

Effect of Propofol on Carotid Body Chemosensitivity and Cholinergic Chemotransduction

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Background: **Propofol decreases the acute hypoxic ventilatory response in humans and depresses *in vivo* carotid body chemosensitivity. The mechanisms behind this impaired oxygen sensing and signaling are not understood. Cholinergic transmission is involved in oxygen signaling, and because general anesthetics such as propofol have affinity to neuronal nicotinic acetylcholine receptors, the authors hypothesized that propofol depresses carotid body chemosensitivity and cholinergic signaling.**

Methods: **An isolated rabbit carotid body preparation was used. Chemoreceptor activity was recorded from the whole carotid sinus nerve. The effect of propofol on carotid body chemosensitivity was tested at three different degrees of Po_2 reduction. Nicotine-induced chemoreceptor response was evaluated using bolus doses of nicotine given before and after propofol 10–500 μM. The contribution of the γ-aminobutyric acid $_A$ receptor complex was tested by addition of γ-aminobutyric acid $_A$ receptor antagonists.**

Results: **Propofol reduced carotid body chemosensitivity; the magnitude of depression was dependent on the reduction in Po_2. Furthermore, propofol caused a concentration-dependent (10–500 μM) depression of nicotine-induced chemoreceptor response, with a 50% inhibitory concentration (propofol) of 40 μM. Bicuculline in combination with propofol did not have any additional effect, whereas addition of picrotoxin gave a slightly more pronounced inhibition.**

Conclusions: **It is concluded that propofol impairs carotid body chemosensitivity, the magnitude of depression being dependent on the severity of Po_2 reduction, and that propofol causes a concentration-dependent block of cholinergic chemotransduction *via* the carotid sinus nerve, whereas it seems unlikely that an activation of the γ-aminobutyric acid $_A$ receptor complex is involved in this interaction.**

THE intravenous anesthetic agent propofol (2,6-diisopropylphenol) is extensively used for anesthesia and conscious sedation. Despite relatively few side effects, propofol may cause respiratory depression and hypoventilation,[1,2] and it is well established that propofol depresses acute hypoxic ventilatory response in humans.[3–5] Notably, it is not clear to what extent propofol interferes with central or peripheral regulation (or both) of respiration. In a recent study of the effect of propofol on ventilation using the dynamic ventilatory response to carbon dioxide, the results indicate a depression of central chemoreceptors as opposed to peripheral chemoreceptor signaling.[6] Two decades ago, Ponte *et al.* showed that propofol markedly depresses carotid body chemoreceptor response to hypoxia in an *in vivo* single-fiber preparation in rabbits.[7] Hence, propofol also seems to be a potent depressant of oxygen sensing in the carotid bodies, whereas the nature of this inhibition is still unknown. We therefore believe it is of interest to more directly study the pharmacological properties of propofol on the carotid body.

There is evidence that propofol acts by a positive modulation of the inhibitory action of γ-aminobutyric acid (GABA) through $GABA_A$ receptors.[8,9] The $GABA_A$ receptor channel belongs to the structurally and genetically related superfamily of fast neurotransmitter-gated ion channels that have the muscle type acetylcholine receptor as a prototype but also includes neuronal acetylcholine, glycine and serotonin type 3 receptor channels. Propofol acts as a competitive antagonist on the major brain neuronal nicotinic acetylcholine receptor (nAChR), $\alpha4\beta2$,[10,11] but not on the $\alpha7$ subunit.[10] It has also been found that propofol dose-dependently and reversibly inhibits neuronal nAChR-mediated currents in cultured PC12 cells.[12] The carotid bodies contain the majority of peripheral chemoreceptors and there is strong evidence that acetylcholine and neuronal nAChRs play a key role for oxygen sensing and signaling.[13] Nondepolarizing neuromuscular blocking agents inhibit nicotinic carotid body chemoreceptor responses in an isolated carotid body preparation.[14–16] Because propofol depresses hypoxic ventilatory response and has an affinity to neuronal nAChR subtypes we now hypothesize that propofol may act as an antagonist on neuronal nAChRs in the carotid body. We therefore propose that propofol impairs carotid body chemosensitivity by interference with either neuronal nAChRs or the $GABA_A$ receptor complex in the carotid body.

