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Short communication

Evidence for peripherally antinociceptive action of propofol in rats: Behavioral and spinal neuronal responses to subcutaneous bee venom

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Abstract

In the present study, behavioral and in vivo electrophysiological methods were used to examine the peripheral effects of propofol on tonic ongoing pain-related responses produced by subcutaneous bee venom-induced inflammatory pain state. Local administration of 0.5 μg propofol produced significant suppression of the well-established ongoing pain responses in both conscious rats and dorsal horn nociceptive neurons. The locally antinociceptive action of propofol is not caused by systemic effect, because contralateral administration of the same dose of drug did not produce any effect. This result indicates that besides central actions, propofol has peripherally antinociceptive action as well.
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Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Propofol; Bee venom; Spinal dorsal horn; Wide-dynamic-range neuron; Tonic ongoing pain; Inflammatory pain state

Propofol (2, 6-diisopropyl phenol) is one of the most widely used intravenous anesthetics in clinic and thought to exert its pharmacological actions at both spinal and supraspinal level of the central nervous system [11,21,23]. For the antinociceptive actions of propofol, it has been demonstrated to be effective in modulation of pain-related behavioral motor responses [2,18,19,25], of nociceptive ventral root potential in neonatal rat spinal cord preparation in vitro [12], and of spinal sensory neuronal responses in rats, cats, and goats in vivo and vitro [1,17,23,24]. Based upon the above behavioral and electrophysiological studies, it is likely that propofol can modulate spinally-organized nociceptive flexion reflexes via acting at both the sensory input (dorsal horn) and the motor output (ventral horn) of the spinal cord. Although propofol is believed to modulate sensory processing in the spinal cord, it is still reported to cause venous pain in 28–90% of patients in clinic when intravenously administered [14,20]. So it is intriguing to know whether the effect of peripheral propofol is analgesic or algogenic. In the present study, we used behavioral and in vivo electrophysiological methods to test the actions of peripheral propofol in a well-established inflammatory pain model by which we can evaluate the effect of drugs on both pain-related behaviors and spinal nociceptive neuronal activities [4–8,16,27–29].

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 use of the animals was reviewed and approved by the FMMU Animal Care and Use Committee. The IASP's ethical guidelines for pain research in conscious animals were followed [30]. Animals were housed under a 12-h light/dark cycle at 22–26 °C, with the lights on at 8:00 am to 8:00 pm. Food and water were available ad libitum.

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During the whole process of the experiments, a volume of 50 μl saline containing 0.2 mg bee venom (BV), 50 μl 100% dimethyl sulfoxide (DMSO), and a single dose of propofol (0.5 μg of 99.99% 2, 6-diisopropyl phenol dissolved in 50 μl DMSO) was subcutaneously injected into the posterior surface of one hind paw, respectively. To study the local effects of DMSO or propofol under both normal and abnormal states, the vehicle and the drug were administered in naive rats (without any insult) or in inflamed rats receiving BV injection on the same site of ipsilateral or contralateral hind paw. The timing for DMSO or propofol administration was 5 min after BV when the behavioral ongoing pain and the tonic spinal neuronal discharges had been well established.

All behavioral testing was done between 9:00 am and 6:00 pm. The rats were acclimatized to the laboratory and habituated to the test boxes for at least 30 min each day for 5 days before behavioral testing. The spontaneous nociceptive behavioral response of the rat was determined by counting the number of paw flinches during each 5-min interval for 1 h following subcutaneous injection. In electrophysiological testing, the rats were initially anesthetized by intraperitoneal injection with ketamine (100 mg/kg) and general anesthesia was maintained by an intravenous dose of urethane–chloralose solution (urethane 125 mg/ml and chloralose 10 mg/ml) at 5 ml/kg 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 pancuronium bromide (2–4 mg/kg/h) and artificially ventilated with oxygen at a tidal volume of 15 ml/kg. Core body temperature was monitored through a thermistor probe inserted into the rectum and maintained at 37.5 ± 0.5 °C. A laminectomy was performed from the T_{13} to L_2 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 L_{4-5} with glass capillary microelectrodes (10–15 MΩ filled with 0.5 M sodium acetate). Explorations with microelectrodes were made in the dorsal horn using an electronically controlled microstepping manipulator. 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. Wide-dynamic-range (WDR) unit was identified on the basis of its characteristic responses to mechanical stimuli applied to the cutaneous receptive field [for details see Refs. [6,29]]. During each trial, we tried our best to make the WDR neuronal activity distinctly separated from the background noise to ensure that the recording was from a single unit [27–29].

