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Invited review

Pharmacology of acid-sensing ion channels – Physiological and therapeutical perspectives

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ABSTRACT

Development of the pharmacology of Acid-Sensing Ion Channels (ASICs) has become a key challenge to study their structure, their molecular and cellular functions and their physiopathological roles. This review provides a summary of the different compounds that directly interact with these channels, either with inhibitory or stimulatory effect, and with high selectivity or poor specificity. They include drugs and endogenous regulators, natural compounds of vegetal origin, and peptides isolated from animal venoms. The *in vivo* use of some of these pharmacological modulators in animal models and a few small clinical studies in humans have provided substantial data on the physiological and physiopathological roles of ASIC channels. Modulation of these channels will certainly provide new therapeutic opportunities in neurological and psychiatric diseases including pain, stroke, epilepsy, anxiety, depression or traumatic injury, as well as in some non-neurological pathologies.

This article is part of a Special Issue entitled 'ASIC Channels'.

1. Introduction

ASIC channels are voltage-insensitive, proton-gated cation channels activated by extracellular acidosis (Waldmann et al., 1997b). They share the same topology of their subunits (two transmembrane domains, a large extracellular loop, and short intracellular N and C-termini), and form amiloride-sensitive cation channels. In rodents, four genes encode at least six different ASIC subunits, ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4. ASICs are highly expressed in neurons, but are also present in non-neuronal tissues. ASIC1a and ASIC2 (both variants a and b) are widely expressed in the central nervous system, while almost all subunits (except ASIC4) are present in sensory neurons of the peripheral nervous system (for more detail, see reviews of Deval & Lingueglia and Lin et al. in this special issue; Deval and Lingueglia, 2015; Lin et al., 2015). Their activation induces neuronal depolarization, sometimes associated with direct and indirect Ca^{2+} entry as for homomeric ASIC1a channels. ASICs have been involved in several physiological and physiopathological processes such as nociception and pain, neuronal acidotoxicity, synaptic function and plasticity, and other conditions that are covered in different reviews of this special issue.

Crystallization of the ASIC1a channel from chicken by the group of Eric Gouaux (Jasti et al., 2007) has shown that three subunits are required to form a functional channel that can be either homo- or heteromeric. Interestingly, it has been shown recently that the recombinant ASIC1a and ASIC2a subunits can assemble with a flexible stochiometry, *i.e.*, no preference for homo- or heteromeric combination of subunits (Bartoi et al., 2014). ASIC2b and ASIC4 are not forming functional proton-gated channels on their own but can contribute to heteromeric channels with other ASIC subunits to modulate, at least for ASIC2b, the properties and the regulation of the channels (Noël et al., 2010). Based on the chicken ASIC1a structure, a model has been proposed where each subunit is represented as a hand holding a ball and divided into finger, thumb, palm, knuckle, β-ball, wrist, and forearm as the transmembrane domains TM1 and TM2. An "acidic pocket" containing several pairs of acidic amino acids is present at the interface between two subunits and has been proposed to be one of the pH-sensor of ASIC channels, whereas cations may access the ion channel by lateral fenestrations in the wrist region, then moving into a broad extracellular vestibule (Gonzales et al., 2009; Jasti et al., 2007) (Fig. 1).

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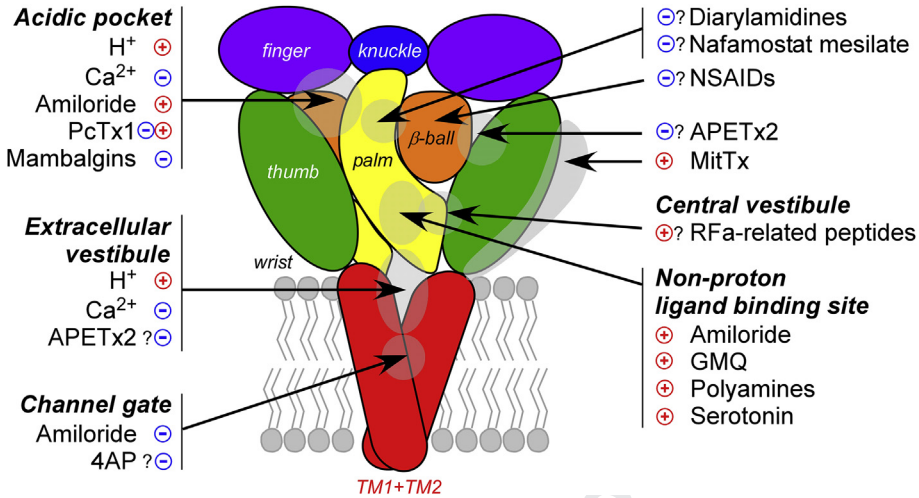

Fig. 1. Sites of interaction of the ASIC-targeting compounds. Schematic ASIC channel based on the chicken ASIC1a structure according to the model of a hand holding a ball (Jasti et al., 2007). Only two subunits out of three are shown for clarity. The inhibitory (−) and stimulatory (+) effects of the different compounds are indicated. Psalmotoxin 1 (PcTx1) can inhibit or activate ASIC channels depending of the channel subtype and the animal species. Amiloride has different binding sites associated with its inhibitory and stimulatory effects. An interrogation mark indicates that the binding site has not been formally demonstrated, with sometimes different possibilities as for APETx2. Note that the listed compounds are not necessarily targeting the same ASIC channel subtypes (see text for details).