The aim of this study was to investigate the effect of propofol on carotid body chemosensitivity and responses to nicotine and furthermore to evaluate to what extent this response is mediated though the nAChR or the $GABA_A$ receptor complex.

Materials and Methods

Animals and Anesthesia

The study was approved by the Local Animal Care and Use Committee of the Karolinska Institute, Stockholm,

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Sweden. Experiments were performed on carotid bodies surgically removed from anesthetized male New Zealand White rabbits (n = 26; weight, 2690 ± 290 g). Anesthesia was induced with thiopentone 50–60 mg intravenous given *via* a 24-gauge cannula in a left marginal ear vein. A continuous infusion of thiopentone was given at a rate of 90–180 $mg \cdot kg^{-1} \cdot h^{-1}$ and adjusted to provide adequate surgical anesthesia. Muscle relaxants were not given to the animal. A tracheotomy was performed *via* an anterior midline incision after 5 ml of lidocaine to the skin (Xylocain® 5 mg/ml; AstraZeneca, Södertälje, Sweden). The animals were then mechanically normoventilated at a respiratory rate of 27 breaths/min using an animal ventilator (model 16/24; CF Palmer, London, UK) with a F_{IO_2} of 0.25 – 0.30.

Preparation of Carotid Bodies

Trachea and esophagus were divided and retracted cranially to expose the carotid bifurcation on both sides. Under the microscope, the carotid sinus nerve (CSN) and glossopharyngeal nerve were identified, carefully dissected, and cut proximally to their confluence. The carotid artery was then identified and the animal was heparinized using 1500 U heparin intravenously (Heparin Leo®, Leo Pharma, Helsingborg, Sweden). The carotid arteries were ligated and cut above the carotid bifurcation. The carotid body with its arterial supply and the CSN were then removed *en bloc*. The common carotid artery was flushed with a few milliliters of modified Tyrode's buffer solution before it was put into the perfusion chamber. All preparations were immediately used in the experiment. In the perfusion chamber, the common carotid artery was attached to a small plastic tube and continuously perfused by gravity at a constant pressure (45 cm H_2O) with modified Tyrode's buffer solution equilibrated with 5% carbon dioxide + 95% oxygen. The carotid body was also superfused *via* the chamber bath which received the same buffer as above *via* a separate plastic tubing. The perfusate volume in the chamber was approximately 2.5 ml; the exact volume was dependent on the size of the preparation placed in the chamber. The flow through the chamber was constant during each experiment ranging from 4 to 6 ml/min.

The composition of the modified Tyrode's buffer solution was as follows (in mM): 120.0 NaCl, 4.0 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 21.4 $NaHCO_3$, 1.9 NaH_2PO_4, and 10.0 D-glucose. The temperature of the perfusate was maintained at 37.0° ± 0.5°C by means of a regulated heating system (Heating Immersion Circulator; MP, Julabo, Germany). The buffer solution was equilibrated with 95% oxygen and 5% carbon dioxide. Repeated samples were taken from the chamber and analyzed (ABL 505; Radiometer, Copenhagen, Denmark) for pH, P_{O_2}, P_{CO_2}, and electrolyte content to ensure stable experimental conditions.

Sinus Nerve Recording

In the perfusion chamber the CSN was cut from the glossopharyngeal nerve and desheathed. The sinus nerve was placed onto a platinum electrode and covered by mineral oil to prevent drying. A reference electrode was placed in the tissue near the carotid body. Chemosensory discharges were recorded extracellularly from the entire CSN. The activity was amplified and filtered (100–10kHz, notch filter 60 Hz) (A-M systems, differential AC amplifier model 1700; Carlsborg, WA). The signal was then digitized and transferred onto a computer for continuous sampling and online analysis using a Digidata 1320A and pClamp 8 system (Axon Instruments, Foster City, CA). As previously described,[17,18] we used an electronic amplifier discriminator that allows selection of action potentials of a given amplitude above the baseline noise.

The selected chemosensory impulses were counted with a frequency meter to measure f_x expressed in Hz. The response was defined as the peak chemoreceptor discharge frequency compared with a baseline recording immediately before hypoxia or nicotine injection (Δf_x = maximal f_x − basal f_x).

