FEBS Letters 353 (1994) 37–42

FEBS 14620

Cloning provides evidence for a family of inward rectifier and G-protein coupled K^+ channels in the brain

Florian Lesage, Fabrice Duprat, Michel Fink, Eric Guillemare, Thierry Coppola, Michel Lazdunski*, Jean-Philippe Hugnot

Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France

Received 11 July 1994; revised version received 29 August 1994

Abstract MbIRK3, mbGIRK2 and mbGIRK3 K^+ channels cDNAs have been cloned from adult mouse brain. These cDNAs encode polypeptides of 445, 414 and 376 amino acids, respectively, which display the hallmarks of inward rectifier K^+ channels, i.e. two hydrophobic membrane-spanning domains M1 and M2 and a pore-forming domain H5. MbIRK3 shows around 65% amino acid identity with IRK1 and rbIRK2 and only 50% with ROMK1 and GIRK1. On the other hand, mbGIRK2 and mbGIRK3 are more similar to GIRK1 (60%) than to ROMK1 and IRK1 (50%). Northern blot analysis reveals that these three novel clones are mainly expressed in the brain. *Xenopus* oocytes injected with mbIRK3 and mbGIRK2 cRNAs display inward rectifier K^+-selective currents very similar to IRK1 and GIRK1, respectively. As expected from the sequence homology, mbGIRK2 cRNA directs the expression of G-protein coupled inward rectifyer K^+ channels which has been observed through their functional coupling with co-expressed δ-opioid receptors. These results provide the first evidence that the GIRK family, as the IRK family, is composed of multiple genes with members specifically expressed in the nervous system.

Key words: Inward rectifier K^+ channel; G protein effector; *Xenopus* oocyte

1. Introduction

K^+-selective channels represent the most diverse ion channel class known and numerous distinct members were identified by electrophysiology in the nervous system [1]. At the molecular level, the study of these channels has begun with the cloning of voltage-dependent K^+ channels. Following the original cloning of the *Drosophila Shaker* gene, more than 16 mammalian genes were isolated encoding A-type and delayed-rectifier outward K^+ channels (for reviews see [2–4]). More recently, expression cloning has led to the molecular identification of inwardly rectifying K^+ channels in plant [5,6] and mammals (for reviews see [7,8]). In contrast with the outward K^+ channels which display six putative membrane-spanning domains (S1 to S6), the mammalian inward rectifier K^+ channels including the mild rectifying ROMK1 [9] and the more rectifying IRK1 [10] and G-protein coupled GIRK1/KGA [11,12], have two potential transmembrane domains (M1 and M2). Despite primary sequence differences, both families display a well conserved domain (H5) which is believed to form the K^+-selective pore.

Molecular biology has helped to elucidate many of the mechanisms underlying the diversity of outward K^+ currents in the brain such as gene duplication, alternative splicing, heteropolymerisation, β subunits, density of expression, etc. (for reviews see [2–4,13–15]). In order to elucidate the possibility that brain inward rectifier potassium channels are encoded by a multigenic family like the outward channels, we have screened under low stringency conditions a mouse brain cDNA library with IRK1 and GIRK1 probes. cDNAs encoding three novel channels essentially expressed in brain were isolated and designated mbIRK3, mbGIRK2 and mbGIRK3. The electrophysiological properties of mbIRK3 and mbGIRK2 were examined using the *Xenopus* oocyte system.

2. Materials and methods

2.1. cDNA cloning and sequencing

An oligo(dT)-primed cDNA λZAPII library derived from poly(A)$^+$ RNA isolated from 20-day-old mouse brain (Stratagene) was screened sequentially with random primed ^{32}P-labeled DNA probes. The GIRK1 and IRK1 probes were amplified by polymerase chain reaction (PCR) using primer sequences corresponding to published sequences [10,11]. Filters were hybridized in 30% formamide, 5 × SSC, 4 × Denhardt's solution, 0.1% SDS, 100 μg denatured salmon sperm DNA at 42°C for 18 h. Filters were then washed stepwise to a final stringency of 0.5 × SSC, 0.3% SDS at 45°C. The positive λZAPIIs were converted to plasmid cDNAs by rescue excision (Stratagene) and the cDNA inserts were sequenced on both strand by the dideoxy nucleotide chain termination method by using the dye terminator kit and automatic sequencing (Applied Biosystems model 373A). Deletion clones for sequencing were prepared with the Erase-A-Base system according to the manufacturer's protocol (Promega).

