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Differential expression of two-pore domain potassium channels in rat cerebellar granule neurons

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ABSTRACT

Two pore domain potassium (K2P) channels are mostly present in the central nervous system (CNS) where they play important roles in modulating neuronal excitability. K2P channels give rise to background K^+ currents (IK_{SO}) a key component in setting and maintaining the resting membrane potential in excitable cells. Here, we studied the expression and relative abundances of K2P channels in cerebellar granule neurons (CGNs), combining molecular biology, electrophysiology and immunologic techniques. The CGN IK_{SO} was very sensitive to external pH, as previously reported. Quantitative determination of mRNA expression level demonstrated the existence of an accumulation pattern of transcripts in CGN that encode K2P9 > K2P1 > K2P3 > K2P18 > K2P2 = K2P10 > K2P4 > K2P5 subunits. The presence of the major K2P subunits expressed was then confirmed by Western blot and immunofluorescence analysis, demonstrating robust expression of K2P1 (TWIK-1), K2P3 (TASK-1), K2P9 (TASK-3) and K2P18 (TRESK) channel protein. Based, on these results, it is concluded that K2P1, -3, -9 and -18 subunits represent the majority component of IK_{SO} current in CGN.

1. Introduction

Two pore domain potassium channels (K2P) give rise to the leak or background potassium currents which are voltage independent and constitutively active in many excitable cells. In mammals, K2P channels are formed from 15 different subunits, which are divided in six subfamilies based on the structural and functional properties [10,17,19]. Structurally, each K2P subunit contains four transmembrane domains and two pore forming domains in tandem [5,18,22]. A functional channel is composed of two identical (homomeric) or different subunits (heterodimeric), that each imparts different functional properties to the channel [1,6,15,26,30]. The heterodimeric configuration of K2P channels increases the functional diversity thereby conferring versatility and dynamic adaptation to fulfill the physiological roles which are implicated [26].

K2P channels are highly regulated by several molecules or stimuli such as kinases, phosphatases, lipids, G proteins, internal and external pH [9,19]. Extracellular pH modulates K2P channels by acting on the upper gate [16]. At the molecular level, the mechanism that confer pH sensitivity has been identified for several members of the K2P family [16]. In the case of the pH-sensitive channels K2P1 (also called TWIK-1 or KCNK1), K2P3 (also called TASK-1 or KCNK3) and K2P9 (also called TASK-3 or KCNK9), acidification blocks the pore of the channel by protonating a histidine at position 98 for K2P3 and 9 and 122 for K2P1. These histidine residues are located adjacent to the potassium selectivity sequence in the first pore forming loop of each subunit of the channel [23,27,29].

In neurons, where K2P channels are highly expressed, the currents generated by these proteins have been well characterized and are known as IK_{SO} (for standing outward potassium current) [21,32]. The IK_{SO} current is strongly modulated by extracellular pH [12,15,21]. And the inhibition of IK_{SO} by acidosis has been associated with an increased of excitability [24,25].

CGNs are glutamatergic interneurons that provide an excitatory input in the molecular layer of the cerebellum. The IK_{SO} in these cells has been correlated with the expression of four K2P genes: K2P1, K2P10 (TREK-2, KCNK10), K2P3 and K2P9 [1,4,11,12,14,21,26,28,31]. Of these subunits, K2P3, K2P9 and K2P1 can also combine to form heterodimeric channels [1,6,15,26,30]. Furthermore, the regulation of K2P1, K2P3 and

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K2P9 heterodimers has been recently studied in CGNs [26]. However, at present, the full contribution of the different K2P channels in determining the resting membrane potential in CGNs remains unknown.

The aim of our study was to explore the K2P channel contribution to IK_{SO} currents using molecular biology, electrophysiology and immunological approaches. We present evidence that K2P1, -3, -9 and -18 subunits contribute the majority of the IK_{SO} in rat CGN.

2. Materials and methods

2.1. Cerebellar granule neurons cultures

CGNs dissociated from 7 to 8 day-old Sprague-Dawley rat cerebellum were isolated as previously described [2]. At the end of the isolation procedure, cerebella were triturated and the dissociated neurons plated onto glass coverslips coated with poly-L-lysine (1 μM/ml) at a density of 2.5×10^5 cells/cm^2. Cultured cells were incubated at 37 °C in a 5% CO_2 in DMEM medium supplemented with 10% fetal calf serum, 5 mM glutamine, 39 mM glucose, 25 mM KCl, and 1% antibiotic (Penicillin/Streptomycin). The medium was renewed every 4 days. All experiments were carried out using CGN cultured for 7–8 days. The experimental procedures were approved by our Institutional Bioethical and Biosafety Committee and by the local government bioethics advisory committee (Fondecyt–Conicyt).

2.2. Extraction and quantification of mRNA from CGN neurons for PCR assays

Total RNA from CGN neurons was extracted from cell cultures using TRIzol Reagent (Life technologies). The RNA quality and integrity were evaluated by spectrophotometric analysis (OD 260/280) and visualized by agarose gel electrophoresis. First-strand cDNA was primed with oligo(dT) from 1 μg of RNA and synthesized using the RevertAid First Strand cDNA Synthesis Kit (Fermentas) at 42 °C for 50 min. Conventional PCR experiments were performed using a reaction mixture consisting of 0.75 U Taq polymerase (Fermentas), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 50 pmol of each primer. The following PCR protocol was used: 15 min at 95 °C, 35 cycles: 15 s at 95 °C, 1 min at 58 °C, 1 min at 72 °C, 7 min at 72 °C, using a LifePro Thermal Cycler (Bioer Technology). The PCR amplification product was visualized by agarose gel electrophoresis (1%) containing ethidium bromide and the images were digitally acquired.