Protocol

Propofol and Chemosensitivity. Carotid body chemosensitivity was tested at three degrees of step reduction in P_{O_2} that typically resulted in a change in P_{O_2} (ΔP_{O_2}, control measurements) of respectively 134 ± 27 (mild reduction), 223 ± 65 (moderate reduction), and 343 ± 62 mmHg (severe reduction). Corresponding gas mixtures and matching P_{O_2} are for all test situations are presented in table 1. All experiments started with a step decrease in P_{O_2} using one of the three degrees, and the subsequent order in which the three degrees were tested was alternated in a balanced fashion. Chemosensitivity was tested before and during perfusion with 100 μM propofol and after 30-min washout (fig. 1A). Before and at chemoreceptor peak response a sample from the perfusion chamber was taken for analysis of P_{O_2}, P_{CO_2}, pH, sodium, and potassium. The resultant spike frequency was used, comparing baseline recordings with peak response.

Propofol and Nicotine-induced Chemoreceptor Responses. Nicotine-induced responses were recorded after injection of a 500 μg nicotine (nicotine hydrogen tartrate salt; Sigma Chemical, St. Louis, MO) bolus (corresponding to a peak concentration of 360 μM). This dose was chosen because it produced an increase in CSN activity similar to the most severe reduction in P_{O_2}. Nicotine was dissolved in 0.5 ml of modified Tyrode's buffer and injected over 5 s into the perfusate as previously described.[15,16] Injection of 0.5 ml of modified Tyrode's buffer solution did not result in any change of the chemoreceptor activity.

The carotid body preparation was perfused with propofol 10, 30, 100, and 500 μM (2,6-diisopropylphe-

Table 1. The Effect of Propofol (100 μM) on Carotid Body Chemosensitivity

	Control			Propofol (100 μM)			After wash-out		
	ΔCSNA (Hz)	P_{O_2} Baseline (mmHg)	ΔP_{O_2} (mmHg)	ΔCSNA (Hz)	P_{O_2} Baseline (mmHg)	ΔP_{O_2} (mmHg)	ΔCSNA (Hz)	P_{O_2} Baseline (mmHg)	ΔP_{O_2} (mmHg)
Severe 95% N_2, 5% CO_2 (n = 10)	630 ± 143	547 ± 41	343 ± 62	563 ± 155*	501 ± 39	287 ± 51	598 ± 179	522 ± 47	343 ± 66
Moderate 25% O_2, 70% N_2, 5% CO_2 (n = 8)	559 ± 206	519 ± 48	223 ± 65	471 ± 178*	499 ± 43	208 ± 43	533 ± 237	516 ± 51	237 ± 54
Mild 60% O_2, 35% N_2, 5% CO_2 (n = 6)	460 ± 186	541 ± 14	134 ± 27	345 ± 116*	524 ± 53	113 ± 29	470 ± 87	529 ± 58	131 ± 48

ΔCSNA = increase in carotid sinus nerve activity; ΔP_{O_2} = change in oxygen tension.

Data are presented as mean ± SD.

* $P < 0.05$, propofol *vs* control.

nol) (Propofol Fresenius, Fresenius Kabi, Uppsala, Sweden) in modified Tyrode's buffer solution. The concentrations were given in alternating order. Before each propofol concentration, a control response to 500 μg nicotine was recorded. Thereafter, one of the propofol concentrations was perfused during a 30-min period followed by nicotine administration. A third nicotine bolus administration was performed after a 30-min washout. Each concentration of propofol was tested in the same manner (fig. 1B). Control injections of nicotine before and after each concentration of propofol were used for comparison and to document the stability of the preparation over time.