2.2. RNA isolation and analysis

Poly(A)$^+$RNAs were isolated and blotted onto nylon membranes as previously described [16]. The blots were probed with *Eco*RI/*Xho*I inserts of pBlueScript-IRK3 (pBS-IRK3), pBS-GIRK2 and pBS-GIRK3 in 50% formamide, 5 × SSPE (0.9 M sodium chloride, 50 mM sodium phosphate (pH 7.4), 5 mM EDTA), 0.1% SDS, 5 × Denhardt's solution, 20 mM potassium phosphate (pH 6.5) and 250 μg denatured salmon sperm DNA at 55°C for 18 h and washed to a final stringency of 0.1 × SSC, 0.3% SDS at 65°C.

2.3. Synthesis of cRNA and functional expression in Xenopus ocytes

The coding sequence of mbIRK3, mbGIRK2 and mbGIRK3 were subcloned into the plasmid pBTG [17]. A DNA fragment corresponding to the coding sequence of the δ-opioid receptor was amplified by PCR and subcloned into pBS [18]. Capped cRNAs were synthetized using the T3 RNA polymerase (Stratagene). Preparation of oocytes, cRNA injections and electrophysiological measurements have been previously described [17]. The 98 mM K^+ solution was made by substituting NaCl with KCl in the normal ND96 solution. Similarly, a 2 mM K^+, sodium free solution, was obtained by substituting sodium with *N*-methyl-D-glucamine (NMDG). Both solutions were mixed in order to obtain solutions of intermediate K^+ concentrations. Deltorphin I was purchased from Neosystem.

*Corresponding author. Fax: (33) 93 95 77 04.

SSDI 0014-5793(94)01007-2

3. Results

3.1. Cloning and sequencing of inward rectifier potassium channels from brain

In order to isolate new brain inwardly-rectifying K^+ channel cDNAs, the coding sequences of GIRK1 and IRK1 channels [10–12] were amplified by RT-PCR from rat heart and mouse muscle, respectively, and used to probe a mouse brain cDNA library under low stringency conditions. The screening with the IRK1 probe resulted in the isolation of cDNAs of approximately 3 kb encoding a 445 amino acids polypeptide designated **mbIRK3**. This protein is almost identical to the K^+ channel recently characterized by Morishige et al. [19]. However nucleotidic differences are observed between the two sequences. This leads to the following changes at the protein level: W79C (within the M1 membrane spanning region), D86E, V96A, E105A, R239G, R444A. These divergences are likely to correspond to polymorphic variations among murine species (data not shown, genbank accession number U11075). cDNAs clones with different 5′-untranslated regions (UTR) were also isolated indicating the existence of alternative splicing from the mbIRK3 gene (data not shown).

The screening with the GIRK1 probe resulted in the isolation of two classes of cDNAs which were distinguished by restriction analysis. From the first group, we sequenced a cDNA of 2.7 kb (Fig. 1A). The predicted product consists of a 414 amino acids (aa) polypeptide designated **mbGIRK2** with a calculated molecular weight (MW) of 47,400 Da which is in good agreement with the MW of 48,000 Da obtained by SDS-PAGE analysis of in vitro translation product (data not shown). We isolated a second 2.7 kb cDNA with a 5′ sequence identical to mbGIRK2 and diverging in the 3′ part of the coding sequence. The predicted product presents a COOH terminus 11 aa longer than that of mbGIRK2 (manuscript in preparation). In the second group of cDNAs isolated by GIRK1 screening, four differents cDNAs of 1.5, 2.6, 2.65 and 3.1 kb were characterized. These sequences shared an identical open reading frame predicting a protein of 376 aa designated **mbGIRK3** (Fig. 1B). The calculated MW of 42,500 Da corresponds to the apparent MW of 44,000 Da of the in vitro translation product (data not

