Characteristics of propofol-evoked vascular pain in anaesthetized rats

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Background. In this study we have assessed vascular pain caused by the i.v. anaesthetic agent, propofol, using the flexor reflex response and compared this with that of capsaicin in anaesthetized intact rats.

Methods. Experiments were performed on 133 male Sprague–Dawley rats weighing 280–340 g. The animals were anaesthetized with urethane (1.3 g kg\(^{-1}\), i.p.), and an arterial cannula was inserted to the level of the bifurcation of the femoral artery. The magnitude of the flexor reflex was examined by recording the electromyogram from the posterior biceps femoris/semitendinosus muscles.

Results. Our data show that the flexor reflexes evoked by intra-arterial (i.a.) injection of propofol (1%, 25–100 \(\mu\)l) and capsaicin (0.05–0.2 \(\mu\)g) were dose dependent. An initial i.a. injection of procaine (2%, 200 \(\mu\)l) blocked both responses. Furthermore, the flexor reflex induced by these chemical stimuli were inhibited by morphine (5 mg kg\(^{-1}\), s.c.) and restored with naloxone (1.5 mg kg\(^{-1}\), s.c.). Pre-treatment with capsazepine (20 \(\mu\)g, i.a.), a selective VR1 antagonist, inhibited the capsaicin-evoked response, but not that of propofol. Indomethacin (10 mg kg\(^{-1}\), i.p.), a non-selective cyclo-oxygenase inhibitor, inhibited only the propofol-evoked response and this recovered with arterial PGE\(_2\) (5 \(\mu\)g).

Conclusions. Collectively our data suggest that propofol-evoked vascular pain is mainly initiated by prostanoids.

Materials and methods

Drugs

The following drugs were used: propofol (1% Diprivan, Astra Zeneca), capsaicin (0.5 mg ml\(^{-1}\), Sigma) dissolved in vehicle (ethanol 10%, 10% TWEEN 80, 80% Ringer’s solution) and diluted with Ringer’s solution (5 \(\mu\)g ml\(^{-1}\)) just before the experiment, urethane 1.3 g (Sigma) dissolved in 10 ml H\(_2\)O, lidocaine hydrochloride (5% Xylocaine, Astra Zeneca), procaine hydrochloride (2% Rocaine, Fuso Pharmaceutical Industries, Ltd), morphine hydrochloride (Sankyo Co. Ltd) and naloxone hydrochloride (Sigma) dissolved in saline, capsazepine (VR1 antagonist, Sigma) dissolved in dimethyl sulfoxide 1% (DMSO, Sigma), indomethacin (Sigma) suspended in 0.5% TWEEN 80, and prostaglandin E\(_2\) (Sigma) dissolved in 0.05 ml ethanol and then diluted with Ringer’s solution (10 \(\mu\)g ml\(^{-1}\)).
Dose and route of administration

Propofol (25, 50, and 100 μl), capsaicin (0.05, 0.1, and 0.2 μg), procaine (200 μl), prostaglandin E_2 (5 μg), capsazepine (20 μg) and its vehicle were injected into the artery at a constant rate (0.8 ml min^-1) in Ringer’s solution. Lidocaine (500 μg 10 μl^-1) was given by intrathecal (i.t.) injection with artificial cerebrospinal fluid (CSF; 126.7 mM NaCl, 2.5 mM KCl, 2.0 mM MgCl_2, and 1.3 mM CaCl_2, 20 μl min^-1). Morphine hydrochloride (5 mg kg^-1), naloxone hydrochloride (1.5 mg kg^-1) and its vehicle were injected subcutaneously. Urethane (1.3 g kg^-1), indomethacin (10 mg kg^-1) and its vehicle were administered intraperitoneally.

Animal preparation

The studies were approved by the Committee on Animal Experiments of Tohoku Pharmaceutical University. All experiments were performed on male Sprague–Dawley rats (n=133, Japan SLC), weighing 280–340 g, which were housed in standard stainless steel cages (30.0×40.0×20.0 cm, width×depth×height) at a constant temperature [23 (1)°C] and relative humidity [53 (2)%] under a 12-h light–dark cycle, with food (CE-2, CLEA Japan, Inc.) and water ad libitum. Arterial and i.t. cannulae were made of silicon-coated polyethylene tubing (PE-10) tapered to an appropriate size by heating. The arterial and i.t. cannulations were performed simultaneously under urethane anaesthesia. To reduce spinal cord stimulation, before making the incision for cannula insertion, the skin was anaesthetized with lidocaine. The arterial cannula was inserted about 1 cm into the left superficial caudal epigastric artery, so that the tip of the cannula reached the bifurcation of the femoral and superficial caudal epigastric arteries. The spinal cord was exposed via a laminectomy at the L3–4 level. An i.t. cannula filled with artificial CSF was inserted caudally through an opening in the dura, and its tip was carefully placed in the subarachnoid space at L5–6. One hour after surgery, the animal was used for the experiment.

