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Kinetic properties and open probability of α7 nicotinic acetylcholine receptors

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ABSTRACT

The alpha7 nicotinic acetylcholine receptor (nAChR) has some peculiar kinetic properties. From the literature of α7 nAChR-mediated currents we concluded that experimentally measured kinetic properties reflected properties of the solution exchange system, rather than genuine kinetic properties of the receptors. We also concluded that all experimentally measured EC_{50} values for agonists must inherently be inaccurate. The aim of this study was to assess the undistorted kinetic properties of α7 nAChRs, and to construct an improved kinetic model, which can also serve as a basis of modeling the effect of the positive allosteric modulator PNU-120596, as it is described in the accompanying paper. Agonist-evoked currents were recorded from GH4C1 cells stably transfected with pCEP4/rat α7 nAChR using patch-clamp and fast solution exchange. We used two approaches to circumvent the problem of insufficient solution exchange rate: extrapolation and kinetic modeling. First, using different solution exchange rates we recorded evoked currents, and extrapolated their amplitude and kinetics to instantaneous solution exchange. Second, we constructed a kinetic model that reproduced concentration-dependence and solution exchange rate-dependence of receptors, and then we simulated receptor behavior at experimentally unattainably fast solution exchange. We also determined open probabilities during choline-evoked unmodulated and modulated currents using nonstationary fluctuation analysis. The peak open probability of 10 mM choline-evoked currents was 0.033 ± 0.006, while in the presence of choline (10 mM) and PNU-120596 (10 μM), it was increased to 0.599 ± 0.058. Our kinetic model could adequately reproduce low open probability, fast kinetics, fast recovery and solution exchange rate-dependent kinetics.

1. Introduction

The α7 subunit containing nicotinic acetylcholine receptor (α7 nAChR) is known as the second most abundant nicotinic receptor type in the CNS (after the α4β2 type receptor). These receptors mostly but not exclusively are homopentamers of the α7 subunit. Their most prominent role within the central nervous system is probably the pre- and postsynaptic modulation of synaptic function and plasticity (Albuquerque et al., 2009; Alkondon and Albuquerque, 2001; Gray et al., 1996; Gu and Yakel, 2011; Lendvai and Vizi, 2008; Lozada et al., 2012; Rozsa et al., 2008; Vizi and Lendvai, 1999), which is the basis of their role in cognition (Hurst et al., 2012; Lendvai et al., 2012; McKay et al., 2007).

The α7 nAChR has some unique properties: a high Ca^{2+} permeability, which is counterbalanced by extremely fast desensitization, causing a fast onset and decay kinetics and an extremely low open probability (Williams et al., 2012, 2011).

The aim of this study was to refine the kinetic analysis of the receptor. In order to clearly see the unusual aspects of α7 nAChR receptor kinetics, we need to briefly review what is known about the kinetic properties of this receptor (Section 3.1.). This re-examination of reported properties, makes it evident that the kinetics depends both on agonist concentration and on solution exchange rate. The nature of these dependences, and their interaction will be discussed, together with their possible mechanism. From the apparent solution exchange rate dependence it follows that measurements of the intrinsic kinetic properties of the receptor may be problematic, and experimentally measured kinetics may misrepresent intrinsic kinetics of the receptor.

Abbreviations: α7 nAChR, α7 subtype nicotinic acetylcholine receptor; PNU-120596, 1-(5-chloro-2,4-dimethoxyphenyl)-3-(5-methylisoxazol-3-yl)urea; SXT, 10–90% solution exchange time.

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One of our aims was to give a more accurate estimation of intrinsic kinetic properties of α7 nAChRs, i.e., receptor kinetics undistorted by insufficient solution exchange rate.