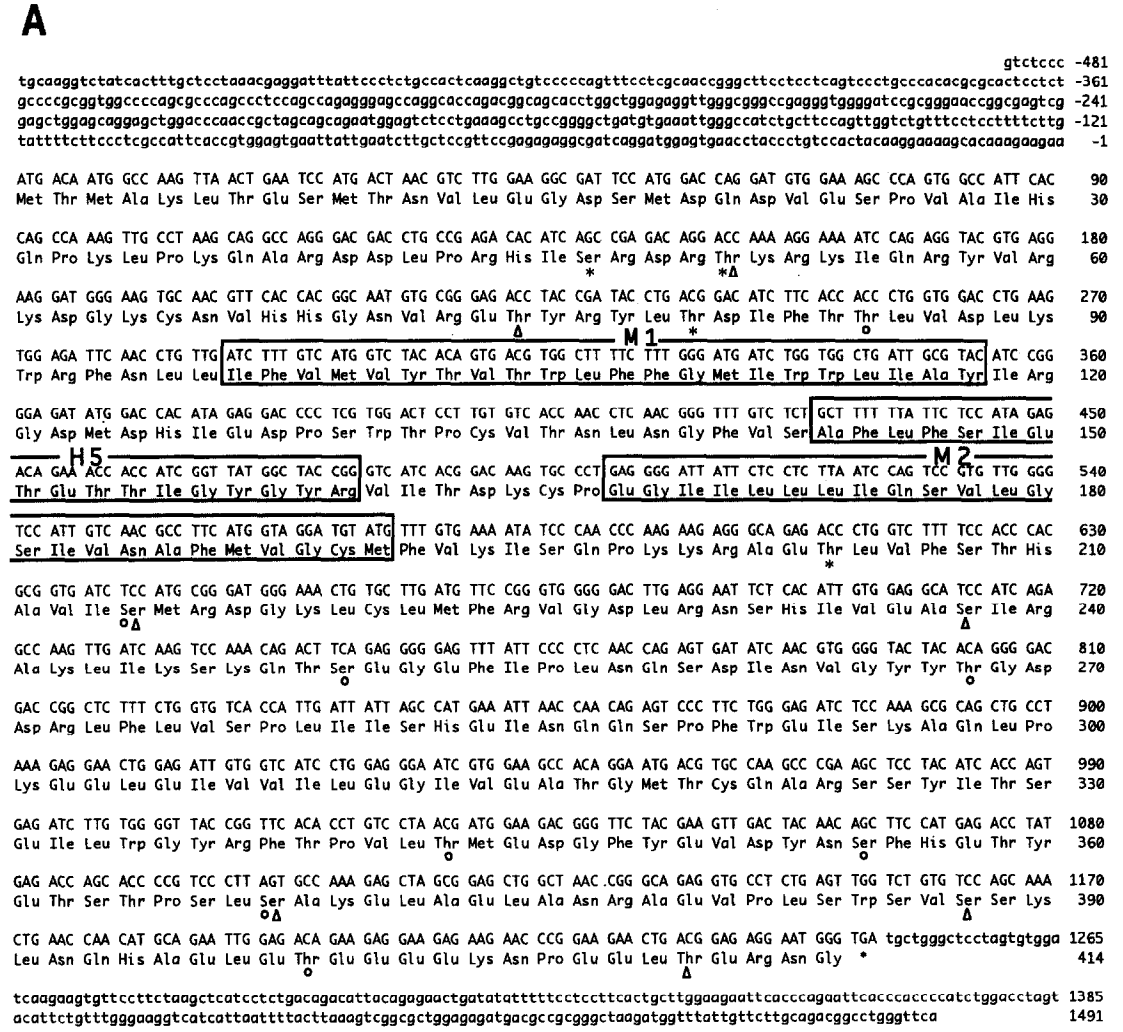

Fig. 1. Nucleotide and deduced amino acid sequences of mbGIRK2 (A) and mGIRK3 (B) cDNA. Nucleotide residues are numbered from the first nucleotide of the initiating ATG codon. Amino acids are numbered beginning with the initiating methionine. The non-sense codons TGA at the end of ORFs are marked by an asterix. The boxed areas delimit the two putative transmembrane domains M1 and M2 and the potential pore-forming domain H5. Putative protein kinase C (△), caseine kinase II (*) and Ca^{2+}-calmodulin kinase (○) phosphorylation sites are shown.

shown). The observed cDNA clones variety arised from alternative splicing in the 5′ UTR and from two polyadenylation sites (data not shown).

Alignment of the mouse brain IRK3, GIRK2 and GIRK3 proteins with the rat ROMK1 [9], GIRK1 [11] and IRK2 [20] and the mouse IRK1 [10] inward rectifier K^+ channels is reported on Fig. 2. All these channels present a common homologous core of approximatively 320 aa. This core contains the hallmarks of inwardly rectifying K^+ channels, i.e. two putative membrane-spanning domains M1 and M2 and a potential pore-forming region H5. Amino acid sequence identities determined from this homologous region indicate that the two novel channels mbGIRK2 and mbGIRK3 are more similar to GIRK1 (57.3–61% identity) than to ROMK1 (44.5–43%) and IRK1 (48.7–53%). On the other hand, mbIRK3 shares 61.5% and 70% identity with IRK1 and rbIRK2, respectively, and only 48% and 46% with GIRK1 and ROMK1, respectively. Amino and carboxyl terminus are homologous between members of the IRK subfamily. However, mbIRK3 has unique characteristics like a shorter N-terminus and additional sequences in the extra-cytoplasmic M1-H5 interdomain and in the C-terminus. No homology was found between the C- and the N-termini of members of the GIRK family except in the C-termini of mbGIRK2 and mbGIRK3. Based upon the topology proposed for these channels, several potential sites for phosphorylation by protein kinase C, Ca^{2+} calmoduline-dependent kinase and caseine kinase II are found in mbGIRK2 (Fig. 1A) and mbGIRK3 (Fig. 1B).

