Different actions of volatile and intravenous anesthetics on interneurons in organotypic spinal cord slices

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Abstract

Background: Immobility is an important aspect of anesthesia. It is now well accepted that ablation of spontaneous and stimulus-induced movements by general anesthetics is spinally mediated. Comparing the effects of sevoflurane and propofol on spontaneous action potential firing, we have recently shown that the capacity of propofol, but not sevoflurane, in depressing spinal neurons is limited.[1] This finding is explained by the observation that the effects of propofol were almost exclusively mediated by $GABA_A$ receptors, whereas sevoflurane acted predominantly via glycine and $GABA_A$ receptors. Two questions are addressed in this study: (1) Do isoflurane and enflurane have a greater capacity in depressing spontaneous action potential firing than diazepam and etomidate? (2) Are the depressant effects of diazepam and etomidate restricted to $GABA_A$ receptors and thus differ from the effects of isoflurane and enflurane?

Methods: Organotypic spinal cord tissue slices were achieved from pregnant Sprague-Dawley rats (day 13-15) according to the method described by Braschler,[2] and used for experiments after 12 days in vitro. The effects of isoflurane, enflurane, diazepam, and etomidate on spontaneous action potential firing were investigated by extracellular voltage recordings from ventral horn interneurons. All procedures were approved by the animal care committee and were in accordance with the German law on animal experimentation.

Results: Isoflurane, enflurane, diazepam, and etomidate reduced spontaneous action potential firing of neurons. Concentrations causing half-maximal effects (isoflurane: 0.17 mM; enflurane: 0.50 mM; diazepam: 1.41 µM; etomidate: 0.21 µM) were smaller than the EC_{50}-immobility (isoflurane: 0.32 mM; enflurane: 0.62 mM; etomidate:1.5 µM). At higher concentrations, complete inhibition of action potential activity was observed with the volatile anesthetics isoflurane and enflurane but not with the intravenous anesthetics diazepam and etomidate. Effects of isoflurane were mediated predominantly by glycine receptors (39%) and GABAA receptors (36%), whereas the effects of enflurane were mediated to 26% by $GABA_A$ receptors and to 29% by glycine receptors. Diazepam and etomidate almost exclusively acted via $GABA_A$ receptors.

Conclusions: Our results suggest that glycine and $GABA_A$ receptors are the most important molecular targets mediating depressant effects of isoflurane, like it was reported previously for sevoflurane. For enflurane, $GABA_A$ and glycine receptors mediated approximately only half of its depressant capacity. The enflurane results are consistent with recent findings obtained from whole spinal cords in mice.[3] Furthermore, our results provide evidence that volatile anesthetics cause immobility by a mechanism distinct from the actions of the intravenous anesthetics diazepam and etomidate, which exclusively seems to act via $GABA_A$ receptors. Our results are consistent with the hypothesis that volatile anesthetics produce immobility via multiple molecular targets in the spinal cord, whereas effects of intravenous anesthetic are restricted to $GABA_A$ receptors.

References:

1. Grasshoff C, Antkowiak B. Anesthesiology 2004 (in press)
2. Braschler UF, Iannone A, Spenger C, Streit J, Luscher HR. J Neurosci Methods 1989; 29: 121-9
3. Wong SM, Cheng G, Homanics GE, Kendig JJ. Anesthesiology 2001; 95: 154-64

Methods

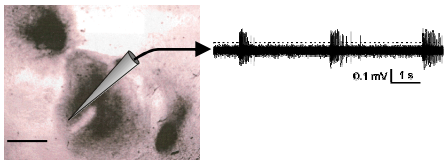

Fig. 1: Extracellular recording from an interneuron in a spinal cord-dorsal root ganglia coculture after two weeks in vitro. Action potentials appeared in bursts, separated by silent periods. The broken line indicates the threshold for event detection.

Results

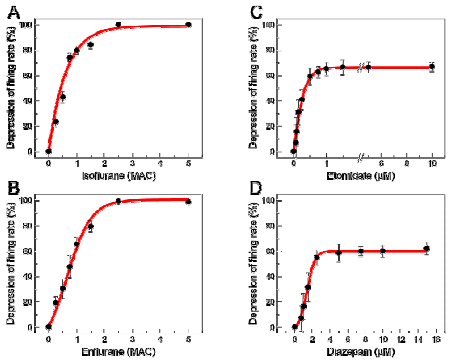

Fig. 2: Concentration-dependent effects of isoflurane (A), enflurane (B), etomidate (C), and diazepam (D) on spontaneous action potential firing. For each concentration, mean and standard error were obtained from 6-12 cells. Curves were fitted with Hill equations. Table 1 shows the median effective concentrations (EC_{50}) and upper limits.