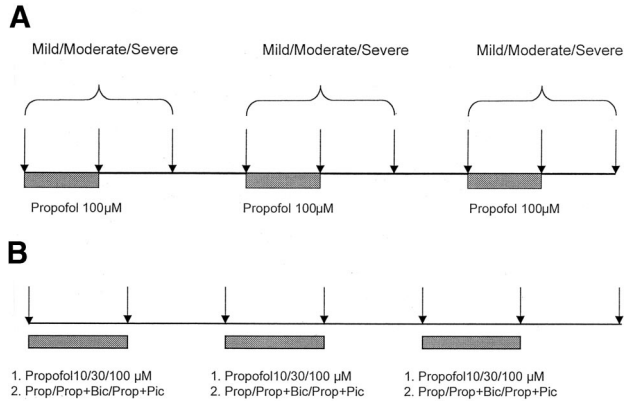

Fig. 1. (*A*) A schematic presentation of the experimental protocol testing propofol and carotid body chemosensitivity using three degrees of step reduction in P_{O_2}: mild, moderate, and severe. The three degrees of P_{O_2} reduction were tested in different sequence between different animals. *Black line* represents perfusion with modified Tyrode's buffer. *Black arrows* indicate reduction in P_{O_2}. *Shaded bars* show 30-min perfusion with 100 μM propofol. (*B*) A schematic presentation of the experimental protocol testing nicotine-induced carotid body chemoreceptor responses to 500 μg nicotine. *Black line* represents perfusion with modified Tyrode's buffer. *Black arrows* indicate administration of 500 μg of nicotine. *Shaded bars* show perfusion with 1) propofol in one of the concentrations 10, 30, or 100 μM, 2) propofol 100 μM (Prop), propofol 100 μM + bicuculline 100 μM (Prop+Bic), propofol 100 μM + picrotoxin 100 μM (Prop+Pic). The order of the concentrations/drugs was rotated between different preparations.

Nicotine-induced responses were also evaluated during perfusion with propofol (100 μM) in the absence or presence of $GABA_A$ antagonists, bicuculline 100 μM, or picrotoxin 100 μM (Sigma Chemical) (fig. 1B).

Because commercially available propofol is dissolved in a vehicle, we also tested this emulsion vehicle (Intralipid® 200 mg/ml; Fresenius Kabi, Uppsala, Sweden) in a concentration of 1:560 in modified Tyrode's buffer solution. This concentration corresponds to the emulsion concentration during perfusion with 100 μM propofol in modified Tyrode's buffer. The emulsion vehicle (1:560) was tested in the same way as described for propofol.

The modified Tyrode's buffer solution and all drugs that were used during the experiment were prepared immediately before each experiment.

In the series of nicotine-induced carotid body chemoreceptor responses, a short duration hypoxic test was performed before the start and after the end of each experiment to confirm the responsiveness and validity of each preparation. During these challenges P_{O_2} of the perfusate was reduced from 95% oxygen + 5% carbon dioxide to 95% nitrogen + 5% carbon dioxide. The resultant increase in chemoreceptor discharge was recorded and spike frequencies were compared before and after the end of the experimental protocol.

Statistical Analysis

We applied off-line analysis of the spike frequencies (f_x). Absolute changes (Hz) in spike frequencies were calculated before and after exposure to hypoxia and nicotine. We normalized the data by construction of a

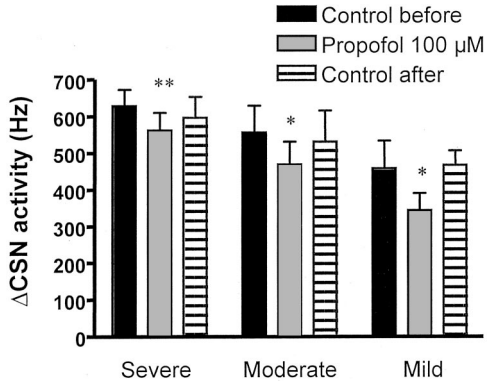

Fig. 2. Increase in carotid sinus nerve activity (ΔCSN activity) during three degrees of reduction in Po_2 (severe, moderate, and mild), before and during perfusion with 100 μM propofol and after washout. *P < 0.05, **P < 0.01. Data are presented as mean ± SEM.

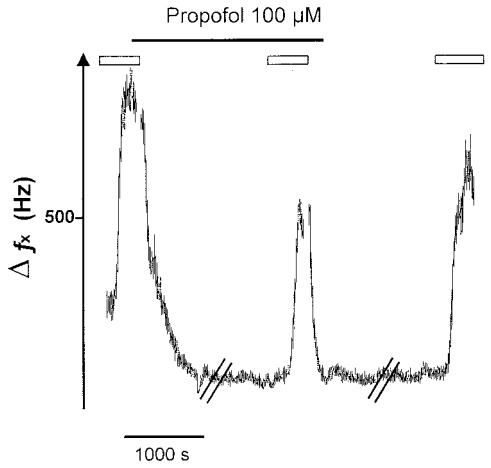

Fig. 3. The effect of 100 μM propofol on carotid body chemosensitivity, Δf_x (Hz) at 95% N_2/5% CO_2 (*shaded bars*) in one carotid body preparation. *Solid line* represents perfusion with 100 μM propofol for 30 min.