Measuring the flexor reflex

The magnitudes of the flexor reflexes in response to arterial propofol, capsaicin, and a pinching stimulus of the skin on the hind limb were measured using an EMG of the left posterior biceps femoris/semitendinosus muscle (Fig. 1). EMG activity was recorded using concentric needle electrodes (26-gauge, Medtronic Inc.) inserted into the muscles and a DAT data recorder (RD-135T, TEAC Co.) after amplification with a polygraph (System 360, NEC Co., Japan). Each EMG was analysed with a signal processor (DP1100, NEC Co.), which summed the amplitudes (mV) of the collected action potentials every 50 ms and displayed the result in rectified form. For quantitative analysis, the area of the rectified form within the EMG was integrated (mV s^-2) and used as the EMG response. In addition, the latency and duration of the EMG responses were measured for propofol and capsaicin stimuli. During the experiments, the rats were maintained at 37 (1±1°C) with a heating sheet. All animals were only used once.

Initial dose–response studies

To determine the doses of propofol and capsaicin to be used a dose–response curve for the flexion reflex with each agent was constructed. Increasing doses of propofol (25 μl, 50 μl,
100 µl) and capsaicin (0.05 µg, 0.1 µg, 0.2 µg) were injected at 60 min intervals in 3 rats in each group (a total of 6 rats).

Main experimental protocol

The effects of local anaesthetics

The effects of i.a. injection of procaine (200 µl) on propofol- and capsaicin-evoked EMG responses were examined in two groups of six rats (a total of 12 rats). Propofol or capsaicin were injected at 3, 60, and 120 min after the initial procaine injection. The effect of i.t. injection of lidocaine (500 µg 10 µl⁻¹) was performed using a similar protocol (a total of 12 rats). Propofol or capsaicin were injected at 5, 60, and 120 min after the initial lidocaine injection.

Desensitization

The potential for desensitization was evaluated with the administration of three repeated injections of propofol followed by one of capsaicin; or three repeated injections of capsaicin followed by one of propofol, within a short time interval (2 min) in six rats in each group (a total of 12 rats).

The effects of opioids

Rats were pre-treated with either morphine (5 mg kg⁻¹, s.c.) and naloxone (1.5 mg kg⁻¹, s.c.), morphine alone (5 mg kg⁻¹, s.c.) or saline (1 ml kg⁻¹, s.c.) (total 36 rats). Propofol or capsaicin were then injected at 30, 90, 150, and 210 min after pre-treatment.

The effect of capsazepine

In the experiments that used capsazepine (20 µg, i.a.), measurements of the propofol- and capsaicin-evoked flexor reflex were carried out at 1 and 60 min after arterial infusions of capsazepine or its vehicle (500 µl, i.a.) in five rats per group (a total of 20 rats).

The effect of indomethacin

Five rats were pre-treated with either indomethacin (10 mg kg⁻¹, i.p.) alone or, indomethacin (10 mg kg⁻¹) and prostaglandin E₂ (5 µg, i.a.) (injected 55 min after the indomethacin) (20 rats). Propofol or capsaicin was injected at 60 and 120 min after pre-treatment with indomethacin. In addition, propofol was injected at 60 and 120 min after a further five rats pre-treated with TWEEN 80 (0.5%, 1 ml kg⁻¹, i.p.).

The effect of prostaglandin E₂

The effects of prostaglandin E₂ (5 µg) on the propofol- or capsaicin-evoked flexor reflex were performed 5 min after pre-treatment in five rats in each group (10 rats).

Statistical analysis

All data were expressed as the mean (sd). Unless mentioned specifically in the text, the data were subjected to one-way or repeated-measures ANOVA. When appropriate, the analysis included Fisher’s Protected LSD post-hoc test. A significance level of P<0.05 was applied to all data.

Results

In this study a total of 133 rats were used.

