Coadministration of the AMPAKINE CX717 with Propofol Reduces Respiratory Depression and Fatal Apneas

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ABSTRACT

Background: Propofol (2,6-diisopropylphenol) is used for the induction and maintenance of anesthesia in human and veterinary medicine. Propofol’s disadvantages include the induction of respiratory depression and apnea. Here, the authors report a clinically feasible pharmacological solution for reducing propofol-induced respiratory depression via a mechanism that does not interfere with anesthesia. Specifically, they test the hypothesis that the AMPAKINE CX717, which has been proven metabolically stable and safe for human use, can prevent and rescue from propofol-induced severe apnea.

Methods: The actions of propofol and the AMPAKINE CX717 were measured via (1) ventral root recordings from newborn rat brainstem–spinal cord preparations, (2) phrenic nerve recordings from an adult mouse in situ working heart–brainstem preparation, and (3) plethysmographic recordings from unrestrained newborn and adult rats.

Results: In vitro, respiratory depression caused by propofol (2 μM, n = 11, mean ± SEM, 41 ± 5% of control frequency, 63 ± 5% of control duration) was alleviated by CX717 (n = 4, 50–150 μM). In situ, a decrease in respiratory frequency (44 ± 9% of control), phrenic burst duration (66 ± 7% of control), and amplitude (78 ± 5% of control) caused by propofol (2 μM, n = 5) was alleviated by coadministration of CX717 (50 μM, n = 5). In vivo, pre- or coadministration of CX717 (20–25 mg/kg) with propofol markedly reduced propofol-induced respiratory depression (n = 7; 20 mg/kg) and propofol-induced lethal apnea (n = 6; 30 mg/kg).

Conclusions: Administration of CX717 before or in conjunction with propofol provides an increased safety margin against profound apnea and death.

P R O P O F O L (2,6-diisopropylphenol) is used for the induction and maintenance of anesthesia in human and veterinary medicine. Propofol’s favorable attributes are its pharmacokinetic properties that result in a rapid, clear emergence. Its disadvantages include the induction of respiratory depression, apnea, and blood pressure reductions.1,2 Furthermore, there are increasing reports

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◆ This article is accompanied by an Editorial View. Please see: Dahan A, Roozekrans M, van der Schrier R, Smith T, Aarts L: Primum non nocere or how to resolve drug-induced respiratory depression. Anesthesiology 2013; 118:1261–3.
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regarding the use of propofol as a drug of abuse and accidental death.\textsuperscript{3,4} Part of the propofol-induced respiratory depression is due to the activation of γ-aminobutyric acid (GABA) receptors in respiratory brainstem neuronal networks.\textsuperscript{5,6} Here, we performed a study toward the development of pharmacological means for minimizing propofol-induced depression of respiration. Specifically, we used \textit{in vitro}, \textit{in situ}, and \textit{in vivo} rat models to show that the pre- or coadministration of the AMPAKINE CX717 can reduce respiratory depression induced by propofol. CX717 is a member of the AMPAKINE family of compounds that modulate amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPARs) by increasing the duration of glutamate-induced AMPAR-gated inward currents.\textsuperscript{7,8} Glutamate-mediated neurotransmission, acting \textit{via} AMPARs, is a critical component for the generation of respiratory rhythm in respiratory brainstem neuronal networks, which is depressed by propofol.\textsuperscript{9–11} Thus, we hypothesized that accentuation of AMPAR-mediated conductances with CX717 will counter respiratory depression induced by propofol. Indeed, previous studies have demonstrated that AMPAKINEs are very effective in alleviating opioid-induced respiratory depression without interfering with analgesia in rodents\textsuperscript{12,13} and humans.\textsuperscript{14} The AMPAKINE CX717 analyzed in this study is of particular interest because it is metabolically stable and has been deemed safe in primate studies and clinical trials.\textsuperscript{14–17}