Our second major goal was to propose a plausible kinetic scheme for major conformational states of the receptor. This is important if we want to understand the mechanism of receptor gating behavior: its agonist concentration-dependence, its solution exchange rate-dependence, and the interaction of the two. As we will describe in Section 3.3.1., none of the kinetic models thus far constructed by our group or others could sufficiently reproduce experimental behavior. Furthermore, an acceptable kinetic model allows the estimation of intrinsic properties, *i.e.*, determination of approximate rate constants of agonist association/dissociation, receptor opening, closing and desensitization.

Constructing an acceptable kinetic model was important for another reason: One of our aims was to study the mode of action of the positive allosteric modulator PNU-120596.

The mode of action of a modulator can be quantitatively described if when we can explain *which* exact conformational transitions are modified, and *how much* they are modified. Does the modulator affect agonist association, agonist dissociation, activation, deactivation, desensitization or recovery? In terms of Markov models: which specific rate constants are increased or decreased in modulator-bound states, and by what factor? Results of this study are presented in the accompanying paper (Szabo et al., 2014).

Finally, the low open probability during agonist evoked currents are one of the unique properties of α7 nAChRs, and it is essential that the kinetic model we construct can reproduce this attribute also. We therefore aimed to determine peak open probability values during agonist evoked receptor activation. The number of open receptors could be deduced from amplitude-variance plots of agonist-evoked currents, but in the case of the α7 nAChRs the mean open time is so low, that a significant part of the variance is lost due to unresolvable fast openings. Because the positive allosteric modulator PNU-120596 has been shown to radically prolong channel open times, and thereby to induce prolonged tail currents in the absence of the agonist (daCosta et al., 2011; Williams et al., 2011), we were able to construct amplitude-variance plots undistorted by channel block, or by unresolvably fast openings and determine open probability values. Details of the mechanism of action for PNU-120596 are described in the accompanying paper (Szabo et al., 2014).

2. Materials and methods

2.1. Materials

Methyllycaconitine and PNU-120596 were obtained from Tocris Bioscience (Bristol, UK). Cell culture products were obtained from Life Technologies™. All other chemicals were obtained from Sigma.

2.2. Cell culture

GH4C1 cells stably transfected with pCEP4/rat α7 nAChR were obtained from Siena Biotech S.p.A. (Siena, Italy). Cells were cultured in poly-L-lysine coated Petri dishes using HAM's F10 medium supplemented with 15% horse serum, 2.5% fetal bovine serum, 1% penicillin–streptomycin, 1 mM GlutaMAX, 100 μg/ml Hygromycin B.

2.3. Electrophysiology

Experiments were performed in whole-cell or outside-out patch configurations, using an Axopatch 200B amplifier and the pClamp software (Molecular Devices, Sunnyvale, CA). Currents were recorded at −70 mV; digitized at 100 kHz (in short protocols where currents were evoked by choline alone) or at 20 kHz (in longer protocols where the effects of PNU-120596 were studied) and filtered at 10 kHz. For illustration and data analysis some of the traces were further digitally filtered off-line at 2 kHz. Borosilicate glass pipettes (1.3–4.8 MΩ) were filled with pipette solution of the following composition (in mM): CsCl 55, CsF 65, EGTA 10, HEPES 10 (pH = 7.2). The extracellular solution contained the following (in mM): NaCl 140, KCl 5 $CaCl_2$ 2, $MgCl_2$ 1, Glucose 5, and HEPES 5, (pH = 7.3). Solution exchange was performed by the "liquid filament switch" method (Franke et al., 1987; Jonas, 1995) using 1.5 mm OD theta glass tubes (Harvard Apparatus, Holliston, MA) and a Burleigh LSS-3200 ultrafast solution switching system. Theta tubes were pulled and broken to have a tip diameter between 150 and 250 μm. A drop of Sylgard® was injected close to the tip of both theta glass channels, half-cured, penetrated by a 0.35 mm OD Microfil™ capillary (WPI Inc., Sarasota FL), and cured fully. Tubing was connected to the inlets of Microfil™ micropipettes. The solution reservoirs were connected to the pressure control unit of a DAD-12 solution exchange system (ALA Scientific Instruments Inc., Farmingdale, NY), this allowed optimization of flow rate (typically ~0.2–0.3 ml/min, which corresponded to 5–20 cm/s flow velocity), and fast exchange of solutions (flow rate increased up to ~1 ml/min). The total dead volume of the tubing from the reservoir to the tip of the theta tube was ~150 μl. Voltage command waveforms for the piezoelectric actuator were written in the pClamp software, and delivered through an analog output of the Digidata 1322A interface. In a few experiments the pressure-controlled dual U-tube system (Szasz et al., 2007) was used for solution exchange, pressure-control was provided by the same DAD-12 instrument. In addition to the fast drug application systems, a permanent laminar flow of extracellular solution at a rate of ~2.5 ml/min was present in the recording chamber throughout the experiments. The osmolarity values of the control extracellular solution, as well as the 10 mM ACh and 10 mM choline solutions were set to 320 mOsm, and all other agonist concentrations were made as a mixture of these.