Real-time PCR was performed using the KAPA SYBR® FAST UNIVERSAL kit (Kapa Biosystems). The cDNA was added to a 20 μl well containing 12.5 μl 2 × buffer, 0.5 μl of 50 × SYBR Green master mix and 5 pmol K2P specific primers, described previously [20] or designed (Table 1). The real-time PCR conditions were: a cycle of 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, 60 °C for 15 s, 72 °C for 20 s and a final cycle at 95 °C for 1 min, 55 °C for 30 s, 95 °C for 30 s. These assays were performed in triplicate with Mx 3000P Agilent-Stratagene thermal cycler and analyzed with MxPro qPCR software (Agilent technologies). All primer pairs were tested and the efficacies evaluated (Those giving 90–100% were selected). Additionally, gel electrophoresis and melting curve analyses, were done to confirm the specific PCR product sizes and the absence of non-specific bands. The expression of each gene was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.3. Protein extraction and Western blotting

K2P channel protein levels in CGN neurons were assessed using Western blot analysis. Briefly, cultured neurons grown in 35 mm plates were lysed using a RIPA lysis buffer supplemented with inhibitors of proteases and phosphatases. The whole cell lysates were stored at −20 °C until the Western blot analysis.

For Western blot assays, 5−25 μg of protein was loaded on to a 10% SDS–polyacrylamide gel and separated by electrophoresis. Proteins were then transferred onto nitrocellulose membranes (Thermo Scientific, USA) and incubated with primary antibodies against K2P1, K2P3, K2P9 and K2P18 channels at the proper dilution. Then the membranes were incubated with the appropriate peroxidase-conjugated secondary antibody and the protein band was visualized using ECL Plus Kit and a hyper film MP (GE Healthcare). The following antibodies were used: anti K2P1 (sc-11481), K2P3 (sc-32067), K2P9 (sc-11317) and K2P18 (sc-51240) and anti-β-adaptin (sc-10762), used as control. All antibodies were obtained from Santa Cruz biotechnology, Inc.

Table 1
Genes and primer sets used for quantitative real time RT-PCR analysis of K2P K^+ channels.

Gene	Common name	GenBank accession	Abbreviation	Primer pair, sense (5′–3′)	Product size (bp)
Kcnk1	K2P1, TWIK-1	NM_021688.3	qRnP1_F qRnP1_R	5′-CTC AGC AAC GCC TCG GGG AAT-3′ 5′-TGA ACG GGA TGC CAA TGA CAG AG-3′	157
Kcnk2	K2P2, TREK-1	NM_172041.2	qRnP2_F qRnP2_R	*5′-GTG GAG GAC ACA TTT ATT AAG T-3′ *5′-GAA GAG GAC ACA GCC AAA CA-3′	93
Kcnk3	K2P3, TASK-1	NM_033376.1	qRnP3_F qRnP3_R	*5′-TCA TCA CCA CAA TCG GCT AT-3′ *5′-AGC GCG TAG AAC ATG CAG AA-3′	76
Kcnk4	K2P4, TRAAK	NM_053804.2	qRnP4_F qRnP4_R	*5′-TGT AGG CTT TGG CGA TTA TGT-3′ *5′-TGA GGC CAC CCA TCT CT-3′	179
Kcnk5	K2P5, TASK-2	NM_001039516.2	qRnP5_F qRnP5_R	*5′-CTA TTC CTT CAT CAC CAT CTC-3′ *5′-AGC CCC AGG TAG ATC CAA A-3′	120
Kcnk9	K2P9, TASK-3	NM_053405.2	qRnP9_F qRnP9_R	*5′-CCT TCT ACT TCG CTA TCA C-3′ *5′-CCA GCG TCA GAG GGA TAC-3′	120
Kcnk10	K2P10, TREK-2	NM_023096.2	qRnP10_F qRnP10_R	*5′-GCT GTC CTC AGT ATG ATT-3′ *5′-CTT TGA TCT CAC CCA CCT CTT-3′	76
Kcnk18	K2P18, TRESK	NM_001003820.1	qRnP18_F qRnP18_R	*5′-CTC ACT TCT TCC TCT TCT TCT C-3′ *5′-TAG CAA GGT AGC GAA ACC TCT-3′	148
Gapdh	GAPDH	NM_017008.4	qRnGAPDH_F qRnGAPDH_R	*5′-CGC ATC TTC TTG TGC AGT-3′ *5′-AAT GAA GGG GTC GTT GAT GG-3′	149

K2P, two-pore domain potassium channels; TWIK-1, tandem of P domains in Weak Inward rectifier K^+ channel; TREK-1 and TREK-2, TWIK-related K^+ channel; TRAAK, TWIK-related arachidonic acid-stimulated K^+ channel; TASK-1, TASK-2 and TASK-3, TWIK-related acid-sensitive K^+ channels; TRESK, TWIK-related spinal cord K^+ channel; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase. Asterisk (*) indicate primers described previously in Marsh et al. [20].

2.4. Immunocytochemistry

The cellular localization of K2P channels in CGN neurons was analyzed by immunofluorescence using a fluorescence microscope. Briefly, CGN neurons seeded on coverslips were fixed using 4% paraformaldehyde (PFA) for 10 min at room temperature, followed by permeabilization with 2% bovine serum albumin in PBS containing 0.1% Triton X-100 for 30 min. Cells were incubated with blocking buffer, followed by incubation with goat polyclonal anti-K2P antibodies overnight at 4 °C. The cells were then incubated with donkey polyclonal secondary antibody to goat IgG – H&L (Alexa Fluor® 594, ab150132; Abcam Inc.) at 1/1000 dilution for 1 h at room temperature. DAPI was used to stain the cell nuclei at a concentration of 0.1 μg/ml for 5 min. Images were digitally acquired with an Olympus BX53 fluorescence microscope (Olympus), coupled to a CCD camera. Digital images were taken using Image-Pro Plus software (Media Cybernetics). Each staining was repeated 3 times in 3 different cultures.