3.2. *Tissue distribution and developmental expression of IRK3 mRNA in the mouse brain*

Using DNA fragments corresponding to the sequence of mbIRK3, mbGIRK2 and mbGIRK3 cDNA clones, we investigated the tissue distribution of these channels by Northern blot analysis (Fig. 3). The mbIRK3 probe strongly hybridized to a transcript of 2.9 kb and less intensively to a transcript of approximatively 6.6 kb. MbIRK3 mRNA is highly expressed in the brain and only moderately in the skeletal muscle. No messenger was detected in the heart, lung, kidney or liver. The mbGIRK3 message is also highly expressed in brain RNA in

B

```
                                       ctgagctgccgttacattcaggagaaacagcagtgtcggcggctcccaatctcagagggaacctagg -241
gtactggggggagatggtgtcagggacatggacgccaacccccaagggtttctgctgctggctactcttctctccaggctctacttctgttcatacggtccatatctcctaggggaccctg -121
aaagcctaggaaccgactctggccatccatctctccgggaagattataacccagagtgcttctcaggggggaagaatttgaagcaaaaccagaccccgcaggatccccgctgcggccgcc   -1

ATG GCG CAG GAG AAC GCC GCT TTC TCT CCC GGG TCG GAG GAG CCG CCA CGC CGC CGC GGT CGC CAG CGC TAC GTG GAG AAG GAC GGT CGC   90
Met Ala Gln Glu Asn Ala Ala Phe Ser Pro Gly Ser Glu Glu Pro Pro Arg Arg Arg Gly Arg Gln Arg Tyr Val Glu Lys Asp Gly Arg   30

TGT AAC GTG CAG CAG GGC AAC GTC CGC GAG ACC TAC CGC TAC CTG ACC GAC CTG TTC ACC ACG CTG GTG GAC CTG CAG TGG CGC CTC AGA  180
Cys Asn Val Gln Gln Gly Asn Val Arg Glu Thr Tyr Arg Tyr Leu Thr Asp Leu Phe Thr Thr Leu Val Asp Leu Gln Trp Arg Leu Arg   60
                                         Δ    M1              *              o
CTG CTC TTC TTC GTG CTC GCC TAC GCG CTC ACT TGG CTC TTC TTC GGT GTC ATC TGG TGG CTC ATC GCC TAC GGT CGC GGC GAC CTG GAG  270
Leu Leu Phe Phe Val Leu Ala Tyr Ala Leu Thr Trp Leu Phe Phe Gly Val Ile Trp Trp Leu Ile Ala Tyr Gly Arg Gly Asp Leu Glu   90
                                                                                                              H5
CAC CTG GAG GAC ACC GCG TGG ACC CCG TGC GTC AAC AAC CTC AAC GGC TTC GTG GCC GCC TTC CTC TTC TCC ATC GAG ACG GAG ACC ACC  360
His Leu Glu Asp Thr Ala Trp Thr Pro Cys Val Asn Asn Leu Asn Gly Phe Val Ala Ala Phe Leu Phe Ser Ile Glu Thr Glu Thr Thr  120
                                                                                   M2
ATC GGC TAT GGG CAC CGC GTC ATC ACC GAC CAG TGT CCC GAG GGC ATC GTG CTG CTG CTG CTG CAG GCT ATC CTG GGC TCC ATG GTG AAC  450
Ile Gly Tyr Gly His Arg Val Ile Thr Asp Gln Cys Pro Glu Gly Ile Val Leu Leu Leu Leu Gln Ala Ile Leu Gly Ser Met Val Asn  150

GCT TTC ATG GTG GGC TGC ATG TTC GTC AAG ATC TCG CAG CCC AAC AAG CGC GCC GCC ACT CTC GTC TTC TCC TCG CAC GCC GTG GTG TCT  540
Ala Phe Met Val Gly Cys Met Phe Val Lys Ile Ser Gln Pro Asn Lys Arg Ala Ala Thr Leu Val Phe Ser Ser His Ala Val Val Ser  180
                                                                             *                                       Δ
CTG CGC GAC GGG CGC CTC TGT CTC ATG TTT CGC GTG GGC GAC CTG CGA TCC TCA CAC ATC GTC GAG GCC TCC ATC CGA GCC AAG CTC ATC  630
