# SUBCONDUCTANCE CURRENT TYPES AT THE NICOTINIC ACETYLCHOLINE RECEPTOR ARE DEPENDENT ON AGONIST STRUCTURE

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At the nicotinic acetylcholine receptor (nAChR) three activated states have been observed; two full conductance ones distinguished by open channel duration, and a subconductance state that has been largely overlooked in recent years. Previously we reported that the incidence of subconductance currents was inversely proportional to agonist concentration. However, with bischoline esters of maximum interonium distance (N-N) greater than 13Å, subconductance currents were fewer than full conductance currents at all concentrations studied (Dryden *et al*., 2002a,b). We speculated that bis-quaternary molecules bound to a pair of binding sites separated by this distance, and that partial occupation of the pair by a monoquaternary ligand such as ACh might account for the subconductance currents. Bis-quaternary ligands on the other hand are anticipated to bind at each end in close succession, and therefore the 20 to 30% of total current events that were subconductances seemed incompatible with our speculation. To determine if bisonium-evoked subconductances were qualitatively different we have categorized the subconductance currents evoked at different concentrations of ACh, oxalyldicholine (OxCh, N-N, 10.64Å) and adipyldicholine (AdpCh, N-N, 15.74Å).

Native nAchR enriched membrane fragments were obtained from *Torpedo californica* electroplax and incorporated into giant liposomes (Riquelme *et al*. 1990). Single channel recordings were made from inside-out patch configurations using an Axopatch 200B amplifier, and Strathclyde WinEDR software. Ligands were applied to the receptors from the lumen of the recording electrodes. Records were collected from at least 3 patches for each drug concentration and pooled provided that the proportions of activated states conformed to the population norms. Fully activated channels manifested a mean point conductance of 30pS, while the smaller currents indicated a mean conductance of 19.4pS. We identified four types of subconductance. Type 1: a single event in isolation from any other, Types 2 & 3: subconductance currents that rose or fell to or from a full conductance value with a clear separation of the current levels, and type 4: a subconductance event interposed between two full conductance currents. At 1μM ACh, type 1 subconductances accounted for 90.7% of all subconductances, with the other three types sharing equally in the remainder. At 10 and 30μM ACh, type 1 subconductances fell to about 80% of the total, while type 4 rose to contribute about 14% of subconductances. Results from OxCh revealed a similar distribution of subconductance types. With AdpCh, the distributions were reversed with type 4 subconductances forming around 80% of all subconductances at all concentrations, while type 1 contributed 25.2% at 300 nM falling to 5.9% at 10μM. Types 2 and 3 were at the 2% level. We propose that subconductance currents arise when only one of a pair of binding sites is occupied, and with bis-onium agonists (N-N>13Å), the virtual simultaneous occupation of both sites ensures full opening of the channel with the interposed subconductances arising when one end of the bisfunctional ligand temporarily dissociates from binding.

Dryden WF *et al*. (2002a) *Br.J. Pharmacol*., **137**, 98P.
Dryden WF *et al*. (2002b) *Soc. Neurosci Abstr*., **28**, 538.5.
Riquelme *et al*. (1990) *Biochemistry*, **29**, 11215-11222.