Materials and Methods

\textbf{In Vitro Brainstem–Spinal Cord Neonatal Preparations}

All experimental procedures were approved by University of Alberta Faculty of Medicine Animal Welfare Committee (Edmonton, Alberta, Canada). Neonatal Sprague–Dawley rats, aged postnatal day (P)0 to P1, were anaesthetized with metofane, decerebrated and the brainstem–spinal cord was dissected according to previously described procedures.\textsuperscript{18,19} The neuraxis was continuously perfused at 27° ± 0.5°C (perfusion rate, 5 ml/min; chamber volume, 3 ml) with modified Kreb solution that contained: NaCl, 128 mM; KCl, 3.0 mM; CaCl\textsubscript{2}, 1.5 mM; MgSO\textsubscript{4}, 1.0 mM; NaHCO\textsubscript{3}, 24 mM; NaH\textsubscript{2}PO\textsubscript{4}, 0.5 mM; and D-glucose, 10 mM plus 1.3% ficoll (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada) bubbled with 95% O\textsubscript{2}–5% CO\textsubscript{2} at a flow rate of 15–22 ml/min sufficient to generate and maintain arterial pressure at 60 mmHg (less than 8 min from initial submersion). Note that 3 mM K\textsuperscript{+} (instead of 5.3–6.3 mM K\textsuperscript{+} regularly used for perfusing WHBP) was optimum for studying of GABA\textsubscript{A}-mediated modulation of respiratory rhythmic discharge.\textsuperscript{21} When perfused with 3 mM K\textsuperscript{+} saline, adult rat (P40–P50) WHBP had much slower respiratory frequency (3–5/min), whereas adult mouse (P40–P50) WHBP had a relatively faster respiratory rhythm (15–20/min) in our experimental conditions. Thus, the mouse was chosen for WHBP experiments. Once perfusion was initiated and arterial pressure stabilized, the preparation was gradually warmed to 29°C by heating the perfusate. The preparation was then allowed to stabilize for 1 h during which time the left phrenic nerve was dissected to monitor inspiratory activity (frequency and burst amplitude). After the 1-h stabilization period, baseline respiratory output was recorded. Respiratory rhythm in WHBP was monitored from the phrenic nerve that was placed over two platinum hook electrodes. Signals were amplified, rectified, low-pass filtered, and recorded to a computer, using an analog–digital converter (Axon Instruments Digidata 1200) and data acquisition software (Axoscope for recording and Clampfit for data analyses; Axon Instruments, Molecular Devices).