All results were expressed by mean ± SEM. The data between drug- and vehicle-treated groups were compared by using ANOVA and multiple comparisons of post hoc analysis. *P* values < 0.05 were considered to be statistically significant.

In the BV test, subcutaneous injection of BV could produce paw flinching response for 1–2 h. In comparison with DMSO, local injection of propofol began to suppress the number of BV-produced paw flinches 5 min after administration and such local antinociceptive effect lasted for 40 min (Fig. 1A). To exclude the systemic effect of propofol, the same dose of the drug was subcutaneously administered on the contralateral hind paw to the BV-treated side. Contralateral injection of propofol did not influence the BV-produced paw flinches (Fig. 1A). The averaged number of paw flinches per 5 min of 1 h time course following treatment of DMSO (n = 8), contralateral (n = 7), and ipsilateral (n = 8) propofol was shown in Fig. 1B.

For the electrophysiological study, a total of 30 WDR neurons were identified and recorded from the spinal dorsal horn of the L_{4-5} segments in 30 anesthetized rats. All neurons in the present study had cutaneous receptive field on the ipsilateral hind paw. As shown in Fig. 2A, injection of BV into the neuronal cutaneous receptive field produced an immediately robust increase in ongoing spike discharges lasting for about 50 min (n = 6). Local injection of DMSO 5

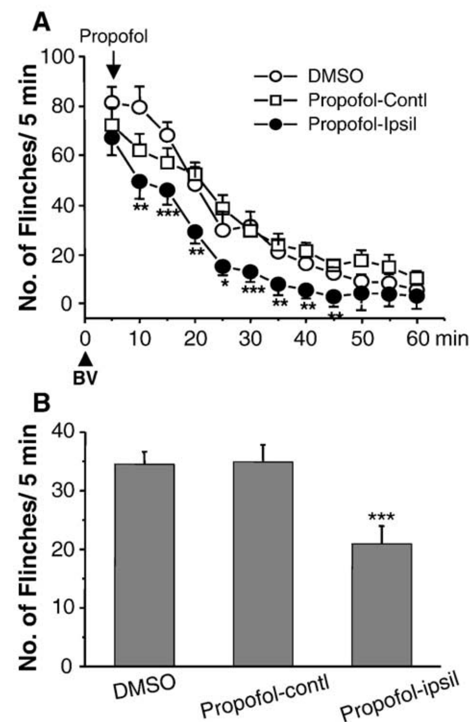

Fig. 1. Effects of subcutaneous administration of propofol or vehicle (dimethyl sulfoxide, DMSO) on the bee venom (BV)-induced persistent ongoing pain-related behaviors, e.g., paw flinching reflex. (A) Curve graph shows time courses of the rats' paw flinches recorded at each 5-min time block for a period of 1 h following local injection of DMSO (ipsilateral, n = 8), Propofol-Contl (contralateral, n = 7), and Propofol-Ipsil (ipsilateral, n = 8) 5 min after subcutaneous BV injection. (B) Bar graph shows the mean number of paw flinches per 5 min in a period of 1 h for DMSO, Propofol-Contl, and Propofol-Ipsil groups. Upright arrow indicates the starting time of BV and the reverse arrow indicates that of drug administration. *P < 0.05; **P < 0.01; ***P < 0.001. Error bars: ±SEM.

