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Monoamine NMDA receptor channel blockers inhibit and potentiate native and recombinant proton-gated ion channels

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ABSTRACT

Acid-sensing ion channels (ASICs) are widely distributed in the peripheral and central nervous system. Although they are involved in many physiological functions, the actual processes that activate ASICs remain unclear. This is particularly true for brain ASICs, which produce only a transient response to a fast drop in pH and cannot mediate sustained current. Therefore, the search for ASIC inhibitors and, especially, potentiators/activators is important. We report that NMDA receptor channel blockers with a comparatively simple structure (9-aminoacridine, memantine, IEM-2117 and IEM-1921) potentiate and/or inhibit ASICs in submillimolar concentrations. The experiments were performed using the patch clamp technique on native ASICs from rat hippocampal interneurons and recombinant ASICs of different subunit compositions expressed in CHO cells. Native ASICs were potentiated by IEM-1921 and IEM-2117, and inhibited by memantine and 9-aminoacridine. Homomeric ASIC1a were inhibited by memantine, IEM-2117 and 9-aminoacridine while IEM-1921 was ineffective. In contrast, homomeric ASIC2a were potentiated by IEM-2117, memantine and IEM-1921, whereas 9-aminoacridine was inactive. The compounds caused a complex effect on ASIC3. 9-aminoacridine and IEM-1921 potentiated the steady-state response of ASIC3 and inhibited the peak component. IEM-2117 not only potentiated ASIC3-mediated currents caused by acidification but also evoked steady-state currents at neutral pH. Our results demonstrate that, depending on the subunit composition, ASICs can be activated or inhibited by simple compounds that possess only amino group and aromatic/hydrophobic moieties. This opens up the possibility to search for new ASIC modulators among a number of endogenous ligands.

1. Introduction

Acid-sensing ion channels (ASICs) represent a subgroup of degenerin epithelial sodium channels (DEG/ENAC) (Kellenberger and Schild, 2002; Waldmann et al., 1997a). These channels are closed at neutral pH but open for sodium ions (ASIC1a channels are also permeable for calcium) upon extracellular acidification. The group of ASICs includes subunits ASIC1a, −1b, −2a, −2b, −3 and −4, which can form either homo- or heterotrimers (see Deval et al., 2010 for review).

ASICs are widely distributed in the nervous system. ASIC1a, ASIC2a and ASIC2b subunits have been shown to be abundant in the brain, particularly in the cerebral cortex, hippocampus, cerebellum, striatum, habenula, amygdala and olfactory bulb (Alvarez de la Rosa et al., 2002; Baron et al., 2002; Bolshakov et al., 2002; Garcia-Anoveros et al., 1997; Lingueglia et al., 1997; Price et al., 1996; Wemmie et al., 2003). Dorsal root ganglion neurons express different ASIC subunits (Alvarez de la Rosa et al., 2002; Chen et al., 1998; Krishtal and Pidoplichko, 1981; Lingueglia et al., 1997; Ugawa et al., 2005; Voilley et al., 2001) including ASIC3 subunits, which form homomeric channels that produce sustained currents. The role of ASIC4 is still not known, although it is expressed in the nervous system and can be targeted to the cell membrane (Donier et al., 2008). A large body of accumulated data shows the involvement of ASICs in various physiological and pathological processes. In addition to pain (Deval et al., 2010; Wemmie et al., 2013), ASICs participate in synaptic plasticity, learning and memory (Wemmie et al., 2002), fear and depression (Wemmie et al., 2004), chemosensing (Ziemann et al., 2009) and vision (Ettaiche et al., 2006; Lilley et al., 2004). Recently, the first evidence of the direct involvement of ASICs in synaptic transmission was published (Du et al., 2014).