\textbf{In Situ Working Heart–Brainstem Preparation}

The \textit{in situ} working heart–brainstem preparation (WHBP) generates a robust respiratory discharge very similar to what is observed \textit{in vivo}\textsuperscript{20} and is well-suited for analyzing basic respiratory rhythmogenesis in reduced adult rodents, without modulatory state-dependent inputs from suprapontine structures. We followed our previously described protocol.\textsuperscript{12} Briefly, mice were anesthetized with isoflurane, submerged in ice-cold oxygenated perfusate, decerebrated and transected caudal to the diaphragm. The torso and brainstem were then transferred to a recording chamber, where the descending aorta was cannulated with a double-lumen cannula (one line to deliver perfusate and the second to monitor blood pressure), and perfused with saline of NaCl, 125 mM; KCl, 3 mM; MgSO\textsubscript{4}, 1.25 mM; NaHCO\textsubscript{3}, 24 mM; NaH\textsubscript{2}PO\textsubscript{4}, 1.25 mM; CaCl\textsubscript{2}, 2.5 mM; and d-glucose, 10 mM plus 1.3% ficoll (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada) bubbled with 95% O\textsubscript{2}–5% CO\textsubscript{2} at a flow rate of 15–22 ml/min sufficient to generate and maintain arterial pressure at 60 mmHg (less than 8 min from initial submersion). Note that 3 mM K\textsuperscript{+} instead of 5.3–6.3 mM K\textsuperscript{+} regularly used for perfusing WHBP was optimum for studying of GABA\textsubscript{A}-mediated modulation of respiratory rhythmic discharge.\textsuperscript{21} When perfused with 3 mM K\textsuperscript{+} saline, adult rat (P40–P50) WHBP had much slower respiratory frequency (3–5/min), whereas adult mouse (P40–P50) WHBP had a relatively faster respiratory rhythm (15–20/min) in our experimental conditions. Thus, the mouse was chosen for WHBP experiments. Once perfusion was initiated and arterial pressure stabilized, the preparation was gradually warmed to 29°C by heating the perfusate. The preparation was then allowed to stabilize for 1 h during which time the left phrenic nerve was dissected to monitor inspiratory activity (frequency and burst amplitude). After the 1-h stabilization period, baseline respiratory output was recorded. Respiratory rhythm in WHBP was monitored from the phrenic nerve that was placed over two platinum hook electrodes. Signals were amplified, rectified, low-pass filtered, and recorded to a computer, using an analog–digital converter (Axon Instruments Digidata 1200) and data acquisition software (Axoscope for recording and Clampfit for data analyses; Axon Instruments, Molecular Devices).
(infusion pump; KD Scientific Inc., Holliston, MA). With the infusion approach, all drug deliveries can be performed with continuous monitoring of plethysmographic recordings without physical handling of the animal. Fresh room air is then circulated through the chamber for 3 min before the propofol infusion to reduce residual isoflurane from the chamber. It should be noted that the plethysmographic recording setup is effective for studying respiratory frequency \( f_r \) and detection of apneas. However, it is not suitable for precise quantification of tidal volume. The physical principle underlying the whole body plethysmography is the detection of pressure changes in the chamber resulting from the heating and humidification of inspired gas. However, tidal volume \( V_T \) measurement may also be influenced by gas-compression effects related to the airway resistance. Because of these limitations, whole body plethysmography only provides semi-quantitative measurements of \( V_T \) and detection of changes relative to control state (before drug administration). \( V_T \) (minute ventilation: \( ml \text{ min}^{-1} \text{ g}^{-1} \)) equates \( f_r \times V_T \), providing semi-quantitative measurements. Therefore, relative value (to control), but not absolute value, for \( V_T \) and \( f_r \) are provided. A pulse oximeter (Nonin 8600V; Nonin Medical Inc., Plymouth, MN) was placed on the tail to monitor oxygen saturation levels. The sedation state was assessed by monitoring the rat’s ability to right itself into the prone position (righting reflex).

**Pharmacological Agents**

Propofol (trade name: Diprivan 1%, 10 mg/ml, purchased from AstraZeneca; Mississauga, Ontario, Canada) was injected intraperitoneally into the left abdomen of P3-P4 rats or injected intravenously into tail vein of adult male rats. Propofol for *in vitro* and *in situ* experiments was dissolved in 100% dimethyl sulfoxide (DMSO, 50 \( \mu l \)). CX717 was dissolved in 100% DMSO (10 mM; Sigma-Aldrich) in 0.45% saline solution for all *in vivo* and *in situ* experiments, with a final DMSO concentration less than 0.1% in volume. Vehicle (100% DMSO) was dissolved in 0.45% saline solution for all *in vitro* experiments, with a final DMSO concentration less than 0.1% in volume. Vehicle (HPCD for *in vivo* and DMSO for *in vitro* and *in situ*) has no effect on baseline respiratory activity or propofol-induced respiratory depression. Bicuculline (free base) and strychnine (Sigma-Aldrich) were dissolved in 100% DMSO (10 \( \mu l \)) for *in vitro* experiments. Bicuculline and strychnine were administered by bath application with a final DMSO concentration less than 0.1% in volume.

