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An evaluation of neuronal nicotinic acetylcholine receptor activation by quaternary nitrogen compounds indicates that choline is selective for the $\alpha 7$ subtype

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Abstract

The agonist properties of acetylcholine (ACh), tetramethylammonium, ethyl-trimethylammonium and choline were evaluated for muscle and neuronal nicotinic receptors in *Xenopus* oocytes. The only essential feature for a neuronal receptor agonist appears to be the charged nitrogen. For specific receptor subtypes, other structural elements appear permissive (neither increasing nor decreasing activity) or non-permissive (decreasing activity). Choline was a full agonist for $\alpha 7$, but a hydroxyl group was strongly non-permissive for other receptor subtypes ($\alpha 1\beta 1\gamma\delta$, $\alpha 3\beta 4$, $\alpha 3\beta 2$, and $\alpha 4\beta 2$). The binding of these ligands to brain membranes is consistent with the electrophysiological results. Physiological concentrations of choline desensitize $\alpha 7$ receptors to ACh suggesting that, in vivo, choline may regulate both the activation and inactivation of this receptor.

Keywords: Nicotinic acetylcholine receptor; Choline; *Xenopus* oocyte; Alpha7 receptors; Desensitization

The concept of the nicotinic pharmacophore established for muscle-type receptors [2], holds that an effective nicotinic agonist requires both a charged nitrogen and a hydrogen bond acceptor group separated by 5.9 Å, features shared by many nicotinic agonists. The concept that at the neuromuscular junction synaptically released acetylcholine (ACh) is rapidly broken down into functionally inert components through a hydrolysis reaction, which splits the pharmacophore, has been supported by a comprehensive literature. Therefore, the notion that ligand-gated channel activators are rapidly eliminated from the synapse was amongst the early essential elements of our understanding of synaptic function.

Molecular cloning and electrophysiological studies have indicated that numerous nicotinic receptor subtypes exist [14] in the nervous system. While the extensive homology and virtual interchangeability of the neuronal and muscle nicotinic receptor subunits is consistent with an initial hypothesis that these different receptor subtypes share the essential pharmacophore [15], challenges to this hypothesis exist. Recently choline [12] and tetramethylammonium (TMA) [11] have been reported to be effective agonists at some neuronal receptor subtypes. Therefore, in the present study we sought to reexamine the concept of a nicotinic pharmacophore as it applies to neuronal receptors.

Cells were recorded in the two-electrode voltage-clamp configuration [6]. All agonist solutions were made in frog Ringers and pH adjusted to 7.3. Radioligand binding assays were performed using [^{125}I]α-bungarotoxin or [^{3}H]nicotine [11]. ETMA was purchased from TCI, other reagents from Sigma and radio-ligands from NEN.

As an initial step in describing the profile of the neuronal nicotinic ACh receptor (nAChR) pharmacophore, TMA was evaluated for its agonist activity for five putative nicotinic receptor subtypes, based on the expression of specific combinations of cloned mammalian receptor subunits, expressed in *Xenopus* oocytes. Receptor subtypes tested and their putative in vivo analogues were: $\alpha 1\beta 1\gamma\delta$, muscle-type receptors; $\alpha 3\beta 4$, ganglionic receptors; $\alpha 3\beta 2$, thalamic nicotinic receptors [3]; $\alpha 4\beta 2$, brain

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high affinity nicotine receptors; and $\alpha 7$, α-bungarotoxin (α-BTX)-sensitive brain nicotinic receptors.

TMA activity was compared to a standard ACh concentration of 1 mM since our previous studies indicated that 1 mM ACh gave maximal or near maximal responses for each of the receptor subunit combinations [6]. Though seemingly high, effects of 1 mM agonist concentrations are of physiological interest, since the local concentration of transmitter at the synapse reaches concentrations as high as 1 mM for transient intervals [10], analogous to the form of stimulation in the present experiments. Prolonged or cumulative desensitization that might be associated with these high agonist concentrations was not a concern in the present experiments, since each oocyte was exposed to only a single high concentration of either ACh or an experimental compound. To control for the variability between oocytes, responses to high agonist concentrations were normalized to the same oocyte's earlier response to the application of a low concentration of ACh.

While TMA (1 mM) stimulated significantly less current through the muscle-type receptor than did 1 mM ACh ($P < 0.05$), TMA was an effective activator of all neuronal receptor subtypes (Fig. 1). For $\alpha 3\beta 4$, $\alpha 4\beta 2$, and $\alpha 7$ receptors, the responses to TMA were not significantly different from those with 1 mM ACh.

