Propofol directly depresses lumbar dorsal horn neuronal responses to noxious stimulation in goats

Joseph F. Antognini MD,*
Xiao Wei Wang MD,*
Marla Piercy BA,*
Earl Carstens PhD†

Purpose: We tested the hypothesis that propofol, acting in the brain, would either enhance, or have no effect, on lumbar dorsal horn neuronal responses to a noxious mechanical stimulus applied to the hindlimb. We recorded the response of lumbar dorsal horn neurons during differential delivery of propofol to the brain and torso of goats.

Methods: Goats were anesthetized with isoflurane and neck dissections performed which permitted cranial bypass. A laminectomy was made to allow microelectrode recording of lumbar dorsal horn neuronal activity. Isoflurane was maintained at 0.8 ± 0.1% to both head and torso throughout the study. During cranial bypass propofol was separately administered to the torso (1 $mg \cdot kg^{-1}$, n=7; 3.75 $mg \cdot kg^{-1}$, n=8) or cranial (0.04 $mg \cdot kg^{-1}$, n=7; 0.14 $mg \cdot kg^{-1}$, n=8) circulations.

Results: Propofol administered to the torso depressed dorsal horn neuronal responses to noxious stimulation: low dose: 500 ± 243 to 174 ± 240 impulses$\cdot min^{-1}$ at one minute post-injection, $P < 0.001$; high dose: 478 ± 204 to 91 ± 138 impulses$\cdot min^{-1}$ at one minute post-injection, $P < 0.05$). Propofol administered to the cranial circulation had no effect: low dose: 315 ± 150 to 410 ± 272 impulses$\cdot min^{-1}$, $P > 0.05$; high dose: 462 ± 261 to 371 ± 196 impulses$\cdot min^{-1}$, $P > 0.05$.

Conclusions: These data indicate that propofol has a direct depressant effect on dorsal horn neuronal responses to noxious stimulation, with little or no indirect supraspinal effect.

Objectif : Vérifier si le propofol, qui agit sur le cerveau, stimulera les réponses neuronales de la corne supérieure lombaire, ou n'aura aucun effet, après l'application d'un stimulus mécanique nocif aux pattes arrières des chèvres. Les réponses ont été enregistrées pendant l'administration différentielle de propofol au cerveau et à la région thoracique.

Méthode : Les chèvres ont été anesthésiées avec de l'isoflurane et la dissection du cou a été réalisée pour permettre une dérivation crânienne. Une laminectomie a été faite pour faciliter l'enregistrement de l'activité neuronale de la corne supérieure lombaire par microélectrode. L'isoflurane a été maintenu à 0,8 ± 0,1 % à la tête et au tronc tout au long de l'étude. Pendant la dérivation crânienne, le propofol a été administré séparément dans la circulation thoracique (1 $mg \cdot kg^{-1}$, n=7; 3,75 $mg \cdot kg^{-1}$, n=8) ou à la tête (0,04 $mg \cdot kg^{-1}$, n=7; 0,14 $mg \cdot kg^{-1}$, n=8).

Résultats : Le propofol administré au niveau thoracique a réduit les réponses neuronales à un stimulus nocif: faible dose: 500 ± 243 à 174 ± 240 impulsions$\cdot min^{-1}$ à une minute postinjection, $P < 0.001$; forte dose: 478 ± 204 à 91 ± 138 impulsions$\cdot min^{-1}$ à une minute postinjection, $P < 0,05$). Le propofol dans la circulation crânienne n'a pas eu d'effet: faible dose: 315 ± 150 à 410 ± 272 impulsions$\cdot min^{-1}$, $P > 0.05$; forte dose: 462 ± 261 à 371 ± 196 impulsions$\cdot min^{-1}$, $P > 0,05$.

Conclusion : Ces données indiquent que le propofol a un effet dépresseur direct sur les réponses neuronales de la corne supérieure à une stimulation nocive, avec un léger effet supraspinal indirect ou sans effet supraspinal.

From the Department of Anesthesiology and Pain Management* and Section of Neurobiology, Physiology, and Behavior,† University of California, Davis, Davis, California USA.

