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Major role of $GABA_A$-receptor mediated tonic inhibition in propofol suppression of supraoptic magnocellular neurons

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ABSTRACT

Using slice patch clamp recording, we examined the effects of general anesthetic propofol (2,6-diisoprophlphenol) on dual modality of $GABA_A$ inhibition in supraoptic nucleus (SON) magnocellular neurosecretory cells (MNCs): conventional quantal synaptic transmission (IPSCs, I_{phasic}) and persistent tonic form of inhibitory current (I_{tonic}). Propofol (10 μM) enhanced I_{tonic} as shown by an inward shift in $I_{holding}$ (16.46 ± 2.93 pA, n = 27) and RMS increase (from 3.37 ± 0.21 pA to 4.68 ± 0.33 pA, n = 27) in SON MNCs. Propofol also prolonged the decay time of IPSCs with decreased IPSCs frequency but no significant changes in IPSCs amplitude. Overall, propofol (1–10 μM) caused much smaller increase in mean I_{phasic} than mean I_{tonic} at all tested concentrations. In consistent with the enhancement of $GABA_A$ currents, propofol attenuated ongoing firing activities of SON MNCs by ~65% of control. Selective inhibition of I_{phasic} by a $GABA_A$ antagonist, gabazine (1 μM), failed to block the propofol suppression of the firing activities, while inhibition of I_{tonic} and I_{phasic} by bicuculline (20 μM) efficiently blocked the propofol-induced neurodepression in SON MNCs. Taken together, our results showed that propofol facilitated I_{tonic} with marginal increase in mean I_{phasic}, and this could be a mechanism reducing the intrinsic SON MNCs excitability during propofol anesthesia.

The magnocellular neurosecretory cells (MNCs) in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON) project to the neurohypophysis [1] where they release oxytocin (OT) or vasopressin (VP) into the bloodstream, and play fundamental roles in reproduction and fluid balance homeostasis. GABA, through activation of $GABA_A$ receptors, is a major inhibitory neurotransmitter modulating neuronal excitability in the nuclei [13,20,28]. $GABA_A$ receptors, pentameric GABA-operated ion channels, produce inhibition in at least two modes in the PVN and SON [23–25]: via conventional quantal synaptic transmission (IPSCs, I_{phasic}) and via a persistent tonic form of inhibitory current (I_{tonic}) [6,19].

The hypnotic effects of a general anesthetic, propofol (2,6-diisopropylphenol), are primarily attributed to the extended $GABA_A$ channel open times [16] and slowing desensitization [2], both attributes of I_{phasic}. There are also emerging evidences that I_{tonic}, like I_{phasic}, is facilitated by many clinically used anesthetics. I_{tonic} is even more sensitive to anesthetics than actions on I_{phasic} [10] and seemingly plays a substantial role in suppressing neuronal excitability [3].

Propofol is often associated with adverse cardiovascular effects, such as decreases in cardiac output [18] and arterial blood pressure [5]. The enhancement of I_{phasic} by propofol in the PVN and SON has been known to be a possible mechanism causing cardiovascular and sympathetic depression during propofol anesthesia [12,32]. However, the functional role of I_{tonic} during anesthesia has not been elucidated in the neurons, although I_{tonic} plays an important role in controlling neuronal excitability in the PVN and SON [23–25] as in cerebellum [4,9] and hippocampus [31]. Here, we showed propofol facilitates tonic as well as phasic modality of $GABA_A$ inhibition in SON MNCs, and the former may play a major role in the neurodepression by the anesthetic.

Male Sprague–Dawley rats (180–220 g) were housed in a 12/12-h light/dark schedule and allowed free access to food and water. All animal experimentation adheres to the policy of the Chungnam National University regarding the use and care of animals.

Patch-clamp recordings from SON neurons were obtained in hypothalamic slices (300 μm) as previously described [25]. Slices

