Inhibitory effects of spinal propofol on the responses of spinal dorsal horn neurons in normal rats

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Abstract: Spinal dorsal horn neurons play an important role in the processing of sensory information and are also targets of modulation by both endogenous and exogenous drugs. Propofol is an intravenous anesthetic and whether it has direct modulatory actions on sensory neuronal responses of the spinal cord dorsal horn has not been well studied. In the present study, a single dose (0.5 µmol) of propofol dissolved in dimethyl sulfoxide (DMSO) was directly applied onto the dorsal surface of the spinal cord and its effect was evaluated in 25 wide-dynamic-range (WDR) neurons and 10 low-threshold mechanoreceptive (LTM) neurons by using extracellular single unit recording technique in sodium pentobarbital anesthetized rats. Compared with the DMSO treatment, propofol produced a significant inhibition of WDR neuronal activity evoked by both noxious heat (45, 47, 49 or 53°C, 15 s) and mechanical (pinch, 10 s) stimuli applied to their cutaneous receptive fields (cRF) on the ipsilateral hind paw skin. To investigate whether propofol exerts a modulatory effect on non-nociceptive afferent-mediated activity, the responses of WDR or LTM neurons to non-noxious brush and pressure were also evaluated. The non-noxious mechanically-evoked responses of both WDR and LTM neurons were significantly suppressed by propofol. The present results indicate that propofol has direct actions on the dorsal horn neurons of the spinal cord in rats. However, since both non-nociceptive and nociceptive afferent-mediated activity can be suppressed, the spinal effects of propofol are not likely to be specifically associated with anti-nociception.

Key words: propofol; dorsal horn of spinal cord; nociception; anti-nociception

异丙酚对正常大鼠脊髓背角感觉神经元反应性的抑制作用

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摘要：脊髓背角感觉神经元不仅在感觉信息的传递和调节中起到重要作用，也是各种内源性和外源性药物的作用靶位。为了了解麻醉药物异丙酚是否对脊髓感觉神经元的反应性具有调节作用，本实验采用在体细胞外单细胞记录技术，观察了脊髓背表面直接滴注0.5 µmol 异丙酚对正常大鼠脊髓背角广泛动态范围（WDR）神经元和低阈值机械感受器（LTM）神经元反应性的影响。实验发现，异丙酚能抑制背角 WDR 神经元由施加于外周感受器的伤害性热刺激（45、47、49 和 53°C，15 s）和夹捏机械刺激（10 s）诱发的反应性，与 DMSO 对照组比较具有显著性统计学差异（P<0.05）；同样，异丙酚对非伤害性机械刺激诱发的 WDR 或 LTM 神经元的反应性也具有显著的抑制作用（P<0.05）。本结果提示，异丙酚可直接作用于正常大鼠脊髓背角神经元，对由非伤害性和非伤害性纤维介导的伤害性反应性均产生抑制作用，因此异丙酚的脊髓背角反应作用可能不是特异性的。

关键词：异丙酚；脊髓背角；伤害性；抗伤害性。

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The intravenous anesthetics have long been known to exert their pharmacological effects mainly via acting at the supraspinal level of the central nervous system, including cerebral cortex, hippocampus, thalamus and brainstem[1].

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However, the actions of intravenous anesthetics in the spinal cord have also been suggested since 1990s[2]. Propofol (2,6-diisopropyl phenol) is one of the most widely used intravenous anesthetics in clinic[3] and is thought to be involved in the modulation of motor responses to peripheral noxious stimulation in behavioral surveys[4-6]. In electrophysiological studies, although propofol has been demonstrated to be effective in suppression of nociceptive ventral root potential (VRP) in neonatal rat spinal cord preparation in vitro[7] and of spinal dorsal horn sensory neuronal responses in cat and goat in vivo[8,9], the results are not conclusive and required to be further studied due to differences in experimental models and animal species. Moreover, regarding the spinal effects of propofol, so far it has only been evaluated by systemic administration of the drug and the supraspinal effects of the drug can not be excluded.