ratio between the response after perfusion with propofol compared with the control response immediately before. Concentration-response curves were adjusted using non-linear regression and one-site competition; $Y = \text{bottom} + (\text{top} - \text{bottom})/(1 + 10^{(x - \log IC_{50})})$, where y is the chemoreceptor response expressed as a ratio. The IC_{50} (50% inhibitory concentration) was calculated using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA). Comparison between two groups was made using Wilcoxon signed rank test. Results are expressed as mean ± SD. A P value of less than 0.05 was considered statistically significant.

Results

Effect of Propofol on Chemosensitivity

Propofol (100 μM) reduced carotid body chemosensitivity at each of the three-step reductions in Po_2 (table 1); we observed a decrease in CSN activity from 630 ± 143 to 563 ± 155 (−11.5%; $P < 0.01$), from 559 ± 206 to 471 ± 178 (−15%; $P < 0.05$), and from 440 ± 178 to 354 ± 108 (−17.5%; $P < 0.05$), in mild, moderate, and severe reduction, respectively (fig. 2). An original registration is presented in figure 3. In all groups the depressed chemoreceptor responses were reversed after the 30-min washout period with modified Tyrode's buffer solution (table 1).

Dose-response Relationship between Propofol and Nicotine-induced Chemoreceptor Discharge

During 10 μM propofol perfusion (n = 7) the carotid body chemoreceptor discharges in responses to bolus nicotine were unchanged from 531 ± 221 to 560 ± 196 Hz (not significant). The chemoreceptor responses after perfusion with 30 μM propofol (n = 9) decreased from 555 ± 234 to 462 ± 268 Hz ($P < 0.05$); 100 μM propofol (n = 7) reduced the response from 529 ± 233 to 240 ± 280 Hz ($P < 0.05$) (figs. 4 and 5), and 500 μM propofol gave a reduction from 248 ± 107 to 40 ± 69 (n = 3, no statistics done). There was a concentration-dependent reduction in CSN activity to 500 μg nicotine after perfusion with propofol (10–500 μM); the IC_{50} was 40.00 μM (95% confidence interval, 8–198 μM) (fig. 6).

Control responses were the same during the whole experimental period (not significant), and there was no effect of the order of which the concentrations were given.

During perfusion with the vehicle (1:560) the response to 500 μg nicotine was unchanged (634 ± 241 versus 602 ± 256 Hz, not significant) (fig. 4).

Effect of Propofol and $GABA_A$ Receptor Antagonists

During perfusion with 100 μM propofol the chemoreceptor response to nicotine decreased from 571 ± 276 to 343 ± 266 Hz ($P < 0.05$), whereas propofol (100 μM) in combination with bicuculline (100 μM) perfusion gave a similar reduction in chemoreceptor response from

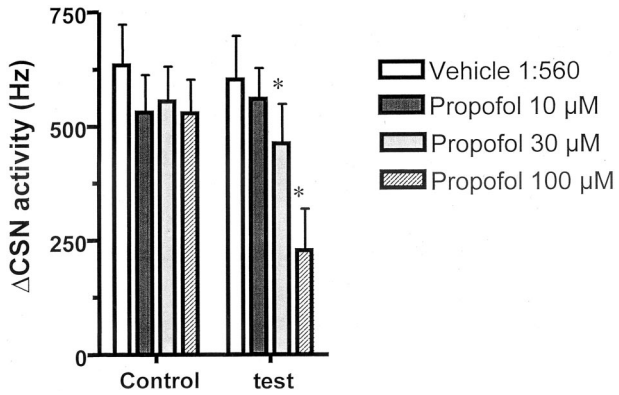

Fig. 4. Increase in carotid sinus nerve activity (ΔCSN activity) to 500 μg nicotine before and during perfusion with propofol (10, 30, and 100 μM) and vehicle (1:560), respectively. Data are presented as mean ± SEM * P < 0.05.