**Analyses**

Data are expressed as mean \( \pm \) SEM. Respiratory parameters were calculated by an average of 2 min continuous recording data, except for an average of 1 min data in adult *in vivo* experiments. Specifically, propofol-induced maximal respiratory depression in adult *in vivo* was calculated by an average of the second minute of propofol administration (2-min protocol). For *in situ* and *in vitro* experiments, values of respiratory frequency, duration, and peak inspiratory burst amplitude were measured from the integrated nerve recording and reported as mean relative to control values. For *in vivo* experiments, values of respiratory frequency, tidal volume, and minute ventilation were reported as mean relative to control values. We normalized and reported control values as 1. However, raw data were used for the analytical test. SigmaPlot 11 (Systat Software, Inc., Chicago, IL) was used to conduct the statistical analyses. There is a similar level of variances in the control (before drug application). The pairwise tests are conducted using two-tailed testing. For figures 1 and 2, the significance of changes in the respiratory parameters (including figs. 1E and 2C) was evaluated by paired *t*-test. All comparisons between propofol and coadministration of CX717 with propofol (including figs. 1H, 2D, 3, and 4) were made by unpaired *t*-test. The significance of changes in oxygen saturation and sedation observation (righting reflex) was evaluated by unpaired *t*-test. The significance of changes in the respiratory parameters in figure 1, F and G was conducted with one-way repeated measures ANOVA (Holm–Sidak methods). The significance of changes in survival rate was evaluated by a proportion z test. *P* value less than 0.05 was taken as significant difference for paired and unpaired *t*-tests. For figure 1, F and G, *P* value less than 0.0083 (0.05/6, Bonferroni correction) was taken as significant difference.

**Results**

**In Vitro Newborn Rat Brainstem–Spinal Cord Preparation**

Perinatal rodent brainstem–spinal cord preparations have been well-characterized and shown to generate complex, coordinated patterns of respiratory-related activity. \(^{18,19}\) Figure 1 shows representative examples of the respiratory discharge of ventral fourth cervical nerve roots (C4) produced by P1 rat brainstem–spinal cord preparations. Application of propofol (2 \( \mu M \); fig. 1A) caused a depression in the frequency and duration of rhythmic respiratory discharge, with a maximum suppression occurring within ~60 min of perfusion. The respiratory depression persisted for ~30 min after washout of propofol from the bathing medium. The effects of propofol (1–5 \( \mu M \)) on respiratory parameters were dose-dependent (fig. 1E). Specifically, after ~60 min of propofol perfusion, respiratory frequency decreased to 69 ± 10% of control, 41 ± 9% of control, and 16 ± 7% of control for 1 \( \mu M \) (n = 5; *P* = 0.042), 2 \( \mu M \) (n = 11; *P* < 0.001), and 5 \( \mu M \) (n = 8; *P* < 0.001) propofol, respectively; respiratory duration decreased to 82 ± 8% of control (*P* = 0.063), 63 ± 5% of control (*P* < 0.001), and 41 ± 6% of control (*P* < 0.001) for 1, 2, and 5 \( \mu M \) propofol, respectively. Propofol completely blocked the respiratory activity after ~60 min of perfusion in 3 of 11 (2 \( \mu M \)), 5 of 8 (5 \( \mu M \)), and 0 of 5 brainstem–spinal cord preparations
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**Fig. 1.** CX717 and bicuculline (BIC) alleviate propofol-induced depression of the rhythmic respiratory activity generated by *in vitro* brainstem–spinal cord neonatal rat preparations. 

A, Long-term recording (>1 h) from a P1 brainstem–spinal cord preparation showing rectified and integrated fourth ventral cervical nerve root (C4) activity. Administration of propofol (2 μM, over 60-min perfusion) caused a marked depression of respiratory frequency and duration. Subsequent addition of BIC (1 μM over 15-min perfusion, followed by 3 μM over 5-min perfusion in the presence of propofol) alleviated the propofol-induced respiratory depression. Note that bath application of bicuculline induced nonrespiratory (N) high amplitude, long duration bursts of motor activity.

