Neuropharmacology 101 (2016) 549–565

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

A single polycystic kidney disease 2-like 1 channel opening acts as a spike generator in cerebrospinal fluid-contacting neurons of adult mouse brainstem

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ARTICLE INFO

Article history:
Received 24 April 2015
Received in revised form
3 July 2015
Accepted 24 July 2015
Available online 26 July 2015

CHEMICAL COMPOUNDS:
Acetylcholine (PubChem CID: 187)
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (PubChem CID: 158397)
DL-2-amino-5-phosphonopentanoic acid sodium salt (AP-V) (PubChem CID: 1216)
6,7-dinitroquinoxaline-2,3-dione disodium salt (DNQX) (PubChem CID: 45073428)
Gama-Amino butyric acid (GABA) (PubChem CID: 119)
Gabazine (SR-95531) (PubChem CID: 107896)
Glutamate (PubChem CID: 33032)
Kainate (PubChem CID: 22880)
N-methyl-D-aspartic acid (NMDA) (PubChem CID: 22880)
D-Tubocurarine (D-TC) (PubChem CID: 16000)

Keywords:
Cerebrospinal fluid contacting neurons
Sensory
Alkalinization
PKD2L1
ASIC
AMPA
Kainate
nAChRs
Brainstem

ABSTRACT

Cerebrospinal fluid contacting neurons (CSF-cNs) are found around the central canal of all vertebrates. They present a typical morphology, with a single dendrite that projects into the cavity and ends in the CSF with a protuberance. These anatomical features have led to the suggestion that CSF-cNs might have sensory functions, either by sensing CSF movement or composition, but the physiological mechanisms for any such role are unknown. This hypothesis was recently supported by the demonstration that in several vertebrate species medullo-spinal CSF-cNs selectively express Polycystic Kidney Disease 2-Like 1 proteins (PKD2L1). PKD2L1 are members of the 'transient receptor potential (TRP)' superfamily, form non-selective cationic channels of high conductance, are regulated by various stimuli including protons and are therefore suggested to act as sensory receptors.

Using patch-clamp whole-cell recordings of CSF-cNs in brainstem slices obtained from wild type and mutant PKD2L1 mice, we demonstrate that spontaneously active unitary currents in CSF-cNs are due to PKD2L1 channels that are capable, with a single opening, of triggering action potentials. Thus PKD2L1 might contribute to the setting of CSF-cN spiking activity. We also reveal that CSF-cNs have the capacity of discriminating between alkalinization and acidification following activation of specific conductances (PKD2L1 *vs.* ASIC) generating specific responses. Altogether, this study reinforces the idea that CSF-cNs represent sensory neurons intrinsic to the central nervous system and suggests a role for PKD2L1 channels as spike generators.

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http://dx.doi.org/10.1016/j.neuropharm.2015.07.030
0028-3908/

1. Introduction

Medullo-spinal cerebrospinal fluid contacting neurons (CSF-cNs) are present around the ependymal layer along the central canal from the *filum terminale* to the brainstem of all vertebrates examined so far (Vigh et al., 2004). CSF-cNs are GABAergic neurons and have a characteristic morphology with a unique dendrite that ends in the CSF with a ciliated protuberance (Vigh et al., 1983; Shimosegawa et al., 1986; Bruni and Reddy, 1987; Dale et al., 1987; Stoeckel et al., 2003; Orts-Del'Immagine et al., 2014). Although, little information is available regarding their function(s), CSF-cNs were suggested to have sensory functions by sensing either CSF movement within the central canal or variations in its composition, notably its pH (Huang et al., 2006). To support this hypothesis, recent data demonstrated that the Polycystic Kidney Disease 2-Like1 protein (PKD2L1), a channel with putative sensory functions (Nauli et al., 2003; Huang et al., 2006; Shimizu et al., 2009), represented a selective marker for medullo-spinal CSF-cNs in several vertebrate species (Djenoune et al., 2014; Orts-Del'Immagine et al., 2014).

PKD2L1 (or TRPP3 and initially named Polycystin-L) is a member of the polycystin family of TRP proteins (Clapham et al., 2010). Mutations of *pkd1* and *pkd2* genes coding for, polycystin-1 and polycystin-2, respectively, account for almost all cases of autosomal dominant polycystic kidney disease (ADPKD), the most common form of inherited polycystic kidney disease (Harris and Torres, 2014). *pkd2l1* and *pkd2l2* are two homologs of the *pkd2* gene identified so far (Veldhuisen et al., 1999; Wu et al., 1998) but are unlikely to be directly linked to ADPKD (Basora et al., 2002; Nomura et al., 1998). Although the functional role of PKD2L1 is still unclear, it was suggested that PKD2L1 might be involved in sensory physiology. Indeed, PKD2L1 has a wide expression pattern especially in several brain nuclei and sensory organs such as retina, taste bud and inner ear (Basora et al., 2002; Huang et al., 2006; Ishimaru et al., 2006; LopezJimenez et al., 2006; Cuajungco et al., 2007; Li et al., 2007; Takumida and Anniko, 2010). Second, in expression systems, PKD2L1 forms a non-selective cationic channel of high conductance and was shown to be regulated by several stimuli such as: voltage, calcium (Chen et al., 1999; Liu et al., 2002), protons (Shimizu et al., 2011), extracellular osmolarity (Shimizu et al., 2009) and temperature (Higuchi et al., 2014). Finally, the levels of channel insertion in the plasma membrane as well as its functional properties were shown to depend on its association with other proteins in particular of the polycystin 1 type (Inada et al., 2008; Ishii et al., 2009; DeCaen et al., 2013; Delling et al., 2013).

