Isoflurane Suppresses the Excitability and Synaptic Transmission of Spinal Nociceptive Pathway in Rats

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ABSTRACT

Background: Isoflurane (IFL), one of the most widely used volatile anesthetics, has an anti-nociceptive effect at the spinal cord level. However, the reported spinal mechanisms of IFL analgesic action remain controversial. The aim of this study was to examine the effect of IFL on the excitability of spinal substantia gelatinosa (SG, lamina II) neurons and the synaptic transmission from primary nociceptive fibers to SG neurons.

Methods: Parasagittal spinal slices with a dorsal root attached were cut from the Sprague-Dawley rats (4-6 weeks old). Whole-cell patch-clamp recordings were made from SG neurons at room temperature. Dorsal root stimulation was used to evoke excitatory postsynaptic currents (eEPSCs). Depolarizing current injection was used to display the firing pattern of action potentials. IFL was given as volume percent (3%) by passing the gas mixture (flow rate, 0.5 L/minute) into equilibrated artificial cerebral spinal fluid (ACSF) for at least 15 minutes at room temperature.

Results: IFL significantly suppressed the peak amplitude of monosynaptic eEPSCs mediated by primary Aδ and C fibers. Regardless of neuron types, IFL significantly decreased the frequency of action potentials in all SG neurons tested.

Conclusions: IFL may play analgesic role through inhibiting or weakening the excitability and synaptic transmission of spinal nociceptive pathway.
rons play an important part in the transmission and modulation of nociceptive signals (22-24).

To further investigate the mechanisms underlying the analgesic action of IFL, the present study utilized patch-clamp technique to test the hypothesis that IFL can inhibit the monosynaptic events from primary C and Aδ fibers to SG neurons, and suppress the action potential discharge of SG neurons.

MATERIALS AND METHODS

Ethics and Animals

Male Sprague Dawley (SD) rats (weighing 90 ± 10 g, 4-6 weeks postpartum) were provided by the Fourth Military Medical University Experimental Animal Center. The animal experiments were approved by the Institutional Animal Management Committee of Fourth Military Medical University. Animals were kept under controlled laboratory conditions: temperature 23-25°C, relative humidity 58-62%, a light/dark cycle 12:12 hours and free access to food and water.

Spinal Slice Preparation

The details of spinal slice preparation were described in previous publications (9, 10, 22-27). Briefly, under deep anesthesia by intraperitoneal injection of 10% chloral hydrate (0.3 g/kg, intraperitoneal [i.p.]), the SD rats were rapidly perfused through the left ventricle with ice-cold sucrose artificial cerebrospinal fluid (sucrose ACSF, mmol/L): sucrose, 75; NaCl, 80; KCl, 2.5; CaCl2, 2.5; MgCl2, 1.2; NaH2PO4, 1.25; NaHCO3, 25; Ascorbate, 1.3; Pyruvate, 3.0. Then the thoracic and lumbar vertebrae was intercepted and fixed on the gel layer of culture dish which was full of 4°C sucrose ACSF. After a lumbosacral laminectomy, the lumbosacral spinal cord with dorsal roots was removed. Vibrating microtome (3000 sectioning system, Vibratome, UK) was used to cut a spinal sagittal slice (400-500 μm thick) with an attached segmental dorsal root (L4 or L5). Spinal cord slice was maintained in standard artificial cerebrospinal fluid (normal ACSF, mmol/L): NaCl, 125; KCl, 2.5; CaCl2, 2.0; MgCl2, 1.0; NaH2PO4, 1.25; NaHCO3, 26; D-glucose, 25; Ascorbate, 1.3; Pyruvate, 3.0, which was equilibrated with 95% O2 + 5% CO2 at 24 ± 1°C for 30 minutes.

Drug Application

Concentration of IFL was given as volume percent (3%) by passing the gas mixture (flow rate, 0.5 L/minute) into equilibrated ACSF through the respective vaporizers (Penlon Limited, UK) for at least 15 minutes at room temperature (24 ± 1°C) (28). Solutions with IFL were contained in pyrex erlenmeyer flasks covered tightly with parafilm. The solubility of IFL at room temperature in ACSF is approximately two fold greater than at 37°C. The concentration of IFL used in this study was equal approximately to twice the concentration of in vivo anesthesia (29, 30).

