Thiopental's effects on GABAergic currents in spinal ventral horn networks in vitro: concentration-dependent shift of impact from phasic to tonic currents

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Abstract

Interneuronal networks in the ventral horn of the spinal cord are plausible substrates for mediating anesthetic-induced immobility. Thiopental, a barbiturate in clinical use, is known to depress neuronal activity and act on $GABA_A$ receptors, among other targets, in the brain and the spinal dorsal horn, but its effects on the spinal ventral horn are largely uncharacterized. Here, we investigated thiopental's effects on spontaneous action potential firing and on GABAergic and glycinergic synaptic transmission in interneurons in the ventral horn of cultured spinal cord slices from mice. Thiopental reduced action potential activity concentration-dependently with an EC_{50} of 16.6±2.4 µM to an uppermost limit of 87.3±5.0 %. Drug interaction experiments as well as recordings of $GABA_A$ and glycine receptor-mediated inhibitory currents indicated that this effect was largely mediated by $GABA_A$ receptors. Specifically, at 20 µM thiopental prolonged the decay time of GABAergic inhibitory postsynaptic currents (IPSCs). Although this prolongation of IPSCs increased the inhibitory charge per IPSC, the concomitant strong reduction of IPSC frequency resulted in less inhibitory current entering the neurons via this route than under control conditions. However, thiopental also induced a robust tonic current of 54±23 pA. This current could be blocked by bicuculline (100 µM), indicating that it was mediated by $GABA_A$ receptors. At higher concentrations of thiopental, IPSC activity nearly collapsed, whereas tonic currents continued to rise, reaching 67±34 pA at 50 µM thiopental. Furthermore, at concentrations exceeding 20 µM thiopental, action-potential depressing mechanisms independent of $GABA_A$ receptors came into play. However, glycine receptors, which form part of the other main inhibitory system in spinal cord, could be excluded as relevant targets.

The results suggest that in the ventral horn thiopental acts mostly, but not exclusively, via $GABA_A$ receptors. With increasing concentrations of thiopental, inhibition via IPSCs is limited by negative feedback manifest in the strong depression of action potential and IPSC frequency whereas action-potential independent inhibition due to tonic currents progressively gains in impact.