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The diuretic amiloride is a classical inhibitor of ASICs (Waldmann et al., 1997b), but it is non-selective and inhibits other members of the degenerin-ENAC family (Kellenberger and Schild, 2002). Many antagonists that possess one or two amidine groups were designed using amiloride as a starting template structure (see, e.g., Chen et al., 2010; Dube et al., 2005; Kuduk et al., 2009). Millimolar concentrations of tetraethylammonium inhibit ASIC1a/ASIC2b heteromeric channels in a voltage-dependent manner resembling the pore block of potassium channels (Sherwood et al., 2011). The non-steroid anti-inflammatory drugs inhibit ASICS in the range of therapeutically used concentrations (Dorofeeva et al., 2008; Voilley et al., 2001). ASICs are also modulated by heavy metal and bivalent ions (Allen and Attwell, 2002; Babini et al., 2002; Babinski et al., 2000; de Weille and Bassilana, 2001; Paukert et al., 2004; Staruschenko et al., 2007; Wang et al., 2006, 2007). Two peptide toxins, PcTx1 and APETx2, isolated from the venom of the spider (Escoubas et al., 2000) and the sea anemone (Diochot et al., 2007), have been shown to specifically inhibit ASIC1a and ASIC3 channels, respectively.

Recently, it has been shown that amiloride causes a dual action on ASICs: It not only inhibits but also at neutral pH potentiates ASIC3 currents (Li et al., 2011). Extracellular spermine reduces the desensitization of ASIC1a, shifting the steady-state desensitization to more acidic pH, and accelerating the recovery from desensitization (Duan et al., 2011). Arachidonic acid also potentiates ASIC-mediated currents (Smith et al., 2007). ASIC3 can be activated at pH = 7.4 by the synthetic compound 2-guanidine-4-methylquinazoline, the endogenous compound agmatine, and related chemicals (Yu et al., 2010). Agmatine can synergize with other ASIC modulators, such as arachidonic acid, to enhance the ASIC3 current (Li et al., 2010). A coral snake toxin, MitTx, can activate ASICs at neutral pH (Bohlen et al., 2011). MitTx induces large sustained currents from ASIC1a and ASIC1b as well as ASIC3 at higher concentrations. FMRFamide and neuropeptide SF do not directly activate ASICs but potentiate the proton-induced current through the ASIC1a and ASIC3 channels (Askwith et al., 2000; Catarsi et al., 2001; Deval et al., 2003; Lingueglia et al., 2006).

Ligands that have been derived from amiloride usually contain one or two amidine groups. It remains unclear if this group is mandatory for the drug action. In the present work, we attempted to find new ASIC ligands among the monoamines. We studied the action of four NMDAR channel blockers (Fig. 1) on native and recombinant ASICs. 9-aminoacridine (9AA) (Benveniste and Mayer, 1995; Costa and Albuquerque, 1994), IEM-1921 (Bolshakov et al., 2005; Rogawski et al., 1989) memantine (Bormann, 1989) and IEM-2117 (Barygin et al., 2009) were selected for the study. The compounds represent distinct groups of NMDAR channel blockers that exhibit different kinetics of action and different degrees of the "trapping" effect (Barygin et al., 2009; Bolshakov et al., 2003). Although the initial expectation was to extend the list of ASIC blockers, we found that these compounds potentiate and/or inhibit native and recombinant ASICs depending on the subunit composition.

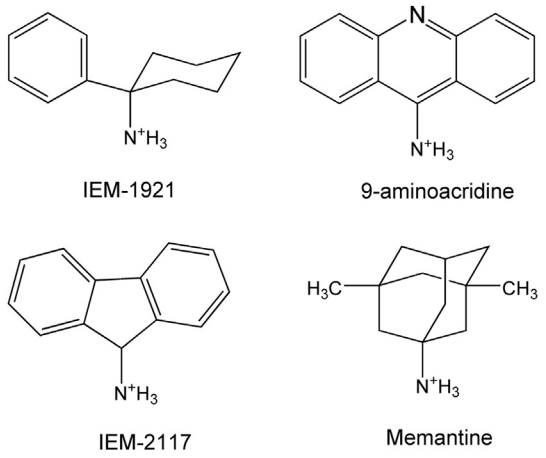

Fig. 1. Chemical structures of the monoamine NMDA receptor channel blockers tested.

2. Methods

2.1. Native receptors

Experiments were conducted in agreement with the Rules of Animal Care and Use Committee of the Sechenov Institute of Evolutionary Physiology and Biochemistry (IEPHB) of the Russian Academy of Sciences, which is fully compatible with European Community Council directives 86/609/EEC. Outbred male Wistar rats 12–17 days old and 25–35 g were obtained from a local (IEPHB) animal facility. Rats were anesthetized with urethane and then decapitated. Maximum efforts were made to minimize the number of animals used and to minimize discomfort.