2.5. Electrophysiological recordings

Macroscopic currents elicited from cultured CGN neurons were evaluated using the whole-cell configuration (voltage clamp) of the patch-clamp recording technique with a PC-501A amplifier (Warner Instruments) as described in a previous study [33]. Voltage protocols and data acquisition were controlled by pCLAMP10 with an acquisition card (DigiData 1440, Molecular Devices). Membrane capacitive currents were electronically subtracted and series resistance compensated during on-line acquisition. Glass microelectrodes (3–5 MΩ) were made from borosilicate capillaries using a horizontal puller (P97 model, Sutter Instruments). The intracellular solution was composed of (in mM): 140 KCl, 0.5 $CaCl_2$, 5 EGTA, 10 Hepes, 2 K_2-ATP, 1 $MgCl_2$; adjusted to pH 7.4 with KOH. The extracellular solution contained (in mM): 120 NaCl, 4 KCl, 2 $MgCl_2$, 0.5 $CaCl_2$, 10 glucose, 10 Hepes; pH 6.4, 7.4 and 8.4 were adjusted with NaOH. To isolate K2P-mediated currents, the extracellular solution was supplemented with the Na^+ channel blocker tetrodotoxin (TTX) at 0.2 μM.

3. Results

3.1. Characterization of pH sensitive IK_{SO} currents in CGN

Extracellular pH changes, in a physiological range from pH8.4 to 6.4, were used to demonstrate the contribution of pH-sensitive K2P channels to IK_{SO} in cultured rat CGN, as previously reported [16]. IK_{SO} was evoked using a holding of −20 mV for 20 s followed by a ramp of 200 ms of duration to −120 mV (Fig. 1B, inset). The depolarized holding potential inactivates the majority of the voltage-gated conductances in the CGN and the ramp protocol is sufficiently slow to allow the membrane current to reach steady state at each membrane potential such that only the constitutively open channels pass currents that follow the ramp [21,32]. In agreement with the previous studies [1,12,15], IK_{SO} was strongly inhibited in presence of external pH of 6.4, with a decrease of 42.3 ± 5.4%, compared to currents studied in the same cell at pH

