Effects of Noxious Stimuli and Anesthetic Agents on Substance P Content in Rat Central Nervous System

Yoshito KOTANI, Yasuaki HIROTA, Kazuna SUGIYAMA, Shigeharu JOH, Tohru SHIBUTANI, Hideo MATSUURA and Reizo INOKI*

Department of Dental Anesthesiology and *Department of Dental Pharmacology, Faculty of Dentistry, Osaka University, Suita 565, Japan

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Abstract—Effects of noxious electrical tooth stimulations and intraarterial administration of bradykinin or inhalation of volatile anesthetics on substance P content in the diencephalon-mesencephalon, pons-medulla and the spinal cord were examined in the rat. Noxious stimulation by electrical long duration stimulation (type 2) of tooth pulp caused an increase of substance P content in the pons-medulla. Inhalation of volatile anesthetics such as methoxyflurane and diethyl-ether produced an increase of substance P content in the spinal cord; and in addition, methoxyflurane produced a decrease of substance P content in pons-medulla. However, halothane did not produce any changes in substance P content in any parts of the central nervous system (CNS). These results suggest that volatile anesthetics such as diethyl-ether and methoxyflurane act on the substance P neuron and may modulate pain transmission through the action of substance P in the pons-medulla or the spinal cord.

Substance P, a potent neuroactive peptide, is widely distributed throughout the central and peripheral nervous systems. Substance P was originally considered to be a neurotransmitter in certain primary afferent neurons. Hence, substance P may play a significant role in pain perception. In addition, intracerebral administration of substance P was reported to produce naloxone-reversible analgesia in rats (1) and mice (2). These reports suggested that substance P had a physiological role for pain control at supraspinal levels. Therefore, it is possible that substance P may have dual function as a transmitter of nociceptive afferents and a modulator for noxious stimulation (3). Meanwhile, anesthetics cause a reduction in synaptic transmission through a particular synaptic pathway in intact animals (4); it, however, remains obscure that effects of anesthetics on transmission of noxious stimuli are involved in the contribution of peptidergic neurons, particularly substance P neuron.

In the present study, effects of various noxious stimuli and volatile anesthetics on substance P content in various parts of rat CNS were examined.

Materials and Methods

Male Sprague-Dawley rats, weighing 200–250 g, were used.

Application of noxious stimuli: Under ether anesthesia, the bipolar stimulating electrode (0.1 mm enamel stainless wire with tip separation of 1 mm) was inserted into the lower incisor tooth pulp in one group (group 1), and a polyethylene cannula was inserted into the femoral artery in another group (group 2). Two hours after these operations, conscious rats in group 1 were restrained in a rat holder, and then the tooth pulp was stimulated by 5 trains of 200 Hz of square wave with 0.5 msec duration, 5V amplitude, repetition rate of 0.5 sec for 5 sec (type 1) or repetition rate of 5 sec for 30 min (type 2); in group 2, bradykinin (2 μg/each 3 min.) was administered through the cannula for 30 min.
All animals responded to these noxious stimuli, revealing pseudoaffective responses such as vocalization, bending or stretching. During application of continuous stimuli, there was little reduction of these responses.

**Inhalation of volatile anesthetics**: Rats were put in the plastic chamber which was filled up with the volatile anesthetics in the concentration of about 1.5 MAC with a constant flow (3L/min) of compressed air and were left to breathe spontaneously for 1 hour in the chamber. MAC used in this study is the equipotent anesthetic dose to abolish the righting reflex or suppress the responses to noxious stimuli such as tail clamp and electrical stimulation. In this context, all animals under inhalation of each anesthetic lost righting reflex and showed a regular slow breathing within 1 hour. Little difference in anesthetic depth was observed among the anesthetics used in this experiment.

**Extraction and measurement of substance P**: Immediately after the noxious stimulation or inhalation of volatile anesthetics, rats were decapitated, and the heads were heated in a microwave oven for 45 sec (5). CNS was then divided into three parts, diencephalon-mesencephalon, pons-medulla and spinal cord (C1–3). Each tissue was homogenized in 2N-acetic acid. After centrifugation at 10,000×g for 20 min at 4°C, aliquots of the supernatant were used for radioimmunoassay of substance P by the method of Powell et al. (6). Antiserum of substance P prepared from rabbits according to Goodfriend et al. (7) was used at a final dilution of 1:5,000. \(^{125}\text{I-Tyr}^8\)-substance P with a specific activity of 50 μCi/μg served as a tracer. The sensitivity of the assay was 0.1–10 pmole of substance P in each tube (0.8 ml). Related peptides such as somatostatin, leucine-enkephalin and bradykinin failed to show a cross-reaction with this antiserum for substance P. Contents of substance P were expressed as means±S.D. and were analyzed for significance by Student’s unpaired t-test.

