons.

A part of this work was described in Japanese with abstract in English (2).

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