Address correspondence to: Joseph F. Antognini MD, TB-170, University of California, Davis, Davis, CA 95616 USA. Fax: 530-752-7807; E-mail: jfantognini@ucdavis.edu

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THE spinal cord is emerging as an important site of anesthetic action.[1,2] A likely target of anesthetic action are neurons within the spinal cord dorsal horn, since they are involved in the transmission of stimuli to other central nervous system sites.[3] Anesthetics, including propofol, depress dorsal horn neuronal responses to innocuous and noxious stimuli.[4–6] Supraspinal sites, such as the rostroventral medulla, modulate dorsal horn neurons.[3] Thus, anesthetic effects on spinal dorsal horn neurons might be expressed directly at the spinal level, indirectly at supraspinal levels, or both.

Propofol has been reported to have no antinociceptive properties,[7] or to be hyperalgesic.[8] Kishikawa *et al.*[6] showed that propofol depressed dorsal horn neuronal responses to innocuous tactile stimuli, but they did not determine effects on responses to noxious stimuli, as no wide-dynamic range (WDR) cells were studied. In another study from the same laboratory, Uchida *et al.*[9] determined that propofol depressed dorsal horn neuronal responses to noxious stimuli, although few WDR cells were studied. Taken together, these studies suggest that propofol depresses dorsal horn neurons, but the relative contributions of spinal and supraspinal actions to the total depressant effect of propofol remain unclear. If propofol is associated with hyperalgesia, then its supraspinal action might be enhancement of dorsal horn cell activity. This might occur as the result of propofol ablating descending inhibition of dorsal horn neurons. We hypothesized that propofol, acting in the brain, would either enhance (hyperalgesia) or have no effect on dorsal horn neuronal responses to a noxious mechanical stimulus applied to the hindlimb.

Methods

The local animal care and use committee approved this study. Nine adult goats (weight 44 ± 4 kg) were anesthetized with isoflurane by mask, their tracheas intubated and lungs mechanically ventilated. After bilateral neck dissection, the carotid arteries and jugular veins were isolated, and the occipital arteries ligated.[10,11] The neck muscles were ligated to minimize any cross-over from the torso to the cranial circulation, or vice versa.[11] A peripheral intravenous catheter was placed and lactated Ringer's solution was infused. A carotid arterial catheter was inserted for determination of systemic blood pressure and for glucose, blood gas and hematocrit analyses. Pancuronium (0.1-0.2 $mg \cdot kg^{-1}$, repeated every 1 - 2 hr) was administered to provide muscle relaxation. Rectal (37.9 ± 0.7 °C) and nasopharyngeal (37.7 ± 0.8 °C) temperatures were adjusted using a heating lamp, and during bypass, the heat exchanger of the oxygenator.

After lumbar laminectomy, the spine was secured using four vertebral clamps. The dura was slit and a tungsten recording microelectrode (resistance ≈ 10 mΩ, F. Haer, Inc., Brunswick, ME) was inserted into the lumbar dorsal horn (approximate L_5 level) using a hydraulic microdrive (D. Kopf Instruments, Tujunga, CA). We sought neurons that had receptive fields that included the dew-claws and/or hoof of the hindlimb. Extracellular action potentials were amplified, displayed on an oscilloscope, and relayed to a personal computer for off-line analysis.[12] Wide-dynamic range and nociceptive-specific type neurons were sought. We only studied units that exhibited reproducible responses to a standard noxious mechanical clamp stimulus (10-inch hemostat applied to the dew-claw or a hoof bulb for 10 sec). Dorsal horn neuronal activity was determined for one minute prior to, and for one minute after the onset of each stimulus, except in one animal that had high spontaneous activity, in which case we used the 10 sec period prior to, and the 10 sec period during, application of the noxious stimulus. Control responses were obtained by applying the stimulus 1-5 times (usually three); the interstimulus interval was five minutes. Propofol was administered (4 $mg \cdot kg^{-1}$ *iv*) and dorsal horn neuronal activity (evoked by the noxious clamp) determined 1, 5, 10 and 15 min after propofol injection. Blood samples were obtained at each stimulus (and in three animals at three minutes as well) and stored for later propofol analysis. End-tidal isoflurane was maintained at 0.8 ± 0.1% to the head and torso throughout the study.