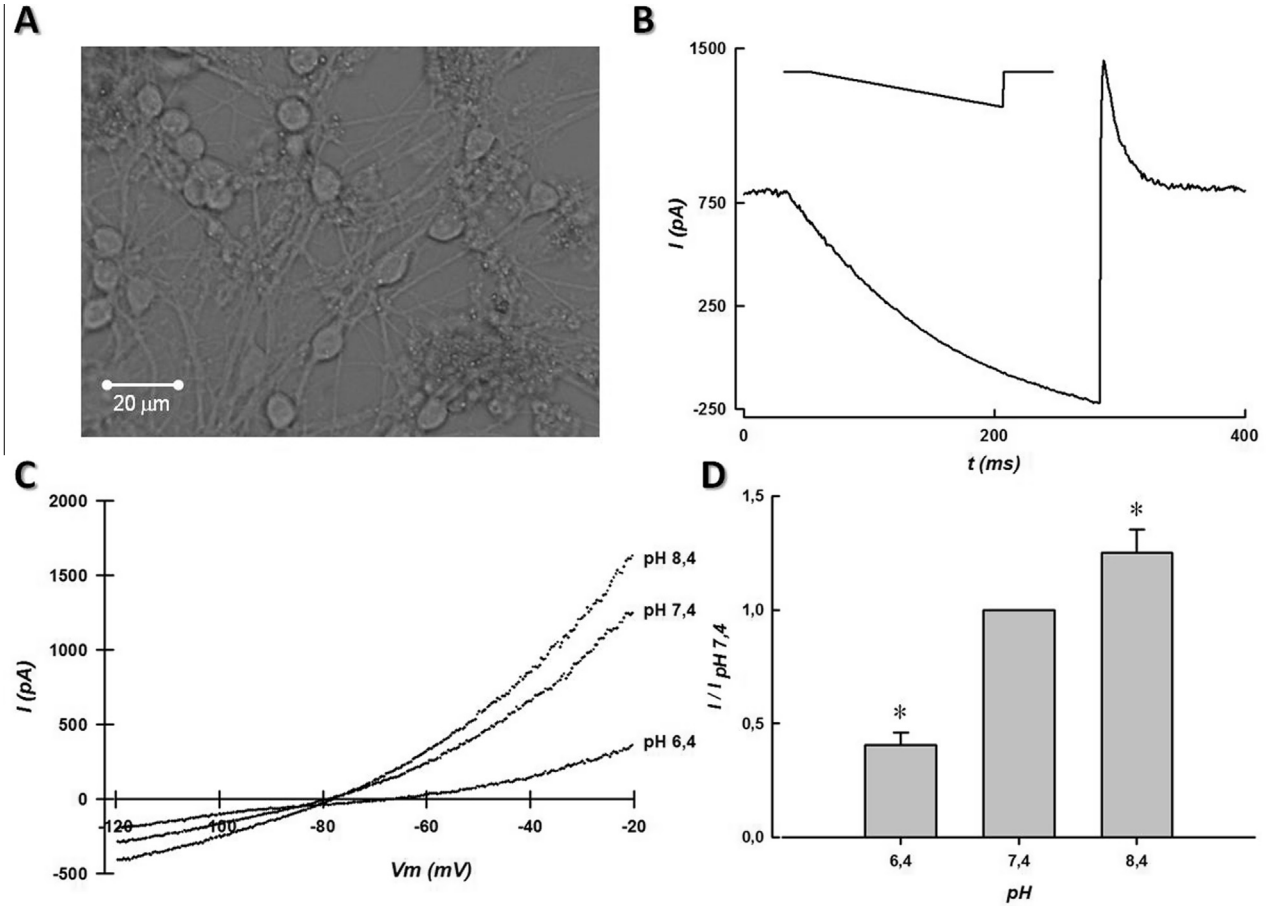

Fig. 1. The standing outward 'background' potassium current (IKso) in CGN. (A) An example of a CGN culture highlighting the bipolar morphology of these cells. (B) Whole-cell current studied using the ramp protocol shown in the inset. The cell membrane was ramped from −20 to −120 mV over 200 ms. (C) *I*/*V* curve relationship of the pH-sensitive component of IKso recorded at different extracellular pH solutions. (D) A bar graph shows the effect of extracellular pH on the whole-cell current measured at −20 mV. Each bar represents the mean ± SEM of five different experiments. *Significant difference from control value observed at pH 7.4 ($P < 0.05$).

7.4. In presence of an external alkaline pH (8.4), the opposite effect was observed. Thus, when the external pH was raised from 7.4 to 8.4 IKso increased by 18.2 ± 7.9% (Fig. 1C and D) (n = 5). The I–V relationship of the IK_{SO} pH-sensitive current was characterized by an outward rectification with an E_K (reversal potential for K^+) of −72.3 ± 2.4 mV at both pH 7.4 and 8.4 and −60.0 ± 4.2 mV for pH 6.4 (Fig. 1C; n = 5), as previously reported for rat CGN [21,25,26]. These results are consistent with those reported for the K2P3 (TASK-1), K2P9 (TASK-3) and K2P1 (TWIK-1) channel currents [23,27,29].

3.2. Expression of K2P channels transcripts in rat CGN

To identify the K2P subunits expressed in rat CGN cultures, which might be an important component in IK_{SO} currents, we evaluated the mRNA transcripts accumulation level by conventional RT-PCR and quantitative real-time PCR (qPCR). Here, we limited our studies to those K2P channels previously detected in CGN or the granule cell layer of the cerebellum and to which functional activity has been corroborated.

By conventional RT-PCR, strong amplification products of different K2P subunits were detected, principally K2P1, 18 (also known as TRESK, KCNK18), 9, and 3 whereas K2P2 and 5 (TASK-2, KCNK5) were only detected at low levels (Fig. 2A; n = 5). All the amplification products detected were of the correct theoretical predicted size (Table 1). Next, in order to determinate the K2P subunit expression on a more sensitive and quantitative level, mRNA transcripts were evaluated by real-time PCR. The mRNA expression level of each K2P transcript was compared with transcript levels for the GAPDH housekeeping gene. As shown in Fig. 2B, strong signals were detected for K2P1, 3, 9, and 18 with these isoforms representing 31.4 ± 0.2%, 5.4 ± 1.1%, 58.6 ± 2.2% and 3.1 ± 1.0% of the K2P transcripts respectively. K2P2, 4, 5 and 10 were less expressed and minimally detected (sum of 1.5%) (Fig. 2C). The declining order obtained for the K2P transcript accumulation (median values), was: K2P9 > K2P1 > K2P3 > K2P18 > K2P2 = K2P10 > K2P4 > K2P5.

Although the presence of mRNA detected by qPCR for the K2P channels, confirmed that K2P9, K2P1, K2P3 and K2P18 are the dominant transcripts present in cultured CGN, the levels of each protein might not correlate. To study this, K2P protein expression was evaluated by Western blot analysis.

3.3. K2P channels protein expression

To corroborate the major expression of the K2P mRNA transcripts in CGN, we performed a Western blot study (Fig. 3A). The four K2P subunits that represent the majority of the mRNA transcripts detected in the cells (K2P1, 3, 9 and 18), were studied using specifics antibodies to assess protein expression for each K2P subunit. As expected the K2P9 and K2P1 proteins were the most abundant subunits with a strong signals at ∼56 kDa, representing 32.7 ± 4.7% and 24.2 ± 1.4% of total amount of immunoreactive proteins detected, respectively (Fig. 3B). K2P3 and K2P18, also were detected with strong signals at approximately the same molecular weight, representing 19.8 ± 2.6% and 23.4 ± 3.1% of immunoreactive proteins detected, respectively (Fig. 3B; n = 4).

These results are in agreements with previous report regarding to the presence of K2P1, 3, and 9 .However, the fourth channel