Electrophysiology

The electrophysiological recording procedures and neuronal type characterization were consulted from our previous publications (22-24). For recordings, spinal cord slice was transferred into a small volume laminar flow perfusion chamber and continuously perfused at 4-6 ml/minute with normal ACSF. SG neurons were directly visualized under a fluorescent microscope (BX51WI, Olympus, Japan) and displayed on a video moniter (SCH-14H, Stonesonic, Chaozhou, China). Pipette microelectrode (Sutter instrument, Novato, CA, USA) was filled with internal solution (mmol/L: K gluconate, 130; KCl, 5; Mg-ATP, 4; phosphocreatine, 10; Li-GTP, 0.3; HEPES, 10; pH, 7.3; 300 mOsm) with resistances of 10-15 MΩ.

Voltage-clamp whole-cell recordings were obtained from SG neurons by holding neurons at -70 mV to record excitatory postsynaptic currents (EPSCs) events. The firing pattern of neurons was tested in current clamp model with holding potential of -60 mV with 1 second long depolarizing pulses from the recording electrode. The attached dorsal root was stimulated by a suction electrode which connected with a stimulator (S88X Stimulator, Astro-Med, West Warwick, RI, USA). Graded 0.1-0.5 ms pulses stimulation of dorsal root was applied to evoke EPSCs (eEPSCs) in SG neurons. If dorsal root evoked response had a constant conduction velocities in several trials, repetitive stimulation (20 Hz for Aδ fiber response; 1 Hz for C fiber response) was used to further judge the monosynaptic or polysynaptic responses (22, 23).
Each eEPSC was recorded for 5 minutes, averaged from 15 eEPSCs sweeps. Action potentials evoked by depolarizing current injection were recorded under current clamp mode with clamping current 0 pA.

Series resistances typically were less than 10 MΩ and were monitored throughout the recording period. Recording from a cell was discarded if the series resistance changed significantly (±25%) and the original value could not be recovered by manipulation of suction applied to the recording pipette. For reasons of recording stability, series resistance compensation was not used. Because series resistance was at least an order of magnitude less than the cell input resistances, voltage or current measurement errors attributable to series resistance were presumed to be less than 10% (22, 23).

Axopatch 200B amplifier (Axon instruments, Union City, CA, USA), a Digidata 1440A (Axon instruments, Union City, CA, USA) and pClampfit10 software (Axon instruments, Union City, CA, USA) were used for data acquisition and analysis. The entire recording process was made at room temperature (24 ± 1 °C). Chemical compounds used in this study were obtained from Sigma Company.

Characterization of Fiber Types

The types of primary fibers forming monosynaptic connection with SG neurons were categorized by the conduction velocity according to the latency and conduction distance. Details for the characterization were described in our previous publications (3, 31, 32). In this study, the Aδ and C fiber mediated eEPSCs were evoked by the intensity of stimulation at 0.98 ± 0.52 V and 5.13 ± 1.45 V, respectively. The conduction velocities of Aδ and C fibers were 1.37 ± 0.42 m/s and 0.28 ± 0.03 m/s, respectively, which were consistent with our previous studies (3, 31, 32).

Statistical Analysis

Prism 6.0 software was used to the statistical data analysis and plotting, including the test of normality of the distribution and homogeneity of variance. Data were presented as means ± standard deviation (SD). The difference of eEPSCs amplitude changes before and after dosing was analyzed by paired t-test. Difference was defined as significant at P<0.05.

RESULTS

IFL Significantly Inhibited the Peak Amplitude of Monosynaptic eEPSCs Mediated by Primary Aδ Fibers

Ten minutes after stable whole-cell recordings, Aδ fiber-mediated monosynaptic eEPSCs were recorded as control. After superfusion with IFL equilibrated ACSF for 1 minute, the amplitude of monosynaptic eEPSCs was significantly inhibited about 37.24 ± 14.31%, compared with controls (**P<0.01, IFL vs control; P>0.05, Wash vs control; N=8). This inhibitory effect could be washed out by continuous perfusion of normal ACSF for about 10 minutes (Figure 1), suggesting that the suppressive effect of IFL on synaptic transmission from Aδ fibers to SG neurons is reversible in a short time schedule.