Initial dose–response studies

On the basis of the EMG value, latency and duration (Fig. 2), we decided to perform the subsequent experiments using propofol (50 µl) and capsaicin (0.1 µg).

When the propofol (50 µl) and capsaicin (0.1 µg) evoked responses were compared, EMG values of the flexor reflex were similar (Table 1). Whilst the duration was similar for both treatments, propofol latency was about 2 s shorter than that of capsaicin (Table 1).

Effects of i.a. and i.t. treatment with local anaesthetics

An initial arterial infusion of procaine through the same cannula transiently blocked the flexor reflex of the infused hind limb to propofol and capsaicin administered within 3 min (P=0.0001, one-way ANOVA) (P<0.01, Fig. 3). Similar depression was found in rats injected with i.t. lidocaine 5 min after treatment (propofol, P=0.0026, one-way ANOVA; capsaicin, P=0.0001, one-way ANOVA) (P<0.01, Fig. 3). Lidocaine i.t. blocked the pinching-evoked flexor reflex at 5 min, while procaine i.a. at 3 min did not (data not shown).

Desensitization

Repeated injection of propofol and capsaicin at 2-min intervals produced desensitization in which the response was attenuated or disappeared. Cross-desensitization was not observed between propofol and capsaicin (Fig. 4).

Effects of opioids

The effects of morphine and the antagonist naloxone on propofol- and capsaicin-evoked flexor reflexes were studied. As shown in Figure 5, morphine pre-treatment caused a significant decrease in the EMG response to propofol at 30, 90, 150 (P<0.01), and 210 (P<0.05) min compared with the vehicle-treated group (P=0.0003, repeated-measures ANOVA). The EMG latencies were significantly prolonged at 30 (P<0.05), 90 (P<0.01), 150 (P<0.05), and 210 (P<0.05) min compared with the vehicle-treated group (P=0.0127, repeated-measures ANOVA, Table 2). In addition, the duration was significantly reduced at 30, 90, 150, and 210 (P<0.01) min compared with the vehicle-treated group (P=0.0001, repeated-measures ANOVA, Table 2). Similar effects of opioids on capsaicin-evoked EMG response and its latency and duration were observed. Morphine also significantly decreased the EMG responses of
capsaicin at 30–210 (P<0.01) min compared with the saline-treated group (P=0.0001, repeated-measures ANOVA, Fig. 5). Overall, morphine significantly prolonged the latency (P=0.0089, repeated-measures ANOVA) and reduced the duration (P=0.0008, repeated-measures ANOVA, Table 2). Naloxone antagonized the effects of morphine (Fig. 5 and Table 2).

Effects of capsazepine
To determine whether propofol- and capsaicin-evoked flexor reflexes were modulated via the vanilloid receptor (VR1), the VR1 antagonist capsazepine was pre-infused. Figure 6 shows the effects of capsazepine on propofol- and capsaicin-evoked EMG responses. Pre-infusion of capsazepine into the artery significantly reduced the capsaicin-evoked EMG responses at 1 min [30.4 (5.3)%, P<0.01] compared with the value of the vehicle-infused group [100.6 (11.0)%] (P=0.0027, repeated-measures ANOVA). No difference was seen between the capsazepine-infused group [106.3 (38)%] and the vehicle-infused group [104.8 (3.8)%] at 60 min. Simultaneously, capsazepine significantly prolonged the latency at 1 min [160.1 (22.3)% P<0.05] (P=0.0332, repeated-measures ANOVA) and reduced the duration at 1 min [58.1 (13.1)% P<0.05] and 60 min [93.2 (10.6)% P<0.05] (Table 2) compared with the vehicle-infused group (P=0.0025, repeated-measures ANOVA), whereas the propofol-evoked EMG responses, latencies, and durations were not affected by capsazepine at any time (Fig. 6 and Table 2).

Effects of indomethacin and prostaglandin E₂
We attempted to determine whether the flexor reflexes to propofol and capsaicin were related to prostanoids. The effects of a biosynthesis inhibitor, indomethacin and prostanoid prostaglandin E₂ were therefore examined. Pretreatment with indomethacin significantly reduced propofol-evoked EMG responses at 60 min [28.8 (8.2)%, P<0.01], whilst that at 120 min recovered [97.2 (8.9)%, P=0.2806] compared with the EMG responses the vehicle-treated group (P=0.0008, repeated-measures ANOVA) (Fig. 7). Although indomethacin significantly prolonged the

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**Table 1** Comparison in the modalities of propofol- and capsaicin-evoked flexor reflex responses

