POSTER DISCUSSION VI

A1035

TITLE: AMOBARBITAL AND PROCAINE HAVE DIFFERENT EFFECTS ON THE NICOTINIC RECEPTOR ION CHANNEL

AUTHORS: C.S. Yost, M.D., B.A. Dodson, M.D.
AFFILIATION: Dept. of Anesthesia, Univ. Calif, San Francisco, San Francisco, CA 94143-0648

Barbiturates and local anesthetics are both allosteric inhibitors of nicotinic acetylcholine receptor (nAChR) function.[1,2] However, it is unclear whether these drugs have the same or different sites of action. The present study investigates the ability of amobarbital and procaine to inhibit nAChR channel opening as a probe for examining possible differences in their mechanisms of action.

The effect of amobarbital and procaine on ligand-gated channel conductance was examined using mouse muscle-type nAChR expressed in *Xenopus* oocytes. Briefly, mRNA, transcribed *in vitro* [3] from plasmids encoding the appropriate α, β, γ and δ subunits, was injected into oocytes dissected from *Xenopus* ovaries. Expression required approximately 24-48 hrs, after which the functional channels were assayed using the specific nicotinic agonist dimethylphenylpiperizinium iodine (DMPP) (50 μM, which both produced an adequate signal and minimized desensitization). The ability of amobarbital and procaine (10^{-6} M to 10^{-3} M) to inhibit channel opening was determined by whole cell, single electrode, discontinuous voltage clamp at holding potentials of -45 mV and -90 mV. All determinations were in at least triplicate. Maximum response (i.e., current), corrected for desensitization, was determined for each measurement with results expressed as % inhibition of maximal response.

Both amobarbital and procaine inhibited nAChR channel conductance in a concentration-dependent fashion with K_d values for half maximal inhibition of approximately 27 μM and 70 μM, respectively, at a transmembrane potential of -90 mV. However, the two agents differed considerably in the voltage-dependence of their inhibition. Increasing the transmembrane potential from -90 mV to -45 mV produced a 20% decrease in procaine-dependent inhibition of channel conductance (see below). These results are consistent with the previously described voltage-dependent, channel blocking properties of the local anesthetics.[4] In contrast, changes in oocyte transmembrane potential produced no alterations in amobarbital-induced inhibition, suggesting a lack of voltage-dependence in the barbiturate site.

In conclusion, the present study supports a site-specific mechanism of action for both the barbiturates and the local anesthetics. However, the findings also support the hypothesis that barbiturates exert their effect through a barbiturate binding site distinct from the voltage-dependent local anesthetic site.

(Supported by Univ. Ca. TRDRP Grant 1RT-352)

References

1. Molec Pharmacol 32:119-126, 1987.
2. J Biol Chem 257:8106-8114, 1982.
3. Nuc Acids Res 12:7035-7056, 1984.
4. Molec Pharmacol 15:559-580, 1979.

EFFECT OF TRANSMEMBRANE POTENTIAL ON % INHIBITION OF CHANNEL CONDUCTANCE

TRANSMEMBRANE POTENTIAL	AMOBARBITAL		PROCAINE	
mV	50 μM	100 μM	50 μM	100 μM
-45	62 ± 3.3 %	69 ± 2.8 %	48 ± 8.5 %	57 ± 11.2 %
-90	62 ± 3.2 %	69 ± 4.6 %	70 ± 5.2 %	84 ± 5.1 %

A1036

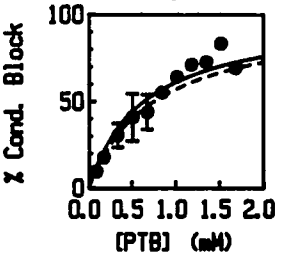
TITLE: DIFFERENTIAL EFFECTS OF PENTOBARBITAL ON SODIUM CHANNELS DERIVED FROM NERVE AND MUSCLE

AUTHORS: H.C. Wartenberg*, M.D., J. Wang*, M.D., B.W. Urban#, Ph.D.,D.S. Duch*, Ph.D. AFFILIATION:*Depts. Anes. and Phys., Cornell Univ. Med. Coll., N.Y. and #Inst. f. Anäst., Univ. Bonn, F.R. of Germany

INTRODUCTION: Previous studies with human brain sodium channels[1] have indicated that, at clinically relevant concentrations, barbiturates interfere with at least two channel functions: they reduce the fractional channel open-time as well as destabilize membrane potential-dependent channel activation. To further investigate the molecular mechanisms and sites of barbiturate action, we examined the effects of pentobarbital on purified sodium channels from a muscle-derived tissue, the electric organ of the electric eel. These channels are structurally and functionally different from brain channels, with similar conductance properties, but differing in their activation behaviors[2]. These channels thus provide a useful experimental system for examining differential anesthetic sensitivities on ion channels.

METHODS: Eel electroplax sodium channels were purified, reconstituted and fused with planar lipid bilayers in the presence of batrachotoxin, as described[2]. Sodium channel currents were recorded under steady-state voltage clamp conditions[1]; the time-averaged channel conductance and voltage dependent activation properties were examined for the same channels before (control) and after the addition of the pentobarbital isomers (PTB) at various concentrations. Activation gating results were fitted with a two-level Boltzmann distribution by a least squares fitting procedure[1]. Control and experimental results were compared using the paired Student's t-test.

RESULTS: After adding PTB, channels opened and closed rapidly, spending progressivly more time in the closed state with increasing PTB concentration (Fig. 1, solid line). The dose-response relationship was indistinguishable from that of human brain channels (dashed line). Although steady-state activation became progressively hyperpolarized, shifting by -1.9 mV ± 6.9 (sd; 0.34 mM PTB, $p<0.5$) and -8.8 mV ± 5.3 (sd; 0.67 mM PTB, $p<0.1$). These shifts were not significant. In contrast, human channel gating in the presence of PTB was shifted in the negative region beyond recording capabilities. The effective gating charge significantly decreased at both concentrations (0.34 mM: $p<0.005$; 0.67 mM: $p<0.005$).



CONCLUSIONS: The similar PTB dose-response curves for the fractional open-time of human and electroplax sodium channels were consistent with the reported similarities in the single channel conductances. However, the shift and slope changes in the muscle-derived channel steady-state activation curves were much less sensitive to anesthetic interactions. These results indicate at least two differential effects of PTB on sodium channel function, related to differences in sodium channel structure. Either PTB interacts with the channel at two or more sites (e.g. lipids, surface charges, protein binding), or a single interaction can affect channel function differentially, dependent on protein structure.

REFERENCES: 1) Frenkel et al., Anes., 72:640, 1990; 2) Recio-Pinto et al., J. Gen. Phys., 94:813, 1987.