2.4. Simulations

The simulation was based on a set of differential equations with the occupancy of each receptor state (i.e., the fraction of the receptor population in that specific state) given by the following equation

$$\frac{dS_i(t)}{dt} = \sum_{j}^{n} (S_j(t)*k_{ji} - S_i(t)*k_{ij})$$

where $S_i(t)$ is the occupancy of a specific state at the time t, $S_j(t)$ is the occupancy of a neighboring state, n is the number of neighboring states, and k_{ij} and k_{ji} are the rate constants of transitions between neighboring states. All simulations were performed using Berkeley Madonna v8.0.1 (http://www.berkeleymadonna.com/), to solve the differential equations using a fourth-order Runge–Kutta method. All parameters were adjusted manually.

2.5. Analysis of data

Curve fitting was performed by the Solver function of Microsoft Excel. The Bateman function: $I(t) = k_1/(k_1-k_2) * \exp(-k_2 * t) - \exp(-k_1 * t)$ was used to fit choline-evoked currents and to obtain apparent rate constants of activation and desensitization. Decay phases of evoked currents were fit with either monoexponential or biexponential functions: $I(t) = (I_{max}-I_{min}) * \exp(-t/\tau) + I_{min}$ and $I(t) = (I_{max}-I_{min}) * [A_1 * \exp(-t/\tau_1) + A_2 * \exp(-t/\tau_2)] + I_{min}$, where τ_1 and τ_2 are the time constants, and A_1 and A_2 are their respective contribution to the amplitude. Exchange rate dependence plots were fit with linear ($y = a^*t + b$), monoexponential ($y = a^*\exp(t^*\tau) + b$) biexponential ($y = a_1^*\exp(t^*\tau_1) + a_2^*\exp(t^*\tau_2)$) or power ($y = a^*(x + b)t$) functions; where a, a_1, a_2, b, τ, τ_1 and τ_2 are constants, and t is the 10–90% solution exchange time (SXT). Paired Student's t test was used for statistical analysis. A probability level of 0.05 or less was considered to reflect a statistically significant difference.

3. Results and discussion

3.1. Background: concentration-dependent and solution exchange rate-dependent kinetics of α7 nAChR-mediated currents

Rat α7 nAChR expressing GH4C1 cells were used at 2–5 days after passage. Agonist application was performed by a pressure-controlled theta tube perfusion system. Whole cells or outside-out patches were lifted to the mouth of the theta-tube.

3.1.1. Concentration-dependent kinetics

The effect of different concentrations of ACh and choline was investigated. As it has been previously observed we found that with increasing agonist concentration: i) the onset kinetics is accelerated, ii) the decay kinetics is accelerated, and iii) the current amplitude is increased. Examples for the concentration-dependent kinetics has been shown in several papers, but typically has not been quantified. For this reason, we have reviewed these figures, measured rise times, and estimated decay time constants (exponential curves were overlaid with the figures after adjusting axes to the calibration bars, and time constants were adjusted until the fit