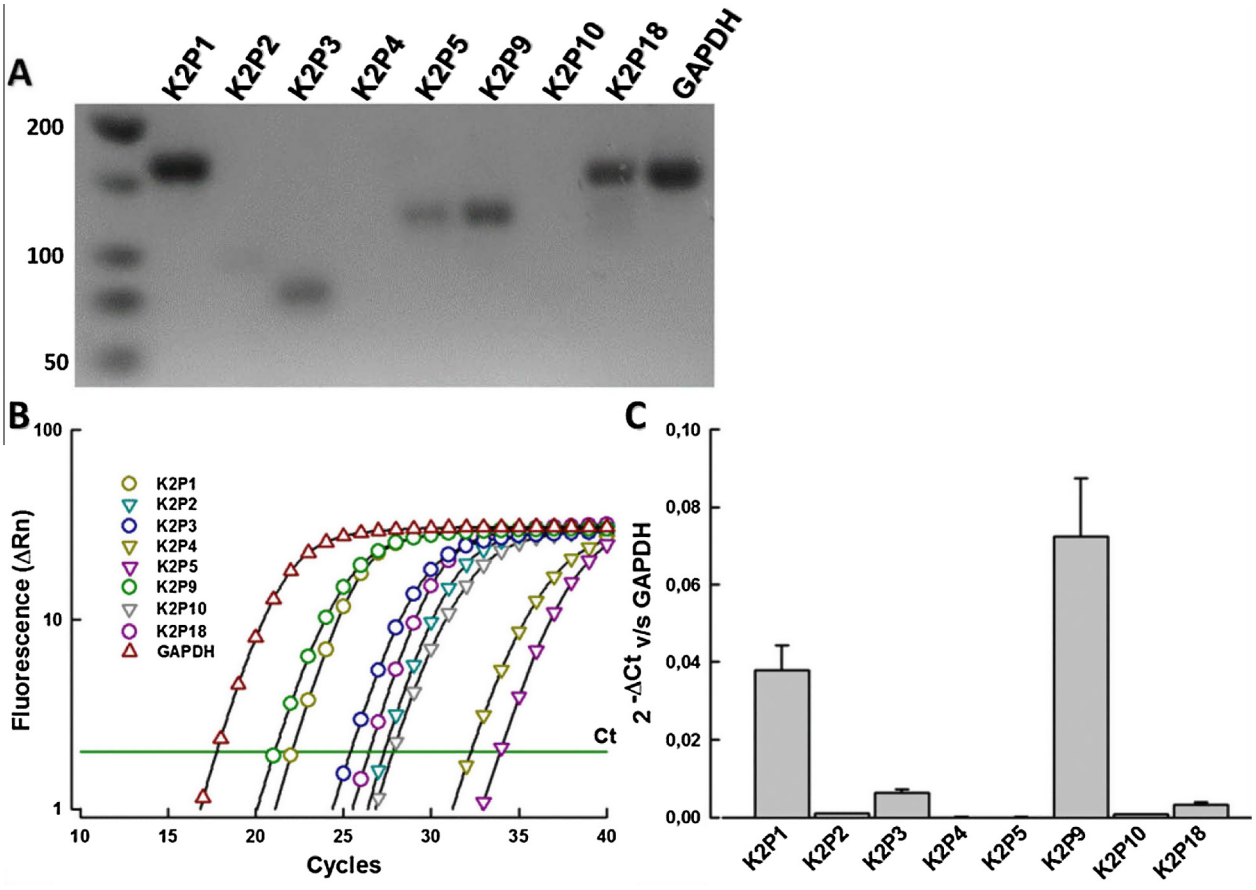

Fig. 2. Expression of K2P subunit transcripts in rat CGN revealed by RT-PCR and q-PCR. (A) Convectional RT-PCR analysis of K2P expression in comparison to GAPDH (n = 5). (B) Real-time PCR amplification plot (number of cycles plotted vs the fluorescence (ΔRn)). (C) A bar graph showing the quantities of each transcript relative to GAPDH expression in cerebella (n = 3). For quantitative analysis of K2P isoform mRNA transcripts (Panel C) GAPDH was used as housekeeping gene.

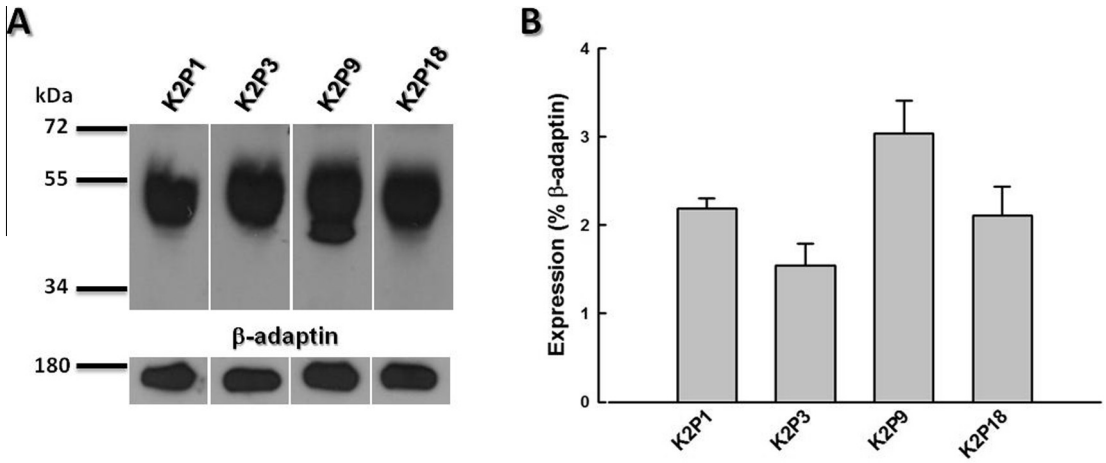

Fig. 3. Protein expression of K2P subunits in CGN cells. The presence of K2P subunits was confirmed in the CGN cells by Western blotting. (A) Representative immunoblots of K2P channels detected in total cells lysates. The positions and the MW's of the respective bands are indicated. (B) Relative abundance of K2P expression, expressed as the ratio of β-adaptin band which was used as protein loading control. Data are expressed as mean ± SEM of 4 Experiments.

detected in this study (K2P18), has only previously been identified in mouse cerebella tissue using conventional RT-PCR [7], and, the best of our knowledge, has not been previously proposed to contribute a significant fraction of IK_{SO} to cultured rat CGN.

