Thiopental inhibits glycine receptor function in acutely dissociated rat spinal dorsal horn neurons

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Abstract

Whole-cell patch-clamp was used to assess the modulatory effect of thiopental (Thio) on glycine (Gly) receptor in mechanically dissociated rat spinal dorsal horn neurons. It was found that Thio inhibited the amplitude, accelerated the desensitization and prolonged the deactivation of Gly-induced currents ($I_{\text{Gly}}$) in a concentration-dependent manner. In addition, a rebound current occurred after washout of the co-application of Gly and Thio in most neurons tested. Moreover, the inhibitory effect of Thio was not the result of cross-inhibition between Gly and GABAA receptors. Furthermore, taurine-induced currents, a low-affinity agonist for Gly receptors, were also markedly inhibited by Thio in a similar way to $I_{\text{Gly}}$. These results indicate that Thio suppresses Gly receptor function and suggest that Thio anesthetic actions might not be mediated by Gly receptors. We speculate that the weak muscle relaxation and the limited analgesic effects observed during Thio anesthesia may attribute to its inhibitory effects on Gly receptors.

Keywords: Acutely dissociated neuron; Thiopental; Glycine receptors; Spinal cord dorsal horn; Whole-cell patch-clamp recording

As central nervous system (CNS) depressants, general anesthetics are used widely in both clinical medicine and neuroscience. We have, however, only recently begun to understand how they exert their effects. Emerging as major contributors to sedative and hypnotic effects of intravenous anesthetics, GABAA receptors, the major inhibitory neurotransmitter receptors in the CNS, have attracted considerable attention [20]. Conversely, another inhibitory neurotransmitter, glycine (Gly) and its receptors, are less studied because they primarily express in the spinal cord and brain stem [17], while the brain is considered the main target for general anesthetics. On the other hand, accumulated evidence suggests that the spinal cord plays a critical role in at least two anesthetic end-points: analgesia and immobility in response to noxious stimulus [10] in which both Gly and GABAA receptors are likely involved. Thus, understanding how general anesthetics modulate Gly receptors might help us to understand the potential mechanisms of general anesthetic-induced analgesia and immobility. Such progress will eventually prompt us to uncover the mystery of how analgesia and locomotion are modulated in the CNS.

Because of its rapid onset of action and recovery, thiopental (Thio), a barbiturate, has been widely used in anesthesia since it was first introduced into clinical practice in the 1950s [3]. However, little information is available up to now about the effect of Thio on receptors and ion channels in vitro. In the present study, therefore, we explored the effect of Thio on Gly receptors in acutely dissociated rat spinal dorsal horn neurons. At a free aqueous concentration of about 25 $\mu$M Thio (corresponding to a total plasma concentration of 40 $\mu$g/ml), rats fail to respond to noxious stimulus [7]. Thus, we used 30 $\mu$M as the clinical relevant concentration of Thio in the present study.

The care and use of animals used for these experiments followed the guideline and protocol approved by the Care and Use of Animals Committee of the University of Science and Technology of China. The spinal dorsal horn neuron was mechanically dissociated as described previously [16]. In brief,
Wistar rats (2-week-old) were anesthetized with urethane (1 g/kg, intraperitoneally) and a segment of the lumbosacral (L4-S2) spinal cord was dissected out and transverse slices (400 μm) of spinal cord were sectioned using a vibratome tissue slicer (VT1000S, Leica Instruments Ltd., Wetzlar, Germany). After incubation at room temperature (22–25°C) for 50 min in artificial cerebrospinal fluid aerated with 95% O2 + 5% CO2, the slice was transferred into standard external solution in a culture dish. A vibration-isolation system[16] was then used to mechanically dissociate the dorsal horn neurons. The dissociated neurons were attached to the bottom of the culture dish and made ready for electrophysiological experiments within 20 min. Consistent with our previous reports[16], the acutely isolated neurons were medium-sized (10–15 μm in diameter), with oval or triangular soma and one to three apical stem dendrites. Under the present experimental conditions, application of Gly evoked inward chloride currents in all neurons tested at a holding potential (VH) of −50 mV, with an EC50 of 30 μM[13].

Whole-cell patch-clamp recordings in voltage-clamp mode were made at room temperature (22–25°C) using an Axopatch 200B patch-clamp amplifier (Axon Instruments, Foster City, CA, USA), which was connected to a Pentium III computer equipped with a Digidata 1320A analog-to-digital converter and pClamp7.0 software (Axon Instruments, Foster City, CA, USA) for data acquisition and analysis. All drugs used in the present experiments were purchased from Sigma (St. Louis, MO, USA) except Thio, which was obtained from Shanghai New Asiatic Pharmaceutical Co. Ltd. The recording solutions were prepared as described[16]. In most experiments, about 80% series resistance compensation was applied. Unless otherwise noted, the membrane potential was held at −50 mV.