B, Rectified and integrated recording from C4 of P1 rat brainstem–spinal cord preparation after bath application of propofol (2 μM over 80 min). Respiratory depression of frequency and duration was alleviated by additional bath application of CX717 (50 μM for 15 min followed by 150 μM for 5 min) in the presence of propofol. 

C, Rectified and integrated recording from C4 ventral roots of P0 rat brainstem–spinal cord preparations after 15-min bath application of CX717 (150 μM).

D, Rectified and integrated recording from C4 ventral roots of P1 rat brainstem–spinal cord preparation after bath application of BIC (3 μM). Note that bath application of bicuculline induced nonrespiratory (N) high amplitude, long-duration bursts of motor activity.

E, Population data showing respiratory burst frequency and duration relative to control after 1-h bath application of varied concentrations of propofol (1 μM, n = 5; 2 μM, n = 11; and 5 μM, n = 8). 

F, Population data showing respiratory burst frequency and duration relative to control after 1-h bath application of propofol (2 μM) and then additional application of BIC (+BIC); n = 4 each.

G, Population data showing respiratory burst frequency and duration relative to control after 1-h bath application of propofol (2 μM, n = 11) and coadministration of CX717 and propofol (Co, n = 4). *P < 0.05, **P < 0.01, ***P < 0.001, NS P > 0.05. All animals tested were postnatal day (P)0 to P1.
There was no significant effect on respiratory amplitude after propofol application ($P > 0.05$).

Data in figure 1A show that the respiratory suppression induced by propofol (2 $\mu$m) was partially alleviated (72 ± 6% of control frequency, $P < 0.001$; 81 ± 6% of control duration, $P = 0.001$) by the GABA$_A$ receptor antagonist bicuculline (1 $\mu$m, administered for 15 min), whereas administration of 3 $\mu$m bicuculline for 5–15 min completely blocked the respiratory suppression induced by propofol (2 $\mu$m), with a respiratory frequency of 95 ± 6% of control ($P < 0.001$) and duration of 103 ± 5% of control ($P < 0.001$). As shown in previous studies,$^{21}$ bicuculline (3 $\mu$m) alone at these doses does not significantly alter baseline respiratory frequency ($P > 0.05$) but can induce long-duration (>3 s), high-amplitude nonrespiratory, seizure-like motor discharge (fig. 1D; observed in 4 of 5 preparations). The nonrespiratory discharge was also observed in the bicuculline (3 $\mu$m) in the presence of propofol (fig. 1A). Population data are shown in figure 1E. Bath application of the glycine receptor antagonist strychnine (1 $\mu$m) did not change propofol-induced respiratory depression ($n = 3$; data not shown; $P > 0.05$). Note that chloride-mediated conductances in the respiratory rhythm generating preBötzinger complex (preBötC) are inhibitory postembryonic day (E)19 in rats.$^{21}$

We then examined the effects of CX717 on reversing the respiratory depression caused by propofol (2 $\mu$m), by applying two doses of CX717 (50 or 150 $\mu$m) to the bathing medium. After 15-min exposure, the lower dose of 50 $\mu$m CX717 partially alleviated the propofol-induced respiratory depression (66±7% of control frequency, $P = 0.006$ and 81±5% of control duration, $P = 0.002$; fig. 1, B and G), whereas 150 $\mu$m CX717 had a more pronounced effect (94±5% of control frequency, $P < 0.001$ and 93±6% of control duration, $P < 0.001$; fig. 1, B, C, and G). Note that a low dose

