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RESPONSE OF HUMAN CNS SODIUM CHANNELS TO MIDAZOLAM

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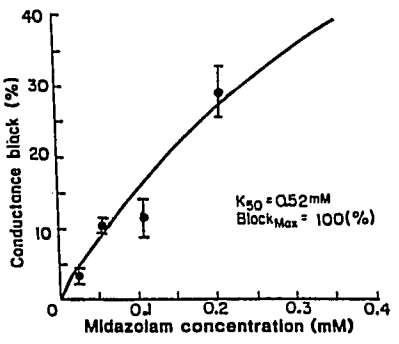
Introduction: Human brain sodium channels, important integral membrane proteins with key functions in neuronal signal integration and transduction, were used to test whether CNS ion channels are selective in their response to anesthetics. Recent studies demonstrated specific effects of the hypnotics propofol[1] and pentobarbital[2] on single human brain sodium channels at clinically significant concentrations. By contrast, the dissociative anesthetic ketamine did not affect this membrane protein at clinically relevant doses[3]. The main site of action for the benzodiazepine midazolam is thought to be the GABA-/benzodiazepine-receptor complex with which it interacts at nanomolar concentrations. Midazolam should not have a major impact on sodium channels in this dose range. To rule out unspecific drug-channel interactions it was of interest therefore, to quantify midazolam effects on sodium channels - demonstrating the ability of the human sodium channel to discriminate between different anesthetic substances on the molecular level.

Methods: With approval of the CUMC Committee on Human Rights in Research sodium channels from synaptosomal fractions, originating from human brain cortex samples, were incorporated into planar lipid bilayers. Under our standard steady-state voltage-clamp conditions, currents from single sodium channels were recorded[2] under control conditions and in presence of the same control channel after the addition of midazolam (concentrations between 0.025 and 0.2 mM) to either the intra- or extracellular electrolyte.

Results: A total of 21 membranes containing 49 channels (range 1-6 channels/membrane) were studied on average for 100 minutes/membrane (± 12 minutes; SEM). Midazolam was active in 36% of this time and caused a dose-depedent increase of channel closing events. This led to a membrane potential independent decrease of the time-averaged channel conductance. The resulting dose-response curve could be approximated by a rectangular hyperbola and a weighted computer fit yielded a maximal average conductance block of 100% and a K_{50} of 0.52 mM (Fig. 1). Midazolam introduced to either side of the channel gave similar results (intrac.: K_{50} 0.53 mM; exc.: K_{50} 0.50 mM). No midazolam mediated effect on the maximal single channel amplitude was apparent. Furthermore midazolam destabilized normal sodium channel steady-state activation behaviour, producing a significant increase of the variability of successive activation curves with a larger range of midpoint potential values.



Discussion: Midazolam affects at least two major sodium channel functions: a membran potential independent reduction of the fractional channel open-time (flickering, long complete closures) and an interaction with the voltage-dependent channel steady-state activation process. However, these effects occur at concentrations far beyond those accleved under anesthesia or long term sedation (0.01 mM at most[4]). In contrast to pentobarbital and propofol the human CNS sodium channel, demonstrating a selective "non-response" to midazolam, does not provide a primary molecular target site for midazolam. Future work will show the extent to which different types of clinical anesthesia can be correlated with differential anesthetic actions on the molecular level.

References: 1. Anesthesiology 71: A590, 1989 2. Anesthesiology 72: 640-649, 1990 3. Anesth Analg 68: S61, 1989 4. Drug Int Clin Pharm 20: 805-806, 1986

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TITLE: DIFFERENTIAL EFFECTS OF PENTOBARBITAL AND HALOTHANE ON BRAIN C-FOS AND JUN-B MESSENGER RNA

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Immediate-early genes (IEG) have been implicated in neuronal signal transduction, memory, and analgesia.[1] Since these neurochemical functions are relevant to the anesthetic state, we investigated the effects of pentobarbital and halothane anesthesia on stimulation-induced expression in the brain of the IEGs c-fos and jun-B.

Institutionally-approved studies were performed in male Sprague-Dawley rats divided into control, pentobarbital, or halothane-treated groups of 10 animals each. All animals received an intraperitoneal (ip) injection prior to drug administration as a means of stimulating c-fos and jun-B expression. Control and halothane-treated animals received vehicle ip only; the pentobarbital group received ip vehicle plus pentobarbital, 65 mg/kg. Animals were then placed in a plexiglass box continuously flushed with 100% O_2; in the halothane group, the O_2 was supplemented with 1.5% halothane. Five animals from each group were sacrificed at 30 and 120 min post-injection and their brains were rapidly removed and frozen. RNA was extracted from whole cerebral hemispheres and the mRNA transcription products of the genes c-fos, jun-B, and beta-actin were quantified by Northern blot hybridization with ^{32}P-labeled DNA probes. c-fos and jun-B mRNA levels were determined from optical density measurements of the autoradiographic bands using measurement of beta-actin to correct for sample to sample variation in the total amount of mRNA analyzed.

Drug administration produced anesthesia within minutes. Neither anesthetic agent modified a stimulus-induced 2-3 fold increase in brain c-fos and jun-B mRNA at 30 as compared to 120 min ($P < 0.01$ for all comparison, unpaired t-test). At 120 min post-injection, however, differential anesthetic effects were apparent. Jun-B mRNA levels were 25% higher than control in pentobarbital-treated animals and 38% lower in rats that received halothane ($P < 0.05$ and $P < 0.01$, respectively, Dunnett's test). Neither anesthetic affected c-fos. These data indicate that general anesthetics selectively and differentially alter the rate at which certain genes are transcribed in the CNS and, hence, imply that general anesthetics change neurons in a fundamental way.

	CONTROL	PENTOBARBITAL	HALOTHANE
c-fos			
30 min	1.10 ± 0.08	1.14 ± 0.20	1.25 ± 0.05
120 min	0.51 ± 0.05	0.39 ± 0.05	0.50 ± 0.04
jun-B			
30 min	2.24 ± 0.19	2.44 ± 0.31	2.14 ± 0.21
120 min	0.75 ± 0.08	0.94 ± 0.04*	0.46 ± 0.03†

Mean ± SEM of the optical density ratio of c-fos or jun-B to beta-actin for 5 animals/group at each time. * $P < 0.05$; †$P < 0.01$

1. Ann Rev Neurosci 14: 421-451, 1991.
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