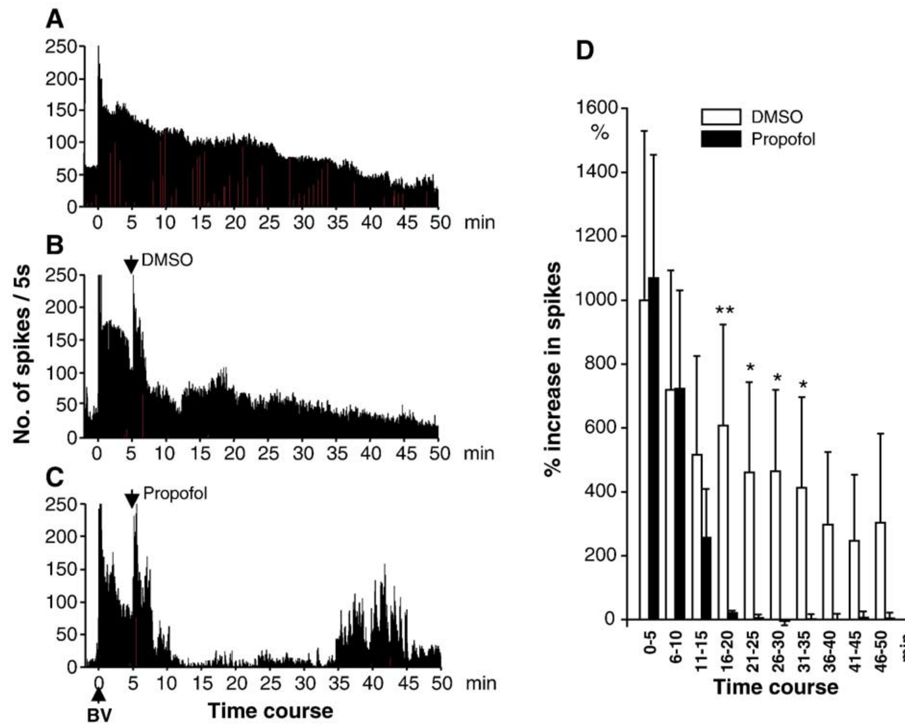

Fig. 2. Effects of subcutaneous administration of propofol or vehicle (dimethyl sulfoxide, DMSO) on the bee venom (BV)-induced increased neuronal activity in anesthetized rats. (A) A typical example of time course recording of the BV-induced tonic spike discharges of a spinal wide-dynamic-range (WDR) neuron; (B and C) representative examples of the effect of DMSO or propofol on BV-induced increase in tonic spike discharges recorded in two single WDR units. (D) Bar graph shows comparison of the mean percent increase in spike discharges per 5 min time block of a 50-min period between DMSO (n = 6) and propofol (n = 6) administration. *P < 0.05; **P < 0.01. Error bars: ±SEM.

min after BV administration produced a transient increase in spikes first and followed by a slight depression for less than 5 min (Fig. 2B), while in contrast, the same treatment with propofol resulted in a distinct suppression of the BV-produced increase in neuronal firing lasting 20–25 min although a transient increase in spikes was also evoked (Fig. 2C). Pooled time course data and statistical comparisons between DMSO- (n = 6) and propofol-treated (n = 6) groups were also performed. It was shown that the percent increase in spikes was not of statistical significance at 0–5 min, 6–10 min, and 11–15 min time blocks between the DMSO- and propofol-treated groups; however, in the drug-treated group, the increased activity was significantly decreased compared with the vehicle-treated group at 16–20 min, 21–25 min, 26–30 min, and 31–35 min time blocks (Fig. 2D). Although the neuronal activity was still lower at 36–40 min, 41–45 min, and 46–50 min time blocks in the drug-treated group, the statistical difference was not observed due to individual difference in activity among neurons (Fig. 2D). Contralateral administration of propofol did not produce any visible change in neuronal activity of the BV-treated side (n = 3, data not shown).