![Fig. 2. CX717 prevents and counters propofol-induced depression of respiratory rhythmic discharge generated by perfused mouse working heart–brainstem preparations. A and B, Recording of raw (bottom trace) and integrated (∫, top trace) phrenic nerve discharge in two preparations (postnatal day P40, P41 mice). Time points at which propofol (2 $\mu$m) and CX717 (50 $\mu$m) were added to the reservoir containing oxygenated perfusion medium are indicated by arrows. Note that it took approximately 1 min for solution in the reservoir to reach the preparation. C, Population data ($n = 5$ each from P40–P50) showing the relative effects (compared with control period) of propofol and CX717 (+CX717 in the continued presence of propofol) on frequency, duration and amplitude of integrated phrenic nerve discharge. D, Population data ($n = 5$ each from P40–P50) showing the relative effects (compared with control period) of propofol and codadministration (of 2 $\mu$m propofol with 50 $\mu$m CX717) on frequency, duration, and amplitude of integrated phrenic nerve discharge. * $P < 0.05$, ** $P < 0.01$.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/930993/...
of CX717 (50 μM; n = 5; P > 0.05) did not cause a significant change in baseline respiratory frequency when added on its own to the media whereas a higher dose (150 μM; n = 4) increased baseline respiratory frequency to 123 ± 5% of control (P = 0.023; fig. 1C). Neither dose induced non-respiratory, seizure-like activity. Furthermore, we examined whether CX717 could prevent the propofol-induced respiratory depression. Coadministration of CX717 (150 μM; n = 4) with propofol (2 μM) blocked the propofol-induced respiratory depression (96 ± 7% of control frequency, P = 0.004; 91 ± 7% of control duration, P = 0.009; fig. 1H).

**Perfused In Situ WHBP**

Rhythmically active brainstem–spinal cord and medullary slice preparations in rodents are not viable beyond the newborn period. However, by using WHBP it is possible to examine central respiratory control and its pharmacology in reduced preparations of older rodents. These preparations generate an in vivo–like breathing motor pattern that includes augmenting phrenic bursts. They are also oxygenated throughout and have uniform brain tissue pHi. Figure 2A shows a representative example of phrenic nerve discharge recorded from a P40 mouse in situ WHBP perfused with 3 mM K⁺ saline. Administration of propofol (2 μM; n = 5) to the perfusate produced a significant decrease in respiratory frequency (44 ± 9% of control), phrenic burst duration (66 ± 7% of control), and amplitude (78 ± 5% of control). Subsequent administration of CX717 (50 μM; n = 5) significantly alleviated propofol-induced depression of respiratory parameters (74 ± 11% of control frequency, P = 0.002; 84 ± 5% of control duration, P = 0.04; and 84 ± 6% of control amplitude, P = 0.036) in the presence of propofol. We then examined whether coadministration of CX717 (50 μM) with propofol (2 μM) could alleviate the propofol-induced respiratory depression. Figure 2B shows a representative example of phrenic nerve discharge recorded from a P41 mouse in situ WHBP perfused with 3 mM KCl saline. CX717 (50 μM) coadministered with propofol (2 μM) alleviated the propofol-induced respiratory depression (76 ± 10% of control frequency, P = 0.042; 88 ± 6% of control duration, P = 0.035; and 93 ± 4% of control amplitude, P = 0.042; n = 5). Population data are presented in figure 2, C and D. Vehicle (0.1% DMSO; n = 3; P > 0.05) coadministered with propofol did not affect the propofol-induced respiratory depression.

**In Vivo Neonatal Rats**

The next stage of the study was to examine propofol in vivo. Toward correlating with the in vitro studies, we first examined coadministrations of CX717 (20 mg/kg) and propofol (30 mg/kg) in newborn rats in vivo (P3-P4). In the control group, vehicle (10% HPCD) solution was administered with 30 mg/kg propofol (fig. 3A). This caused a marked depression in the respiratory frequency within 5 min after propofol injection and lasted for ~30 min (n = 5). Respiratory frequency decreased to 38 ± 7% of control. Relative VT decreased to 56 ± 9% of control. Relative V̇E decreased to 24 ± 6% of control. Coadministration of CX717 (20 mg/kg) with propofol (30 mg/kg) significantly prevented propofol-induced respiratory depression (77 ± 8% of control frequency, P = 0.018; 83 ± 7% of control VT, P = 0.012; and 64 ± 9% of control V̇E, P = 0.021; n = 5; fig. 3, B and C). CX717 (20 mg/kg) had no effects on baseline respiratory activity (n = 4, data not shown; P > 0.05).

