Propofol inhibits carbachol-induced chloride secretion by directly targeting the basolateral K⁺ channel in rat ileum epithelium

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Abstract
Background: Propofol is a widely used intravenous general anesthetic. Acetylcholine (ACh) is critical in controlling epithelial ion transport. This study was to investigate the effects of propofol on ACh-evoked secretion in rat ileum epithelium.

Methods: The Ussing chamber technique was used to investigate the effects of propofol on carbachol (CCh)-evoked short-circuit currents (Isc).

Key Results: Propofol (10⁻²–10⁻⁶ mol/L) attenuated CCh-evoked Isc of rat ileum mucosa in a dose-dependent manner. The inhibitory effect of propofol was only evident after application to the serosal side. Pretreatment with tetrodotoxin (TTX, 0.3 μmol/L, n=5) had no effect on propofol-induced inhibitory effect, whereas serosal application of K⁺ channel inhibitor, glibenclamide, but not, an ATP-sensitive K⁺ channel inhibitor, largely reduced the inhibitory effect of propofol. In addition, pretreatment with either hexamethonium bromide (HB, nicotinic nACh receptor antagonist) or Cl⁻ channel blockers niflumic acid and cystic fibrosis transmembrane conductance regulator (inh)-172 did not produce any effect on the propofol-induced inhibitory effect.

Conclusions & Inferences: Propofol inhibits CCh-induced intestinal secretion by directly targeting basolateral K⁺ channels.

KEYWORDS
acetylcholine receptor, intestinal secretion, K⁺ channels, propofol, short-circuit current

1 | INTRODUCTION

Acetylcholine (ACh) is a central molecule involved in the maintenance of fluid and electrolyte balance. It regulates the absorption-secretion pathways of epithelial cells and its actions are believed to be mediated via muscarinic acetylcholine receptors (mAChRs).¹⁻³ Five muscarinic receptor subtypes (M₁–M₅) have been identified, each exhibiting different tissue distribution and signal transduction pathways.⁴ Some literatures show that rat ileum epithelial cells express M₁, M₂, or M₃ muscarinic receptor subtypes.⁵,⁶ M₃ receptor is thought to predominantly localize on the surface of the intestinal epithelium to mediate the actions of ACh directly.¹ Either chloride ion secretion or smooth muscle contraction in ileum is effectively antagonized by a selective ligand for M₃ receptors.⁷ Electrophysiological studies in Ussing chambers have shown that ACh and cholinergic agonists stimulate fluid and chloride ion secretion in the mammalian gut.⁸⁻¹⁰ Cholinergically induced intestinal anion secretion is generally believed to be caused by stimulation of epithelial muscarinic M₃ receptors, whereas muscarinic M₁ receptors are thought to be localized primarily on enteric neurons. Interestingly, it has been reported that M₁ receptor is a negative regulator of secretory function in the gut, in contrast to M₃ receptor.¹¹ The authors of that study proposed that p38 mitogen-activated protein kinases were involved in M₁ receptor-induced negative regulation of intestinal epithelium secretion.¹¹

Propofol is a widely used intravenous general anesthetic. Although it is known that the anesthetic actions of propofol are mediated by
GABA<sub>A</sub> receptors, it has been suggested that, in Xenopus oocyte, propofol also attenuates M<sub>1</sub> receptor-mediated ACh signal transduction at the receptor site and/or at the site of interaction between the receptor and its associated G protein. A therapeutic concentration of propofol was shown to inhibit nicotinic ACh ion channels in mouse tumor cells. Interestingly, propofol also protects the intestine epithelial cells from injury by heat stress and lipopolysaccharides. We propose propofol protects the epithelial barrier function through intestinal epithelial ACh receptors.