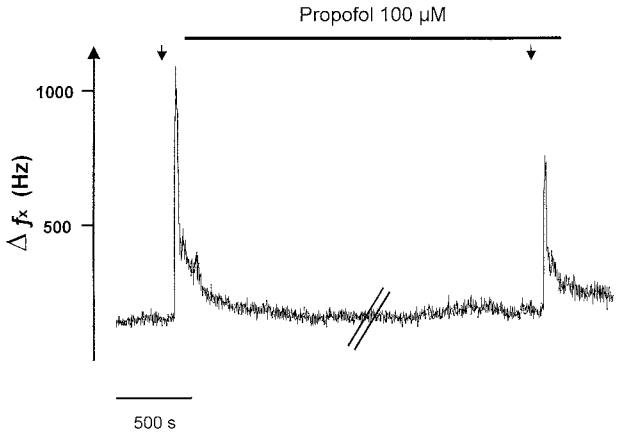

Fig. 5. Recording from an individual experiment showing carotid sinus nerve activity, Δf_x (Hz), to 500 μg nicotine before and during perfusion with 100 μM propofol. *Arrows* indicate administration of 500 μg nicotine. *Solid bar* shows 30 min perfusion with 100 μM propofol.

532 ± 324 to 291 ± 306 Hz ($P < 0.05$). However, propofol (100 μM) and picrotoxin (100 μM) reduced the response to nicotine from 618 ± 308 to 232 ± 207 Hz ($P < 0.05$). A comparison of the reduction in chemoreceptor responses to nicotine after perfusion with propofol and in combination with one of the two $GABA_A$ antagonists was done. This comparison was made by construction of a ratio between the control value before and after drug perfusion. The nicotine-induced chemoreceptor response was reduced to 56 ± 29% after 100 μM propofol; addition of 100 μM bicuculline or 100 μM picrotoxin gave a reduction of 46 ± 30% or 33 ± 22%, respectively (fig. 7). Hence, addition of picrotoxin to propofol reduced the chemoreceptor response further ($P < 0.05$), whereas there was no difference after addition of bicuculline (not significant). This indicates a slight chemodepressant effect of picrotoxin.

The hypoxic tests before and at the end of nicotine test protocols were unchanged; in the propofol group the values were 524 ± 299 and 590 ± 156 Hz, respectively, in the vehicle group the values were 756 ± 102 and

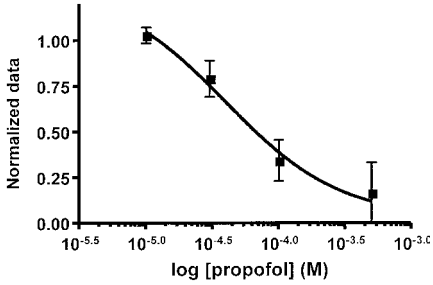

Fig. 6. Propofol blocks the nicotine-induced chemoreceptor activity in a concentration-dependent manner. Concentration-response relationship for nicotine-induced (500 μg nicotine) carotid sinus nerve activity at increasing concentrations of propofol. The 50% inhibitory concentration is 40.00 μM (95% confidence interval: 8–198 μM) for propofol. $R^2 = 0.60$. Normalized data (control/test response) are presented as mean ± SEM.

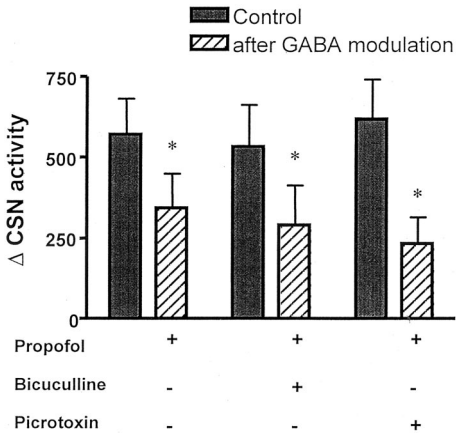

Fig. 7. Summary of the effect of γ-aminobutyric acid $(GABA)_A$ antagonists on the reduction in nicotine-induced carotid sinus nerve activity (ΔCSN activity) by propofol (n = 6). Data are presented as mean ± SEM *$P < 0.05$.

742 ± 144 Hz, respectively, and in the propofol/$GABA_A$ antagonist group the values were 635 ± 300 and 625 ± 352 Hz, respectively, before and after experiments.