The dorsal horn of the spinal cord is the major target of nociceptive primary afferent input from the body and is important in the mediation of pain information. Based on electrophysiological characteristics, three classes of dorsal horn neurons are identified and referred to as: (1) low-threshold mechanoreceptive (LTM) neurons, which only receive Aβ fiber input; (2) wide-dynamic-range (WDR) neurons, which receive Aβ, Aδ and C fiber input; (3) nociceptive specific (NS) neurons, which receive Aδ and C fiber input[10]. Among the three dorsal horn neurons, WDR neurons are believed to be the major ones involved in the mediation of spinoally-organized nociceptive flexion reflex and play important roles in the development of pain hypersensitivity (hyperalgesia and allodynia)[11]. In the present study, we dissolved 99.99% propofol in dimethyl sulfoxide (DMSO) and applied 5 µl solution containing 50 mmol/L sodium acetate. Explorations with microelectrodes were made in the dorsal horn using an electronically controlled microstepping manipulator. To minimize the central changes caused by searching stimuli, electrical current pulse at Aβ strength (100 µA, 50 µs, 1 Hz) was applied to the skin of the hind paw ipsilateral to the recording site as a search stimulus to identify dorsal horn neurons. After Aβ response was induced, 2 or 3 electrical stimuli at C fiber strength (5 mA, 500 µs, 1 Hz) were applied to test whether the neuron was multireceptive. The dorsal horn neuron was identified as LTM or WDR unit on the basis of its characteristic responses to mechanical stimuli applied to the receptive field. The LTM units used for the present study were characterized by: (1) having a cutaneous receptive field (cRF) consisting of a small low threshold area ipsilateral to the recording site; (2) only responding to brush, but not responding to pressure and noxious pinch. The WDR units used for the present study were characterized by: (1) having a cRF consisting of a small low threshold center and a large high threshold surround ipsilateral to the recording site; (2) responding with an increasing firing rate to brush, pressure and noxious pinch applied to the low threshold center, but for stimuli applied to the high threshold surround, only noxious pinch evoked spike response; (3) showing no apparent accommodation when continuous noxious stimulation was applied. After successful iden-

1 METHODS

1.1 Animals. Experiments were performed on male Sprague-Dawley albino rats (180~250 g). Animals were provided by Laboratory Animal Center of the Fourth Military Medical University (FMMU) and the use of the animals was reviewed and approved by the FMMU Animal Care and Use Committee. Animals were housed under a 12:12 h light/dark cycle at 22–26°C, with the lights on at 8:00 am–8:00 pm. Food and water were available ad libitum.