**Results**

**Effects of noxious stimuli**: Type I stimulation did not produce any significant changes in substance P content in any parts of the CNS (Fig. 1). On the other hand, type 2 stimulation produced an increase in substance P content of the pons-medulla (17% P<0.05) and a tendency to decrease substance P content in the spinal cord (26%) (Fig. 2). Noxious stimulation by the intraarterial administration of bradykinin produced a tendency to increase substance P content in the pons-medulla (22%) (Fig. 3).

**Effects of volatile anesthetics**: Inhalation of halothane (1%) did not produce any
remarkable changes in substance P content in any parts of the CNS (Fig. 4), while inhalation of methoxyflurane (0.3%) produced a significant increase of substance P content in the spinal cord (61% P<0.05) and a significant decrease of substance P content in the pons-medulla (50% P<0.05) (Fig. 5). Inhalation of diethyl-ether (5%) also produced a significant increase of substance P content in the spinal cord (19% P<0.05) (Fig. 6).

Discussion

Effects of volatile anesthetics on the substance P neurons in rat CNS have not been well understood. In the present study, effects of various volatile anesthetics on substance P content in various parts of the CNS were studied. Since Stewart et al. (2) reported that intracerebral or intraperitoneal injections of substance P in nanogram doses induced analgesia when measured by the hot plate test and its analgesia was antagonized by naloxone, many investigators revealed that substance P had an analgesic effect in rodents, and substance P-induced analgesia was mediated by opioid peptides (1, 8, 9). Since the analgesic effect of substance P was blocked by the concomitant intraventricular injection of specific antibody against an opioid peptide, methionine-
enkephalin, the result suggests that substance P produces an analgesic effect in rats by releasing methionine-enkephalin at the supraspinal levels involved in pain control (10). Recently, substance P was reported to induce a Ca**+-dependent release of methionine-enkephalin from slices of the periaqueductal gray matter and striatum of rats (3). Substance P (10^-6 M) added into the fluid perfusing the subarachnoidal spaces in the spinal cord was also reported to release methionine-enkephalin from the spinal cord (11).

Substance P-positive cells were distributed to the pons, that is, the periventricular central gray, dorsal raphe nucleus and medulla-oblongata, that is, raphe nucleus and reticulo-gigantcellularis (12). Furthermore, intracerebral injection of substance P into the dorsal nucleus of the raphe in the midbrain periaqueductal gray had an analgesic effect in rats when tested by the tail flick test (1). These recent studies suggest that the pons-medulla area must be an important site for the analgesic action of substance P. Results obtained in the present experiment also implied that the increase of substance P content in the pons-medulla might participate in pain transmission. According to the hypothesis that accumulation or depletion of peptidergic neurotransmitter in a certain brain region is respectively due to a decrease or increase of the release from the neuron terminals (13), effects of noxious stimulation of short duration (type 1) on substance P neuron were so transient that its content was not influenced to make prominent changes in any area, while those of long duration (type 2) were so effective that release of substance P was significantly decreased in the pons-medulla. The same tendency was shown after the noxious stimulation due to bradykinin administration. Although anesthetics may have a general action on both excitatory and inhibitory synaptic transmissions, the excitatory synaptic transmission is preferentially depressed by anesthetics, while the inhibitory synaptic transmission may be either depressed or enhanced depending on the pathway involved (4). Furthermore, the analgesic potency of anesthetics was partly related to the activation of the descending system for control of pain transmission (14). This descending system inhibits synaptic transmission of sensory impulse at the dorsal horn of the spinal cord by their inhibitory function of biogenic amines or endogenous opioids released (15). From our present study, it was speculated that substance P neuron activity in the spinal cord might be inhibited by activating the descending system under inhalation of diethyl-ether or methoxyflurane, and in addition, the activity of substance P neurons in pons-medulla might be enhanced, resulting in activation of the descending system under inhalation of methoxyflurane. Diethyl-ether and methoxyflurane were clinically evaluated to have a potent analgesic activity, but halothane was not. Clinical evaluation of halothane with less analgesic potency may attribute to no remarkable changes in substance P content under inhalation of halothane.

Results obtained in this experiment suggest that volatile anesthetics such as diethyl-ether and methoxyflurane act on the substance P neuron in the pons-medulla and the spinal cord to modulate pain transmission.

References
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