In murine medullar CSF-cNs, we recently reported the presence of a spontaneously active unitary current bearing all the functional properties of PKD2L1 currents and we suggested that its activation could modulate CSF-cN excitability (Orts-Del'immagine et al., 2012). Nevertheless, the nature of the channels expressed in CSF-cNs could not be definitively demonstrated because of the lack of a selective blocker for PKD2L1 channels.

Here, using patch-clamp recording techniques on brainstem slices prepared from PKD2L1 mice lacking the channel and their wild type littermates (Horio et al., 2011), we demonstrate that functional PKD2L1 channels are indeed expressed in CSF-cNs. They are capable, at a single channel level, to generate a depolarization large enough to trigger action potentials and would act as spike generator. They play a role in the setting of basal excitability and in sensing extracellular variations in pH. Finally, and because of the lack of any excitatory synaptic entries, PKD2L1 appears to represent an important excitatory input to these peculiar neurons.

2. Materials and methods

2.1. Animals

PKD2L1$^{+/+}$ (wild type) and PKD2L1$^{-/-}$ (mutant) mice were obtained by breeding heterozygous PKD2L1$^{+/-}$ mice (B6.Cg-Pkd2l1tm1.1^{Yuni}/J; http://jaxmice.jax.org/strain/016853.html and Horio et al., 2011) while PKD2L1:EGFP transgenic mice were obtained by crossing PKD2L1-IRES-Cre with Z/EG reporter transgenic mice (Huang et al., 2006; Orts-Del'immagine et al., 2012). All animals were housed at constant temperature (21 °C) under a standard 12 h light–12 h dark cycle, with food (pellet AO4, UAR, Villemoisson-sur-Orge, France) and water provided *ad libitum*. Experiments were conducted in conformity with the rules set by the EC Council Directive (2010/63/UE) and the French "Direction Départementale de la Protection des Populations des Bouches-du-Rhône" (licence N°13.435 held by JT and N°13.430 by NW). Every precaution was taken to minimize animal stress as well as the number of animals used. All our experiments were conducted on animals whose genotype was determined following PCR on DNA extracted from the tail to asses EGFP and CRE expression or PKD2L1$^{+/+}$ (wild type) and PKD2L1$^{-/-}$ genotype (see Table 1 for details). The breeding of heterozygous PKD2L1$^{+/-}$ mice also

Table 1
Summary of the experimental procedure used for PCR on tail genomic DNA. Table indicating the sequence of the sense and antisense primers used in our PCR experiments to determine the genotype of PKD2L1$^{+/+}$ (wild type) and PKD2L1$^{-/-}$ mice (mutant see B6.Cg-Pkd2l1tm1.1^{Yuni}/J; http://jaxmice.jax.org/strain/016853.html and Horio et al., 2011) as well as of the PKD2L1:EGFP transgenic mice. The molecular weight of the different amplicons (Amplicon size) is indicated as well as the experimental parameters for each set of PCR reaction. PCR reaction for PKD2L1 animals were adapted from the protocol published by the Jackson Laboratories (http://jaxmice.jax.org/strain/016853.html).

GENE	Sense primers sequence	Anti-sense primers sequence
CRE	5′-CGT ACT GAC GGT GGG AGA AT-3′	5′-CCC GGC AAA ACA GGT AGT TA-3′
eGFP	5′-GCC ACA AGT TCA GCG TGT CC-3′	5′-GCT TCT CGT TGG GGT CTT TGC-3′
PKD2L1	5′-GAT CTG CAA TGC AAT GAA CC-3′	Wild type: 5′-GAC CCT CTG CCT TGT GTC TC-3′ Mutant: 5′-ACA CCG GCC TTA TTC CAA G-3′

	CRE	eGFP	PKD2L1
Amplicon size	166 bp	573 bp	Wild type: 725 bp Mutant: 575 bp
PCR reaction	Initial denaturation (5 min, 95 °C), Denaturation (30 s, 95 °C), Primers hybridization (30 s, 62 °C), Elongation (30 s, 72 °C), Final elongation (7 min, 72 °C), Number of Cycles: 36	Initial denaturation (5 min, 95 °C), Denaturation (30 s, 95 °C), Primers hybridization (30 s, 64 °C), Elongation (45 s, 72 °C), Final elongation (7 min, 72 °C), Number of Cycles: 36	Initial denaturation (5 min, 95 °C), Denaturation (30 s, 95 °C), Primers hybridization (30 s, 62 °C), Elongation (45 s, 72 °C), Final elongation (7 min, 72 °C), Number of Cycles: 36