1.2 Electrophysiological recording. The rats were initially anesthetized by intraperitoneal injection with sodium pentobarbital (50 mg/kg) and the general anesthesia was maintained by an intravenous dose of pentobarbital-pipercuronium solution (pentobarbital 2.5 mg/kg·h⁻¹ and pipercuronium 0.4 mg/kg·h⁻¹) and supplemented when required. A tracheal cannula was inserted and the animal was placed in a stereotaxic frame. The animal was then paralyzed by an intravenous injection of pipercuronium (0.4 mg/kg·h⁻¹) and artificially ventilated with oxygen at a tidal volume of 15 ml/kg. Adequate anesthesia was confirmed intermittently during the whole experiment by examining whether the animal made spontaneous movements or had arousal responses to noxious pinch applied to the skin at the time when the muscle relaxant wore off. Core body temperature was monitored through a thermister probe inserted into the rectum and maintained at 37.5 ± 0.5°C by means of a feedback-controlled heating pad under the ventral surface of the abdomen. A laminectomy was performed from the T1 to L2 vertebrae to expose the lumbosacral enlargement of the spinal cord. The dura mater was longitudinally opened and the exposed cord was covered with warm fluid paraffin oil (37°C) to prevent it from drying. Extracellular single unit recordings were made from L4-5 with glass capillary microelectrodes (10–15 ΜΩ filled with 0.5 mol/L sodium acetate). Explorations with microelectrodes were made in the dorsal horn using an electronically controlled microstepping manipulator. To minimize the central changes caused by searching stimuli, electrical current pulse at Aβ strength (100 µA, 50 µs, 1 Hz) was applied to the skin of the hind paw ipsilateral to the recording site as a search stimulus to identify dorsal horn neurons. After Aβ response was induced, 2 or 3 electrical stimuli at C fiber strength (5 mA, 500 µs, 1 Hz) were applied to test whether the neuron was multireceptive. The dorsal horn neuron was identified as LTM or WDR unit on the basis of its characteristic responses to mechanical stimuli applied to the receptive field. The LTM units used for the present study were characterized by: (1) having a cutaneous receptive field (cRF) consisting of a small low threshold area ipsilateral to the recording site; (2) only responding to brush, but not responding to pressure and noxious pinch. The WDR units used for the present study were characterized by: (1) having a cRF consisting of a small low threshold center and a large high threshold surround ipsilateral to the recording site; (2) responding with an increasing firing rate to brush, pressure and noxious pinch applied to the low threshold center, but for stimuli applied to the high threshold surround, only noxious pinch evoked spike response; (3) showing no apparent accommodation when continuous noxious stimulation was applied. After successful iden-
tification of a single LTM or WDR unit, the unit responsiveness to 15 s thermal stimuli or 10 s mechanical was recorded. The mechanical stimuli were (1) brush, performed by stroking on the center of the cRF at a frequency of 1 time/s with a hairy paint brush; (2) pressure, performed by picking up a fold of skin with a flattened alligator clip to produce a consistent strength that was not painful when tested on the experimenter’s skin; and (3) noxious pinch performed by pinching a fold of skin with a small serrated clip to produce a consistent strength which was obviously painful when tested on the experimenter’s skin. The forces of pressure and pinch stimuli applied to the cRF were uniform although the precise strength of the stimulus was not available. The unit responsiveness to heat stimuli was measured with a DPS-705 thermal stimulator (Inter Medical Co., Japan). The spike trains were monitored with a memory oscilloscope and the numbers of neuronal firing were simultaneously recorded and saved on a PC computer via an A/D converter following spike discriminator and counter.

1.3 Drug administration. A volume of 5 µl DMSO or propofol (0.5 µmol) was used (99.99% propofol was provided by Xi’an LiBang Pharmaceutical Co., dissolved in DMSO). Drug was directly applied onto the dorsal surface of the spinal cord in site of the recorded neurons.

1.4 Data analysis. All results are expressed by mean ± SEM. The data between groups treated with drugs and vehicle were compared by using ANOVA post hoc analysis (Fisher’s PLSD test). P values <0.05 were considered to be statistically significant.

2 RESULTS

A total of 35 neurons were recorded from the spinal dorsal horn of the L₄₋₅ segments in 35 anesthetized rats. Twenty five of 35 neurons were identified as WDR neurons, while the other 10 neurons were LTM neurons.

2.1 Effect of propofol on responses of spinal WDR units to noxious heat stimuli

A period of 15 s noxious heat stimulus (45°C, 47°C, 49°C and 53°C) was applied to the cRF of 25 WDR units prior to or 10 min after DMSO or propofol administration. The heat response of a WDR unit increased gradually along with the increase in skin temperature (Fig. 1A) and those of 13 WDR units were not significantly changed by DMSO administration (Table 1). In contrast, the heat responses of 12 WDR units were all significantly reduced by propofol administration when compared with the values of baseline and DMSO administration (Table 1). The inhibitory rate of 12 WDR units in response to the above four temperatures by propofol was 86%, 75%, 66%, 72%, respectively. Figure 1 shows the responses of a typical WDR units prior to or 10 min after propofol administration.