<table>
<thead>
<tr>
<th>Substances</th>
<th>Latency [s (sd)]</th>
<th>Duration [s (sd)]</th>
<th>EMG value [mVs² (sd)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol 50 µl</td>
<td>3.10 (1.42)</td>
<td>18.67 (7.78)</td>
<td>150.99 (35.47)</td>
</tr>
<tr>
<td>Capsaicin 0.1 µg</td>
<td>5.31 (0.89)</td>
<td>13.24 (2.90)</td>
<td>168.60 (78.66)</td>
</tr>
</tbody>
</table>

Fig 2 The effect of increasing doses on i.a. propofol- and capsaicin-evoked rectified EMG (lower) in rats showing EMG value (mVs²), latency (s), and duration (s) for each dose (a, b, c) of propofol and capsaicin (upper).
latency at 60 min [306.2 (69.2)%, P<0.01 (P=0.0184, repeated-measures ANOVA), it did not significantly reduce the duration at any time (Table 2). These effects of indomethacin disappeared with arterial pre-infusion of prostaglandin E₂ (Fig. 7). In contrast, the capsaicin-evoked EMG values, latencies and durations were not affected by indomethacin at any time (Fig. 7 and Table 2). Based on the results with indomethacin, we examined the effects of prostaglandin E₂ alone on the propofol- and capsaicin-evoked flexor reflexes. As Figure 8 shows, arterial pre-infusion of prostaglandin E₂ alone augmented both responses at 5 min after infusion. Prostaglandin E₂ increased the EMG responses obtained with propofol [411.9 (45.1)%, P<0.01] and capsaicin [143.2 (8.9)%, P=0.009, Mann–Whitney U-test] compared with the pre-infusion values, and a significant difference was seen between propofol and capsaicin (P=0.009, Mann–Whitney U-test). Furthermore, prostaglandin E₂ reduced the latency of the propofol response [35.6 (6.1)%, P=0.009, Mann–Whitney U-test] compared with pre-infusion values, while prostaglandin E₂ did not affect the duration of propofol [137.9 (22.1)%, P=0.009, Mann–Whitney U-test] compared with pre-infusion values, and no difference was seen between propofol and capsaicin (Fig. 8).
We observed that arterial pre-infusion of procaine blocked arterial propofol- and capsaicin-evoked flexor reflexes, but not the response to skin pinching. In contrast, i.t. pretreatment with lidocaine caused a transient disappearance of the flexor reflex with all stimuli. These findings explicitly indicate that i.a. injection of propofol and capsaicin evokes the spinal flexor reflex via peripheral vascular chemonociceptors. Repeated exposure of the vascular chemonoceptors to algesic substances triggers acute tolerance.91 0 Similar results were obtained for both the propofol- and capsaicin-evoked flexor reflex responses. However,
cross-desensitization between propofol and capsaicin was not seen, suggesting that the chemonociceptors responding to these substances have different characteristics. A previous investigation showed that vascular chemonociceptors, which are sensitive to several algesics, are widely distributed at peripheral nerve endings, including those between thin myelinated A-fibres and unmyelinated C-fibres. Szolcsanyi and colleagues\textsuperscript{11} reported that i.a. injection of capsaicin

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Fig 6 The effects of arterial pre-treatment with capsazepine (20 μg) on i.a. (A) propofol- and (B) capsaicin-evoked EMG responses. **P<0.01 vs 1% DMSO (n=6, Fisher’s Protected LSD test).

Fig 7 The effects of pre-treatment with indomethacin (INDM, 10 mg kg\textsuperscript{-1}, i.p.) on i.a. (A) propofol- and (B) capsaicin-evoked EMG responses. Prostaglandin E\textsubscript{2} (PGE\textsubscript{2}, 5 μg) was infused arterially 55 min after indomethacin treatment. **P<0.01 vs 0.5% TWEEN 80, indomethacin + prostaglandin E\textsubscript{2} (n=5, Fisher’s Protected LSD test).
mainly activated single discharges of the polymodal C-fibres. Therefore, capsaicin-induced flexor reflexes we observed may be involved in exciting the vascular chemoneceptors of the polymodal C-fibres, while propofol-induced flexor reflex may act mainly to excite polymodal Aδ-fibres, as polymodal stimuli-induced impulses are predominantly conducted by polymodal Aδ-fibres in human veins.\(^{12}\)

In clinical studies, i.v. pre-treatment with the opioid alfentanil reduced the pain that occurred after an injection of propofol.\(^{5}\) In this study, arterial propofol and capsaicin had similar sensitivities to opiate receptors, as s.c. morphine markedly inhibited the flexor reflexes evoked by both i.a. propofol and capsaicin, and the antagonist naloxone restored these depressed responses. The same inhibitory effects of morphine on arterial bradykinin-evoked flexor reflexes have been reported in unanaesthetized rats.\(^{13}\) We have also reported similar results in another animal model of vascular pain.\(^{14}\) Ours and other studies indicate that vascular chemoneceptors are generally sensitive to opioids.

