Effects of thiopental on spinal ventral horn network activity: shift from phasic to tonic GABAergic inhibition

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Abstract

Background: Interneuronal networks in the spinal ventral horn are plausible substrates for mediating anesthetic-induced immobility.1 Here, we investigated how their activity is affected by clinically relevant doses of the barbiturate thiopental.
Methods: Organotypic spinal cord tissue slices were prepared from pregnant wild type mice (day 13-15) according to the method described by Braschler.2 Effects of thiopental on spontaneous action potential firing were investigated by extracellular recordings. Whole cell voltage clamp recordings were performed for quantifying effects of thiopental on GABAergic and glycinergic synaptic transmission. Analysis of base line currents (tonic currents) followed the method proposed by Glykys and Mody.3 All procedures were approved by the animal care committee and were in accordance with the German law on animal experimentation.
Results: In cultured spinal cord slices from mice, thiopental reduced action potential activity with an EC50 of 16.6±2.4 µM. Recordings of GABAA and glycine receptor-mediated inhibitory currents indicated that the effect was largely mediated by GABAA receptors and that glycine receptors were not relevant targets. Specifically, 20 µM thiopental prolonged the decay time of spontaneous GABAergic inhibitory postsynaptic currents (sIPSCs) more than twofold. Although this prolongation of decay time increased the inhibitory charge per sIPSC the concomitant strong reduction of sIPSC frequency resulted in less inhibitory current entering the neurons via this route. However, 20 µM thiopental also induced a tonic current of 30±10 pA, mediated by GABA(A) receptors. 50 µM thiopental nearly abolished sIPSC activity but augmented tonic currents to 69±14 pA. Furthermore, at this concentration, activity-depressing mechanisms independent of GABAA receptors came into play.
Conclusions: The results suggest that in the spinal ventral horn thiopental acts mostly, but not exclusively, via GABA(A) receptors. With increasing concentrations of the drug, inhibition via sIPSCs is limited by negative feedback on interneuronal firing whereas action potential-independent GABAergic inhibition due to tonic currents gains progressively in impact.

References:
1. Kim JB et al. Anesth Analg 2007; 105:1020-26.
2. Braschler UF et al. J Neurosci Methods 1989; 29: 121-9.
3. Glykys J and Mody I. J Physiol 2007; 582, 1163-78.

Thiopental depresses spinal interneuronal network activity

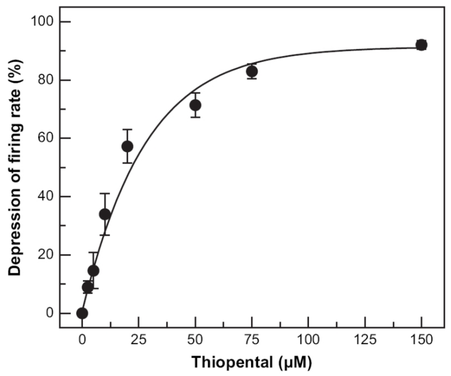

Fig. 1: Depression of firing rates in cultured organotypic spinal slices induced by thiopental. The curve represents a Hill fit to the data (R2 0.987). The half-maximal effect occured at 16.6±2.4 µM and the maximal depression was 87.3±5.0%.

Major contribution of GABAergicsynaptic transmission to action potential depression

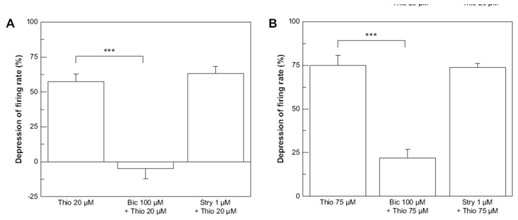

Fig. 2: Effects of two different concentrations of thiopental on firing rates in the presence of blockers of $GABA_A$ (bicuculline) and glycine (strychnine) receptors. Star symbols indicate statistical significance as assessed by ANOVA and post-hoc tests (***; p<0.001). (A) Depression of firing rates 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.

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

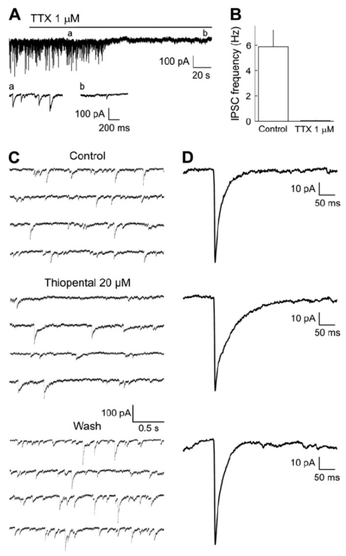

Fig. 3: (A, B) Spontaneous (=action potential-dependent) vs. miniature IPSCs in ventral horn interneurons. (A) Time course of currents in an interneuron exposed to 1 µM TTX. The corresponding excerpt (labeled 'b') shows a miniature IPSC. (B) Summary of IPSC frequency under control conditions (CNQX, APV 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.