When ethyl-trimethyl ammonium (ETMA) was tested, muscle-type receptors ($P < 0.01$), $\alpha 3\beta 2$ receptors ($P < 0.001$), and $\alpha 4\beta 2$ receptors ($P < .01$), all showed decreased responses to ETMA compared with either the smaller compound TMA or the endogenous activator ACh. However, for $\alpha 3\beta 4$ and $\alpha 7$ receptors, responses to 1 mM ETMA were not different from the responses to 1 mM ACh or TMA (Fig. 1).

Extending this structure-activity analysis, the activation profile for choline was examined. The additional hydroxyl group resulted in a decrease in activity at the 1 mM concentration compared to ETMA ($P < 0.01$, unpaired two-tailed *t*-tests) for all of the receptors tested. However, the responses of $\alpha 7$ receptors were less dramatically affected than those of the other receptor subtypes. When choline was tested at a concentration of 10 mM, slight increases from the 1 mM responses were observed for muscle-type and $\alpha 3\beta 4$ receptors. However, for $\alpha 7$ receptors, responses were obtained that were fully equivalent to 1 mM ACh responses (Fig. 1B), consistent with results reported by Mandelzys et al. [12]. In order to confirm that Ca-dependent chloride currents were not biasing our $\alpha 7$ choline responses we conducted some additional experiments in Barium-Ringers (1.8 mM $BaCl_2$ substituted for 1.8 mM $CaCl_2$). Under these conditions, 10 mM choline stimulated responses that were 96 ± 8% the amplitude of 1 mM ACh responses.

Based on these results, the concept of the nicotinic pharmacophore may be revised for the neuronal receptors, and limited to the basic nitrogen. The hydrogen bond acceptor function of the acetyl-group can be seen as a permissive factor for the activation of all neuronal-type receptors rather than a required feature. Both the additional methyl group of ETMA as well as the hydroxyl group of choline are permissive elements only for $\alpha 7$ receptors. For receptor subtypes other than $\alpha 7$, in addition to being strictly a neutral or permissive element, the acetyl-group also serves to suppress non-permissive aspects of ETMA and, most notably, choline.

The binding of nicotinic ligands to brain membranes readily distinguishes two main classes of receptors [4]. One type (primarily $\alpha 4\beta 2$ receptors [7]) binds [^{3}H]nicotine, [^{3}H]ACh or [^{3}H]cytisine with high affinity, while the

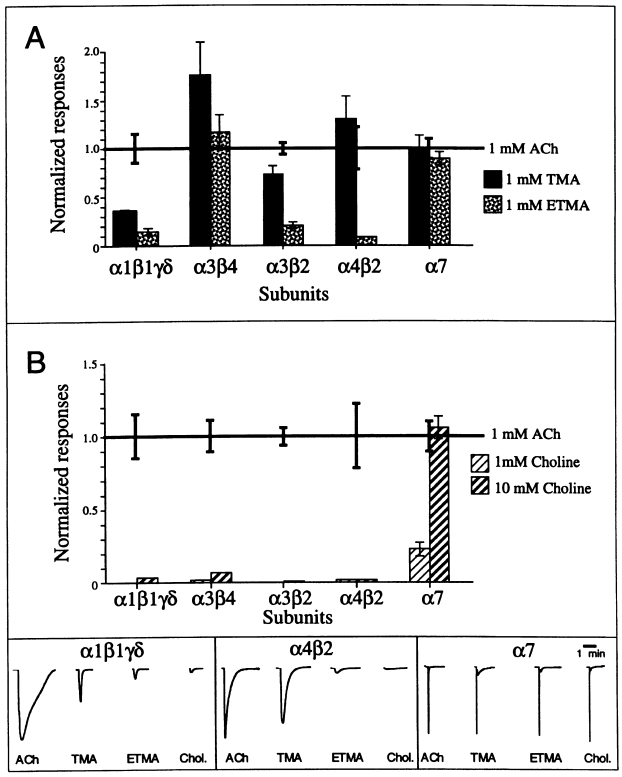

Fig. 1. The responses of defined nicotinic receptor subunit combinations to agonist analogs. The normalized responses of receptors formed in *Xenopus* oocytes from the expression of the indicated subunit cDNA combinations to: (A) 1 mM TMA and 1 mM ETMA; (B) 1 mM and 10 mM choline. The dark line indicates the relative responses of the various subunit combinations to 1 mM ACh, with the heavy error bars representing the SEM of the ACh responses. The normalization procedure was as follows. Each oocyte was first evaluated for its response to a standard low ACh concentration. The oocyte was then tested for its response to 1 mM ACh, TMA, or ETMA. Experimental responses were calculated relative to that cell's response to the ACh standard. These steps generated 'cell-normalized values'. The average cell-normalized values (± SEM, $n \geq 4$) were calculated for each experimental condition, and the values for the TMA and ETMA responses were scaled to the average 1 mM ACh cell-normalized values. The ACh standard concentrations used were 1 μM for $\alpha 1\beta 1\gamma\delta$, 10 μM for $\alpha 3\beta 2$, $\alpha 3\beta 4$ and $\alpha 4\beta 2$, and 300 μM for $\alpha 7$. Representative raw data traces for muscle-type ($\alpha 1\beta 1\gamma\delta$), $\alpha 4\beta 2$, and $\alpha 7$ receptors are illustrated in the insert. Shown are responses to 1 mM ACh, 1 mM TMA, 1 mM ETMA and 10 mM choline, all scaled to the respective ACh controls in the same oocytes.