I. Clinically relevant concentrations of thiopental depress action potential activity in spinal ventral horn in vitro

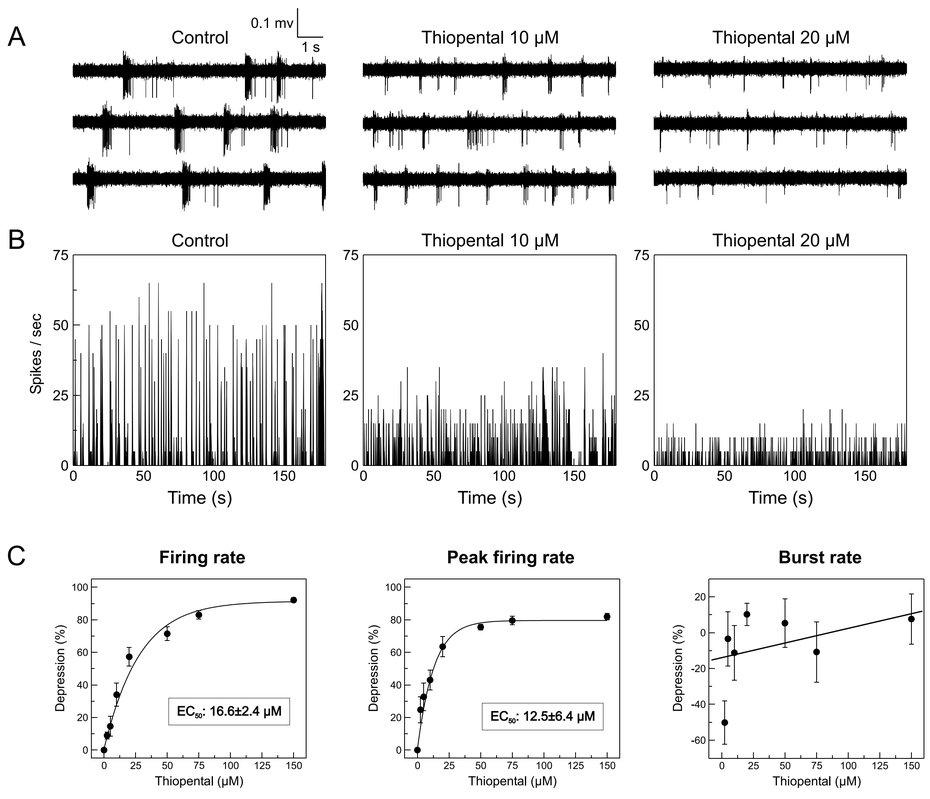

Figure 1. Effects of thiopental on action potential activity in organotypic spinal slices. (A) Excerpts of original recordings under control conditions and in the presence of 10 and 20 µM thiopental. The average firing rates were 8.6 Hz (control), 5.2 Hz (10 µM), and 2.3 Hz (20 µM); burst rates were 0.3 Hz (control), 0.3 Hz (10 µM), and 0.4 Hz (20 µM). (B) Time course of action potential activity over the total recording period of 180 s (data were binned with a width of 200 ms). (C) Depression of various aspects of spontaneous action potential activity by thiopental. For each concentration, the mean value and standard error were obtained from 8-10 cells. The curves and lines represent Hill fits and linear fits, respectively, to the data.

II. Major contribution of GABAergic but not glycinergic synaptic transmission to action potential depression

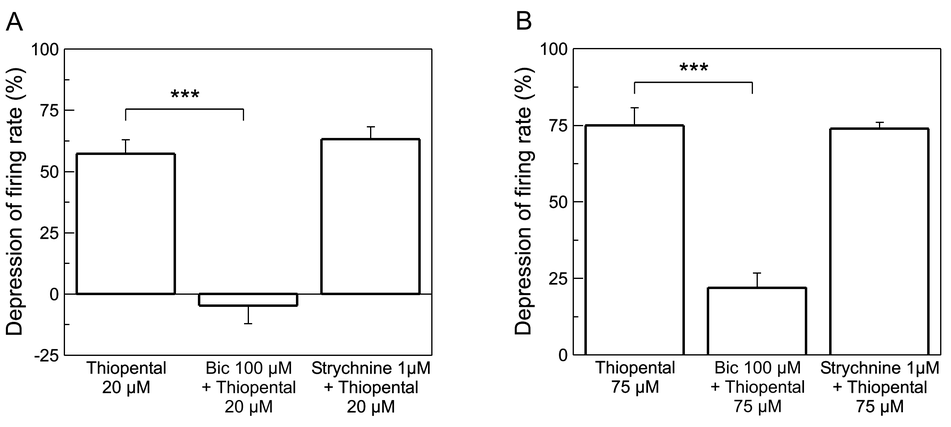

Figure 2. Effects of two different concentrations of thiopental on firing rates in the presence of blockers of $GABA_A$ (bicuculline) and glycine (strychnine) receptors. Star symbols indicate statistical significance as assessed by ANOVA and post-hoc tests (***; p<0.001). (A) Depression of firing rates by 20 µM thiopental under control conditions (left bar), with bicuculline (center) and with strychnine (right). 20 µM thiopental depressed firing rates by ~59% under control conditions but had no effect with bicuculline (n=10). Blockade of glycine receptors did not diminish the action potential-depressant effect of thiopental. (B) Depression of firing rates by 75 µM thiopental in the same conditions as in A. In the presence of bicuculline, 75 µM thiopental depressed firing rates only by 24%, compared to 75% without bicuculline (n=10). Again, strychnine did not abate thiopental's depressant effect.