IFL Significantly Suppressed C Fiber-Mediated Monosynaptic eEPSCs

The whole cell recordings were performed as same as experiment one, the membrane potential was held at -70 mV. The segmental dorsal root was stimulated by a suction electrode. When the C fiber-mediated monosynaptic eEPSCs were challenged by IFL, the peak amplitude of eEPSCs was suppressed by 28.67 ± 1.05% (***P<0.001, IFL vs control; P>0.05, Wash vs control; N=7). The suppressive effect of IFL on synaptic transmission from C fibers to SG neu-
IFL Suppressed the Action Potential Firing Frequency of SG Neurons

To further examine the effect of IFL on the excitability of SG neurons, the whole-cell recordings were switched to current clamp mode. Depolarizing current injection to recorded neurons was used to initiate action potentials. Tonic (Figure 3, upper panel) and phasic firing (Figure 3, lower panel) patterns were evidenced in most recorded SG neurons, only a few neurons expressed delayed firing pattern (data not shown). Perfusion IFL equilibrated normal ACSF for 1 minute significantly decreased the firing frequency of SG neurons. The numbers of APs decreased from 7.28 ± 1.09 to 3.13 ± 1.33 in tonic firing neurons (**P<0.001, IFL vs control; P>0.05, Wash vs control; N=10, Figure 3, upper panel), from 8.09 ± 2.01 to 3.12 ± 2.03 in phasic firing neurons (**P<0.001, IFL vs control; P>0.05, Wash vs control; N=9, Figure 3, lower panel) and from 4 to 2 in delayed firing neurons (**P<0.01, IFL vs control; P>0.05, Wash vs control; N=2; data not shown). These effects were reversible in all cases (Figure 3).

DISCUSSION

Superficial dorsal horn is the spinal center for pain transmission and modulation, which receives the peripheral nociceptive information mainly through primary C and Aδ fibers (33). Electrical stimulation to primary nociceptive afferents can imitate different intensity of pain signals. The present electrophysiological study demonstrated that IFL can reduce the excitability of SG neurons and suppress the synaptic transmission from primary nociceptive fibers to SG neurons in the spinal dorsal horn, suggesting that IFL plays analgesic role through inhibiting or weakening the excitability and synaptic transmission of spinal nociceptive pathway.

An earlier study has used the similar recording techniques and examined the effect of IFL on the spinal excitatory synaptic transmission at 37℃ (34). They did find the suppressive action of IFL on the excitatory polysynaptic responses in SG neurons possibly through the potentiation of the inhibitory synaptic transmission, but failed to detect the direct effects on excitatory monosynaptic responses. Because the solubility of IFL significantly depends on the environmental temperature, the solubility of IFL at room temperature (24℃) is approximately two fold greater than at 37℃. The concentration of IFL used in the present study is about 3%, two fold greater than the previous study (1.5%) (29, 30, 34). Therefore, the different concentration of IFL may be responsible for the discrepancies between our study and the earlier study.
Most studies concerning the effect of IFL on neuronal excitability and synaptic transmission were conducted in the brain neurons. Antkowiak and Helfrich-Forster (15) have reported that IFL can enhance the central inhibitory synaptic response which was mediated by GABA receptors. Jones and Harrison found that IFL can extend GABA receptor-mediated inhibitory postsynaptic currents in cultured rat hippocampal neurons (16). Extracellular recording of hippocampal CA1 neurons showed that 2.0% IFL can reduce NMDA receptor-mediated current (17).

Several studies revealed that IFL can reduce the excitability of central neurons. IFL can increase the chloride ion flow (13, 22), decrease membrane resistance via activation of the two pore leak potassium channels (35, 36), and finally leads to the hyperpolarization of central neuronal membrane and decrease of action potential discharges. Our data suggested that IFL can significantly reduce the action potential firing frequency of SG neurons, which was consistent with the findings obtained from central neurons. Recently, the spinal mechanisms of IFL-induced analgesia have become a new research focus. Zhou et al. (18) reported that emulsified IFL can produce regional anesthesia by inhibiting of voltage-gated Na+ channels, and enhance nociceptive blockade of QX-314 by thermal transient receptor potential vanilloid 1 (TRPV1) channel activation in the spinal level (19, 37).

The limitation of present study would be the nature of in vitro recordings. The isolated spinal cord slices were maintained in a somewhat artificial environment. For instance, the room temperature is much lower than normal physical condition. Therefore, the effects of IFL revealed in this study may not be a mimic of in vivo experiments. Further in vivo study is needed.

In conclusion, the present study illuminated that IFL may directly inhibit the excitability and synaptic transmission of spinal nociceptive pathway, and may contribute to the analgesia action.

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All authors have no other potential conflicts of interest for this work.

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