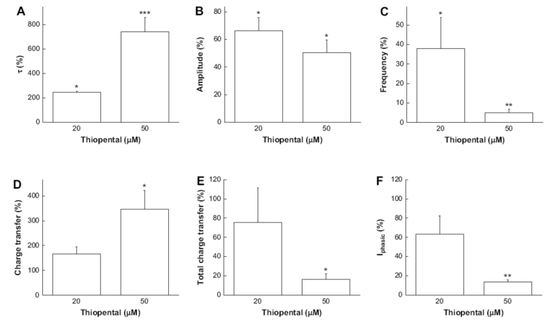

Fig. 4: Quantitative analysis of thiopental's effects on sIPSCs measured in the presence of CNQX (50 µM), APV (50 µM) and strychnine (1 µM). For each concentration the mean value and standard error were obtained from 6-8 cells and are expressed in percent relative to control conditions. Star symbols 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. (F) Iphasic, the average phasic current entering the neurons.

Induction of tonic GABAergic currents by thiopental

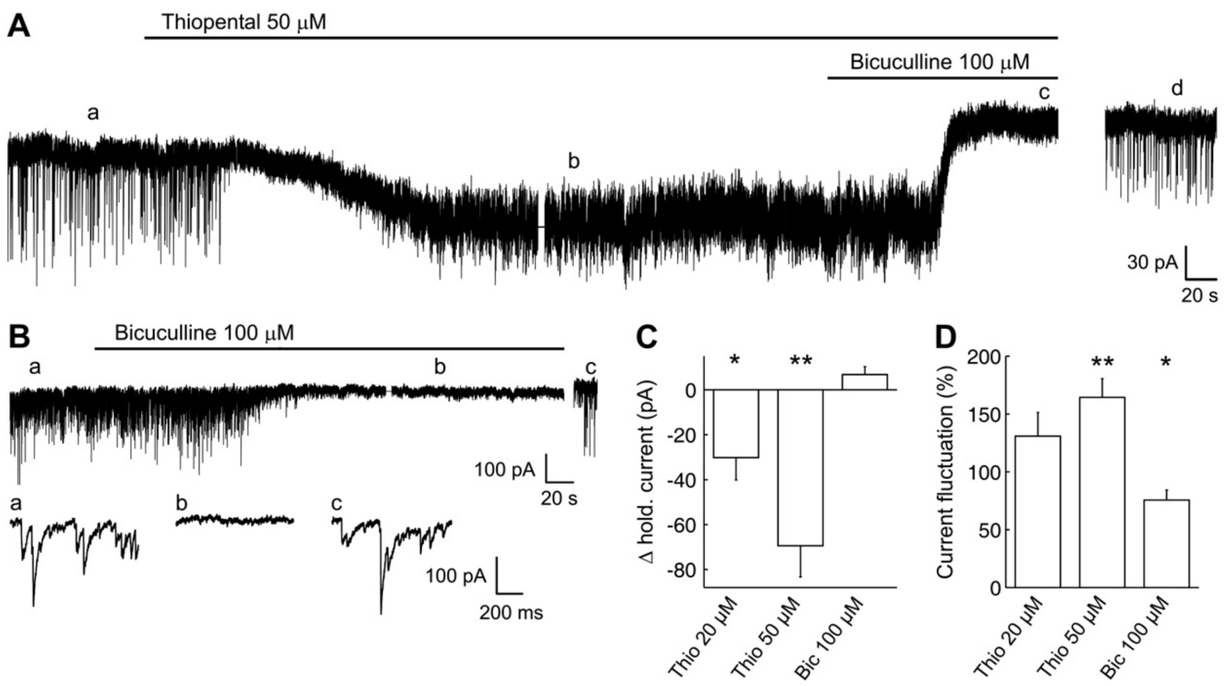

Fig. 5: Membrane currents in spinal interneurons and their modulation by thiopental and bicuculline. CNQX (50 µM), APV (50 µM) and strychnine (1 µM) were in the bath at all times. (A) Time course of currents in an interneuron exposed to 50 µM thiopental 90 s after recording started. Bicuculline (100 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).