COMPARISON OF PHARMACOLOGICAL BLOCKADE CHARACTERISTICS OF INVERTEBRATE ACh RECEPTORS EXPRESSED IN XENOPUS LAEVIS OOCYTES.

I.G. Welsford, Gretchen Kuchenmeister and Kara Hoffman
Dept. of Biology, Bradley University, Peoria, IL 61625

Transmembrane, ligand-gated neurotransmitter receptors are requisite for the reception of extracellular neurotransmitter/neurohormone signals and for the transduction of these signals into alterations in membrane potential and/or second messenger systems. As such, neurotransmitter receptors may be viewed as information processing units within the central nervous system (CNS) of animals. Recent advances in molecular neurobiology have begun to elucidate the structure/function relationships of these molecules through cloning and site-directed mutagenesis studies. However, the bulk of this research to-date has been devoted to analysis of neurotransmitter receptors from vertebrates, specifically mammalian groups. Surprisingly little is known about the structure/function relationships of neurotransmitter receptors from animals from divergent phyla and particularly from marine invertebrates. The lack of such comparative data potentially ignores a broad array of unique structural and physiological adaptations in neurotransmitter receptors in particular and ligand-gated receptors in general. Work ongoing in our laboratory is focused on analyses of structure/function relationships of neurotransmitter receptors, specifically acetylcholine receptors (AChRs), from marine invertebrates from divergent phyla. We report here results on differences in neuronal bungarotoxin (nBGT) sensitivity and forskolin sensitivity between AChRs from the coelenterate Cyanea capillata, the annelid Nereis virens, and the echinoderm Asterias forbesi.

Comparisons between AChRs were conducted using Xenopus laevis oocyte. expression of total RNA extracted from the CNS of each species using the Urea-LiCl method of Auffray and Rougeon (1973. Eur. Jl. Biochem. 107: 303-314). Purity and degradation of extracted RNA was tested by standard methods including examining the absorbance (OD_{280} and OD_{260}) of the extract with a spectrophotometer and by running samples of the extract on agarose-formaldehyde gels. Oocytes were obtained from HCG-treated oocyte-positive female Xenopus laevis (Nasco Inc.), treated with 2% collagenase (Type IA, Sigma Chem. Corp.) for 2 h and manually defolliculated to remove any adherent follicular cells. Oocytes were injected with 200 ng of total RNA suspended in sterile water (50 nL total injected volume) using a positive displacement pippeter (Drummond Inc.). Oocytes were incubated in sterile saline (96 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6, 293 $\pm$ 5 mOsm/kg H_2O), supplemented with 1 μL/mL of gentamycin and 2.5 mM Na-pyruvate, for 72-96 hours prior to recording. Properties of expressed receptors were studied under two-electrode voltage clamp (Dagan Model 8500) using standard techniques. Electrode tip resistances (pulled using a WPI model Pull-I electrode puller) ranged from 0.5-2.0 MΩ. Whole cell ACh currents (Fig. 1) were recorded while superfusing oocytes with 1 μM Acetylcholine chloride, 0.3

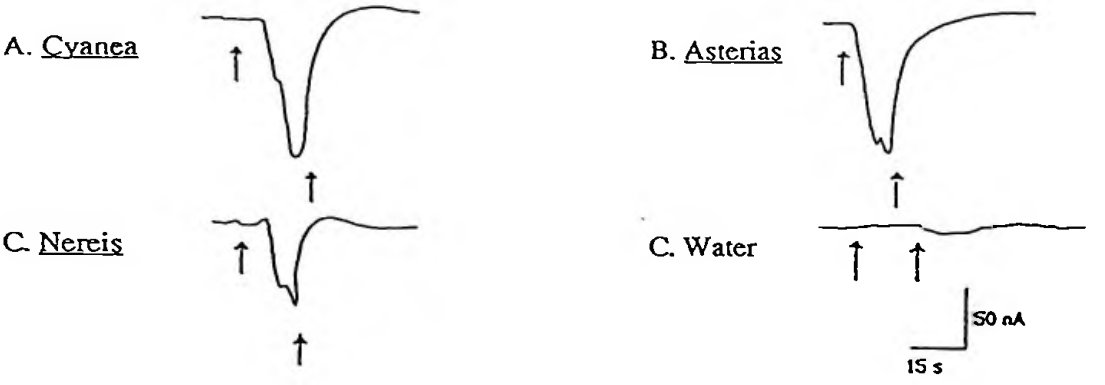

Figure 1. Representative inward ACh-induced currents recorded from oocytes injected with RNA from each of the indicated species (A-C) and control oocytes injected with vehicle only (D). First arrow denotes the beginning of bath application of 1 μM ACh and the second arrow indicates the onset of saline wash.