After determination of the control response to propofol injection, heparin (4 $mg \cdot kg^{-1}$ *iv*, repeated 2 $mg \cdot kg^{-1}$ every 1-2 hr) was administered and a cannula placed into the carotid artery, and Y cannulae were placed into the jugular veins. Blood (500 ml) was drained from the animal to prime the bubble oxygenator (B-10, Bentley, American Edwards, Irvine Ca). Oxygenator gas flow was O_2 95% and CO_2 5% at 5-6 $L \cdot min^{-1}$. An isoflurane vaporizer was placed in-line with the gas flow. Isoflurane concentration in the arterial blood perfusing the head and brain was estimated from the isoflurane concentration in the oxygenator exhaust,[1,10] and torso isoflurane was determined from end-tidal sampling. Oxygenator exhaust and end-tidal gases were monitored with a calibrated agent analyzer. Cranial bypass was initiated by diverting cranial venous blood to the oxygenator, with cranial blood flow initiated at 250-500 $ml \cdot min^{-1}$. The remaining open carotid artery was clamped to achieve complete bypass.[1,10] Glucose was infused (10-20 $mg \cdot min^{-1}$) into the oxygenator. Adequacy of cranial bypass was determined indirectly by monitoring the electroencephalo-

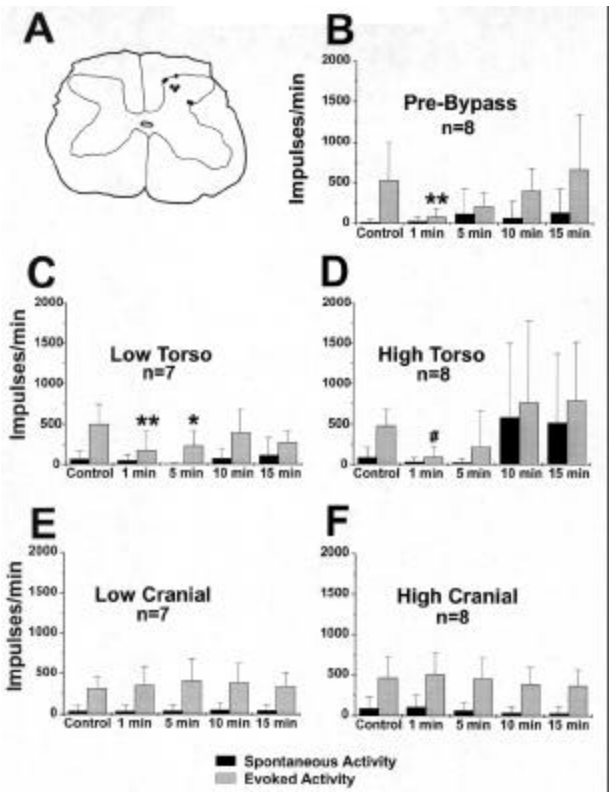

FIGURE 1 (A) Cross sectional view of a representative section of the lumbar cord (approximate L_5 segment) demonstrating the recording sites. Note that most sites were in the superficial-to-mid dorsal horn. (B) Pre-bypass data. Propofol depressed the neuronal responses to noxious stimulation. During bypass, when propofol was administered to the torso in low (C) or high (D) doses, evoked activity decreased. The increased activity at 10 and 15 min after the high dose torso administration was due to two cells that had a marked increased spontaneous activity. Propofol administered to the cranial circulation (low (E) and high (F) dose) did not have any significant effect on the spontaneous or evoked response. Mean ± standard deviation. $^{**}P < 0.001$, $^{*}P < 0.01$ and $\#P < 0.05$ compared to control responses.

gram (A-1050, Aspect Medical Systems, Natick, MA). Once bypass flows had stabilized (usually requiring about 30 min) spontaneous and evoked dorsal horn neuronal activities were recorded with cranial and torso isoflurane at 0.8 ± 0.1%. In three animals, initiation of bypass resulted in loss of the recorded neurons, and new neurons were found. (These three animals had pre-bypass responses similar to the other animals in which the ability to record neuronal responses was not lost.) Neuronal responses to the noxious clamp were determined and propofol administered to the cranial and torso circulations. Propofol was intravenously administered to the torso in low (1 $mg \cdot kg^{-1}$, n=7) and high (4 $mg \cdot kg^{-1}$, and one animal given 2 $mg \cdot kg^{-1}$, n=8) doses. The higher dose is a typical induction dose for goats.[1,3] Propofol was administered to the venous limb of the oxygenator in low (0.025-0.05 $mg \cdot kg^{-1}$, mean 0.04 $mg \cdot kg^{-1}$, n=7) and high (0.1-2 $mg \cdot kg^{-1}$, mean 0.14 $mg \cdot kg^{-1}$, n=8) doses. The order of the propofol injections was varied experiment to experiment. In one animal, only the high propofol doses to head and torso were administered. Neuronal responses to the noxious mechanical clamp were determined at 1, 5, 10 and 15 min after each propofol injection. At each time point (and in three animals at three minutes as well) blood (5 ml) was withdrawn from the torso and cranial arterial circulations for later analysis of propofol concentrations. The individual propofol injections to the cranial and torso circulations were separated by at least 30-45 min to permit return of control neuronal responses and propofol concentrations in the torso and oxygenator blood to decrease to very low concentrations.