Brains were removed quickly and cooled to 2–4 °C in an ice bath. Transverse hippocampal slices (250 μm thick) were prepared using a vibratome (Campden Instruments Ltd., Loughborough, UK) and stored in a solution containing (in mM): NaCl 124, KCl 5, $CaCl_2$ 1.3, $MgCl_2$ 2.0, $NaHCO_3$ 26, NaH_2PO_4 1.24 and D-glucose 10, aerated with carbogen (95% O_2, 5%CO_2). Typically, 6–7 slices were obtained from 1 rat brain.

The neurons were isolated from slices with vibrodissociation (Vorobjev, 1991). The method allows a cell to be isolated from a local part of the slice under visual control using an inverted microscope. The experiments were carried out on hippocampal interneurons isolated from the lacunosum moleculare and radiatum layers of the CA1 region. Interneurons were identified by morphological, pharmacological and electrical characteristics. The neurons were discriminated from glial cells by the appearance of action potentials in response to depolarizing pulses under the current clamp. Pyramidal neurons usually have pyramidal-like somata and preserved apical and basal (in some neurons) dendrites, the interneurons varied in size and shape; however, most were round to oval. Pyramidal neurons express AMPA-type glutamate receptors, which are impermeable for Ca^{2+} and insensitive to selective channel blocker IEM-1460. In contrast, kainate-induced currents in interneurons demonstrate moderate to high sensitivity to IEM-1460 due to the presence of Ca^{2+}-permeable AMPA receptors (Buldakova et al., 1999). ASIC-mediated currents in these neurons were characterized previously by our group (Bolshakov et al., 2002) and by others (Weng et al., 2010). It has been shown that unlike interneurons pyramidal neurons demonstrate very small pH-activated currents (Bolshakov et al., 2002).

The whole-cell patch clamp technique was used to record the membrane currents generated in response to fast acidification. Series resistance of about 20 MΩ was compensated by 70–80% and monitored during the experiments. Currents were recorded using an EPC-8 amplifier (HEKA Electronics, Lambrecht, Germany), filtered at 5 kHz, sampled and stored on a personal computer. Drugs were applied using the RSC-200 perfusion system (BioLogic Science Instruments, Claix, France) under computer control. The extracellular solution contained the following (in mM): NaCl 143, KCl 5, $MgCl_2$ 2.0, $CaCl_2$ 2.5, D-glucose 18, HEPES 10 and MES 10 (pH was adjusted to 7.35 with HCl). The pipette solution contained the following (in mM): CsF 100, CsCl 40, NaCl 5, $CaCl_2$ 0.5, EGTA 5 and HEPES 10 (pH was adjusted to 7.35 with CsOH). All experiments were performed at room temperature (22–24 °C).

2.2. Recombinant receptors

CHO cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2. Cells were maintained with standard culture conditions (Dulbecco's modified Eagle's medium DMEM + 10% fetal bovine serum + 5% streptomycin/penicillin). Plasmids encoding ASIC subunits were transfected using Lipofectamine 2000 (Invitrogen, USA) following the manufacturer's protocol. Expression vectors encoding rat ASIC1a, ASIC2a, and ASIC3 were a kind gift from Dr. A. Staruschenko. For the expression of the homomeric channels, cells were transfected with 0.5 μg rASIC1a, rASIC2a and rASIC3 cDNA per 35 mm^2 dish together with 0.5 μg eGFP. Electrophysiological experiments were performed 48–72 h after transfection. Transfected cells were identified with green fluorescence using the Leica DMIL microscope.

Whole-cell currents were recorded in the voltage-clamp mode. The pipette solution contained the following (in mM): CsF 100, CsCl 40, NaCl 5, $CaCl_2$ 0.5, EGTA 5 and HEPES 10 (pH was adjusted to 7.35 with CsOH). Cells were continuously superfused with an extracellular solution containing the following (in mM): NaCl 143, KCl 5, $CaCl_2$ 2.5, D-glucose 18, $MgCl_2$ 2, HEPES 10 and MES 10, adjusted to pH 7.35. Current recordings were acquired with an EPC10-USB (HEKA