3.4. Immunofluorescence of K2P channels in CGNs

Next, CGN were incubated with specific antibodies for K2P1, 3, 9 and 18 to study the expression pattern for each subunit in intact cells. Robust, positive staining was detected for each channel (Fig. 4A, C, E and G), and exhibited a localization pattern expected for membrane proteins. No signal was observed when the primary antibody was omitted for each channel tested (Fig. 4B, D, F and H), respectively. The staining detected for K2P1, K2P3 and K2P9 was consistent with the previously reported [26]. The cell nuclei were detected by DAPI staining (blue color).

4. Discussion

The importance of background potassium currents to the excitability of neuronal cells was predicted over 70 years ago [8,13]. However, it was not until the late-1990s that K2P channels were first described and shown to pass potassium selective currents in excitable cells. Encoded by fifteen KCNK genes in humans, K2P channels have emerged as a key regulator of the resting membrane potential in excitable cells, determining the cellular input resistance and the shape of action potentials. Because K2P channels have distinct physiological and pharmacological properties, the role of these proteins in determining the electrical properties of specific subsets of neurons is predicated on determining which of the different K2P subunits are expressed in particular cells.

Here, we present evidence for the functional activity, quantitative mRNA transcript accumulation and immunolocalization of different K2P channels subunits expressed in primary cultures of rat cerebellar granule neurons. The functional characterization of the IK_{SO} current passed by these channels revealed a pH-sensitive component of consistent with the expression of K2P1 (TWIK-1), K2P3 (TASK-1) and K2P9 (TASK-3) [23,27,29]. Furthermore, we show that mRNA transcripts for four K2P subunits are prevalent in the cells. Thus, K2P9 representing the 58.6%, K2P1 31.4%, K2P3 5.4% and K2P18 3.1% of the K2P transcripts, normalized with a housekeeping gene (GAPDH). Accordingly, the mRNA expression profile suggests that the channels expressed are K2P9 > K2P1 > K2P3 > K2P18. These results are in line with those previously reported, where K2Ps 9, 3 and 1 has been associated to IK_{SO} currents in CGN cells [26].

K2P9 (also called TASK-3 or KCNK9), was previously identified as the principal component of the IK_{SO} current by several different groups. This channel was detected by *in situ* hybridization in mice and rat models [1,4,14]. K2P9 was also identified, in a quantitative level, by qPCR [15]. Consequently, these results were corroborated by KO mice model where the CGN were depolarized and a small current injection is able to generate action potentials [1,3]. And recently, Plant et al. [26], shows that K2P9 subunits are an important component of IK_{SO} forming both homomeric channels and heterodimers with K2P1 or K2P3.

The second dominant channel identified, K2P1, has been omitted in studies reported by several groups, independently of the strong initially evidence that the channel is expressed in cerebellar tissue [1,4] and single-channel analysis in CGN that identified an unknown 'type IV ion channel' conductance of 30 ± 2 pS at −60 mV [12] which is well correlated with the conductance reported for K2P1 [9].

The third channel, K2P3 (TASK-1), was also previously considered as a key, acid-sensitive component of IK_{SO}. The channel has been identified in cerebellar tissue by *in situ* hybridization [1,4,14], RT-PCR, immunofluorescence [21] and qPCR studies [15]. Nonetheless, the KO mice generated for K2P3, has no a significant effect over electrical properties of CGN cells [1]. These discrepancies would be explained by a compensation mechanism in which K2P9 can replace the functional activity of heteromeric K2P3/K2P9 channels [1].

The fourth K2P subunit identified in our experiments corresponds to K2P18 (also called TRESK). This channel has been identified only at the mRNA level using by conventional RT-PCR [7]. K2P18 has a single channel conductance of 13 pS at −60 mV [9], this conductance can be associated to the ion channel type 1 in CGN [12]. The type 1 conductance was previously associated with K2P3 however, this correlation was not confirmed and was suggested before K2P18 was cloned. We identified the presence of K2P18 using different lines of evidence which support this channel as a dominant component of the background K^+ current in CGN. Thus, we conducted Western blot analysis of the K2P subunits detected by mRNA accumulation analysis to confirm that the transcripts represent protein produced in the neuronal cells. Our results show that K2P9 protein was the most abundant (K2P9 > K2P1 = K2P18 > K2P3). However, the veracity with which