The vanilloid receptor (VR1) is located throughout the central and peripheral sensory nervous systems, mainly on C-fibres,\(^ {15,16}\) and is sensitive to protons, high temperatures, and capsaicin in vitro.\(^ {17}\) Moreover, capsaicin sensitivity is related to the expression of VR1 mRNA in the sensory ganglia of rats.\(^ {18}\) This evidence indicates that many effects of capsaicin on the sensory system are exerted via VR1. Furthermore, capsazepine, a competitive VR1 antagonist,\(^ {19}\) depressed various actions of capsaicin on sensory neurons either in vivo\(^ {20}\) or in vitro.\(^ {21}\) In this study, i.a. pre-treatment with capsazepine markedly depressed the capsaicin-evoked nociceptive reflex and shortened its latency and duration. However, it did not entirely depress that of propofol. Another in vitro study found that propofol did not influence the function of recombinant rat VR1 receptors.\(^ {22}\) Considering these results and the properties of capsaicin, the vascular pain related to capsaicin may result from the activation of intra- or peri-vascular VR1 receptors on C-fibres, while that of propofol may arise from other receptors or mechanisms.

In contrast to the effects of capsazepine on the propofol- and capsaicin-evoked flexor reflex responses, we observed that indomethacin, a non-steroid anti-inflammatory drug (NSAID), strongly depressed propofol-evoked responses. In contrast, indomethacin had little effect on the capsaicin-evoked flexor responses. This is supported by the observation that indomethacin failed to affect the capsaicin-induced nociceptive cardiac reflexes of dogs.\(^ {23}\) We have also already reported that, aspirin, did not inhibit i.a. capsaicin-induced aversive behaviour in guinea pigs.\(^ {14}\) Another clinical study found that i.v. pre-treatment with aspirin alleviated the pain caused by propofol.\(^ {24}\) NSAIDs exert their effects by inhibiting cyclo-oxygenase (COX) and consequently prostaglandin production.\(^ {25}\) COX exists as two isomers, COX-1 and COX-2.\(^ {26}\) In general, analgesic effects of these drugs are attributed to their inhibition of COX-2.\(^ {27}\) Nakane and Iwama documented that propofol activated the plasma kallikrein–kinin system, which resulted in the formation of bradykinin, a potent endogenous algesic, and caused pain.\(^ {7}\) Other studies have demonstrated that NSAIDs block bradykinin-release of prostaglandin Es, which are involved in bradykinin-induced pain.\(^ {28}\) We have also reported that arterial bradykinin excites thalamic nociceptive neurons,
which are inhibited by NSAIDs and restored by arterial infusion of prostaglandin E2. Therefore, our data suggest that the depressive effects of indomethacin on propofol-evoked flexor reflexes resulted from inhibition of the activity of COX-2. In support of this hypothesis, the attenuated propofol responses were restored to control levels by i.a. infusion of prostaglandin E2, which is synthesized from the arachidonate cascade by COX. Moreover, arterial prostaglandin E2 alone augmented the propofol response accompanying a shortened latency greater than that of capsaicin.

In summary, our study revealed several characteristics of i.a. propofol-evoked pain using a flexor reflex model. The propofol response was compared with that of capsaicin. Propofol and capsaicin both evoked the flexor reflex in a dose-dependent, opioid-sensitive manner, although cross-desensitization was not observed between propofol and capsaicin. The VR1 antagonist capsazepine depressed only the capsaicin-evoked responses and not the propofol-evoked responses. The opposite results were seen in indomethacin-treated animals. Furthermore, arterial pre-infusion of prostaglandin E2 potentiated the propofol-evoked responses more strongly than those evoked by capsaicin. These results suggest that propofol characteristically causes vascular pain that occurs in response to prostanoids, particularly prostaglandin E2.

References