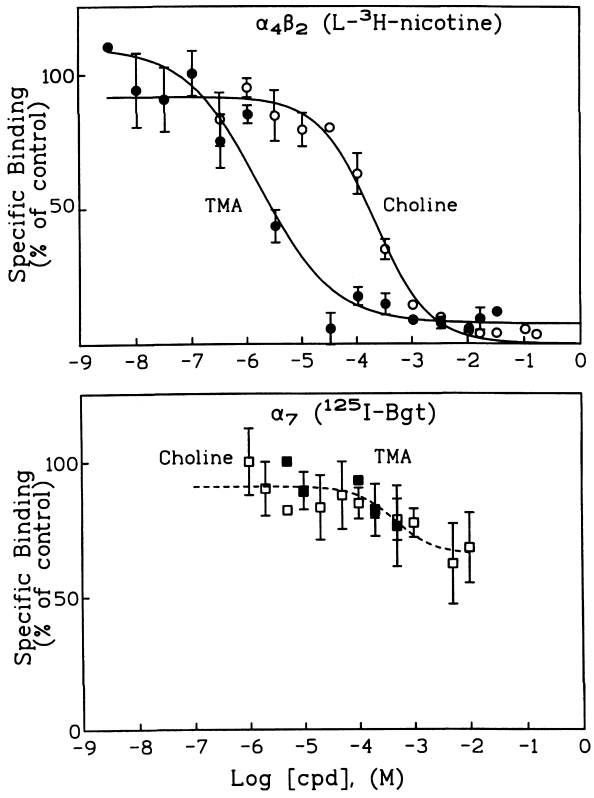

Fig. 2. Competition binding curves for TMA (solid symbols) and choline (open symbols) for displacement of 10 nM l-[^{3}H]nicotine (top panel) or 10 nM [^{125}I]α-BTX (bottom panel) in membranes prepared from rat brain. Symbols represent mean ± SEM (n>3).

other (associated with the α7 gene product [16]) binds α-BTX with high affinity.

Based on the electrophysiological profiles of TMA and choline for the activation of $\alpha 4\beta 2$ and α7 receptors, both TMA and choline might be hypothesized to displace α-BTX binding with relatively low potency, with the potency of choline differing from that of TMA by no more than a factor of 10, corresponding to their relative potencies for activating α7 receptors. On the other hand, it would be predicted that TMA would be far more potent at displacing nicotine binding than would be choline.

Both of these hypotheses were tested with membrane samples prepared from rat brains and found to be valid. TMA (k_i = 0.48 ± 0.2 μM) is 200-fold more potent than is choline (k_i = 112 ± 0.59 μM) for displacing [^{3}H]nicotine. Both choline and TMA displaced [^{125}I]α-BTX with similar low potency (Fig. 2).

Since chronic exposure to low agonist concentrations may also promote an increase in resting desensitization, the effects of low choline concentration on the responses to high concentrations of ACh were investigated. After obtaining control responses to 300 μM ACh, oocytes were incubated in 10 μM choline and tested for changes in their responses to ACh over a 10 min period (Fig. 3). Although no direct response to 10 μM choline could be recorded in this system, responses to 300 μM ACh were depressed by approximately 25–30% in the presence of 10 μM choline. Following the removal of choline, ACh responses returned to the full control levels. Experiments were also conducted under conditions where choline was not present in the agonist solution (data not shown). Under those conditions, we observed a 46 ± 6% inhibition of the 300 μM ACh response when cells were incubated in 10 μM choline for the 5 min prior to the application of ACh alone, and a 53 ± 3% inhibition if the cells were pre-exposed to 50 μM choline.

The relatively short time course of this experiment leaves open the question of whether the chronic exposure of α7 receptors to endogenous choline in vivo might lead to fundamental inhibition of α7-type receptors as mediators of rapid post-synaptic responses in the brain. However, it is tempting to speculate that the general failure to record α-BTX-sensitive post-synaptic nicotinic responses in mammalian central nervous system tissues may be due to a relatively high level of resting desensitization of α7 receptors by endogenous choline, and this effect may also relate to the study of cultured neurons [12], since tissue culture media typically contains 7–10 μM choline.