Because propofol administration to the torso resulted in transient blood pressure decreases, phenylephrine was administered to maintain blood pressure in the normal range. Phenylephrine was chosen because it does not appear to alter dorsal horn neuronal responses.[14,15] Nonetheless, in four animals, we determined what effect, if any, phenylephrine had on neuronal responses. To evaluate possible effects of hypotension, in four animals we used nitroprusside, nitroglycerin and/or phentolamine to decrease the blood pressure to levels associated with propofol administration. The effects of phenylephrine and the hypotensive agents were tested at the peak of the hemodynamic response (generally at around one minute). The peak effect of propofol on blood pressure and dorsal horn neuronal responses also generally occurred at one minute. In five animals we also determined if the propofol vehicle (lipid emulsion) had any effect on neuronal responses.

The spinal recording site was marked with an electrolytic lesion by passing direct current through the recording microelectrode. The goat was killed with potassium chloride and isoflurane. The cord was removed, fixed in formalin, frozen, cut in 50 μm sections, and mounted on microscope slides. The electrolytic lesions were observed under a light microscope and plotted onto a computer video image of the spinal cord section.

Propofol concentration in blood was determined using high-pressure liquid chromatography (HPLC) with a protocol modified from two prior methods.[16,17] In brief, after centrifuging, the plasma was stored at –70°C until analysis. The propofol was extracted from