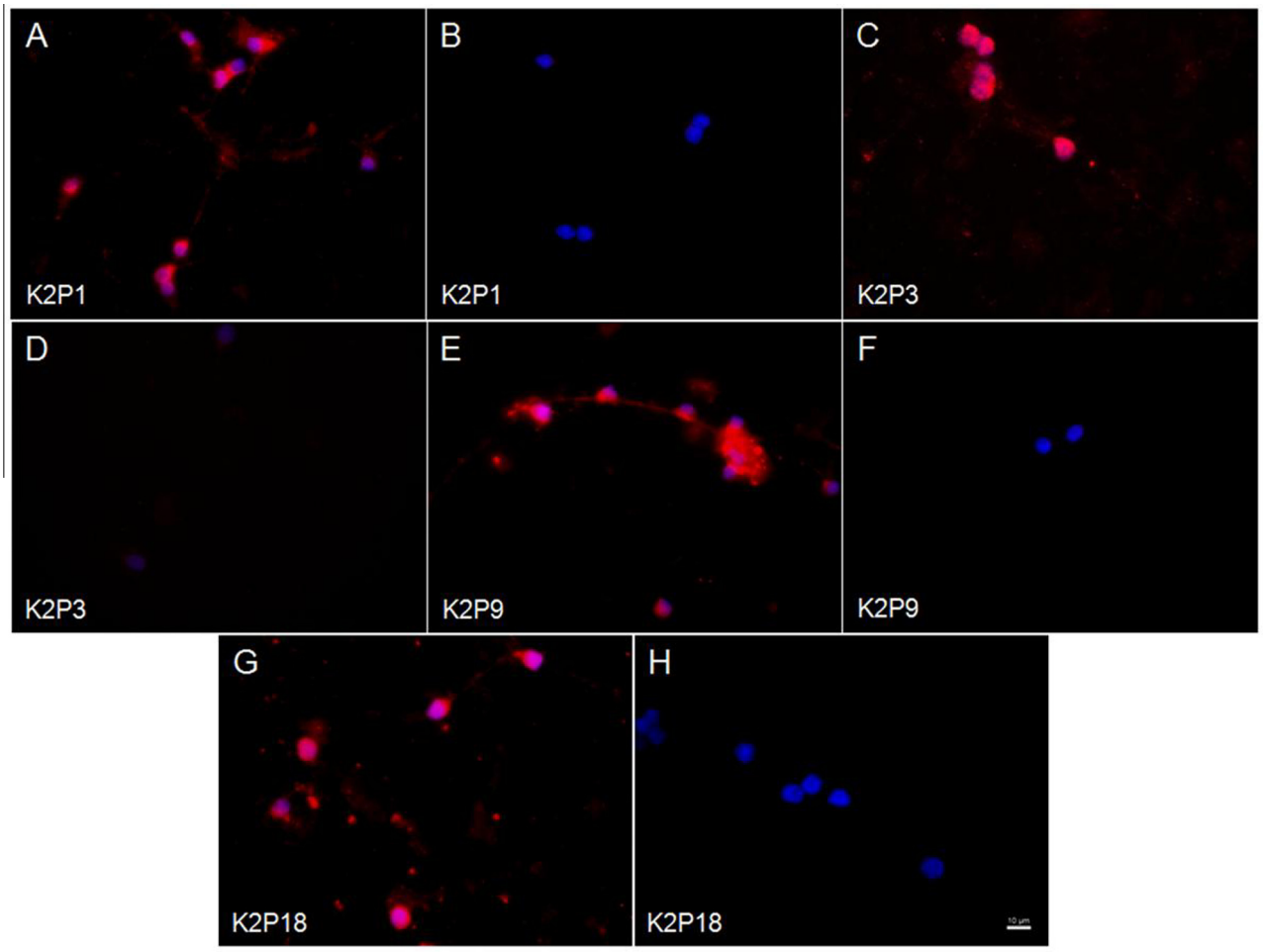

Fig. 4. Immunofluorescence of K2P channels in CGN cells. (A, C, E and G) Immunohistochemical localization of K2P1, 3, 9 and 18 proteins (red fluorescence), respectively. (B, D, F and H) Inmunostaining images for K2P subunits when the primary antibodies were omitted. DAPI was used for cell nuclei staining (blue fluorescence). The scale bar represents 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

protein levels can be quantified by Western blot analysis is limited because each subunit was studied using different primaries antibodies. Although Western blot analysis corroborate the presence of these proteins (K2P9, 1, 3 and 18) and the immunofluorescence revealed their localization pattern in CGN cells.

Acknowledgments

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References

[1] M.I. Aller, E.L. Veale, A.M. Linden, C. Sandu, M. Schwaninger, L.J. Evans, E.R. Korpi, A. Mathie, W. Wisden, S.G. Brickley, Modifying the subunit composition of TASK channels alters the modulation of a leak conductance in cerebellar granule neurons, J. Neurosci. 25 (2005) 11455–11467.
[2] P.M. Bilimoria, A. Bonni, Cultures of cerebellar granule neurons, CSH. Protoc. 2008 (2008). db.
[3] S.G. Brickley, M.I. Aller, C. Sandu, E.L. Veale, F.G. Alder, H. Sambi, A. Mathie, W. Wisden, TASK-3 two-pore domain potassium channels enable sustained high-frequency firing in cerebellar granule neurons, J. Neurosci. 27 (2007) 9329–9340.
[4] S.G. Brickley, V. Revilla, S.G. Cull-Candy, W. Wisden, M. Farrant, Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance, Nature 409 (2001) 88–92.
[5] S.G. Brohawn, M.J. del, R. MacKinnon, Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K+ ion channel, Science 335 (2012) 436–441.
[6] G. Czirjak, P. Enyedi, Formation of functional heterodimers between the TASK-1 and TASK-3 two-pore domain potassium channel subunits, J. Biol. Chem. 277 (2002) 5426–5432.
[7] G. Czirjak, Z.E. Toth, P. Enyedi, The two-pore domain K+ channel, TRESK, is activated by the cytoplasmic calcium signal through calcineurin, J. Biol. Chem. 279 (2004) 18550–18558.
[8] D.E. Goldman, Potential, impedance, and rectification in membranes, J. Gen. Physiol. 27 (1943) 37–60.
[9] S.A. Goldstein, D.A. Bayliss, D. Kim, F. Lesage, L.D. Plant, S. Rajan, International Union of Pharmacology. LV. Nomenclature and molecular relationships of two-P potassium channels, Pharmacol. Rev. 57 (2005) 527–540.
[10] S.A. Goldstein, D. Bockenhauer, I. O'Kelly, N. Zilberberg, Potassium leak channels and the KCNK family of two-P-domain subunits, Nat. Rev. Neurosci. 2 (2001) 175–184.
[11] W. Gu, G. Schlichthorl, J.R. Hirsch, H. Engels, C. Karschin, A. Karschin, C. Derst, O.K. Steinlein, J. Daut, Expression pattern and functional characteristics of two novel splice variants of the two-pore-domain potassium channel TREK-2, J. Physiol. 539 (2002) 657–668.
[12] J. Han, J. Truell, C. Gnatenco, D. Kim, Characterization of four types of background potassium channels in rat cerebellar granule neurons, J. Physiol. 542 (2002) 431–444.
[13] A.L. Hodgkin, B. Katz, The effect of sodium ions on the electrical activity of giant axon of the squid, J. Physiol. 108 (1949) 37–77.
[14] C. Karschin, E. Wischmeyer, R. Preisig-Muller, S. Rajan, C. Derst, K.H. Grzeschik, J. Daut, A. Karschin, Expression pattern in brain of TASK-1, TASK-3, and a tandem pore domain K(+) channel subunit, TASK-5, associated with the central auditory nervous system, Mol. Cell. Neurosci. 18 (2001) 632–648.
[15] I. Lauritzen, M. Zanzouri, E. Honore, F. Duprat, M.U. Ehrengruber, M. Lazdunski, A.J. Patel, K+-dependent cerebellar granule neuron apoptosis. Role of task leak K+ channels, J. Biol. Chem. 278 (2003) 32068–32076.
[16] F. Lesage, J. Barhanin, Molecular physiology of pH-sensitive background K(2P) channels, Physiology (Bethesda) 26 (2011) 424–437.

