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Lidocaine effects on acetylcholine-elicited currents from mouse superior cervical ganglion neurons

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ABSTRACT

Lidocaine is a commonly used local anaesthetic that, besides blocking voltage-dependent Na^+ channels, has multiple inhibitory effects on muscle-type nicotinic acetylcholine (ACh) receptors (nAChRs). In the present study, we have investigated the effects of lidocaine on ACh-elicited currents (I_{ACh}s) from cultured mouse superior cervical ganglion (SCG) neurons, which mainly express heteromeric α3β4 nAChRs. Neurons were voltage-clamped by using the perforated-patch method and I_{ACh}s were elicited by fast application of ACh (100-300 μM), either alone or in presence of lidocaine at different concentrations.

I_{ACh}s were reversibly blocked by lidocaine in a concentration-dependent way (IC_{50} = 41 μM; n_H close to 1) and the inhibition was, at least partially, voltage-dependent, indicating an open-channel blockade. Besides, lidocaine blocked resting (closed) nAChRs, as evidenced by the increased inhibition caused by a 12 s lidocaine application just before its co-application with the agonist, and also enhanced I_{ACh}s desensitisation, at concentrations close to the IC_{50}.

These results indicate that lidocaine has diverse inhibitory actions on neuronal heteromeric nAChRs resembling those previously reported for *Torpedo* (muscle-type) nAChRs (Alberola-Die et al., 2011). The similarity of lidocaine actions on different subtypes of heteromeric nAChRs differs with the specific effects of other compounds, restricted to particular subtypes of nAChRs.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels (LGICs) that mediate fast synaptic transmission in peripheral and central nervous system. An outstanding characteristic of nAChRs is their wide structural heterogeneity, based in both diverse subunit composition and stoichiometry, which associates to subtype-specific functional and pharmacological properties (Gotti and Clementi, 2004; Dani and Bertrand, 2007). So far, the most studied, and yet the prototypic model for these receptors, is the muscle-type nAChR, localised at the neuromuscular junction of vertebrates and at the electrocytes of some electric fishes, and composed of two α1 subunits and one β1, δ and γ or ε subunits. In the peripheral and central nervous system there are different subtypes of nAChRs, generically called neuronal nAChRs. In mammalian autonomic ganglia neurons, including the superior cervical ganglion (SCG), the predominant nAChR is the heteromeric α3β4 subtype, though there are a significant percentage of combinations with α5 and/or β2 subunits (Gotti et al., 2009; David et al., 2010). Moreover, homomeric α7 receptors are also present, but their currents are only evoked in the presence of PNU 120569 (David et al., 2010). Finally, the two main nAChRs subtypes in the mammalian central nervous system are α4β2 and α7 (Flores et al., 1992; Barrera and Edwardson, 2008) though combinations of α (2-5) with β (2-4) subunits and receptors formed by α9 and/or α10 are also present (Dani and Bertrand, 2007).

Though lidocaine is one of the local anaesthetics (LAs) more commonly used in clinical practice, its detailed mechanisms of action on muscle-type nAChRs have been only recently studied (Alberola-Die et al., 2011); in fact most studies used permanent-charged lidocaine-analogues such as QX-314 or QX-222 (Neher and Steinbach, 1978; Pascual and Karlin, 1998; Papke et al., 2001). In contrast to QX-314 or QX-222, the molecule of lidocaine has a tertiary amine group, so that, at physiological pH, a fraction of the lidocaine molecules remains uncharged. The simultaneous presence of both forms of lidocaine, charged and neutral, seems to be responsible for the multiple inhibitory actions of lidocaine on muscle-type nAChRs from *Torpedo marmorata* microtransplanted to *Xenopus* oocytes, which include open-channel blockade,

Abbreviations: ACh, acetylcholine; I_{ACh}, acetylcholine-elicited current; LA, local anaesthetic; LGIC, ligand-gated ion channel; nAChR, nicotinic acetylcholine receptor; QX-222, 2-(trimethylammonio)-*N*-(2,6-dimethylphenyl) acetamide chloride; QX-314, 2-(triethylammonio)-*N*-(2,6-dimethylphenyl) acetamide bromide; SCG, superior cervical ganglion.

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enhancement of desensitisation, and blockade of closed-state (resting) receptors (Alberola-Die et al., 2011). The aim of the present study was to characterize in mouse SCG neurons the effects of lidocaine on acetylcholine-elicited currents (I_{ACh}), which are mainly mediated by heteromeric α3β4 nAChRs.

2. Materials and methods

2.1. Animals

Animal handling and experimental procedures were approved by the Spanish Higher Research Council and the University of Vigo Scientific Committee and they conformed to Spanish and European guidelines for the protection of experimental animals (RD1201/2005; 2010/63/UE).

2.2. Cell culture

Mouse SCG cell culture was carried out as described previously (Martínez-Pinna et al., 2002; Romero et al., 2004; Lamas et al., 2009). Briefly, mice (20–60 d of age) were terminally anesthetized with CO_2 and immediately decapitated. SCGs were removed and cleaned up in cold Leibowitz medium (L-15) under a binocular microscope; then, the ganglia were chopped and incubated in collagenase (2.5 mg/ml in HBSS) for 15 min at 37 °C. After washing, the ganglia were then incubated for 30 min in trypsin (1 mg/ml in Hanks). Finally, neurons were mechanically isolated, centrifuged, and plated in 35 mm Petri dishes previously coated with laminin (10 μg/ml in EBSS). Neurons were cultured for 1–2 d at 37 °C and 5% CO_2 in L-15 medium containing the following: 24 mM $NaHCO_3$, 10% fetal calf serum, 2 mM L-glutamine, 38 mM D-glucose, 100 UI/ml penicillin, 100 μg/ml streptomycin, and 50 ng/ml nerve growth factor.