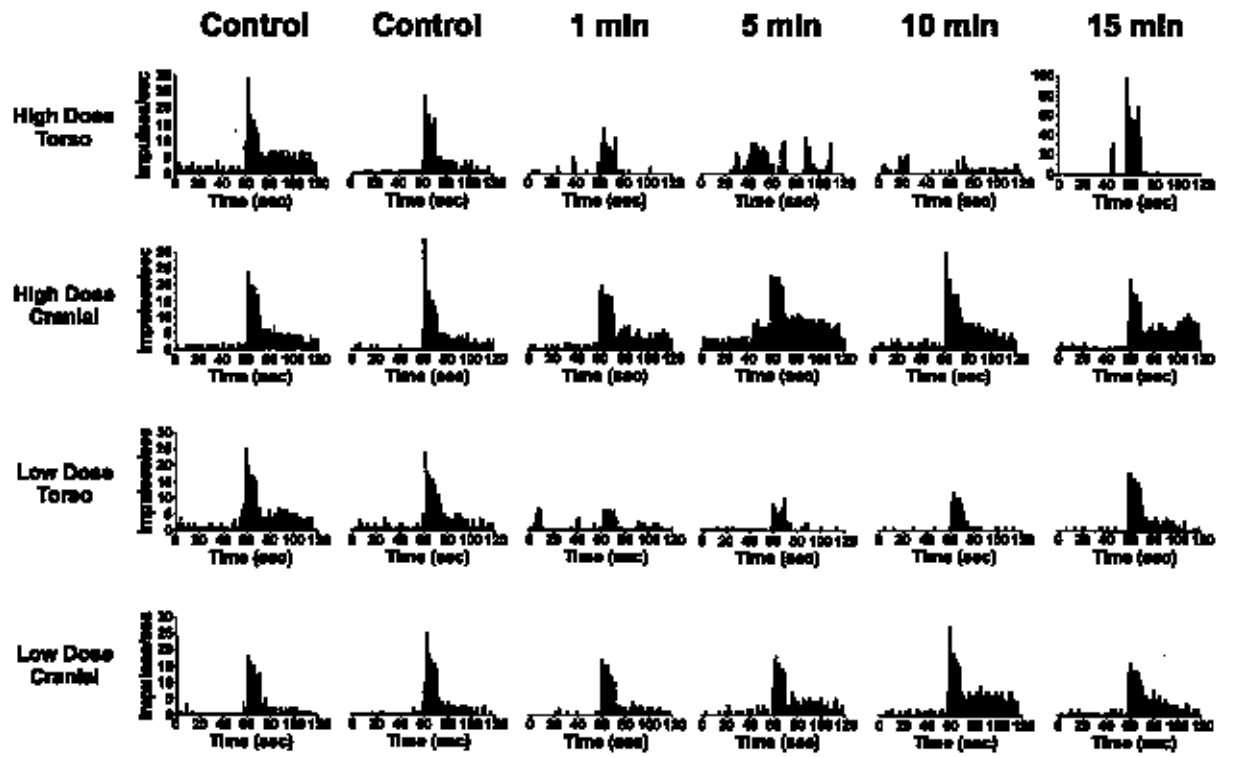

FIGURE 2 Examples of peristimulus-time histograms from one animal. The noxious stimulus was applied for 10 sec at each 60 sec mark. Prior to each propofol administration two control responses are shown. The responses at 1, 5 10 and 15 min after propofol administration are shown. Note that the high and low propofol doses to the torso diminished the response, but that propofol administered to the cranial circulation had no effect. The impulses/sec scale for the 15 min response at the high torso dose differs from the others.

the plasma using a solid-phase extraction column and injected onto a Waters HPLC column (electrochemical detection). Using a range of 0-20 $\mu g \cdot ml^{-1}$, standard curves were constructed that demonstrated high correlation coefficients r = 0.98-1.0). The lower limit of detection was 25 $ng \cdot ml^{-1}$. To determine the propofol concentrations, the propofol peaks of the experimental samples were compared to the standard curves.

The data are expressed as mean ± standard deviation. Because the neuronal response data did not appear to be normally distributed, a log transformation was performed.[18] Repeated measures analysis of variance (ANOVA) of the transformed data was used to detect differences in neuronal responses pre- *vs* post-propofol injection, followed by the Student-Newman-Keuls multiple comparisons test. A $P < 0.05$ was considered to be statistically significant.

Results

Bypass data from one animal was excluded because of a progressive decline in the electroencephalogram during bypass. All cells were WDR cells in that they responded to increased stimulation intensity with increased discharge number. The recording sites were located in the superficial and mid-dorsal horn (Figure 1A). When propofol was administered pre-bypass, there was a marked decrease in mean neuronal responses that returned to control levels at 15 min post-injection (Figure 1B). During bypass, propofol injected into the torso at the low (1 $mg \cdot kg^{-1}$) and high (4 $mg \cdot kg^{-1}$) doses depressed neuronal responses (Figures 1C, 1D). Injection of propofol into the cranial circulation did not affect neuronal responses (Figure 1E, 1F). An individual example shown in Figure 2 demonstrates the depressant effect of propofol when administered to the torso, with minimal effect when administered to the cranial circulation.

Propofol concentrations generally peaked at 1-5 min, with the peak resulting from cranial administration tending to be lesser and occurring later than in torso administration (Figure 3), although the areas under the curves were similar. There was little cross-over of propofol from torso to the head (and vice versa), demonstrating that nearly complete isolation was achieved.

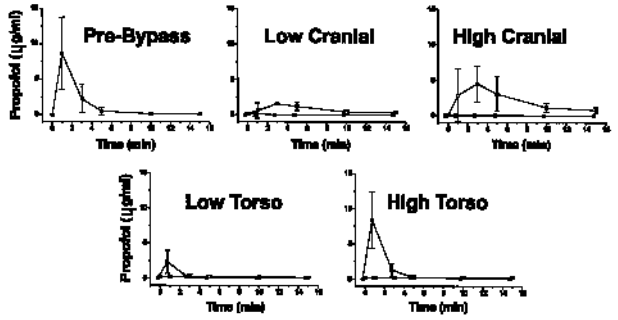

FIGURE 3 Propofol concentrations. Closed squares = torso arterial blood; open squares = cranial arterial blood. The pre-bypass data represents systemic arterial blood. The high torso dose resulted in a mean peak at around 8-9 $\mu g \cdot ml^{-1}$, while the lower torso dose resulted in a mean near 2 $\mu g \cdot ml^{-1}$. The cranial propofol doses resulted in mean peaks that occurred slightly later in time (compared with the torso doses), and in the case of the high cranial dose, the mean peak was only 54% of that resulting from high torso administration. In addition, torso administration of propofol generally resulted in a faster concentration decline when compared to cranial administration. Note that cross-over from torso circulation to cranial circulation (and vice versa) was negligible. The torso data are slightly offset in time for clarity. (n = 7-8 at each time point except three minutes where N=3.)