III. Induction of tonic GABAergic currents by thiopental

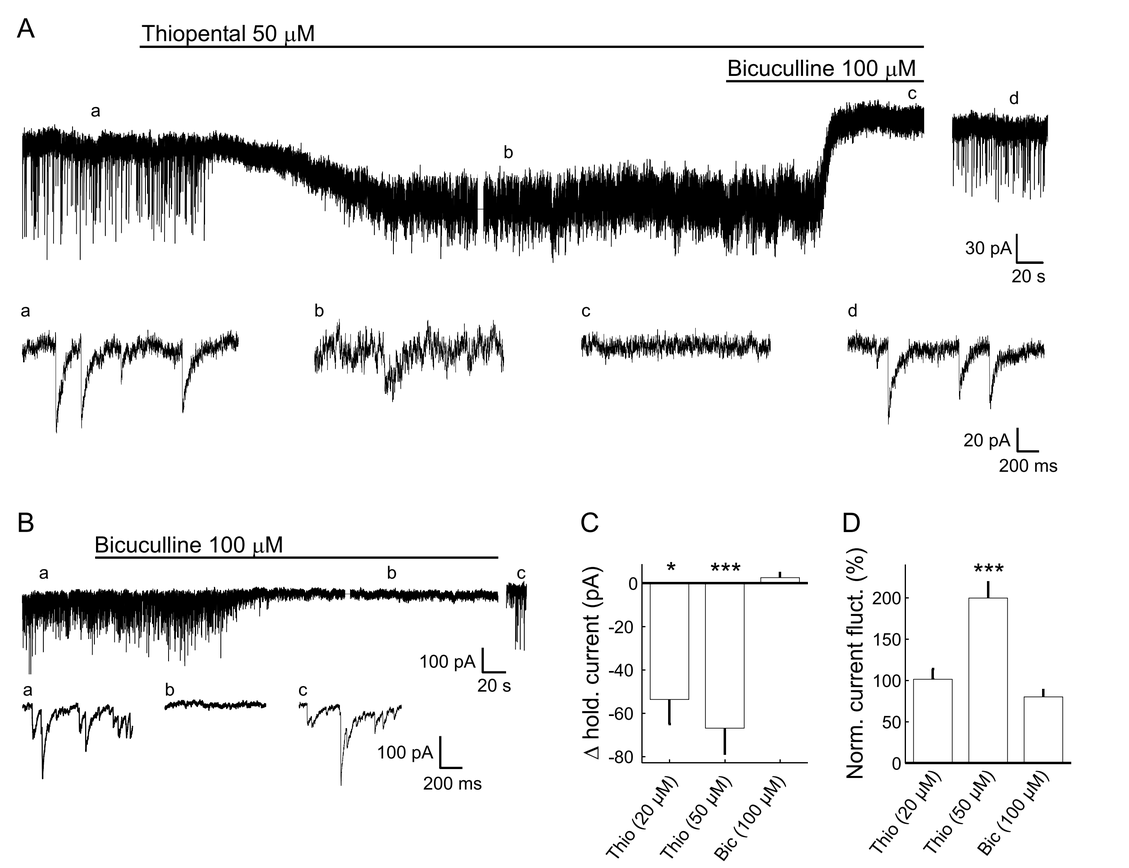

Figure 3. Membrane currents in spinal interneurons and their modulation by thiopental and bicuculline. CNQX (50 µM), APV (50 µM) and strychnine (1 µM) were in the bath at all times. (A) Time course of currents in an interneuron exposed to 50 µM thiopental 90 s after recording started. Bicuculline (100 µM) was added 8 min after wash-in of thiopental. Note the strong inward current and current fluctuations which were induced by thiopental and suppressed by bicuculline. At the end of the experiment, both drugs were washed out for 19 min, leading to a partial recovery of spontaneous inhibitory postsynaptic currents (sIPSCs; visible in the data portion on the right hand side which is disjunct from the rest of the data). The gap in the middle of the data trace (~4 min after wash-in of thiopental) corresponds to a brief (5 s) interruption of the recording for a measurement of the electrode's access resistance. Small letters mark the position of excerpts plotted below the main trace. (B) Current changes induced by blocking $GABA_A$ receptors with bicuculline (100 µM). Bicuculline suppressed sIPSCs but did not alter the holding current. IPSC amplitudes recovered after an extensive wash-out period (30 min). As in A, excerpts were taken from the data at positions marked by small letters. (C) Summary of changes in tonic (holding) currents. The value of the holding current under control conditions was subtracted from the current measured after application of thiopental (left and middle bars) or bicuculline (right bar). Star symbols above bars indicate statistical significance of the effect (paired t-test, *; p<0.05, ***; p<0.001). (D) Summary of changes in current fluctuations as measured by the width of the positive flank in current amplitude distributions. The data are normalized to control conditions. Star symbols above bars indicate statistical significance of the difference to control conditions (paired t-test, same notation as in C).