[17] F. Lesage, M. Lazdunski, Molecular and functional properties of two-pore-domain potassium channels, Am. J. Physiol. Renal Physiol. 279 (2000) F793–F801.
[18] C.M. Lopes, N. Zilberberg, S.A. Goldstein, Block of Kcnk3 by protons. Evidence that 2-P-domain potassium channel subunits function as homodimers, J. Biol. Chem. 276 (2001) 24449–24452.
[19] D.P. Lotshaw, Biophysical, pharmacological, and functional characteristics of cloned and native mammalian two-pore domain K+ channels, Cell Biochem. Biophys. 47 (2007) 209–256.
[20] B. Marsh, C. Acosta, L. Djouhri, S.N. Lawson, Leak K(+) channel mRNAs in dorsal root ganglia: relation to inflammation and spontaneous pain behaviour, Mol. Cell. Neurosci. 49 (2012) 375–386.
[21] J.A. Millar, L. Barratt, A.P. Southan, K.M. Page, R.E. Fyffe, B. Robertson, A. Mathie, A functional role for the two-pore domain potassium channel TASK-1 in cerebellar granule neurons, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 3614–3618.
[22] A.N. Miller, S.B. Long, Crystal structure of the human two-pore domain potassium channel K2P1, Science 335 (2012) 432–436.
[23] M.J. Morton, A.D. O'Connell, A. Sivaprasadarao, M. Hunter, Determinants of pH sensing in the two-pore domain K(+) channels TASK-1 and -2, Pflugers Arch. 445 (2003) 577–583.
[24] R.A. North, Potassium-channel closure taken to TASK, Trends Neurosci. 23 (2000) 234–235.
[25] L.D. Plant, P.J. Kemp, C. Peers, Z. Henderson, H.A. Pearson, Hypoxic depolarization of cerebellar granule neurons by specific inhibition of TASK-1, Stroke 33 (2002) 2324–2328.
[26] L.D. Plant, L. Zuniga, D. Araki, J.D. Marks, S.A. Goldstein, SUMOylation silences heterodimeric TASK potassium channels containing K2P1 subunits in cerebellar granule neurons, Sci. Signal. 5 (2012) ra84.
[27] S. Rajan, L.D. Plant, M.L. Rabin, M.H. Butler, S.A. Goldstein, Sumoylation silences the plasma membrane leak K+ channel K2P1, Cell 121 (2005) 37–47.
[28] S. Rajan, E. Wischmeyer, C. Karschin, R. Preisig-Muller, K.H. Grzeschik, J. Daut, A. Karschin, C. Derst, THIK-1 and THIK-2, a novel subfamily of tandem pore domain K+ channels, J. Biol. Chem. 276 (2001) 7302–7311.
[29] S. Rajan, E. Wischmeyer, L.G. Xin, R. Preisig-Muller, J. Daut, A. Karschin, C. Derst, TASK-3, a novel tandem pore domain acid-sensitive K+ channel. An extracellular histiding as pH sensor, J. Biol. Chem. 275 (2000) 16650–16657.
[30] E.M. Talley, D.A. Bayliss, Modulation of TASK-1 (Kcnk3) and TASK-3 (Kcnk9) potassium channels: volatile anesthetics and neurotransmitters share a molecular site of action, J. Biol. Chem. 277 (2002) 17733–17742.
[31] E.M. Talley, J.E. Sirois, Q. Lei, D.A. Bayliss, Two-pore-domain (KCNK) potassium channels: dynamic roles in neuronal function, Neuroscientist. 9 (2003) 46–56.
[32] C.S. Watkins, A. Mathie, A non-inactivating K+ current sensitive to muscarinic receptor activation in rat cultured cerebellar granule neurons, J. Physiol. 491 (Pt. 2) (1996) 401–412.
[33] L. Zuniga, V. Marquez, F.D. Gonzalez-Nilo, C. Chipot, L.P. Cid, F.V. Sepulveda, M.I. Niemeyer, Gating of a pH-sensitive K(2P) potassium channel by an electrostatic effect of basic sensor residues on the selectivity filter, PLoS ONE 6 (2011) e16141.