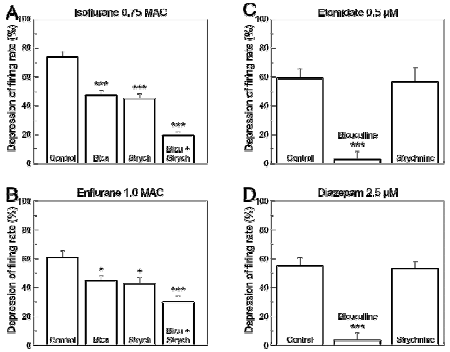

Fig. 3: Effects of 0.24 mM isoflurane (corresponding to 0.75 MAC), 0.62 mM enflurane (corresponding to 1 MAC), 0.5 µM etomidate and 2.5 µM diazepam on ongoing activity of spinal neurons in the presence of bicuculline (Bicu), strychnine (Strych), or bicuculline and strychnine (Bicu + Strych). Anesthetic concentrations were equieffective (60% depression) in the absence of Bicu and/or Strych. (A) Isoflurane depressed mean firing rates to almost the same amount either in the presence of 100 µM bicuculline (36%) or 1 µM strychnine (39%). A combination of both antagonists further reduced the mean firing rate (t-test, ***; p<0.001, n=9-10). (B) Similar results were obtained with enflurane (t-test, *; p<0.05, ***; p<0.001, n=9-10). (C) Depressant actions of etomidate were almost completely prevented in the presence of 100 µM bicuculline (t-test, ***; p<0.001, n=8) but remained unaffected by strychnine (t-test, n=8). (D) Similar to etomidate, reduction of ongoing activity by diazepam was completely prevented in the presence of 100 µM bicuculline (t-test, ***; p<0.001, n=10) and not affected by strychnine (t-test, n=10).

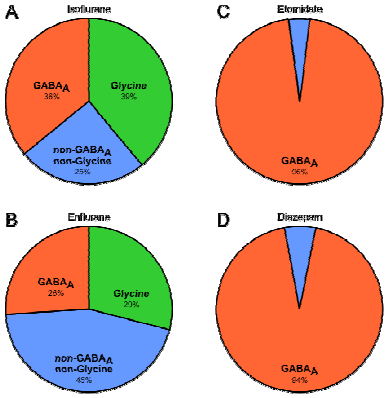

Fig. 4: Estimated contributions of molecular targets to the effects of isoflurane (0.75 MAC), enflurane (1 MAC), etomidate (0.5 µM), and diazepam (2.5 µM) on the mean firing rates. These concentrations were considered equipotent since they depressed spontaneous network activity by approximately 60%. Effects of the anesthetics in the absence of bicuculline and strychnine were taken as 100%.

	EC_{50}	R^2	Upper limit (%)
isoflurane	0.17±0.02 mM (0.52±0.05 MAC)	0.975	100
enflurane	0.50±0.05 mM (0.80±0.08 MAC)	0.983	100
etomidate	0.21±0.01 µM	0.993	66.84±1.13
diazepam	1.41±0.04 µM	0.996	60.68±0.82

Table 1: Half-maximal depression of average spike rates and Upper limits, as calculated from the concentration-response fits in figure 2.

Outlook

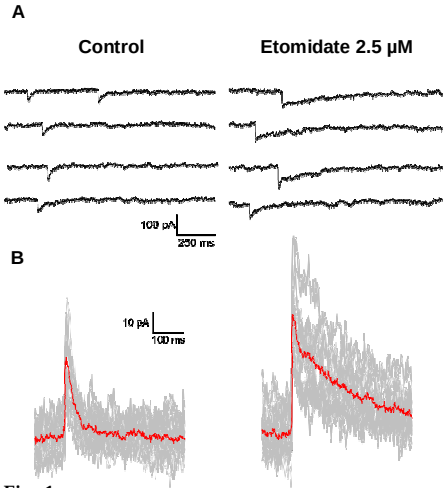

Fig. 1: The effect of 2.5 µM etomidate on action potential-independent $GABA_A$ inhibitory post synaptic currents (miniature IPSCs). CNQX 50 µM, AP5 50 µM, strychnine 1 µM, and TTX 1 µM were added to the bath medium. The cell was held at a membrane potential of -70 mV. Patch pipettes had open-tip resistances of 1.5-3 MΩ when filled with the recording solution (in mM) 145 CsCl, 1 $MgCl_2$, 5 EGTA, 10 HEPES, and 2 MgATP. (A) Original recordings. (B) Overlay of the miniature IPSCs shown in (A).

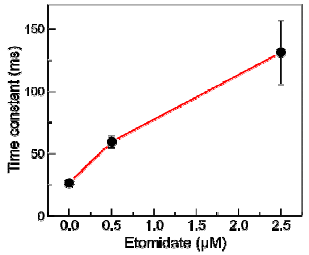

Fig. 2: Time constants (τ) of current decays in the presence of different concentrations of etomidate. Time constants were obtained from monoexponential fits.

Conclusion

Our findings imply that anesthetics acting almost exclusively via $GABA_A$ receptors have a limited capacity in depressing action potential firing of spinal neurons. However, this limited capacity is not explained by corresponding effects on IPSC kinetics.