Fig. 1. Inhibitory effects of spinal propofol on the responses of a WDR neuron to noxious heat stimuli. The spike response of a WDR neuron to radiant heat (45, 47, 49 and 53°C) applied to its cRF for 15 s prior to drug (A) and 10 min after drug treatment (B) with single dose (0.5 µmol /5 µl) of propofol. C: Site of cRF on the rat paw pad ipsilateral to the recording side in the spinal cord.
2.2 Effect of propofol on the responses of spinal WDR and LTM units to noxious and non-noxious mechanical stimuli

A 10 s mechanical stimuli was applied to the cRF of 35 units prior to or 10 min after i.t. propofol or DMSO administration. The mechanical response of a WDR unit increased gradually along with the increase in mechanical intensity (brush, pressure and pinch) (Fig. 2A), but only brush induced responses of LTM unit (Fig. 3A). Similarly, the responses of WDR and LTM units to mechanical stimulus were not significantly changed by DMSO administration (Table 2). Ten min after propofol was directly applied onto the dorsal surface of the spinal cord, compared with baseline values the mechanical responses of the 12 WDR units were significantly reduced (Table 2). The inhibitory rate of the WDR neurons in response to brushing, pressing and pinching by propofol was 27%, 36% and 21%, respectively. The responses of 5 LTM units to brushing were also markedly reduced (Table 2) with 78% inhibitory rate. Figures 2 and 3 show the responses of a WDR unit and a LTM unit prior to or 10 min after propofol administration, respectively.

Table 1. Inhibitory effects of propofol on the responses of spinal cord dorsal horn WDR neurons to noxious heat stimuli

<table>
<thead>
<tr>
<th>Nociceptive heat stimuli</th>
<th>45ºC</th>
<th>47ºC</th>
<th>49ºC</th>
<th>53ºC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO (n=13) Pre-treatment</td>
<td>27.81 ± 10.95</td>
<td>69.13 ± 12.35</td>
<td>108.24 ± 14.78</td>
<td>151.38 ± 29.92</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>23.83 ± 9.59</td>
<td>52.17 ± 13.22</td>
<td>97.33 ± 10.21</td>
<td>149.75 ± 22.24</td>
</tr>
<tr>
<td>Propofol (n=12) Pre-treatment</td>
<td>25.67 ± 9.63</td>
<td>57.20 ± 6.66</td>
<td>104.00 ± 10.15</td>
<td>168.67 ± 44.61</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>3.56 ± 2.13*</td>
<td>14.40 ± 4.43***</td>
<td>34.70 ± 7.30***</td>
<td>46.44 ± 11.94***</td>
</tr>
</tbody>
</table>

The value (mean ± SE) indicates the average number of spikes per second. Propofol post- vs pre-treatment: *P<0.05, ***P<0.001. Post-treatment with propofol vs with DMSO: *P<0.05, **P<0.01, ***P<0.001. DMSO post- vs pre-treatment: P>0.05.
Table 2. Inhibitory effects of propofol on the responses of spinal cord dorsal horn WDR and LTM neurons to mechanical stimuli

<table>
<thead>
<tr>
<th></th>
<th>Mechanical stimuli</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Brush</td>
<td>Press</td>
<td>Pinch</td>
</tr>
<tr>
<td>WDR</td>
<td>DMSO (n=13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-treatment</td>
<td>23.83 ± 9.39</td>
<td>36.43 ± 11.72</td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>21.50 ± 11.11</td>
<td>26.29 ± 11.11</td>
</tr>
<tr>
<td>Propofol (n=12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-treatment</td>
<td>44.00 ± 9.02</td>
<td>48.14 ± 8.65</td>
</tr>
</tbody>
</table>
|        | Post-treatment     | 12.00 ± 5.37* | 17.57 ± 6.52* | 45.57 ± 14.31**#
| LTM    | DMSO (n=5)         |        |        |
|        | Pre-treatment      | 105.14 ± 21.44 |        |        |
|        | Post-treatment     | 97.86 ± 19.53 |        |        |
| Propofol (n=5) |                   |        |        |
|        | Pre-treatment      | 113.80 ± 14.60 | 28.60 ± 15.94***### | 28.60 ± 15.94***### |
|        | Post-treatment     | 28.60 ± 15.94***### |        |        |

The values (mean ± SE) indicate the average number of spikes per second. Propofol post- vs pre-treatment: *P<0.05, **P<0.01, ***P<0.001; Post-treatment with propofol vs with DMSO: *P<0.05, ***P<0.001. DMSO post- vs pre-treatment: P>0.05.