Origin (Microcal Software) and Excel (Microsoft, Seattle, WA, USA) were used for data display and analysis. Current deactivation and desensitization were fitted by exponential functions (Clampfit 8.1, Axon Instruments, Foster City, CA, USA). During the fitting process, the goodness of fit was evaluated by the χ2 value. The current desensitization was fitted beginning shortly after the peak of response and deactivation trace began from the peak of the tail current when the tail current appeared, otherwise the fit begun from the removal of drug(s). Statistical
comparison was carried out using the Student's t-test for comparison between two groups and analysis of variance (ANOVA) for multiple comparison (as noted) with p < 0.05 (*) or 0.01 (**) considered significant. n Represents the number of neurons studied. All data were expressed as mean ± S.E.M.

Whole-cell recordings were obtained from these neurons randomly. At a clinically relevant concentration of 30 μM Thio significantly inhibited the peak amplitude of Gly-induced currents (I_{Gly}) Fig. 1A and B and affected the kinetics of I_{Gly} (Fig. 1C); the time constant of desensitization (τ_{des}) was reduced, but the time constant of deactivation (τ_{dea}) was increased. Thio did not alter the reversal potential of I_{Gly} (data not shown). As shown in Fig. 1B, the modulatory effect of Thio on peak amplitude of I_{Gly} depended on Gly concentrations; the inhibition of Thio of I_{Gly} was more pronounced with low Gly concentration. However, the change in τ_{des} of I_{Gly} increased in response to a raise in Gly concentration, whereas the degree of Thio prolongation of τ_{dea} decreased with increased Gly concentration.

The effect of Thio at various concentrations on 30 μM I_{Gly} was also investigated (Fig. 2). Thio dissolved in ion-free water is strongly alkaline. At pH 7.4, Thio precipitates from standard external solution at concentrations above 300 μM. For this reason, no concentration of Thio over 300 μM was used. Similar modulatory effect of Thio on I_{Gly} was observed at different concentrations. Thio inhibited the peak amplitude, increased the τ_{des} and reduced the τ_{dea} of I_{Gly}. Thio itself induced inward chloride currents at concentrations above 50 μM. Thus, the ratio of inhibition was calculated according to the formula ratio = (I_{Gly+Thio} − I_{Thio})/I_{Gly}, where I_{Gly+Thio} represents the current induced by Gly plus Thio. I_{Thio} represents the Thio-evoked current. The ratio of changes on amplitude, τ_{des}, and τ_{dea} of I_{Gly} all increased with the raise of Thio concentration (Fig. 2B). In addition, a rebound current appeared after washout of the co-application of 30 μM Gly and 300 μM Thio in most neurons tested (Fig. 2A).

A previous study suggests an asymmetric cross-inhibition between Gly and GABA_A receptors [13], and Thio itself activated GABA_A receptors at high concentrations (≥50 μM) (data not shown) in these neurons. To test whether the direct activation of GABA_A receptors by Thio causes subsequent cross-inhibition between Gly and GABA_A receptors, we examined the effect of 300 μM Thio on I_{Gly} in the presence of 10 μM bicuculline, a selective antagonist of GABA_A receptors (Fig. 3). No significant difference was observed between the inhibition produced by 300 μM Thio in the absence or presence of bicuculline (Figs. 2 and 3). Thus, it is likely that Thio inhibits I_{Gly} independent of GABA_A activation.

In order to investigate the mechanism underlying the rebound current observed after washout of the co-application of Gly and Thio, we further tested different drug application protocols (Fig. 3) in the presence of bicuculline. As shown in Fig. 3, co-application of Thio and Gly markedly reduced I_{Gly} amplitude.
and increased $\tau_{\text{dea}}$ either with (Fig. 3B, Pre) or without (Fig. 3B, No-pre) the pre-application of Thio; the inhibition ratios of $I_{\text{Gly}}$ amplitude were $0.20 \pm 0.03$ and $0.32 \pm 0.04$, respectively, and the potentiation ratios of $\tau_{\text{dea}}$ were $2.72 \pm 0.34$ and $3.36 \pm 0.31$, respectively. However, sequentially applied 300 $\mu M$ Thio and Gly reduced the amplitude of $I_{\text{Gly}}$ to a lesser extent, and $\tau_{\text{des}}$ and $\tau_{\text{dea}}$ were not changed significantly (Fig. 3B, Seq). Interestingly, the rebound currents disappeared in the sequential application protocol (Fig. 3A).