**In Vivo Adult Rats**

Coadministrations of CX717 (20–25 mg/kg) or vehicle (10% HPCD) and propofol (20 mg/kg) were performed to determine the efficacy of CX717 to reduce propofol-caused respiratory depression in adult rats (300–400 g). The respiratory frequency before propofol administration was not significantly different between CX717 group (94 ± 3 bursts/min; n = 7) and vehicle group (95 ± 5 bursts/min; n = 7; P > 0.05). Figure 4A shows that coadministration of propofol (20 mg/kg) over a 2-min infusion period and vehicle (10% HPCD) solution caused a marked depression in respiratory frequency and relative amplitude that lasted for ~5 min (n = 7). These data are consistent with the effect reported for a similar propofol dose and infusion rate on respiratory rate in adult rats. The maximal depression was observed during second minute of propofol administration (n = 7; fig. 4A) with
decreased respiratory frequency (49 ± 8% of control), relative
V_T (34 ± 7% of control), and relative V_E (20 ± 6% of control).
Coadministration of CX717 (20 mg/kg, n = 7; fig. 4, B and C)
with propofol (20 mg/kg) over a 2-min perfusion significantly
reduced the propofol-induced respiratory depression in fre-
quency (82 ± 8% of control; P < 0.001), relative V_T (61 ± 8% of
control; P = 0.02), and relative V_E (52 ± 8% of control; P = 0.003).
Oxygen saturation levels at the completion of the propofol injection were significantly increased from 73 ± 6% (vehicle group; n = 5) to 90 ± 3% (CX717 group; n = 5; P = 0.035). CX717 (20 mg/kg) did not significantly decrease the duration of sedation as evident by loss of righting reflex (23 ± 5 min; n = 4; P > 0.05) after the 2-min propofol admin-
istration relative to vehicle administration (29 ± 3 min, n = 4).

Figure 5A shows recordings from an experiment in which
30 mg/kg propofol (with vehicle 10% HPCD solution) was infused rapidly (~20 s). This administration protocol induced profound apnea that was lethal in all animals (n = 6). However, coadministration of CX717 (20–25 mg/kg) with 30 mg/kg propofol minimized the period of apnea and all animals survived (fig. 5B; n = 5). Infusion of the vehicle (10% HPCD) before propofol (30 mg/kg) did not cause a significant change in propofol-induced lethal apnea (n = 3). Preadministration of CX717 (20–25 mg/kg) 1–2 min before delivering the propofol bolus minimized the period of apnea and all animals survived (n = 3). However, administration of CX717 (20–25 mg/kg) 1 min after propofol (30 mg/kg) rescued only two of seven animals tested. Collectively, these


**Data**

Data demonstrate the pre- or coadministration of CX717 (20–25 mg/kg; \(P = 0.001\)) was capable of reducing propofol-induced lethal apnea but postadministration of CX717 (20–25 mg/kg; \(P > 0.05\)) was not a reliable means of rescue.

**Discussion**

Propofol is a sedative-hypnotic that is widely used for the induction and maintenance of anesthesia in a variety of clinical settings including emergency departments, intensive care units, and for diagnostic procedures.23,24 Toward improving patient safety, this study was designed to determine whether administration of the AMPAKINE CX717 would offer protection against propofol-induced respiratory depression. The hypothesis is based on the fact that CX717 increases the strength and efficacy of glutamatergic neurotransmission via AMPARs that is essential for controlling neuronal excitability in key respiratory neuronal populations. Precedence for such an approach arises from previous studies demonstrating that CX717 is very effective in both preventing and rescuing from severe fentanyl-induced respiratory depression in rodent models and humans without interfering with sedation and analgesia.12,13 Furthermore, CX717, at doses similar to those used in this study, does not induce unwarranted side-effects, as shown in primate and human studies.14–17 Collectively, the data from this study indicate that pre- or coadministration of CX717 with propofol diminishes the amount of respiratory depression.

