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Dual effect of lobeline on α4β2 rat neuronal nicotinic receptors

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ABSTRACT

The effect of lobeline on rat α4β2 nicotinic receptors expressed in COS cells was studied using the patch-clamp technique. Currents were recorded in whole-cell mode 2–4 days after cell transfection by plasmids coding the α4β2 combination of receptor subunits. In cells sensitive to acetylcholine, the application of lobeline evoked minor responses (up to 2% of maximal acetylcholine response). When acetylcholine was applied to the background of an already running application of lobeline, acetylcholine responses were inhibited in a concentration- and time dependent manner. However, when lobeline was applied simultaneously with acetylcholine without any prepulse or during an already running application of acetylcholine, the acetylcholine responses were potentiated up to 300–600% of that of the control. The site of lobeline action overlaps with the cholinergic site, as was proven by the partially protective effect of (+)-tubocurarine. Thus, lobeline can apparently desensitize receptors when applied alone (inhibition) whereas its binding to a second agonist site with the first one already occupied by acetylcholine leads to channel opening (potentiation).

1. Introduction

Nicotinic receptors are subject to pharmacological modulation (inhibition or potentiation) from various orthosteric and allosteric binding sites (Arias, 1998, 2000; Maelicke and Albuquerque, 2000; Smulders et al., 2004; Smulders et al., 2005; Zwart et al., 2000; Zwart and Vijverberg, 1997, 2000).

Lobeline (Fig. 1B) is an alkaloid found in the plant *Lobelia inflata* (Felpin and Lebreton, 2004) and has a long history of therapeutic usage, ranging from emetic and respiratory stimulant to tobacco smoking cessation agent (Dwoskin and Crooks, 2002), despite the mechanism(s) of its action being unclear. However, a considerable amount of evidence from binding experiments and various functional studies suggests that there is a direct interaction between lobeline and nicotinic receptors.

Lobeline was initially considered to be generally a nicotinic agonist (Decker et al., 1993; Felpin and Lebreton, 2004). The lobeline molecule possesses some structural features known from other nicotinic ligands — a basic amine and groups tentatively forming hydrogen bonds with the receptor molecule (Fig. 1B); it is, however, unclear whether lobeline fits the actual nicotinic pharmacophore model (Glennon and Dukat, 2000). It competes for binding with other cholinergic drugs with a high affinity (Damaj et al., 1997; Parker et al., 1998; Terry et al., 1998) and displays full or partial agonism or even antagonism, depending on the nicotinic receptor subtype (Wu et al., 2006).

The notion of lobeline interacting directly with nicotinic receptors is supported by an analysis of *Aplysia* and *Lymnaea* acetylcholine binding proteins (A-AChBP, L-AChBP) crystallized in complex with nicotinic ligands, including lobeline and the high affinity- and efficacy agonist epibatidine (Hansen et al., 2005). Although the surfaces involved in epibatidine and lobeline binding do not overlap precisely, both clearly interact with the ligand binding domain. In addition, the large movement of loop C that is thought to wrap around bound agonists, but not antagonists, can be seen in the lobeline-binding A-AChBP crystal.

Lobeline binding at nicotinic receptors was studied via competition with radiolabeled epibatidine (Parker et al., 1998), nicotine (Damaj et al., 1997) and cytisine (Terry et al., 1998). It was found that β2-containing receptors bind lobeline with higher affinity than β4-containing receptors (Parker et al., 1998). The direct agonist behavior of lobeline is strongly dependent not only on the subunit composition, but also on the species and expression system. Lobeline is a full agonist at the human α4β4 nicotinic acetylcholine receptor but only a partial agonist at human α4β2 nicotinic acetylcholine receptor (20% of response to nicotine) expressed in SH-EP1 epithelial cells (Wu et al., 2006). Moreover, lobeline is a partial agonist of rat α3β2 receptors, inducing 21% of the acetylcholine response (better than carbachol, nicotine and cytisine) while at rat α3β4, lobeline elicits 18% of the response to acetylcholine and is a weaker agonist than carbachol (Covernton et al., 1994). Lobeline is also a low-efficacy agonist at fetal rat muscle nicotinic receptor (Cooper et al., 1996) and an antagonist at the human α7 receptor (Briggs and McKenna, 1998). Functional measurements of lobeline interaction with rat α4β2

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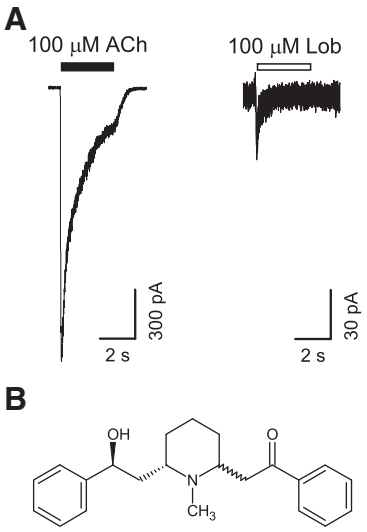

Fig. 1. A) Activation of rat α4β2 nicotinic receptors by acetylcholine (ACh) and lobeline (Lob). Examples of whole-cell ion currents induced by 3 s application of 100 μM acetylcholine or lobeline in COS cell clamped at −40 mV. Note the difference in pA scale. B) Scheme of lobeline molecule, illustrating the existence of two oxygen-containing groups available for a putative H-bond, which has been suggested as an important part of its interaction with an acetylcholine binding site.

receptors are still incomplete. Our goal was to more precisely specify the mode of action of lobeline on rat α4β2 receptors expressed in a COS cellular system.