In this study we investigated the effects of propofol on carbachol (CCh)-induced chloride secretion from the rat ileum epithelium in Ussing chambers and the mechanisms involved. We found that propofol attenuates CCh-mediated signal transduction in the rat ileum mucosa by inhibiting basolateral K<sup>+</sup> channels. Notably, we revealed a new pathway critically involved in propofol-mediated inhibition of CCh-evoked chloride secretion that may yield new therapeutic strategies for the treatment of diarrhea.

2 METHODS

2.1 Animals and tissue preparation

Adult male Wistar rats (200–250 g) were purchased from the Animal Centre of Shandong University (Jinan, China). Rats were allowed free access to water but were fasted overnight before the experiments. On the day of the experiments, rats were anesthetized, decapitated, and tissues prepared as described previously. Briefly, after cutting along the mesenteric border, segments of ileum were pinned flat on a Sylgard-lined Petri dish with the mucosal surface facing down and the serosa and muscularis were stripped away gently to obtain mucosal-submucosal preparations. During this procedure, tissue preparations were bathed in ice-cold Krebs’ solution continuously oxygenated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The composition of Krebs’ solution contained was (in mmol/L): NaCl 120.6; KCl 5.9; CaCl<sub>2</sub> 2.5;KH<sub>2</sub>PO<sub>4</sub> 1.2; MgCl<sub>2</sub> 1.2; NaHCO<sub>3</sub> 15.4; glucose 11.5. All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Shandong University, and the study was approved by the Medical Ethics Committee for Experimental Animals, Shandong University, China (no. ECAESDUSM 2012029).

2.2 Measurement of I<sub>SC</sub>

The I<sub>SC</sub> was measured using the Ussing chamber technique. Tissue preparations were mounted between the two halves of an Ussing chamber, equipped with water-jacketed gas lifts; the exposed area was approximately 0.50 cm<sup>2</sup>. The tissue preparations were bathed on both sides with 5 mL Krebs’ solution, pH 7.4, gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The Krebs’ solution was maintained at 37°C during the experiments by circulation through a reservoir. The tissue was continuously voltage-clamped to zero potential difference by the application of external current, with compensation for fluid resistance. Baseline value of the electrical parameters were determined as a mean value over the 3 minutes immediately prior to drug administration. The tissue was equilibrated to these conditions for 20 minutes to stabilize I<sub>SC</sub>

**FIGURE 1** Inhibitory effects of propofol on carbachol (CCh) evoked changes in the short-circuit current (I<sub>SC</sub>). The representative traces show that serosal addition of 30 μmol/L CCh (30 μM) to the bathing solution evoked an increase in I<sub>SC</sub>. (A) There was no significant difference in the amplitude of the evoked response between the first and second applications of CCh timed 10 minutes apart. (B) In the presence of 10 μmol/L propofol to the serosal side, there was a significant decrease in the CCh-evoked change in I<sub>SC</sub> compared with vehicle (DMSO) treatment. (C) Statistical analysis for B and C. Data are mean ±SEM (n=11). *P<.05 compared with the first application of CCh.

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**Key Points**

- This study demonstrates that propofol inhibits CCh-induced intestinal secretion by directly targeting basolateral K<sup>+</sup> channels. Propofol is a widely used intravenous general anesthetic. The aim of this study was to investigate the effects of propofol on carbachol (CCh)-evoked short-circuit currents (I<sub>SC</sub>). I<sub>SC</sub> was measured using the Ussing chamber technique.
- The results show that propofol dose-dependently attenuated CCh-evoked I<sub>SC</sub> of rat ileum mucosa by directly targeting basolateral K<sup>+</sup> channels.
prior to the addition of drugs. The transepithelial potential difference for each preparation was measured with Ag/AgCl reference electrodes (P2020S; Physiologic Instruments, San Diego, CA, USA) connected to a preamplifier that was, in turn, connected to a voltage clamp amplifier (VCC MC4; Physiologic Instruments). The change in the $I_{SC}$ ($\Delta I_{SC}$) was determined as the difference in values before and after stimulation and was normalized as the current per unit area of epithelium (mA/cm$^2$).