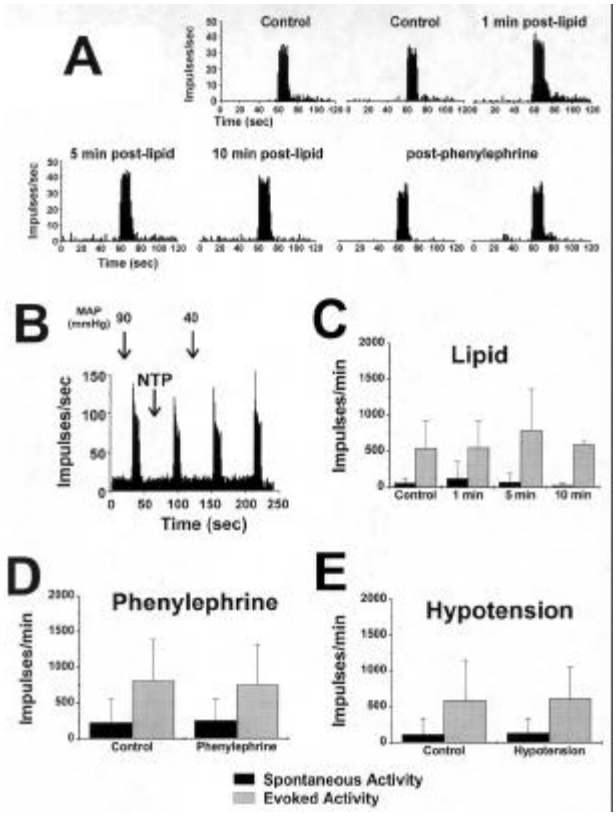

FIGURE 4 (A) Individual peristimulus time histogram from one animal administered lipid vehicle and phenylephrine. The noxious stimulus was applied for 10 sec at each 60 sec mark. The mean arterial pressure (MAP) increased from 130 to 190 mmHg with the phenylephrine injection. No significant effect was seen. (B) Individual response from one animal administered nitroprusside. The MAP decreased from 90 to 40 mmHg, but no effect on the neuronal response was detected. NTP= nitroprusside. Mean (± standard deviation) data are shown for the lipid (C) and phenylephrine (D) injections and for the effect of hypotension (E). None had a significant effect. The dorsal horn neuronal responses to the phenylephrine and hypotensive drugs were determined at the peak of the hemodynamic response, generally at around 1 min. n = 4-5 in C-E.

Injection of lipid vehicle had no effect on evoked responses (Figure 4). Phenylephrine increased mean arterial pressure (MAP) from 104 ± 42 to 172 ± 43 mmHg, but did not significantly alter the responses (Figure 4). Likewise, hypotension (from 90 ± 13 to 49 ± 9 mmHg) had no major effect on neuronal responses. The high torso propofol dose decreased MAP from 110 ± 16 to 53 ± 18 mmHg, with recovery to 78 ± 29 mmHg at five minutes.

Blood gas, glucose and hematocrit data are shown in Table I. There was a mild acidosis that was not progressive.

Discussion

The results of the present study indicate that propofol directly depresses dorsal horn neuronal responses to noxious mechanical stimulation, with little or no indirect effects occurring at supraspinal sites. These data are consistent with results from some, but not all, previous studies that examined propofol's actions. Jewett *et al.*, using a neonatal rat spinal cord preparation, found that propofol had no indirect supraspinal effects on the spinal cord, and that the propofol concentrations that would be associated with analgesia were close to those that would likely cause anesthesia.[19] Uchida *et al.* found that propofol depressed dorsal horn neuronal responses to low threshold and noxious stimulation.[9] Some reports suggest that propofol is associated with increased neuronal responses, e.g., hyperalgesia. Petersen-Felix *et al.* determined that propofol decreased the mechanical pressure threshold in humans,[20] and Anker-Møller *et al.* likewise determined that subhypnotic propofol doses decreased noxious mechanical thresholds.[21] Ewen *et al.* found that, in rats, propofol (in sub-hypnotic doses) decreased nociceptive thresholds.[8] Wilder-Smith *et al.*, on the other hand, found no evidence for hyperalgesia in humans administered sub-hypnotic doses of propofol.[22] The reasons for these discrepancies are not clear.