Fig. 3. Inhibitory effects of propofol on the responses of a LTM neuron to non-noxious mechanical stimuli. The LTM neuron was only activated by brush applied to its cRF for 10 s (A), the spike response of the LTM neuron was decreased 10 min after a single dose (0.5 µmol /5 µl) of propofol (B). C: Site of cRF on the rat paw pad ipsilateral to the recording side in the spinal cord.

3 DISCUSSION

In the present study, we, for the first time, provided a new line of evidence showing direct local action of propofol on the dorsal horn of the spinal cord. The effects of propofol were consistent and we did not observe neurotoxic effect that might be caused by direct infiltration of the cord by the DMSO-propofol solution. Our previous behavioral survey also found no abnormal change in rat behaviors and spinal cord appearance after subarachnoid catheterization and intrathecal administration of the drug to the lumbar cord[12]. Both of our behavioral and electrophysiological studies showed feasibility in study of spinal effects of DMSO-propofol solution by direct administration to the
spinal cord. In this experimental paradigm, we found that propofol could inhibit both nociceptive (WDR) and nonnociceptive (LTM) neuronal activities in response to peripheral heat and mechanical stimuli, suggesting that propofol, on one hand, does have anti-nociceptive effects, but, on the other hand, the effects are not likely specifically associated with spinal analgesia and probably associated with inhibition of other sensorimotor functions observed in clinical anesthesia.

The analgesic effects of propofol were initially discovered in human subjects[13,14] and some recent clinical electrophysiological recordings of the compound muscle action potential and spinal primary afferent depolarization in human subjects suggest a spinal role of propofol in the spinal cord[15,16]. In the acutely isolated neonatal (1~5 d old) rat spinal cord preparation, superfusing 0.5~10 μmol/L propofol resulted in a dose-related, bicuculline antagonized inhibition of nociceptive slow VRP, suggesting γ-aminobutyric acid (GABA) A type receptor-mediated inhibition in the spinal cord[7]. In physiologically intact, awake and drug-free cats, Uchida et al. observed that intravenous injection (i.v.) of propofol (7.5 mg/kg) could suppress the responses and decrease the cRF size of spinal dorsal horn LTM neurons, however, the response of WDR neurons to mechanical and heat stimuli was not well studied[8]. The suppression of i.v. propofol on spinal WDR neuronal responses to clamping the cRF was found by electrophysiological recordings in isoflurane-anesthetized goat, however, the effects of i.v. propofol on heat-evoked responses were not studied[9]. In peripheral tissue injury model, i.v. propofol was also found to inhibit the formalin-induced c-Fos expression in the spinal cord laminae I[17,18]. These indirect data are consistent with our present results. Taken together, it is concluded that the propofol-produced analgesia is likely caused by its direct actions on the spinal cord and WDR neurons are the likely targets of the drug. Although the above results demonstrated a promising spinal cord anti-nociceptive actions of propofol under physiological state, the results of its anti-nociceptive effects in pathological pain state are controversial as shown in two studies by using the same rat formalin-test, namely, Goto and his colleagues did not observe propofol-produced analgesia[19], however, O’Connor and Abram found that it did[20]. The reasons for this discrepancy are not clear and need to be further studied by direct administration of propofol to the spinal cord. More recently, by intrathecal administration of propofol we found that propofol could inhibit persistent nociception induced by peripheral subcutaneous injection of bee venom, the bee venom test, which has been demonstrated to be comparable to the formalin-induced persistent nociception[12,21,22].

Regarding the molecular targets of propofol in the spinal cord, GABA_A receptors is thought to be the major one involved in mediation of propofol-produced anti-nociception, although other targets such as glutamate ionotropic receptors (NMDA or non-NMDA) and opioid receptors are also involved[4-5]. Since the functional binding sites of propofol on GABA_A receptors are associated with α-, β- and γ-subunit subtypes, the diverse distribution of different isoforms of GABA_A receptors in the CNS, might underlie the mechanisms of non-specific anti-nociceptive actions of propofol in the spinal cord[23-29].

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