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Different effects of lobeline on neuronal and muscle nicotinic receptors

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ABSTRACT

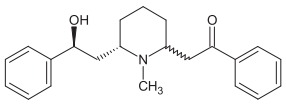
Lobeline is a plant alkaloid known to interact with cholinergic system. The effect of lobeline on neuronal α3β4 receptors expressed in COS cells and muscle embryonic αβγδ receptors naturally expressed in TE671 cells was studied using a patch-clamp technique. Our results show that lobeline inhibited responses to acetylcholine in human embryonic muscle nicotinic receptor in a pseudo-noncompetitive manner. The responses of rat neuronal α3β4 receptors to a low concentration of acetylcholine were potentiated by a mixed occupation mechanism that corresponds to "competitive potentiation". This potentiation turned into voltage-dependent inhibition for α3β4 receptors was activated by a high concentration of acetylcholine.

1. Introduction

Nicotinic acetylcholine receptors are pentameric ligand-gated ion channels comprised of five transmembrane subunits forming the central pore. Nicotinic receptors are subjected to pharmacological modulation via various orthosteric and allosteric binding sites (Hogg et al., 2005; Lena and Changeux, 1993; Maelicke and Albuquerque, 2000).

Lobeline is an alkaloid from the plant *Lobelia inflata* (Felpin and Lebreton, 2004) and has been used for a long time in the therapy of respiratory problems, tobacco addiction and as an emetic. There is a considerable amount of evidence that lobeline interacts with various nicotinic receptors. The precise mechanism of its therapeutic effects is not clear and is thought to be a complex mixture of effects on nicotinic receptors and perhaps also other mechanisms.

The lobeline molecule has the general structural feature of nicotinic ligands (Glennon and Dukat, 2000) and its direct interaction with nicotinic receptors is also supported by its interaction with the acetylcholine binding protein from *Aplysia* (Hansen et al., 2005).



Crystals of the acetylcholine binding protein in complex with lobeline showed that lobeline is positioned in the binding pocket and interacts with loop C in a similar manner as the agonist epibatidine. The physiological, therapeutic and toxic effects of lobeline are caused by complex interactions of lobeline with various subtypes of nicotinic receptors and its various activities are apparently conditioned by the composition of the receptor subunit. The results from laboratory experiments could also depend on the expression system. Lobeline is a full agonist at the human α4β4 nicotinic acetylcholine receptor but only a partial agonist at the human α4β2 nicotinic acetylcholine receptor (20% of the response to nicotine) expressed in SH-EP1 epithelial cells (Wu et al., 2006). Lobeline is also a low-efficacy agonist at fetal rat muscle nicotinic receptors expressed in *Xenopus* oocytes (Cooper et al., 1996) and an antagonist at the human α7 receptor (Briggs and McKenna, 1998).

We recently described the complex behavior of lobeline on the rat brain α4β2 receptor (Kaniakova et al., 2011). We found that lobeline is a low-efficacy partial agonist of the α4β2 receptor (low affinity variant with $(\alpha4)_3(\beta2)_2$ stoichiometry) which can cause high-affinity desensitization of whole-cell transmembrane currents in the prolonged presence of a low concentration of lobeline. Because of the slow rate of unbinding of lobeline, it acts as pseudo-noncompetitive antagonist to acetylcholine. When lobeline is applied together with or during the application of a lower concentration of acetylcholine, it induces a "competitive" potentiation of the response, probably via a mixed occupation of both receptor binding sites. The potentiation closely corresponds to the mathematical model of mixed occupancy with one binding site occupied by the full agonist acetylcholine and the second binding site by the weak partial agonist lobeline (Cachelin and Rust, 1994;

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Zwart et al., 2000). To complete our knowledge about the effects of lobeline, we focused on two model types of nicotinic receptors that might be influenced by the drug during its therapeutic application, in particular on the embryonic muscle receptor in TE671 cells and α3β4 neuronal receptors transiently expressed in a COS cell line.

2. Materials and methods

Experiments on the human embryonic muscle nicotinic receptor αβγδ were performed on the cell line TE671 (kindly provided by Dr. Jan Říčný). These cells were cultivated in Dulbecco's Modified Eagle's Medium (DME) supplemented with 10% fetal calf serum (both from Sigma Aldrich, St. Louis, MO). Nicotine (100 μM) was added to the cultivation medium 2–5 days before measurement to increase the expression of nicotinic receptors (Ke et al., 1998). cDNA coding α3 and β4 subunits of the rat neuronal nicotinic receptor were kindly obtained from Dr. S. Heinemann. cDNAs were subcloned into the pcDNA3.1 (Invitrogen) expression vector at the multiple cloning site. The experiments with α3β4 nicotinic receptors were performed on COS cells transiently transfected with plasmids coding the appropriate subunit combination (1:1 ratio α3:β4) using the Lipofectamine 2000 (Gibco BRL) procedure. COS cells were also cultivated in a DME medium supplemented with 10% fetal calf serum. COS cells were used for measurement more than 48 h after the transfection. Whole-cell patch-clamp measurements were performed using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Cells were held at −40 mV during all recordings, apart from when measuring voltage dependence.