P_{O_2}, P_{CO_2}, pH, sodium, and potassium concentrations were stable during the entire experimental period (table 2).

Discussion

We conclude that propofol depresses carotid body chemosensitivity, the magnitude of depression is dependent on the reduction in P_{O_2}, and propofol causes a concentration-dependent block of nicotine-induced carotid body chemoreceptor response. It seems unlikely that an activation of the $GABA_A$ receptor complex is involved in the depression of nicotine-induced chemoreceptor responses.

Our data confirm previous *in vivo* results showing that propofol depresses carotid body chemosensitivity.[7] During the last decade studies have been done showing that propofol depresses the acute hypoxic ventilatory response in male volunteers.[3,5] To further clarify the site of action of this depression, Nieuwenhuijs *et al.* varied end-tidal carbon dioxide using a multifrequency binary sequence to quantify the effect of propofol on ventilatory control in humans. From this system it was concluded that during sedative concentrations of propofol the depression of ventilation was localized to the central chemoreflex loop at central chemoreceptors.[6] To elucidate the mechanisms behind these reports it is appropriate to investigate the effect of propofol directly on the peripheral chemoreceptors (*i.e.*, the carotid bodies). The isolated carotid body preparation has been extensively used for physiologic and pharmacological investigations of the carotid body function.[14–17,19] The benefits of using an isolated preparation are that local effects of various agents can be studied on the carotid body chemosensing and signaling without interfering with systemic effects.

Table 2. Analysis from the Perfusion Chamber During the Experiments

	P_{CO_2} (mmHg)	pH	Na (mmol/l)	K (mmol/l)
Propofol, 10/30/100/500 μM	32.8 ± 2.0	7.38 ± 0.03	137 ± 1.16	3.9 ± 0.05
Vehicle (1:560)	33.9 ± 2.8	7.37 ± 0.03	136 ± 0.91	3.9 ± 0.05
Propofol ± Bicuculline/Picrotoxin	34.6 ± 3.3	7.36 ± 0.04	135 ± 1.46	3.9 ± 0.06
P_{O_2} reduction	34.3 ± 2.0	7.36 ± 0.03	136 ± 1.35	3.9 ± 0.05

Data are presented as mean ± SD.

P_{CO_2} = partial pressure of carbon dioxide; P_{O_2} = partial pressure of oxygen.

The current experiments were performed directly after removal of the carotid body, which is crucial for the reliability of results. To furthermore assure and document a stable preparation throughout the experiment, pH, P_{CO_2}, P_{O_2}, sodium, and potassium were measured in the perfusate at regular 30-min intervals during the entire experimental period while perfusate temperature and flow pressure were strictly controlled. With the application of a constant perfusion flow governed by gravity *via* the carotid body arterial supply, it is possible to subtract coexisting baroreceptor activity, making it easier to interpret the variation in CSN impulse activity as a reflection of chemosensitivity. Recording from the entire CSN shows the sum effect where the quality of recording may vary over time as a result of recruitment or loss of additional fibers. Therefore, we compared each test situation with the preceding control and furthermore performed another control after each test situation.

The carotid body is the major oxygen sensor in the body, and several recently published reports in this field clarify, to some extent, the mechanisms for oxygen sensing and signaling.[20–22] Key events include inhibition of oxygen-sensitive potassium currents (*e.g.*, TASK-1 and Kv), leading to membrane depolarization and voltage-gated calcium influx,[23–25] causing release of neurotransmitters and depolarisation of the CSN. The most important neurotransmitters seems to be acetylcholine, dopamine, substance P, and nitric oxide,[13,20] and interference with any of these transmitters alters oxygen signaling by a change in CSN activity. The carotid body originates embryologically from the neural crest, consequently, carotid body nAChRs are predominantly of the neuronal type and presence of the neuronal nAChR subunit $\alpha 4$ has been demonstrated in the carotid body and in the adjacent petrosal ganglion in cat[26] whereas the $\alpha 7$ subunits have been found in the carotid body afferent system.[26,27] Recently, the $\alpha 3$, $\alpha 4$, and $\beta 2$ subunit-containing nAChRs were present and functional in cultured glomus cells.[28] In addition, the mRNA from six different nAChR subunits ($\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\beta 2$, and $\beta 4$) have been detected in carotid body total RNA.[29] Hence, our findings add to the current body of information regarding cholinergic signaling of the carotid body.