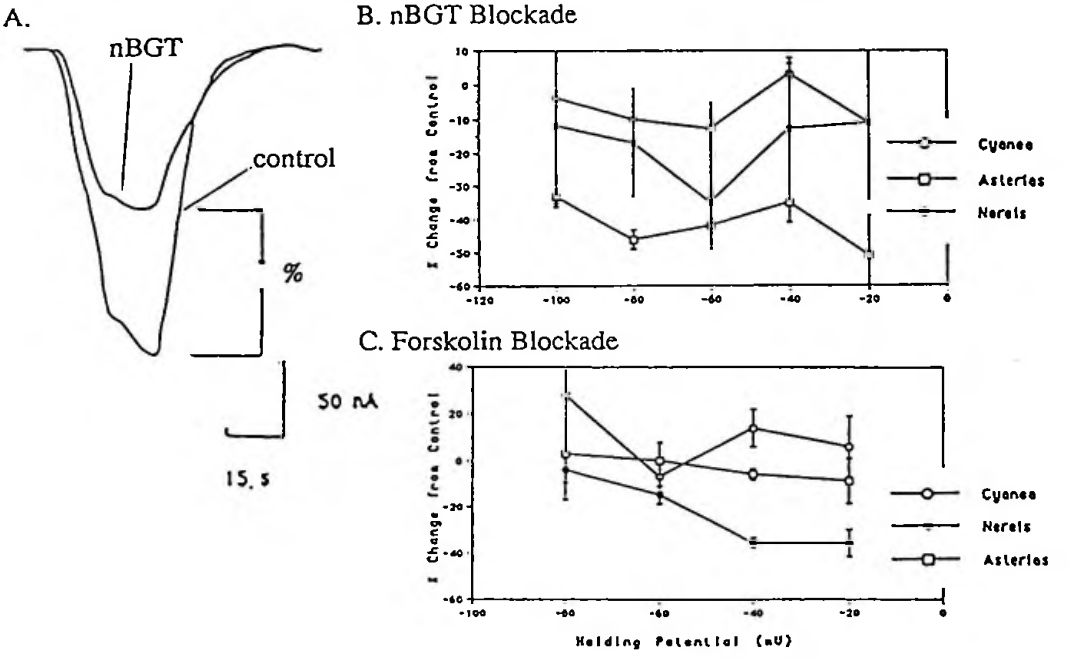

Fig. 2. Figure 2A shows how the pharmacological data were normalized in the present experiments. In the figure a sample inward current trace from Asterias is shown in the absence ("control") and presence of nBGT ("+nBGT"). The percent change from control current in the presence of the blocker was then calculated ("%"). Fig. 2B shows the response of each species to 1 μM nBGT at varying holding potentials. Note the variation in sensitivity between each species and the voltage-sensitivity of Nereis blockade. Each point represents the mean (+ SD) response of trials conducted in 5 separate oocytes. Fig. 2C shows the response of each species to 10 μM forskolin at varying holding potentials. Note that only Nereis demonstrated significant blockade which was voltage-dependent.

μM atropine solution (atropine was added to block ACh conductance due to presence of any remaining adherent follicular cells). Forskolin and nBGT, were applied in the superfusate at a concentration of 1 μM. Trials were separated by 2 min. The effects of blockers on the AChRs of varying species were normalized for comparison by calculating the percentage change (from control) of ACh currents obtained in the presence of each drug (see Fig. 2A) at holding potentials ranging from -100 to -20 mV. Mean responses of at least 5 trials from five separate oocytes were obtained at each holding potential and for each species. Data were square root arcsine transformed and analyzed using a mixed-model ANOVA followed by post-hoc Fisher LSD comparisons (Zar, J.H. 1984. Biostatistical Analysis, Prentice-Hall, NJ).

Figure 1 shows representative whole-cell ACh-induced currents from oocytes injected with total RNA from each species and with vehicle only. Note the long duration of the current and the slow desensitization kinetics characteristic of these receptors expressed in oocytes (Welsford, I.G. 1991. Soc. Neurosci. Abstr. 17:583; Welsford, I.G. submitted). Figure 2A shows a representative normalized measurement. Figure 2B shows the response of the AChRs of varying species to application of nBGT. As can be seen, Cyanea AChRs appear relatively insensitive to nBGT (maximal mean response 11.5 % decrease over controls) at all holding potentials. In contrast, Asterias showed a significant decrease in ACh-induced current in the presence of nBGT (ranging from 35% to 53% decrease from control levels) at all holding potentials. The response of Nereis to nBGT was voltage-dependent, with significant blockade seen at only -60 mV holding potential. There was a similar variability in response of varying species to forskolin application. (Figure 2C). Cyanea and Asterias showed no significant blockade at any holding potential. In contrast, as was the case with nBGT, the response of Nereis AChRs to forskolin was voltage-dependent, with significant blockade seen at only -40 and -20 mV holding potential (Fig. 2C).

These data suggest structural heterogeneity between the AChRs of each species. Specifically, since nBGT is thought to bind at or near the ACh binding site in mammalian AChRs (see for a review, Role, L.W. 1992. Current Opinion Neurobiol. 2: 254-262), differences in toxin efficacy may equate with sequence differences near this region of the AChR. Similarly, differences in sensitivity to forskolin in mammals are thought to correlate to sequence differences at a site distinct from the toxin and ACh binding site of the AChR (e.g. Aylwin, M.L. and M.M. White. 1992. Mol. Pharm. 41: 908-913.). Single channel recording and cloning experiments are underway to determine the number of potential AChR subtypes present in each species.

Supported by a PEW Fellowship to IGW and GMK, Whitehall Foundation Grant awarded to IGW, and a CURSOR Fellowship awarded to KBH.