EFFECTS OF LOCAL ANESTHETICS ON CHOLINERGIC AGONIST BINDING AFFINITY OF CENTRAL NERVOUS SYSTEM α-BUNGAROTOXIN RECEPTORS

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1. Introduction

In general, pharmacological effects of local anesthetics may be attributed to their ability to reversibly block the propagation of nerve and muscle action potentials [1]. At physiologically potent concentrations, local anesthetics (LA) also act as non-competitive antagonists of the physiological response of post-synaptic nicotinic acetylcholine receptors (nAChR) to cholinergic agonists [2], and increase agonist binding affinities of nAChR from electric organ [2–4]. It is postulated that the primary site of LA action on nAChR function is at the receptor-coupled ionophore [2]. Furthermore, LA–nAChR ionophore interactions are thought to accelerate physiological desensitization of nAChR, manifest biochemically as increased affinity of nAChR for agonist [2].

Specific receptors for α-bungarotoxin (α-Bgt), a potent competitive antagonist at nAChR sites in the periphery [5], have been detected in rat central nervous system (CNS) membrane preparations [6–10]. The affinity of these central α-Bgt receptors (α-BgtR) for cholinergic agonists is found to increase on exposure to agonist [10,11]. Nevertheless, on the basis of inconsistent pharmacological and physiological results, uncertainty remains regarding the relationship between α-BgtR and authentic nAChR in the CNS, despite a wide body of biochemical and histological evidence consistent with their identity (see [12]).

Reasoning that if CNS α-BgtR are true nAChR, coupled to functional ion channels, LA might be expected to cause biochemically measurable increases in α-BgtR affinity for cholinergic agonists, we have undertaken a study of the effects of LA on the ability of acetylcholine (ACh) to inhibit interaction of α-BgtR with ^{3}H-labeled α-Bgt.

2. Experimental

Methods for preparation of α-Bgt, ^{3}H-labeled α-Bgt (α-[^{3}H]Bgt), and rat brain crude mitochondrial fraction membranes containing α-BgtR, techniques for chemical modification of brain α-BgtR disulfides with dithiothreitol (DTT), and details of α-[^{3}H]Bgt binding assays were as in [13–17]. The ability of ACh to inhibit toxin binding (in the presence of 100 μM eserine to block acetylcholinesterase activity) was measured using two experimental paradigms. Preincubation assays were conducted by pretreating α-BgtR with ACh for 30 min prior to initiation of toxin binding by addition of α-[^{3}H]Bgt to 10 nM. Coincubation assays were initiated by adding membrane preparations to solutions containing α-[^{3}H]Bgt and ACh. Effects of LA were assessed using coincubation assays where membranes containing α-BgtR were pretreated with LA for 30 min prior to addition of toxin and ACh. Affinity of α-BgtR for ACh was quantitated by determining the concentration of ligand necessary to reduce specific α-[^{3}H]Bgt binding (over a 30 min time course) to 50% of control values (K_i^{30}).

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3. Results and discussion

Local anesthetics, at high concentrations, directly inhibit toxin binding to brain crude mitochondrial fraction membranes. For example, concentrations of tetracaine (tet) and dimethisoquin (dim) necessary to block 50% of specific α-[^{3}H]Bgt binding are ~200 μM. The results suggest that LA interact with α-BgtR at the cholinergic active site at these concentrations.

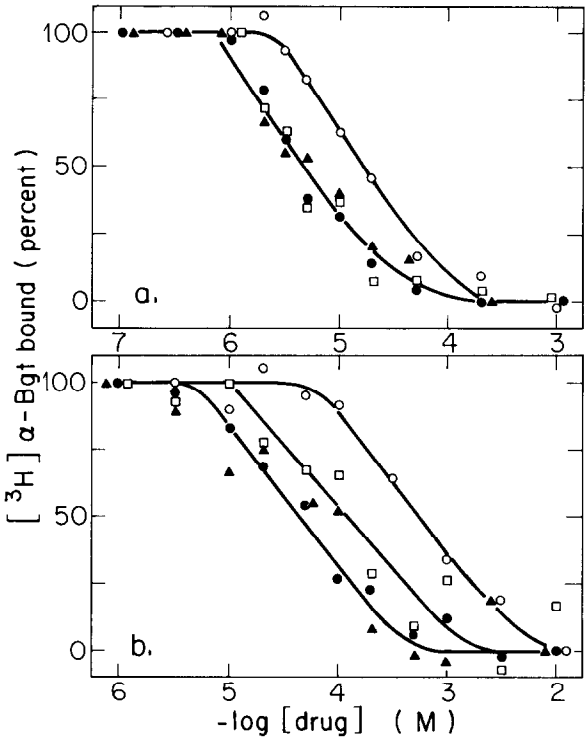

Fig.1. Effects of LA on ACh inhibition of α-[^{3}H]Bgt binding. Specific α-[^{3}H]Bgt binding (%) is plotted against the mole concentration of competing ACh (logarithmic scale). Assays were for native (upper panel) and DTT-treated (lower panel) brain membranes. Membranes were either preincubated for 30 min with ACh prior to the addition of α-[^{3}H]Bgt (•), or are added to ACh and α-[^{3}H]Bgt simultaneously (coincubation), after exposure for 30 min to 20 μM tetracaine (□), 20 μM dimethisoquin (▲), or buffer without local anesthetic (○). Results of an experiment where membranes were added to LA, ACh, and α-[^{3}H]Bgt were indistinguishable from those for coincubation assay in the absence of LA.

Whether brain membranes in the native state or DTT-treated membranes were used, preincubation with cholinergic agonists lead to an increase in the affinity of α-BgtR for agonists, as measured by toxin binding-inhibition assays (fig.1). Values of K_i^{30} for ACh inhibition of α-[^{3}H]Bgt binding were 5 μM and 50 μM for preincubation assays using native and DTT-treated membranes, respectively. In contrast, ACh K_i^{30} values were 16 μM and 550 μM for native and reduced α-BgtR, respectively, on coincubation assays. These results confirm our earlier observations concerning agonist-induced alterations in α-BgtR state, which were interpreted as biochemical correlates of transformation from resting to desensitized states of CNS α-BgtR/nAChR [10,11,17].

The rate of transformation of α-BgtR to the high-affinity state is apparently accelerated after α-BgtR has been treated with LA at low concentrations, where direct competition of LA for α-Bgt binding is eliminated (fig.1). Even on coincubation assay, K_i^{30} values for ACh inhibition of toxin binding were 5 μM for native membranes pretreated with 20 μM dim or 20 μM tet. Corresponding K_i^{30} values for ACh interaction with DTT-treated membranes were 50 μM and 100 μM for reduced α-BgtR preincubated with 20 μM dim and 20 μM tet, respectively. These LA effects were also evident in studies of ACh inhibition of the rate of α-[^{3}H]Bgt binding to brain membranes. The results may be interpreted as demonstrating LA-induced acceleration of transformation of α-BgtR to a high-affinity/desensitized state.

It may be concluded that the α-BgtR in rat brain detected in this study is coupled to an entity that possesses sites for high-affinity interaction with LA, and that LA occupation of those sites influences α-BgtR affinity for cholinergic agonists. The implication is that the CNS α-BgtR-LA binding site complex is a physiological functional ion-conductance regulator, which displays the pharmacological specificity of an ACh receptor–ionophore complex.

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