The anesthetic effect of propofol is most likely attributable to a positive modulation of GABA on $GABA_A$ receptor channels.[8,9] GABA is a well-characterized inhibitory neurotransmitter of the central nervous system, which acts at ionotropic ($GABA_A$ and $GABA_C$) and metabotropic ($GABA_B$) receptors. GABA has been found in glomus cells of mouse[30] and rat.[31] Fearon *et al.* also found a presence of $GABA_B$ receptor subunits in glomus cells,[31] but there is no report of the $GABA_A$ receptor in the carotid body. Because of the presence of GABA in the carotid body we speculated that propofol's inhibitory effect on nicotine-induced chemoreceptor responses might be attributable to an interaction with the $GABA_A$ receptor complex. Therefore, we also applied two different $GABA_A$ receptor antagonists, the competitive antagonist bicuculline and the noncompetitive antagonist picrotoxin, to propofol. The rationale for this was to block the $GABA_A$ receptor in two different ways; however, this did not abolish the reduction of chemoreceptor response to nicotine after propofol and, notably, after picrotoxin the reduction was augmented. Hence, we believe that propofol's depressant effect of nicotine-induced chemoreceptor activity is attributable to a blockade of neuronal nAChRs in the carotid body or the afferent CSN. Because of the homology and similarity between the $GABA_A$ receptors and nAChRs, which belongs to the same ligand-gated ion channel superfamily ($GABA_A$, glycine, nicotinic, and serotonin type 3 receptors), it seems reasonable that propofol have an affinity also to the nAChRs.

Supporting our hypothesis, it was shown that propofol blocks the $\alpha 4\beta 2$ neuronal nAChRs expressed in *Xenopus* oocytes in micromolar concentrations,[10,11] and dose-dependently blocks neuronal nAChRs in PC12 cells.[12] Furthermore, propofol inhibits carbachol-induced catecholamine release in adrenal medullary cells.[32] In humans, physostigmine reverse propofol-induced CNS effects,[33] indicating that inhibition of cholinergic transmission might be part of the anesthetic action of propofol. Altogether, our results are in line with the theory that propofol also blocks nicotinic AChRs. The neuronal nAChRs participate in synaptic transmission, both as a postsynaptic mediator of fast synaptic responses and at presynaptic sites, whereas nAChR activation modulates the release of acetylcholine, dopamine, GABA, glutamate, norepinephrine, and serotonine.[34] As our experimental protocol tests both presynaptic and postsynaptic neurotransmission our results provide no

information as to whether this interaction is attributable to a presynaptic or postsynaptic block (or both).

Plasma concentrations of propofol during anesthesia are in the range of 11–51 μM (2–9 μg/ml), but as the protein-bound fraction is more than 95%, the free plasma concentration of propofol is much smaller.[35] In the previous *in vivo* study,[7] no concentrations were measured but the doses were high. However, a direct extrapolation of data in this study to the clinical situation must be viewed with caution because of possible *in vitro-in vivo* differences, species differences, and the fact that the free plasma concentration of propofol may not be identical with the effect-site concentration of the carotid body *in vivo*.

This study shows that propofol depresses carotid body chemosensitivity and that the depression is inversely related to the reduction in P_{O_2}. From a pharmacological perspective it seems reasonable that a mild physiologic response (*i.e.*, mild reduction in P_{O_2}) is more easily blocked than a severe physiologic response. Chemosensitivity was tested at various degrees of P_{O_2} changes; however, one could argue that the gas mixtures presented (table 1) do not all represent hypoxia. It is important to realize that this isolated preparation represents an artificial system and that the actual P_{O_2} in the glomus cells is most likely lower. Combining the results from previous studies[6,7] with our current findings suggest that propofol has a depressant effect both on central chemoreceptors and peripheral carotid body chemosensitivity, possibly *via* interaction with cholinergic signaling.

We conclude that propofol impairs carotid body chemosensitivity, the magnitude of depression is dependent on the severity of P_{O_2} reduction, and that propofol causes a concentration-dependent block of cholinergic chemotransduction *via* the carotid sinus nerve, whereas it seems unlikely that an activation of the $GABA_A$ receptor complex is involved in this interaction.

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