### 2.3 | Drugs

Carbachol was first added to the serosal bathing solution and changes in $I_{SC}$ were measured (first application). The CCh were then washed out and $I_{SC}$ was allowed to stabilize for 10 minutes prior to the addition of propofol to the serosal bathing solution. After 10 minutes, CCh was added to the serosal bathing solution again and $I_{SC}$ was measured (second application). The difference of two applications in CCh-evoked $\Delta I_{SC}$ indicated the propofol-induced inhibitory effect. To investigate the underlying mechanisms, 10 minutes before propofol, drugs were added to the bathing solution. To evaluate the contribution of ion channels to $\Delta I_{SC}$, cystic fibrosis transmembrane conductance regulator (CFTR) (inh)-172 (10$^{-5}$ mol/L) and niflumic acid (10$^{-4}$ mol/L) were added to the mucosal bathing solution, whereas glibenclamide (10$^{-5}$ mol/L) or tetraethylammonium was added to the serosal bathing solution. Tetrodotoxin (TTX; 10$^{-6}$ mol/L) was used to investigate the role of neural pathways in the responses, whereas hexamethonium bromide (HB, nicotinic nACh receptor antagonist 10$^{-5}$ mol/L) was used to evaluate the role of nicotinic receptors. Both drugs were added to the serosal bathing solution.

### 2.4 | Statistical analysis

All data are expressed as the mean±SEM of $n$ animals. The significance of differences in baseline electrophysiological parameters among tissues was evaluated using one-way analysis of variance (ANOVA) or unpaired Student’s t-tests. IC50 value is subsequently determined using a sigmoidal dose–response curve fit. Two-tailed $P<.05$ was considered significant.

### RESULTS

#### 3.1 | Effects of propofol on CCh-evoked changes in short-circuit currents

Figure 1 shows a representative trace of a recording of a short-circuit current ($I_{SC}$) in the rat ileum mucosa in response to two

**FIGURE 2** Dose-dependent inhibitory effects of propofol on carbachol (CCh)-evoked changes in the short-circuit current ($I_{SC}$). (A) Representative trace of a recording of change in $I_{SC}$ induced by CCh in the presence of Vehicle (DMSO), propofol 10$^{-5}$ mol/L and propofol 10$^{-3}$ mol/L. (B) Based on these results, the IC50 for propofol was calculated to be 32 μmol/L. Data are mean±SEM ($n=6$).

**FIGURE 3** Inhibitory effects of propofol on carbachol (CCh)-evoked changes in the short-circuit current ($I_{SC}$) were observed only after the addition of propofol to the bathing solution on the serosal side. (A) The representative traces show that, in the presence of propofol (10$^{-2}$ mol/L) in the serosal side (upper lane) but not mucosal side (low lane), CCh-evoked $I_{SC}$ was largely decreased (upper lane). (B) Histograms of change in CCh-evoked response in the presence of propofol or not. Data are mean±SEM ($n=7$). ***$P<.001$ compared with before application.
serosal applications of CCh 10 minutes apart. There was no reduction in the amplitude of the $I_{SC}$ with repeated application of CCh (Fig. 1A). Of note, serosal addition of propofol significantly decreased the $I_{SC}$ induced by CCh compared with dimethylsulphoxide (DMSO) treatment (Fig. 1B and C). The inhibitory effect of propofol ($10^{-2} - 10^{-6}$ mol/L) on CCh-evoked responses was dose dependent (Fig. 2). Notably, only serosal addition of propofol showed the inhibitory effect on CCh-evoked responses (Fig. 3).

3.2 Effects of TTX on propofol ($10^{-5}$ mol/L) inhibition of CCh-induced changes in $I_{SC}$

The enteric nervous system (ENS) plays an important role in the regulation of intestinal epithelial ion transport. To investigate the involvement of the ENS in the effects of propofol. Tetrodotoxin ($10^{-6}$ mol/L) was added to the solution bathing the serosal side of the tissue to block the ENS. The addition of TTX to the bathing solution had no effect on the inhibitory effects of propofol on CCh-evoked changes in $I_{SC}$ (Fig. 4A).