2. Materials and methods

cDNA coding the α4 and β2 subunits of rat neuronal nicotinic AChR were kindly obtained from Dr. S. Heinemann. cDNAs were subcloned into the pcDNA3.1 (Invitrogen) expression vector at the multiple cloning site. The experiments were performed on COS cells transiently transfected with plasmids coding the appropriate subunit combination (1:1 ratio α4:β2) using Lipofectamine 2000 (Gibco BRL). COS cells were cultivated in a minimal essential medium which was supplemented with 10% fetal calf serum (both from Sigma Aldrich, St. Louis, MO). More than 48 h after the transfection procedure, whole-cell patch-clamp measurements were performed using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Successfully transfected cells were detected by cotransfection with a CD4 coding plasmid (kindly provided by Dr. G. Westbrook) and Dynabeads M-450 CD4 (Dynal Biotech, Norway) aggregation control. Cells were held at −40 mV during recordings.

Fire-polished glass micropipettes with an outer diameter of approx. 3 μm were filled with a solution of the following composition (in mM): CsF 110, CsCl 30, $MgCl_2$ 7, Na_2ATP 5, EGTA 2, HEPES-CsOH 10, pH 7.4. The resulting resistances of the microelectrodes were 3 to 5 MΩ. The cell bath solution contained (in mM): NaCl 160, KCl 2.5, $CaCl_2$ 1, $MgCl_2$ 2, HEPES-NaOH 10, glucose 10, pH 7.3. Solutions of drugs (α-Lobeline hydrochloride from FLUKA, Germany; all other from Sigma Aldrich, St. Louis, MO) were applied using a rapid perfusion system (Mayer et al., 1989) consisting of an array of ten parallel quartz–glass tubes, each approximately 400 μm in diameter. The tubes were positioned and the flow of various solutions switched on/off under microcomputer control (Dittert et al., 1998). A complete change of the solution around the cell could be carried out in 30 to 60 ms. For signal recording and data evaluation, an Axon Instruments Digidata 1320A digitizer and pCLAMP9 software package (Axon Instruments, Foster City, CA) were used. Data were low-pass filtered at 1 kHz and digitized at 2 kHz.

The values are given as means ± S.E.M. Statistical significance was estimated by *t*-test (SigmaPlot-Systat Software).

3. Results

3.1. Agonist effects

The application of acetylcholine to cells clamped at negative membrane potential produced inward currents which displayed desensitization (Fig. 1A left). The activation curve was monophasic, exhibiting an apparent EC_{50} of acetylcholine on α4β2 receptors of 159 ± 24 μM and Hill coefficient H = 0.93 ± 0.07. These values are in agreement with those reported for mouse α4β2 receptors (Karadsheh et al., 2004), indicating that the receptors had a prevalent subunit stoichiometry of $(\alpha4)_3(\beta2)_2$ (Karadsheh et al., 2004; Moroni and Bermudez, 2006).

When applied alone, lobeline at concentrations up to 100 μM evoked very low current responses which were only detectable in cells with the highest expression of nicotinic receptors and represented less than 1–2% of the maximal response to acetylcholine (Fig. 1A right). Lobeline responses were virtually completely desensitizing with a time constant of 0.3–1 s. More precise estimation of the desensitization parameters was difficult because of the small amplitude of lobeline responses.

3.2. Inhibition

When 1 μM lobeline was preapplied and then coapplied with 100 μM acetylcholine, it inhibited acetylcholine responses in a time- and concentration dependent manner (Fig. 2A, B). The onset of lobeline action was gradual and depended on the drug concentration (Fig. 2 B). 0.01 μM lobeline inhibited the acetylcholine response to 50% after almost 10 s of preapplication (Fig. 2B) while 0.1 μM lobeline reached the same level of inhibition in 1 s (Fig. 2B). The preapplication of 1 μM lobeline resulted in almost complete inhibition in less than 5 s (Fig. 2A). Interestingly, the degree of inhibition was virtually independent of acetylcholine concentration (Fig. 2D). The blocking effect of lobeline could be washed out completely in 180 s and the time course of recovery seemed to be double exponential ($\tau_1 = 62$ s, $\tau_2 = 1.6$ s) (Fig. 2C). The dependence of the inhibition plateau on lobeline concentration and values of on and off rate constants (data not shown) indicate that the IC_{50} for lobeline is approximately 5 nM.

A short preapplication (0.5 s) of 1 μM lobeline deforms the desensitization time course of acetylcholine response while the initial peak is still present. However, after a preapplication lasting 2 s and more, 100 μM acetylcholine-induced responses had no initial peak and only gradually increased to a low plateau (Fig. 2A). The time course of acetylcholine responses after lobeline preapplication is complex, probably because receptor desensitization is modulated by slowly equilibrating proportions of receptors occupied by lobeline and/or acetylcholine.

To verify whether lobeline inhibits responses via acting on acetylcholine binding sites, we used 30 μM (+)-tubocurarine, a classical competitive antagonist (Arias, 2000; Strecker and Jackson, 1989). (+)-tubocurarine preapplication (5 s) was followed by coapplication with lobeline 0.1 μM (10 s). Cells were then washed with bath solution for 20 s. When we applied 100 μM acetylcholine after this wash-out period, we observed a diminution of the inhibiting effect of lobeline compared to the effect of an identical concentration of lobeline applied in the same time schedule but without (+)-tubocurarine (Fig. 3). The protection of acetylcholine receptor responses by (+)-tubocurarine indicates that the lobeline acts at a site overlapping with the (+)-tubocurarine binding site and therefore might act at the same site as acetylcholine.

3.3. Potentiation

Surprisingly, if lobeline was applied simultaneously with the agonist acetylcholine, a strong potentiation was observed, up to 300% of the control responses to acetylcholine (Fig. 4A). The most effective