TABLE Hematocrit, glucose and blood gas values

	Pre-Bypass Arterial	*Bypass-Body Arterial*	*Bypass-Oxygenator-Arterial*	*Bypass-Oxygenator-Venous*
Hct	38 ± 7	26 ± 4	28 ± 5	26 ± 5
glucose ($mg \cdot dL^{-1}$)	180 ± 65	113 ± 31	99 ± 22	93 ± 22
pH	7.41 ± 0.03	7.39 ± 0.04	7.34 ± 0.06	7.29 ± 0.05
PO_2 (mmHg)	560 ± 39	505 ± 92	504 ± 30	87 ± 40
PCO_2 (mmHg)	38 ± 4	39 ± 5	39 ± 10	46 ± 12
BE ($mEq \cdot L^{-1}$)	0 ± 3	-1 ± 2	-3 ± 3	-2 ± 3

Mean ± standard deviation. Hct = hematocrit. PO_2 = partial pressure of oxygen. PCO_2 = partial pressure of carbon dioxide. BE = base excess.

Methodological differences might be partially responsible. In the present study, anesthetic doses of propofol were used, so we might have missed any effects occurring at sub-hypnotic doses.

Although the exact site at which propofol acts is unknown, there is mounting evidence that the $GABA_A$ receptor modulates, at least in part, propofol's effects.[23] The $GABA_A$ antagonists (bicuculline and SR-95531) partially reverse the antinociceptive effect of propofol.[24] Interestingly, opiate-receptor antagonism also reverses propofol's antinociceptive effect, so the relative roles of the $GABA_A$ and opiate receptors in propofol's action remain unclear.[24] Propofol also appears to act on sodium channels.[25]

In our previous study, we determined that isoflurane, similar to propofol, had predominately direct spinal effects.[26] In the present study, we recorded neuronal responses during isoflurane administration (0.8%). It is possible that this isoflurane concentration might have masked any subtle effects of propofol. Studies that documented supraspinal descending inhibition of dorsal horn neurons were performed in anesthetized animals.[27,28] Despite the presence of sufficient anesthesia to block nocifensive reflexes, descending modulation still occurred.[27,28] For example, administration of small doses of pentobarbital reduced tonic descending inhibition in monkeys anesthetized with chloralose supplemented by pentobarbital.[29] Thus, had there been any indirect supraspinal depressive effect of propofol, we should have detected it, although it was likely small compared to the overwhelming direct spinal effect. Furthermore, because we used anesthetic doses, we cannot make any conclusions about effects due to sub-hypnotic propofol doses. Our experimental protocol (baseline isoflurane anesthesia with propofol injection) has application to the clinical situation in which an isoflurane-anesthetized patient moves and propofol is administered to stop the movement.

The peak propofol concentration in the head (following the high dose) did not match the peak occurring after the high dose administered to the torso. The low torso dose, however, depressed the dorsal horn neuronal response to the same degree as the high torso dose. Thus, the maximal direct effect was reached with a peak plasma concentration of 2 $\mu g \cdot ml^{-1}$. Because the peak cranial concentration with the high cranial dose was 4.5 $\mu g \cdot ml^{-1}$, it seems unlikely that we would have missed a large indirect depressive effect. The areas under the curves were similar so that cranial and torso injection of propofol resulted in similar probabilities for propofol to access its sites of action. We cannot discount the possibility that some dorsal horn neurons might have been affected by cranial propofol administration inasmuch as not all dorsal horn neurons are necessarily similarly affected by supraspinal actions of propofol (e.g., some neurons, but not all, might be modulated by propofol's supraspinal effects).

Propofol when selectively administered to the cranial circulation had no effect on dorsal horn neuronal responses to noxious mechanical stimulation, while torso administration markedly depressed these responses. These data indicate that propofol, in anesthetic doses, directly depresses neurons in the dorsal horn, with minimal indirect supraspinal effects.

Acknowledgments

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