3.3 Effects of ion channel blockers on propofol ($10^{-5}$ mol/L) inhibition of CCh-induced changes in $I_{SC}$

In the presence of ion channel blockers, CCh was added to the serosal bathing solution (first application) and then washed out. After 10 minutes of stabilization, CCh was added to the serosal bathing solution in the pretreatment with ion channel blockers and propofol (second application). The difference between the two applications in CCh-evoked $\Delta I_{sc}$ indicated the propofol-induced inhibitory effect. The ion channel blockers alone had no effect on repeated applications of CCh-evoked $\Delta I_{sc}$ (data not shown). Serosal treatment with HB had no effect on propofol-induced inhibitory effect in CCh-evoked $\Delta I_{sc}$ (Fig. 4B). Moreover, mucosal application of either niflumic acid, a blocker of $Ca^{2+}$-activated $Cl^{-}$ channels (CaCC), or CFTR inhibitor CFTR (inh)-172, had no effect on the inhibitory effects caused by propofol (Fig. 4C). However, serosal application of glibenclamide (Gli), a $K^{+}$ channel inhibitor, reversed the propofol-induced inhibitory effect (Fig. 4D and Fig. S1). Interestingly, another $K^{+}$ channel inhibitor TEA did not affect the propofol-induced inhibitory effect (Fig. 4E).

4 DISCUSSION

This study demonstrates that the intravenous general anesthetic propofol attenuates ACh receptor-mediated signal transduction in the rat ileum epithelium by targeting basolateral potassium channels. Of the many factors that affect ion transport, the neurotransmitter ACh is of particular importance. In normal mammalian colon, the ion transport response to ACh or to synthetic derivatives (e.g., CCh) is dominated by activation of epithelial $M_{3}$ receptors, which causes a $Cl^{-}$ efflux from cells. It has been reported that the $mAChR$ and its signal
transduction cascade are targets for general anesthetics. Specifically, several general anesthetics attenuated cloned M subtypes receptor-mediated signal transduction in Xenopus oocytes. The authors of these studies speculated that the target of propofol in AChR-mediated signal transduction is the receptor itself.

However, the effects of anesthetics on AChR-mediated signal transduction in the rat ileum epithelium have not been reported. The results of this study show that propofol attenuates the increase in $I_{SC}$ elicited by the cholinergic agonist CCh.

Most studies support the view that the increase in $I_{SC}$ induced by CCh is due primarily to Cl$^-$ secretion. The Cl$^-$ ions can flow to the lumen through the apical CFTR channel and possibly via CaCC. In epithelia, the Na, K-ATPase, also known as sodium pump, transports 3 Na$^+$ out and 2K$^+$ into the cell. In addition to maintaining the intracellular ion homeostasis, this pumping process generates a transmembrane electrochemical gradient that regulates other ions transport. The intracellular Cl$^-$ concentration is maintained by a Na$^+$-K$^+$-2Cl$^-$ co-transporter that actively accumulates Cl$^-$. After binding to muscarinic cholinoreceptors (mACHR), CCh activates a G-protein to increase intracellular levels of Ca$^{2+}$ and cAMP, which, in turn, drives Cl$^-$ secretion by stimulating Ca$^{2+}$-activated Cl$^-$ channels (CaCC) or cAMP-dependent cystic fibrosis transmembrane conductance regulator (CFTR) in the apical membrane of intestinal epithelial cells. In addition, CCh increases basolateral K$^+$ efflux. Propofol decreases basolateral K$^+$ channels permeability, then depolarizes the basolateral membrane potential and reduces the transmembrane electrochemical gradient, and thus depresses the CCh-evoked Cl$^-$ secretion.

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CONFLICTS OF INTEREST

No competing interests declared.

REFERENCES


SUPPORTING INFORMATION

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