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 between 3 and 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 and Vyklicky, 1989) consisting of an array of 10 parallel quartz-glass tubes, each approximately 400 μm in diameter. The tubes were positioned in the vicinity of the recorded cells and the flow of various solutions was switched on/off under microcomputer control (Dittert et al., 1998). A complete change of the solution around the cell could be carried out in 30–60 ms. For signal recording and data evaluation, an Axon Instruments Digidata 1320A digitizer and pCLAMP9 software package was used (Axon Instruments, Foster City, CA). Data were low-pass filtered at 1 kHz and digitized at 2 kHz.

Concentration–response curves were fitted to the Hill equation:

$$I(C_a) = \frac{Y_{max} \cdot C_a^H}{C_a^H + EC_{50}^H} \quad (1)$$

where C_a is the agonist concentration, Y_{max} is the extrapolated maximal value of the response to a saturating concentration of the agonist, $I(C_a)$ is the relative value of membrane current, EC_{50} is the agonist concentration inducing 50% of the maximal response, and H is the Hill coefficient.

Inhibition curves were fitted to the simple inhibition curve:

$$\frac{Y_L}{Y_{cont}} = \frac{IC_{50}^H}{IC_{50}^H + L^H} \quad (2)$$

where Y_L is current response amplitude after agonist and lobeline application, Y_{cont} is the control response to the agonist, IC_{50} is the lobeline concentration that inhibits 50% of the control response, H is the Hill coefficient for inhibition and L is the concentration of lobeline.

The voltage dependence of lobeline-induced inhibition was fitted to the Boltzmann function (Hille, 1992; Woodhull, 1973; Aidley and Stanfield, 1996):

$$IC_{50}(V_m) = IC_{50}(0) \cdot \exp\left(-V_m \cdot \delta \cdot \frac{F}{R \cdot T}\right) \quad (3)$$

where $IC_{50}(V_m)$ and $IC_{50}(0)$ are the inhibition constants at the given membrane potential V_m and extrapolated value for zero membrane potential, respectively. Universal constants F, R and T have their usual values (F=9.648 10^4 C mol^{-1}, R=8.315 J K^{-1} mol^{-1} and T=298 K) and δ is the relative electrical distance of the lobeline binding site from the outside.

Mixed effects of concentration-dependent potentiation/inhibition were analyzed as partial agonism/competitive antagonism/ion channel block manifesting in the bell-shaped curves of relative response amplitudes (Zwart and Vijverberg, 1997):

$$\frac{Y_L}{Y_{cont}} = \left(1 + \left(\frac{2 \cdot f \cdot L'}{A'}\right)\right) \cdot \left[(1+A')/(1+A'+L')\right]^2 \cdot \left[\frac{1}{1+L''}\right] \quad (4)$$

where Y_L/Y_{cont} is the response with lobeline normalized to the response in the absence of lobeline. A', L' and L'' represent the concentrations of acetylcholine and lobeline, respectively, normalized to their equilibrium binding constants K_A (EC_{50}), K_L and K_i. The factor f describes the difference in response evoked by receptors occupied by the combination of acetylcholine and lobeline and by receptors occupied by 2 acetylcholine molecules. The decay phases of the current responses and unbinding of the modulator were fitted to an exponential function with an added constant plateau component using the program Clampfit 9 and the relationship:

$$A(t) = A \cdot \exp\left(-\frac{t}{\tau}\right) + C \quad (5)$$

where A and τ are the amplitude and time constant of desensitization, respectively. C is the amplitude of the non-desensitizing component of the response, the final plateau. For further analysis, the amplitudes were normalized to the peak value for each response: a=100% · (A/A_{max}); c=100% (C/A_{max}).

Statistical analysis of the data was performed after transforming the concentration data to –log values indicated as pEC_{50}, pIC_{50} or pK_i, since the distribution of errors corresponds better to the Gaussian distribution after the logarithmic transformation. All values of EC_{50}, IC_{50} and K_i are therefore given as means and their −log value (pEC_{50}, pIC_{50} or pK_i) ± S.E.M. Statistical significance was estimated by t-test (SigmaPlot-Systat Software, Chicago, IL). The voltage dependence of the inhibition was investigated by unpaired t-test.

3. Results

3.1. Inhibitory action of lobeline on human embryonic muscle nicotinic receptors

TE671 cells naturally expressing the αβγδ nicotinic receptor responded to the fast application of acetylcholine with desensitizing cationic currents (Fig. 1A). The activation curve was fitted to the Hill activation function (Eq. (1)) with the parameters EC_{50}=7 μM (pEC_{50}=5.16 ± 0.01) and Hill coefficient H=1.47 ± 0.07 (n=6) (Fig. 1B, black circles). The highest concentration of lobeline, 100 μM, induced very small but distinctive responses of up to 0.1% of the maximal response to acetylcholine, only visible in cells with an extremely high sensitivity to acetylcholine (not shown). Lower concentrations of lobeline did not induce any macroscopic response.

When lobeline was applied together with acetylcholine, acetylcholine responses were inhibited compared to the control. The relative degree of inhibition increased with increasing lobeline