Leu Arg Asp Gly Arg Leu Cys Leu Met Phe Arg Val Gly Asp Leu Arg Ser Ser His Ile Val Glu Ala Ser Ile Arg Ala Lys Leu Ile  210
                                                                                         Δ
CGC TCC CGT CAG ACG CTC GAG GGC GAG TTC ATC CCT TTG CAC CAG ACC GAC CTC AGC GTG GGC TTT GAC ACG GGG GAC GAC CGC CTC TTT  720
Arg Ser Arg Gln Thr Leu Glu Gly Glu Phe Ile Pro Leu His Gln Thr Asp Leu Ser Val Gly Phe Asp Thr Gly Asp Asp Arg Leu Phe  240
                                                                                         o
CTC GTC TCA CCT CTC GTC ATC AGC CAC GAA ATC GAT GCC GCC AGC CCC TTC TGG GAG GCA TCG CGC CGC GCC CTC GAG AGG GAC GAC TTC  810
Leu Val Ser Pro Leu Val Ile Ser His Glu Ile Asp Ala Ala Ser Pro Phe Trp Glu Ala Ser Arg Arg Ala Leu Glu Arg Asp Asp Phe  270
                                                                             Δ
GAG ATC GTA GTC ATT CTC GAG GGC ATG GTG GAG GCC ACG GGA ATG ACG TGC CAA GCT CGA AGC TCG TAC CTG GTG GAT GAA GTG TTG TGG  900
Glu Ile Val Val Ile Leu Glu Gly Met Val Glu Ala Thr Gly Met Thr Cys Gln Ala Arg Ser Ser Tyr Leu Val Asp Glu Val Leu Trp  300

GGA CAC CGG TTC ACA TCC GTG CTC ACC CTG GAG GAT GGT TTC TAT GAG GTG GAC TAC GCC AGC TTC CAC GAA ACC TTT GAG GTG CCC ACA  990
Gly His Arg Phe Thr Ser Val Leu Thr Leu Glu Asp Gly Phe Tyr Glu Val Asp Tyr Ala Ser Phe His Glu Thr Phe Glu Val Pro Thr  330
                     *           o                                           o
CCC TCG TGC AGT GCT CGG GAA CTG GCA GAA GCC GCG GCC CGC CTT GAT GCC CAT CTC TAC TGG TCC ATC CCC AGC AGG CTG GAT GAG AAG 1080
Pro Ser Cys Ser Ala Arg Glu Leu Ala Glu Ala Ala Ala Arg Leu Asp Ala His Leu Tyr Trp Ser Ile Pro Ser Arg Leu Asp Glu Lys  360
           oΔ                                                                        o
GTG GAG GAA GAA GGG GCT GGG GAG GGG GGC AGG TGC GGG AGA TGG AGC TGA caaggagcacaatggctgccacccccagagagtgagtccaaggtgtgactgg 1183
Val Glu Glu Glu Gly Ala Gly Glu Gly Gly Arg Cys Gly Arg Trp Ser  *                                                     376
tttcctcccacccctgtggcagaccaggggccggactcaggtacacagaagctgcgagtggaggtggaagaagaggaggcaggcagtgtcccgaggaacagctaaagttgggagaggc 1303
ccgctgagtccaggatcgagtagggaaggctgaggtcctggtttgaagagagagggttgcagggcggggtgagagaacatgtcagtctgtctgtgtttgaccttcacatcggttcatggg 1423
tggatggatggacagaaggatgggctcatgggggttgatcgggaaggtggagcagatagagacagccaatggataatcgctcaggtggtaagtggcttggcagtcgatgatcgtcacctg 1543
cagcacacctttgtgagaaatccatggcatccttttcttccagatataggtagcctcaaaccagggagcgtggcttagggagcaggctgtcaggtggactaccacccccactcacctcc 1663
cctcaactggcctccctgatgtgtgacacgcctgcctaactagagaagagagcactgggtagaggtggacacaggtgtggctgccctccccagtatcactgtcccatggcgagaggtcag 1783
aaaggcaaacaaacaatgggggtagatgctgagcagggaggggccctgaagcaggacctggggacagccaaggacaactatttgtgagagaggaatgaaaccttgcaggtcctgccaca 1903
gaagcaagaagcagaggaaaggccatggagagacttaataaagggttttacaagggaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa                               1994
```

Fig. 1 (*continued*).