Finally, we examined the effect of Thio on the response induced by taurine, a low-affinity agonist of Gly receptors [21].

The unbinding rate of taurine is fast enough that the channel-closing rate becomes the rate-limiting step for deactivation, which is extremely rapid after agonist withdrawal [12]. For this reason, we could test whether Thio altered the channel-closing rate by evaluating the $\tau_{\text{des}}$ of current induced by taurine ($I_{\text{Tau}}$) in the presence of Thio. As shown in Fig. 4, 300 $\mu M$ Thio slightly inhibited the peak amplitude of the current induced by 300 $\mu M$ taurine, leaving $\tau_{\text{des}}$ unaffected. However, Thio, at high concentrations, greatly reduced the peak of $I_{\text{Tau}}$, decreased $\tau_{\text{des}}$, and increased $\tau_{\text{dea}}$. The effect of Thio at various concentrations, on the amplitude, $\tau_{\text{des}}$, and $\tau_{\text{dea}}$ of $I_{\text{Tau}}$ is summarized in Fig. 4B.

Taken together, our results suggest that Thio allosterically inhibits Gly receptors by binding to a distinct site, similar to the effect of $H^+$ on Gly receptors [14], resulting in a decrease of $\tau_{\text{dea}}$ and $\tau_{\text{dea}}$. In addition, previous studies show that pentobarbital, which possess similar chemical structure with Thio, inhibits Gly receptors via open-channel blockage in rat spinal neurons [16] and in HEK293 cells expressing $\alpha_1$-Gly receptors [19]. Our data also support an additional role of Thio, acting as open-channel blocker on the Gly receptors. A rebound current that appeared after washout of Thio and Gly is indicative of an open-channel blocker of Gly receptors [16]. In support of this speculation, the rebound current disappeared when Thio and Gly were applied sequentially (Fig. 3). Furthermore, for the low-affinity agonist taurine, no rebound current was observed probably due to its rapid unbinding rate (Fig. 4).

In contrast to the inhibitory effect on native neurons, however, Thio and pentobarbital were positive allosteric modulators on $\alpha_1$-Gly receptor expressed in Xenopus oocytes [2, 4, 8]. This discrepancy may result from some unknown differences between preparations and/or experimental conditions. In particular, specific factors in cell lines like HEK293 cells and Xenopus oocytes may affect the modulatory properties of Thio and even the expression properties of the Gly receptors. Interestingly, pentobarbital also produces opposite regulatory effects on $\alpha_1$-Gly receptors expressed in Xenopus oocytes [2, 4, 8] and on native Gly
receptors [16] or on αβ Gly receptors expressed in HEK293 cells [19].

In addition to its effect on the I_Gly amplitude, we observed that Thio accelerated the desensitization and slowed the deactivation of I_Gly in a dose-dependent manner (Fig. 2). Similar effects were reported for the volatile anesthetics halothane [12], and benzodiazepines [18] on the GABAA receptors. Li and Pearce [12] showed that halothane slows the agonist-unbinding rate (K_off), leading to an accelerated desensitization and prolonged deactivation of current induced by GABA. It is thus possible that Thio slows Gly unbinding rate from its receptors, resulting in the alteration of I_Gly kinetics. In addition, since the deactivation process is determined by both channel-closing rate and agonist-unbinding rate, we could attribute at least partially the increased effect of Thio on the τ_channel of I_Gly to a prolonged channel-closing rate of Gly receptors.

In summary, we observed that general anesthetic Thio inhibited the amplitude and accelerated the desensitization of I_Gly. These results suggest that Thio would suppress Gly receptor function in vivo. In has been known that Gly receptors are involved in modulating muscle tension [6] and nociceptive signals [11] in spinal cord. More recent evidence demonstrates that αβ-Gly receptor, which locates in the superficial dorsal horn, is an essential target for spinal PGE2-mediated inflammatory pain sensitization [1,9]. These previous studies indicate that Gly receptors, at least some subunits, are very important for the pain regulation. Thus, we suggest that the weak muscle relaxation and analgesic effect during Thio anesthesia [5] may attribute to the inhibitory effect of Thio on Gly receptors. For this reason, anesthetics that combine the ability to potentiate both GABAergic and glycinergic transmissions may be of greater utility. Interestingly, two major inhibitory neurotransmitters GABA and Gly are co-released from the same presynaptic terminals [15], and there is an asymmetric cross-inhibition between Gly and GABAA receptors [13]. That whether and how general anesthetics like Thio may regulate the inhibitory synaptic balance awaits further experiments.

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References
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