The pharmacology of ASIC channels is diverse and has significantly increased in recent years, including synthetic compounds (Table 1, Fig. 2), endogenous regulators (Table 2, Fig. 3), natural products from plants and animals (Table 3, Fig. 4), either with inhibitory or stimulatory effect and of high or poor selectivity.

2. Pharmacology

2.1. Synthetic compounds

2.1.1. Amiloride and derivatives

Amiloride, a K^+-sparing diuretic licensed for hypertension and heart failure, was the first described blocker of ASIC channels (Waldmann et al., 1997a, 1997b, 1996). Amiloride acts as a non-discriminative low affinity pore-blocker of ASIC channels (IC_{50} 5–100 μM), also blocking other ionic channels and exchangers (Frelin et al., 1988; Kleyman and Cragoe, 1988). Interestingly, the sustained-component of the ASIC3 current is resistant to amiloride blockade. The amiloride derivatives benzamil and EIPA are also low affinity, poorly selective, reversible blockers of ASIC channels. Amino acids possibly implicated in the amiloride block are located in the second transmembrane domain on the extracellular side of the channel gate (Kellenberger et al., 2003; Schild et al., 1997). By co-crystallization amiloride was shown to partially occlude the pore of the chicken ASIC1a channel in the extracellular vestibule (Baconguis et al., 2014), probably blocking ion conduction by dipping the amidino group into the pore (Fig. 1). Amiloride also exerts an additional paradoxical enhancing effect at high concentrations, and is able to open homomeric ASIC3 channels (EC_{50} 560 μM) and heteromeric ASIC3 + ASIC1b channels at neutral pH, and also to synergistically enhance the channel activation driven by mild acidosis (Adams et al., 1999; Li et al., 2011; Waldmann et al., 1997a; Yagi et al., 2006). This activating effect, contrary to the inhibitory effect, has been shown to be dependent on the integrity of a non-proton ligand sensing domain present in ASIC3 channel (Li et al., 2011). However, two molecules of amiloride were found within the acidic pocket of ASIC1a after co-crystallization, suggesting the possibility that binding of amiloride to the acidic pocket could also be involved in its paradoxical activating effect (Baconguis et al., 2014; Yu et al., 2011).

A set of small molecules inspired from amiloride and containing a guanidinium group and a heterocyclic ring, has been also shown to activate/modulate ASIC3 channels (Yu et al., 2010), among which the synthetic GMQ (2-guanidine-4-methylquinazoline, EC_{50} ~ 1 mM,). GMQ is able to generate a current at neutral pH that displays little or no desensitization. The binding site of GMQ is distinct from the site involved in pH-sensing (Yu et al., 2010, 2011) (Fig. 1), although the nature of this non-proton binding site is still a matter of debate (Alijevic and Kellenberger, 2012), and GMQ induces an acidic shift of the pH dependence of inactivation of ASIC1a, ASIC1b, ASIC2a, and ASIC3 channels. It also affects the pH dependence of activation of ASIC3 channels (alkaline shift) and of ASIC1a and ASIC1b channels (acidic shift). As a consequence, GMQ creates a window current at pH 7.4 in ASIC3, but is not able to activate the other channel subtypes (Alijevic and Kellenberger, 2012). The ASIC3 channel activation was shown to be regulated by a dynamic interplay between GMQ and extracellular protons and Ca^{2+} (Yu et al., 2010). Recently GMQ was also shown to inhibit $GABA_A$ ligand-gated channels with an IC_{50} of 0.4 μM (Xiao et al., 2013).

2.1.2. A-317567

A-317567 is a small molecule unrelated to amiloride, described to be more selective than amiloride, but however a non-discriminative inhibitor of ASIC currents (IC_{50} 2–30 μM) in sensory and central neurons (Coryell et al., 2009; Dube et al., 2005). The amidine moiety of A-317567 is critical for the inhibitory effect on ASIC3 current. Analog inhibits recombinant human ASIC3 and ASIC1a currents with a better potency (IC_{50} ~ 356 and 450 nM, respectively) (Kuduk et al., 2010). Unlike amiloride, A-317567 blocks both the fast and sustained phases of the ASIC3 current with equal potency. However, this compound also interacts (IC_{50} < 10 μM) with a number of neurotransmitter receptors, suggesting possible off-target effects if used *in vivo* (Kuduk et al., 2010).

2.1.3. Non-steroid anti-inflammatory drugs

Besides their well-known ability to inhibit cyclooxygenases, non-steroid anti-inflammatory drugs (NSAIDs) also directly inhibit the activity of both ASIC1a and ASIC3 channels (Voilley,

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