2.3. Perforated-patch whole-cell recording

Electrophysiological recordings were performed at room temperature and under continuous perfusion (≈10 ml/min) using an Axopatch 200B amplifier (Molecular Devices). Two-step fire-polished patch pipettes, with a tip resistance of 2–4 MΩ, were used to record through perforated patches obtained with amphotericin B (75 μg/ml); patches with series resistance above 20 MΩ were discarded (Rae et al., 1991). Junction potential ≈5 mV was not corrected (Neher, 1992). Data were digitized through a Digidata 1440A and analysed and plotted using the pClamp10 software (Molecular Devices) and Origin 6.1 (OriginLab Corp., Northampton, MA, USA). The sampling frequency was 10 kHz and recordings were low pass filtered at 5 kHz by the amplifier built-in filter. The recording pipette solution contained (in mM): 105 Cs-acetate, 25 CsCl, 3 $MgCl_2$, 0.5 $CaCl_2$, and 20 HEPES, pH = 7.2 with Tris (tris(hydroxymethyl)aminomethane). Standard bath solution contained (in mM): 140 NaCl, 3 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 D-glucose, and 10 HEPES, adjusted to pH 7.2 with Tris. Atropine (0.5 μM) and TTX (0.5 μM) were routinely added in order to block muscarinic receptors and voltage-dependent sodium channels, respectively. Agonists and antagonists were applied using a fast-step perfusion system SF-77B (Warner Instruments, USA).

2.4. Data analysis

To determine the effect of lidocaine on I_{ACh}, we measured I_{ACh} peak evoked by 300 μM ACh alone or together with different lidocaine doses (0.1 μM–10 mM). I_{ACh}s elicited in the presence of lidocaine were normalised to the I_{ACh} evoked by ACh alone (Control) and data were fitted, using the Origin 6.1 software, to the following form of the Hill equation (1):

$$\frac{I}{I_{max}} = \left[1 + \left(\frac{IC_{50}}{[ACh]}\right)^{n_H}\right]^{-1} \quad (1)$$

where I is the I_{ACh} peak elicited at 300 μM ACh (applied either alone or together with lidocaine), I_{max} is the maximum current recorded, IC_{50} is the lidocaine concentration required to inhibit one-half the maximum current, and n_H is the Hill coefficient.

The rate of desensitisation was determined by measuring the I_{ACh} amplitude elicited by 300 μM ACh, either alone or co-applied with 3 or 30 μM lidocaine, at different times after the current peak. The percentages of desensitisation were obtained using Eq. (2)

$$D_{ti} = \left[1 - \left(\frac{I_{ti}}{I_{peak}}\right)\right] \times 100 \quad (2)$$

where D_{ti} is the percentage of desensitisation at the specified time, I_{peak} the peak current amplitude, and I_{ti} the current amplitudes remaining 1, 2, and 3 s after the peak.

2.5. Drugs

ACh, atropine sulphate, lidocaine, HEPES, L-15 Leibovitz medium, collagenase, HBSS, trypsin, laminin, EBSS, penicillin, streptomycin and reagents of general use were purchased from Sigma (St. Louis, MO, USA)

2.6. Statistics

The values given in the text and figures correspond to the mean ± SEM. When comparing two-group means of normally distributed values, the Student's *t*-test was used. Among-group differences were determined by the Kruskal–Wallis analysis of variance on ranks; the comparison of groups was made using the Dunn's test. A significance level of $p < 0.05$ was considered for all cases.

3. Results

3.1. Lidocaine inhibits I_{ACh} in SCG neurons

Cultured SCG neurons were patched and voltage-clamped at −60 mV, unless otherwise stated, by using the perforated-patch whole cell recording. For each experimental condition, recordings were made from neurons of 5-8 independent cell cultures, unless otherwise stated. Fast application of ACh (300 μM) for 5 s to a clamped neuron elicited a desensitising inward current (Lamas et al., 1997), which was due to the activation of nAChRs expressed by these neurons (Fig. 1A, Control). When ACh (300 μM) was co-applied with different doses of lidocaine (0.1 μM–10 mM), to the same neuron, the I_{ACh} peak decreased in a dose-dependent way (Fig. 1A). Similar results were obtained in 7 different cells. The average dose–inhibition relationship (Fig. 1B) was well fitted to a single sigmoid curve, from which could be estimated an IC_{50} of 41 μM and a slope (n_H) of 0.90 ± 0.04. These results are in good agreement with those reported by Gentry and Lukas (2001) by measuring $^{86}Rb^+$ efflux in SH-SY5Y cells expressing α3β4 nAChRs (IC_{50} = 63; n_H = 1.2), in spite of the methodological differences.

3.2. Lidocaine effects on I_{ACh} desensitisation

Besides decreasing I_{ACh} peak, lidocaine modified the I_{ACh} inactivation kinetics of SCG neurons, and this effect was dependent on the lidocaine dose, as previously described for *Torpedo* nAChRs microtransplanted to *Xenopus* oocytes (Alberola-Die et al., 2011).