Recordings from cervical ventral and hypoglossal cranial roots using the *in vitro* brainstem–spinal cord preparation provide information regarding the pharmacology of respiratory rhythm generating networks and the pathways transmitting that respiratory drive to motoneurons, without the confounding changes in cardiovascular function, peripheral chemoreceptors, and supramedullary structures. Propofol induces a slowing of respiratory rhythm. The dose of propofol added to the bathing medium is within the range present in brain tissue after an anesthetic dose of propofol administered to a rat *in vivo*.5 Subsequent administration of CX717 to the bathing medium reverses the propofol-induced depression of respiratory frequency. Note that the propofol-induced respiratory depression in this study was much severe than that in previous report.5 This is likely due to the higher extracellular \(K^+\) ([\(K^+]_o, 6.2 \text{ mM}\)] used in that study compared with the more physiologically relevant 3 mM \(K^+\) concentration used in the current study. The \([K^+]_o\) influences the function of co-transporters that determine transmembrane \(Cl^-\) gradients and thus the reversal potential for chloride-mediated conductances.21 This in turn modulates the amplitude of inhibitory actions via \(GABA_A\) receptors through which propofol is acting.5,6

*In vitro*, we have shown that \(GABA_A\) receptor-mediated enhancement of chloride conductance suppresses respiratory frequency by acting within the putative inspiratory rhythm generator located in the ventrolateral medulla, the preBötC.21,25 AMPAKINEs, including CX717, increase AMPAR-mediated neurotransmission in preBötC neurons12 and hypoglossal motoneurons.26 Thus, we propose that AMPAKINEs will, in part, counter propofol-induced respiratory depression by accentuating AMPAR-mediated conductances to counter-enhanced chloride-mediated conductances within the preBötC. *In vivo*, CX717 administered before or in conjunction with propofol provided an increased safety margin against profound apnea and death. Enhanced AMPAR-mediated transmission by CX717 within multiple respiratory nuclei including the preBötC, retrotropezoïd nucleus, nucleus tractis solitaries, pontine respiratory nuclei, and motoneurons controlling upper airway and ribcage muscles are all potential sites of action *in vivo*. However, unlike the case with fentanyl-induced severe apnea, CX717 was not effective in rescuing severe propofol-induced effects once they were established. In addition to respiratory depression, dose-dependent

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**Fig. 5.** Administration of CX717 (20–25 mg/kg, intravenously) prevented propofol-induced lethal apnea in adult rats. A, Whole body plethysmographic measurements of the frequency and depth of breathing from an adult rat. Administration of a high dose of propofol (30 mg/kg) over a short period (~20 s) induced a profound, lethal respiratory depression. Vehicle 2-hydroxypropyl-\(\beta\)-cyclodextrin (10% HPCD) injection with propofol (30 mg/kg, indicated by arrow) administration had no significant effects on respiratory depression. B, Coadministration of CX717 (20 mg/kg) with propofol (30 mg/kg, indicated by arrow) reduced the severity of propofol-induced apnea and depression of respiratory frequency and prevented death.
hypotension is the commonest complication. Given that there are distinct mechanisms for respiratory- and cardiovascular-related changes induced by propofol, a combination of pharmacological therapies may be necessary to overcome severe disruptions once they are established. Nevertheless, these data from rodent models do suggest that administration of CX717 with propofol will provide protection against reaching an irreversible, lethal perturbation of cardiorespiratory systems as a result of propofol administration.

References
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