The results indicate that beta subunit-containing neuronal nicotinic receptors function in such a way that synaptic activity may be regulated by the metabolism of ACh by acetylcholinesterase. However, these results also indicate that the breakdown of ACh to choline in the brain may not effectively eliminate α7-mediated signals, and questions the very nature of the kinds of signals which α7 receptors may transduce.

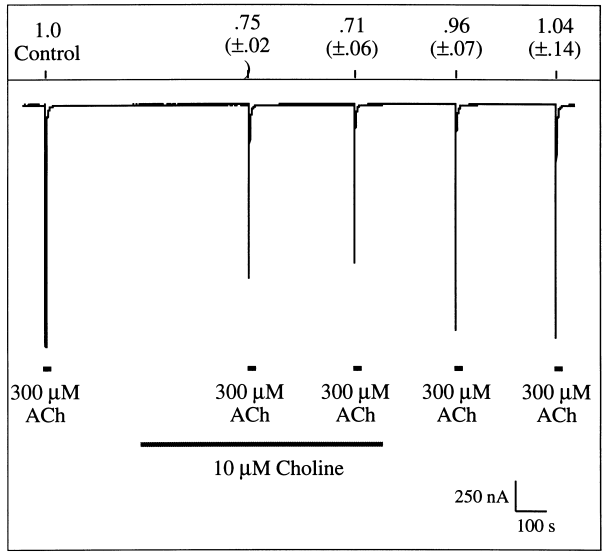

Fig. 3. Cross desensitization of ACh responses by choline. Five minutes after a standard control ACh (300 μM) response, the bath solution was switched to Ringer solution containing 10 μM choline. After a 5 min equilibration in choline-Ringers, responses to the ACh standard were evaluated and a 25–30% decrease in the ACh response was observed. After return to normal Ringers, ACh responses returned to control levels. A representative recording is shown and the numbers above the trace are the means (± SEM) from three such experiments.

The $\alpha 7$ gene is expressed at high levels in the hippocampus and elsewhere in the brain, correlating well with the binding of α-BTX. α-BTX receptors are more abundant in the brain than are high affinity nicotine binding sites [4], and based on their high calcium permeability, such putative $\alpha 7$-type receptors have been suggested to be potential mediators of neuronal plasticity, perhaps involved in learning and memory [14]. They have also been shown to play a role in regulating intracellular calcium signaling [17].

Our results suggest that choline may serve as the endogenous activator of $\alpha 7$ receptors. Free choline concentrations in plasma have been reported to range from 10 to 40 μM, and in cerebrospinal fluid have been estimated to be 4–12 μM [9]. While these are concentrations that are far lower than those used to activate maximal peak responses in oocytes, the exposure of $\alpha 7$ receptors to levels of circulating choline may result in a low levels of tonic activity. The activation properties of $\alpha 7$-type receptors appear to be unique among nicotinic receptors in that, in the presence of high agonist concentrations, desensitization is rapid, yet a high affinity desensitized state is not observed. The macroscopic current stimulated by high agonist concentrations in oocytes and reported for putative $\alpha 7$-like receptors in hippocampal neurons and chick ciliary ganglion cells desensitizes during the application of agonist, yet small and sustained responses have been observed in response to low concentrations of agonist (2 μM nicotine or 10 μM ACh, in ciliary or hippocampal neurons, respectively) [1,18]. It was proposed that such low level activation is responsible for previously reported α-BTX-sensitive increases in intracellular calcium concentrations [18].

Low level calcium signals have been proposed to have trophic functions and promote cell survival under conditions of stress [8]. Although desensitized receptors will not effectively coordinate an evoked response, they will still exhibit intermittent bursts of activity [5]. Based on the high calcium permeability of the $\alpha 7$ receptor subtype, even low levels of tonic $\alpha 7$ activity might have a significant effect on calcium homeostasis, perhaps providing a basis for the cytoprotective [8] and α-BTX-sensitive neuromodulatory effects [13] that have been reported for nicotinic agonists.

In conclusion, these results provide new insights into the function of α-BTX-sensitive nicotinic receptors, the nature of the nicotinic pharmacophore for neuronal receptor subtypes, and the potential importance of choline as a signalling molecule in the brain. As nicotinic compounds are being developed as therapeutics for Alzheimer's disease and have been proposed for schizophrenia, a better understanding of the neuronal nicotinic receptor pharmacophore and the physiological functions of neuronal nicotinic receptors will be of great value for successful drug development.

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