IV. Phasic GABAergic currents (IPSCs) with thiopental - depression of frequency outweighs prolongation

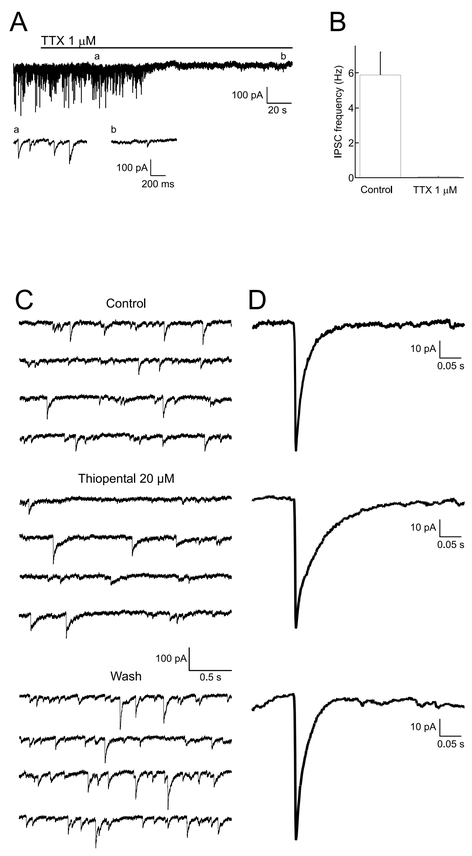

Figure 4. (A, B) Spontaneous (=action potential-dependent) vs. miniature IPSCs in ventral horn interneurons. (A) Time course of currents in an interneuron exposed to 1 µM Tetrodotoxin (TTX). Tetrodotoxin led to a dramatic reduction of IPSCs. The corresponding excerpt (labeled 'b') shows a miniature IPSC. (B) Summary of IPSC frequency under control conditions (CNQX, APV strychnine in the bath) and with additional TTX. (C, D) Effects of thiopental on spontaneous GABAergic IPSCs. The currents were measured in the presence of CNQX (50 µM), APV (50 µM) and strychnine (1 µM). (C) Original recordings of the effects of 20 µM thiopental on sIPSCs. Thiopental caused a prolongation of half-decay times from 24.6 ms (control) to 59.7 ms. The effect was reversible (25.7 ms after washout of the drug). The frequency of sIPSCs was reduced from 0.8 Hz (control) to 0.5 Hz (20 µM thiopental); it reverted to 0.9 Hz after washout. (D) Averaged sIPSCs of the corresponding recordings displayed in C.

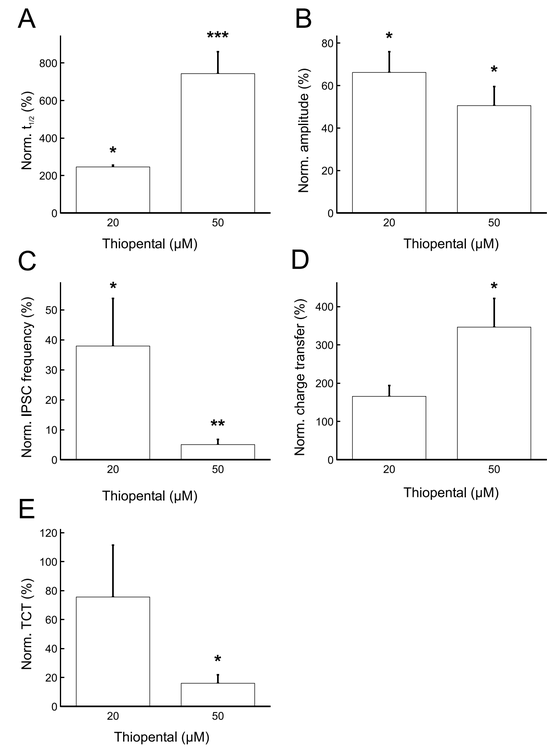

Figure 5. Quantitative analysis of thiopental's effects on sIPSCs measured in the presence of CNQX (50 µM), APV (50 µM) and strychnine (1 µM). For each concentration the mean value and standard error were obtained from 6-8 cells and are expressed in percent relative to control conditions. Star symbols above bars indicate statistical significance of the difference to control conditions (t-test, *; p<0.05, **; p<0.01, ***; p<0.001). (A) Half-decay time of sIPSCs. (B) sIPSC amplitude. (C) sIPSC frequency. Note the strong decrease from 20 to 50 µM thiopental. (D) Average charge transferred per IPSC. (E) Total charge transfer (TCT) in a given time interval. It was calculated as the product of the integral of averaged sIPSCs with the frequency of the events.