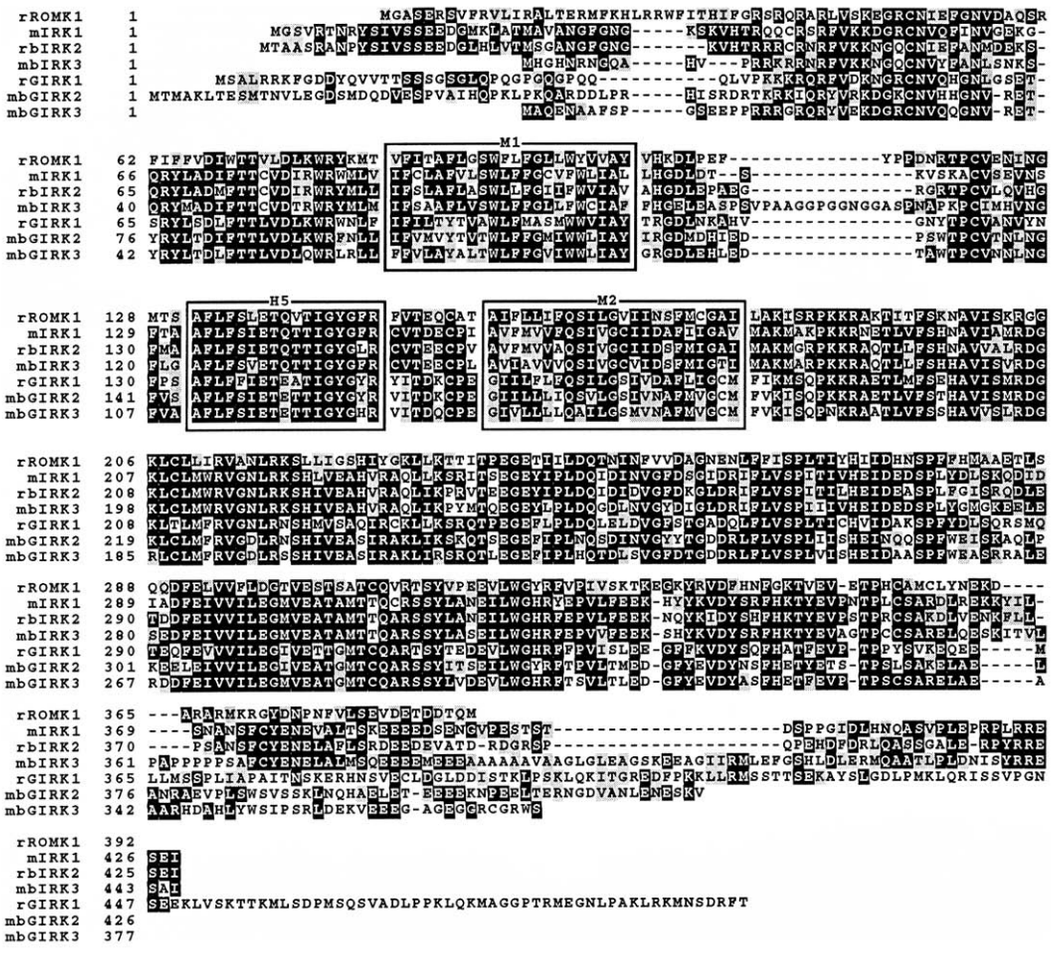

Fig. 2. Amino acid sequence alignment of the rat ROMK1, GIRK1 and IRK2, the mouse IRK1 K^+ channels and of the newly cloned mouse brain IRK3, GIRK2 and GIRK3 channels. Shaded amino acids represent fully conserved moieties between the three channels. The segments M1, H5 and M2 are boxed.

which a transcript of 4.2 kb is detected. A longer exposure of the autoradiogram revealed a low expression in the skeletal muscle and no expression in other examined tissues. Finally, mbGIRK2 is the less expressed of the three examined channels. A transcript of 6.8 kb was only detected in the brain. Because the size of the isolated cDNAs for GIRK2 and GIRK3 are shorter than those observed for mRNAs, it may be suggested that the sequences shown in Fig. 1 do not contain the entire untranslated sequences.

Since these K^+ channels are mainly expressed in brain, we have also examined their expression during brain development (at 2, 8, 15, 45 and 120 days post-partum). Northern blot analysis revealed that these three channels are moderately expressed at 2 days and that messages accumulate regularly from 2 to 45 days. Expressions are then stable from 45 to 120 days (data not shown).