For the above electrophysiological recordings in inflamed rats, we found that both DMSO and propofol produced an immediate, transient increase in spike discharges following subcutaneous administration (Figs. 2B and C). Since this exaggerated neuronal response could be a result of an artifact caused by injection itself and solution diffusion or excitatory

Table 1
Comparison of the local effects of subcutaneous administration of propofol (PPF) and dimethyl sulfoxide (DMSO) on both behavioral and spinal dorsal horn neuronal responses in rats

Time	No. of behavioral flinches/5 min		No. of neuronal discharges/s	
	DMSO (4)	PPF (5)	DMSO (4)	PPF (8)
−2–0 min	0	0	7.97 ± 2.14	8.68 ± 1.96$^{n.s.}$
0–5 min	35.75 ± 3.75	33.80 ± 2.41$^{n.s.}$	14.91 ± 3.78*	17.14 ± 2.18$^{n.s.,*}$
6–10 min	13.50 ± 3.40	6.90 ± 2.30$^{n.s.}$	11.40 ± 2.70	13.56 ± 1.88$^{n.s.}$
11–15 min	0	0	8.23 ± 2.12	7.80 ± 3.20$^{n.s.}$

Note. 1% PPF was dissolved in 50 μl 100% DMSO. ANOVA post hoc analysis was used for comparisons between PPF and DMSO (n.s., no significant) as well as comparisons of values of time points between after-treatment and before-treatment (*P < 0.05). Time −2–0 min is a time block prior to any treatment, while 0–5, 6–10, and 11–15 are time blocks after DMSO or PPF treatment. Numerical in parenthesis indicates the number of animals or single units studied.

effect of the drug and DMSO, we further saw the local effect of propofol and DMSO in both behavioral and electrophysiological recordings in naive rats. Similar to the result of vehicle, local propofol produced transient spontaneous paw flinches and increase in spike discharges of spinal WDR units lasting less than 5–10 min (Table 1). There was no significantly statistical difference in the number of paw flinches and the increased neuronal spike discharges between propofol- and DMSO-treated group ($P > 0.05$, Table 1).

The present result, for the first time, showed that subcutaneous injection of propofol could suppress the established persistent ongoing pain state in both behavioral and electrophysiological examination. Although local propofol produced a transient "irritation" phenomenon, such as short-lasting paw flinches and increased neuronal activity, it was likely to be caused by the vehicle DMSO, but not propofol, since in both behavioral and electrophysiological studies the "irritant" effect of DMSO far more outlasted that of propofol. Moreover, contralateral injection of propofol did not affect the BV-induced persistent responses, suggesting that the local antinociceptive effect of propofol should not be produced by systemic action. This result implicates that propofol might be more useful than previously thought for clinical analgesia by subhypnotic dose.

So far, the molecular targets of propofol at the peripheral site are not clear but are worthy of further studying. It has been suggested that the dorsal horn functional changes are state-dependent and initially evoked and/or driven by activation of peripheral nociceptors following peripheral tissue or nerve injury [13,26]. In our previous studies, we also demonstrated that the BV-induced tonic spike discharges of WDR neurons are primarily evoked and driven by ongoing primary afferent impulses conducted by the capsaicin-sensitive primary afferent fibers [5–7,27]. It has also been proved that long-lasting, tonic firing of the dorsal horn WDR neurons can facilitate spinally-organized nociceptive flexion reflex [4,28,29]. In the induction and maintenance of the BV-induced tonic ongoing spike discharges of the WDR neurons, peripheral excitatory amino acids and their ionic *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors were demonstrated to be involved in the processing [7,27]. Because NMDA-evoked currents can be blocked by propofol [22], glutamate NMDA receptor subtypes are likely to be the acting targets of propofol in periphery. Also because several lines of evidence showed existence of $GABA_A$ receptors in DRG nociceptor cells [9,15,17] and existence of multiple binding sites on $GABA_A$ receptors for propofol [3,10], the local modulatory effect of propofol on tonic pain responses might be associated with peripheral action on $GABA_A$ receptors.

In conclusion, besides its central actions, propofol also has peripherally antinociceptive effects on ongoing pain produced by peripherally inflammatory pain state and the molecular targets of propofol in the periphery of the somatic system remain to be further studied.

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