TITLE : BARBITURATE ACTIONS ON SINGLE HUMAN BRAIN SODIUM CHANNELS

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Introduction: Sodium channels are essential for the generation of fast propagated action potentials, playing a vital role in neuronal signal integration and cell communication. Sodium channels are important as the main site of local anesthetic action, are affected in general anesthesia and are the target for a group of anticonvulsant agents. Our present knowledge of the effects of anesthetics on ion channels is exclusively derived from animal experiments, concentrating on peripheral nerve rather than brain preparations. Existing studies show that sodium channels differ not only between animal species but also between tissues in the same animal; e.g. between the central and peripheral nervous system. Information on anesthetic actions on human brain sodium and other ion channels is lacking, owing to limited tissue availability and the rapid loss of protein function in conventional experimental preparations. In this study, we demonstrate that new planar lipid bilayer technology can overcome these limitations, and provide evidence that human brain sodium channels are affected at clinically relevant doses of pentobarbital.

Methods: With approval of our Committee on Human Rights in Research, human cortical tissue samples were obtained from four patients undergoing craniotomies and immediately frozen; following established techniques, synaptosomal vesicles were prepared and fused with planar lipid bilayers in the presence of batrachotoxin[1]. Single sodium channel currents were recorded under steady-state voltage-clamp conditions. The control behavior of single sodium channels (current-voltage curves, fractional open time, steady-state voltage activation) was established for 30 to 40 minutes. Between 170 μM and 1010 μM of either (R)-(+)- or (L)-(-)-isomer of pentobarbital was subsequently added to either the extracellular or intracellular electrolyte in the presence of the same channel. The electrophysiological measurements were repeated and continued until the membrane broke spontaneously, typically after several hours.

Results: At depolarized potentials, sodium channels remained mostly open under control conditions (fractional open time 0.95, ± 0.03, S.D., single channel conductance 26.1 pS, ± 0.6 pS, S.D.). Following membrane hyperpolarization, channels closed with an average midpoint potential of -87 mV, reproducible within ± 10 mV (S.D.). These properties are within the range expected for batrachotoxin-modified sodium channels from other species and suggest a viable preparation.

1. Less than a minute after pentobarbital addition, the channel began to open and close very rapidly, (at a rate greater than 50 Hz) at all administered doses. This led to the following membrane potential independent decrease in the fractional open time: 15.8% (± 2.08%, S.E.M.) at 170 μM, 34.9% (± 4.1%) at 340 μM, 45.8% (± 5.3%) at 510 μM, 51.4% (±5.2%) at 670 μM and 54.9% at 1010 μM pentobarbital. The dose-response curve followed a rectangular hyperbola. A computer fit yielded a maximal average conductance suppression of 90% and a K_{50} of 560 μM.

2. The actions of the (+) and (-) isomers on the sodium channel were similar, and the reduction of the time averaged conductance at all doses showed no statistically significant difference.

3. Maximal single channel current amplitudes appeared not to be affected by pentobarbital.

4. Barbiturate introduced at either side of the channel gave similar results.

5. Pentobarbital destabilized normal steady-state activation behavior, producing a shallower slope of the activation curve with larger doses. The mid-point potentials ranged much more widely in the presence of barbiturate and sometimes channels did not close at all with hyperpolarization. Both isomers affected the gating modification in a similar way.

Conclusion: This is the first demonstration of the molecular action of a general anesthetic on a single human ion channel. Similar rapid openings and closings have been previously reported for canine brain sodium channels exposed to racemic mixtures of pentobarbital.[2] Effects on steady-state activation have not been previously characterized. The concentrations used are within the range of concentrations encountered during clinical anesthesia[3], demonstrating a major impact on two primary functions of the sodium channel: a voltage-independent reduction of the fractional channel open-time and an interaction with the voltage-dependent steady-state activation behavior of the sodium channel. As the actions described here do not demonstrate any stereospecificity, it is clear that other ion channels must also be involved in the clinical actions of barbiturates. However, the sodium channel would contribute to overall anesthetic depression, supporting the hypothesis that anesthesia results from the superposition and integration of several anesthetic actions on the molecular level[4]. The work presented here demonstrates the lipid bilayer preparation to be a most promising tool for the investigation of anesthetic and other pharmacological properties of human brain ion channels and receptors.

References:
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