3.3. *Functional expression of inward rectifier K^+ channels in Xenopus oocytes*

Large K^+-selective currents were recorded in mbIRK3 cRNA injected oocytes. The electrophysiological properties of these currents are essentially identical to those reported by Morishige et al. [19] and are not illustrated. Briefly, mbIRK3 shows a strong rectification and is sensitive to Ba^{2+} (IC_{50} = 6.4 μM at −150 mV) and to Cs^+ (IC_{50} = 10.9 μM at −150 mV). A slope conductance of 16 ± 4 pS (n = 12), estimated between −50 and −120 mV, was found in the cell-attached configuration (140 mM K^+ in the pipette).

The electrophysiological properties of mbGIRK2 were characterized in oocytes injected with mbGIRK2 and δ-opioid receptor cRNAs. Application of an external solution containing 98 mM K^+ instead of the external normal solution (ND96) revealed an inward current of 511 ± 52 nA (n = 5) at −80 mV (Fig. 4B). This current represented the basal activity of inward rectifier K^+ channels. The application of a δ-opioid agonist (deltorphin I) in 98 mM K^+ solution increased the inward current by 190 ± 16 nA (n = 5) (Fig. 4B). The *I–V* characteristics of the deltorphin activated mGIRK2 current showed strong inward rectification. The current amplitude and the reversal potential were both dependent on the external K^+ concentration (Fig. 4C). The reversal potential of the mbGIRK2 current changed as a function of the external K^+ concentration indicat-

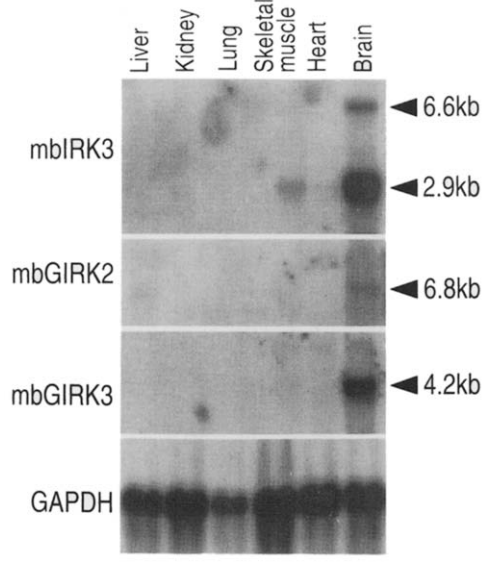

Fig. 3. Northern blot analysis of mbIRK3, mbGIRK2 and mbGIRK3 expressions in adult mouse. Each lane represents 5 μg of poly(A)$^+$RNA. The autoradiographs of mbIRK3 and mbGIRK3 were exposed for 24 h at −70°C and mbGIRK2 for 96 h. A blot was hybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe for relative quantification. The exposure time was 2 h at −70°C.

ing that the current was mainly carried by K^+ (Fig. 4C). In comparison, the control oocytes injected with the δ-opioid receptors showed only low endogenous inward currents (9 ± 4 nA (n = 5)) during application of a solution containing 98 mM K^+ and deltorphin I (Fig. 4A).

Using the same electrophysiological protocols as for mbIRK3 and mbGIRK2 channels, we failed to characterized any exogenous K^+ current in oocytes injected with mbGIRK3 cRNA alone or co-injected with the δ-opioid receptor cRNA.

4. Discussion

In order to isolate and characterize new brain inward rectifier K^+ channels, we have screened at low stringency a mouse brain cDNA library with IRK1 and GIRK1 probes. This resulted in the isolation of three novel sequences. One of these encodes a protein identical to the channel recently cloned by Morishige et al. [19] and designated mbIRK3. With 60 to 70% amino acid identity with the mouse macrophage IRK1 and the rat brain IRK2 and less than 50% with ROMK1 and GIRK1 channels, mbIRK3 is expected to belong to the functional IRK subclass of inward rectifier channels. Effectively, in *Xenopus* oocytes, mbIRK3 cRNA directs the expression of K^+-selective currents with properties typical of the previously expressed IRK1 and rbIRK2 isoforms, i.e. a strong rectification (channels are open at potentials more negative than E_K) and a block by Cs^+ and Ba^{2+}. However, the mbIRK3 channel has a unitary conductance (16 pS) different of that of IRK1 (20 pS) [10] and rbIRK2 (35 pS) [20]. Another major difference between these different isoforms is their tissue distribution. While they are all expressed in the brain, IRK1 is also detected in macrophage, heart and skeletal muscle and rbIRK2 in heart, skeletal muscle and kidney. Only mbIRK3 is highly and almost exclusively expressed in the brain. This diversity of sequences and distributions of IRK channels is expected to be associated with multiple functional roles of these different channels particularly in the brain.