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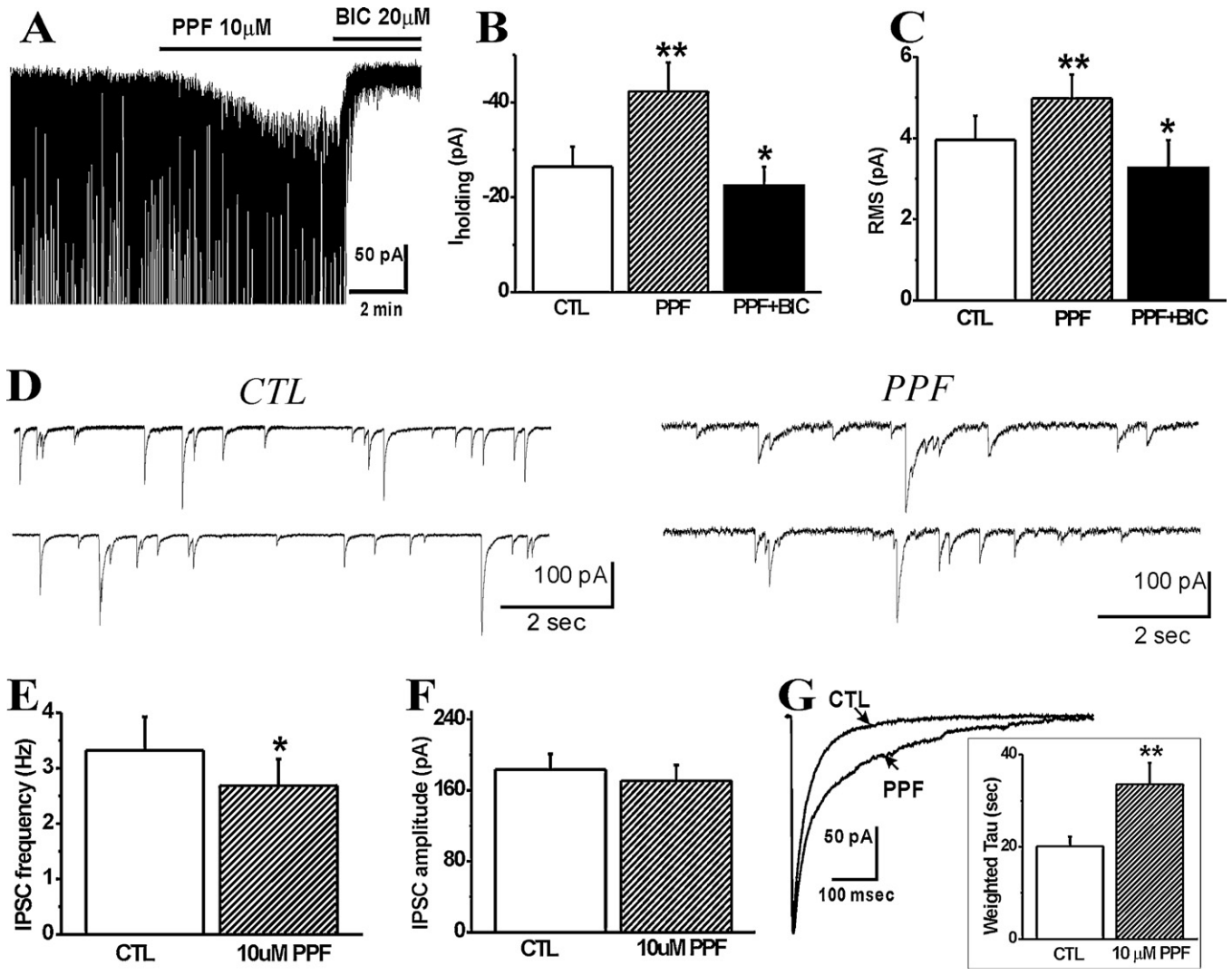

Fig. 1. Effects of propofol on I_{tonic} and I_{phasic} in SON neurons. (A) Representative current trace showing that propofol (10 μM) induced an inward shift in $I_{holding}$, and RMS increase. This effect was blocked by the $GABA_A$ receptor antagonist bicuculline (BIC, 20 μM). Mean changes in $I_{holding}$ (B) and RMS (C) induced by propofol are summarized. (D) Expanded current traces illustrating spontaneous IPSCs obtained from the same neuron as in (A) in the absence and presence of propofol. Summary data showing mean changes in IPSCs frequency (E) and amplitude (F) induced by propofol. (G) Averaged IPSCs ($n = 120$ events) obtained from the same neuron as in (A) before and during bath application of propofol. Summarized data showing propofol significantly increased the decay time of sIPSC are shown (inset). Summarized data shown are means ± SEM. $^*P < 0.05$, $^{**}P < 0.01$ when compared to its respective control.

containing the SON were cut using a vibrosilcer (Leica VT 100s, Leica, Bensheim, Germany) in ice-cold artificial cerebrospinal fluid (aCSF), and placed in a holding chamber containing standard oxygenated aCSF until used. The standard aCSF consists of (in mM): NaCl 126; $NaHCO_3$ 26; KCl 5; NaH_2PO_4 2.4; $CaCl_2$ M 2.4; $MgCl_2$ 1.2; glucose 10; pH was 7.3–7.4. The medium was saturated with 95% O_2–5% CO_2.

Electrophysiological recordings were obtained with using Axopatch200-B amplifier (Axon Instruments, Foster City, CA). Current and voltage output were filtered at 2 kHz and digitized at 10 kHz (Digidata 1322A, Axon Instruments) in conjunction with pClamp 8.2 software. Patch pipettes (borosilicate glass, 3–7 MΩ) were filled with a high Cl^- containing solution (in mM): 140 KCl, 20 HEPES, 0.5 $CaCl_2$, 5 EGTA, and 5 Mg^{2+}ATP, pH 7.3. Spontaneous inhibitory postsynaptic currents (sIPSCs, recorded at −70 mV) were detected in the presence of the glutamate AMPA/kainate receptor antagonist, DNQX (5,7-dintroquinoxaline-2,3-dione, 10 μM), NMDA receptor antagonist AP5 (DL-2-amino-5-phosphonopentanoic acid, 100 μM), and analyzed using MiniAnalysis (Synaptosoft). The decay phase of IPSCs was best fitted with a double-exponential function, and presented in weighted values [8]. The holding current ($I_{holding}$) and RMS noise were measured in 50 ms epochs of traces lacking PSCs, separated by ~800 ms. I_{tonic} was defined as the difference in $I_{holding}$ before and after application of $GABA_A$ receptor blocker picrotoxin or bicuculline. RMS noise was measured in the same epochs using MiniAnalysis software.

Spontaneous firing discharges were recorded in continuous mode. For firing activity, patch pipettes filled with a more physiological concentration of Cl^- were used (in mM): 140 K–gluconate, 10 KCl, 10 HEPES, 0.5 $CaCl_2$, 5 EGTA, and 5 Mg^{2+}ATP, pH 7.3. Firing rate was calculated using MiniAnalysis, by counting the number of action potentials in 10 s bins. Mean values for each condition were then obtained.

Numerical data are presented as means ± SEM. Paired Student's *t*-test and analysis of variance repeated measures (ANOVA-RM), followed by Tukey's post hoc tests, were used to compare the effects of a drug treatment.

To determine if propofol modulate the $GABA_A$ receptor mediated tonic currents in SON neurons, we measured the holding