The isolation of two cDNAs encoding proteins with sequences more similar to GIRK1 than to IRKs and ROMK1 and designated mbGIRK2 and mbGIRK3 argues for the existence of a subfamily of G protein-modulated inward rectifier K^+ channels. The observation that mbGIRK2 cRNA directs in oocytes the expression of a strongly rectifying K^+ channel which is modulated by the δ-opioid receptor probably via an endogenous protein G confirms this view. It is interesting to note that mGIRK2, like GIRK1/KGA [12], displays a basal activity independent of the G protein coupled receptor. This basal activity probably contributes to the resting membrane potential even in the absence of an activated receptor. For unknown reasons, mbGIRK3 failed to give a functional expression in *Xenopus* oocytes and experiments are being carried out to express this putative channel in other cell types and/or with other G coupled receptors. MbGIRK2 and mbGIRK3 are specifically expressed in the central nervous system. In contrast, GIRK1/KGA is expressed as well in the heart as in the brain [11,12].

In conclusion, this work presents further evidence that inward rectifier K^+ channels form a large multigenic family. Cloning of these channels provides molecular probes to explore and define the physiological roles of these different inward rectifier

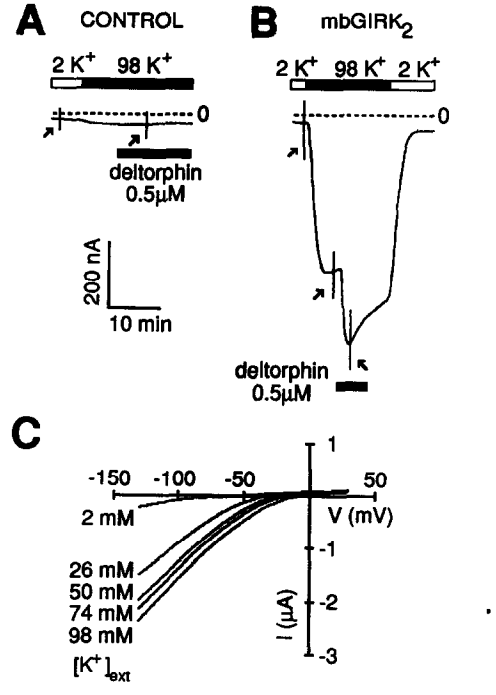

Fig. 4. Functional characterization of mbGIRK2 in *Xenopus* oocytes by the two electrodes voltage clamp technique. Inward currents evoked by external application of 98 mM K^+ and 0.5 μM deltorphin in δ-opioid receptor cRNA injected oocyte (A) and in δ-opioid receptor and mbGIRK2 cRNAs injected oocyte (B). The holding potential was −80 mV. External K^+ concentrations and deltorphin application are shown by horizontal bars (A,B), voltage ramps are indicated by arrows on current traces (reduced for clarity of the plot). (C) I–V curves were recorded in various concentrations of K^+ by using voltage ramps (between −130 to +30 mV) lasting 500 ms. Ramps were recorded at the peak of the deltorphin-evoked current.

K^+ channels in the brain. On the other hand, the characterization of functional G coupled IRK channels (1 and 2) as well as related but uncoupled IRKs channels (1, 2 and 3) will probably provide useful elements for further study of structure-function relationships of G protein-K^+ channel interactions.

Acknowledgements: We are very grateful to B. Attali, G. Romey, E. Honoré, J. Barhanin, R. Waldmann and I. Lauritzen for fruitful discussions. Thanks are due to M.-M. Larroque, N. Leroudier and C. Roulinat for expert technical assistance. This work was supported by the Association Française contre les Myopathies (AFM) and the Centre National de la Recherche Scientifique (CNRS).

References

[1] Rudy, B. (1988) Neuroscience 25, 729–749.
[2] Rudy, B., Kentros, C. and Vega-Saeng de Miera, E. (1991) Mol. Cell. Neurosci. 2, 89–102.
[3] Jan, L.Y. and Jan Y.N. (1992) Cell 69, 715–718.
[4] Pongs, O. (1992) Physiol. Rev. 72, S69–S88.
[5] Anderson, J.A., Huprikar, S.S., Kodrian, L.V., Lucas, N.J. and Gaber, R.F. (1992) Proc. Natl. Acad. Sci. USA 89, 3736–3740.
[6] Sentenac, H., Nonneaud, N., Minet, M., Lacroute, F., Salmon, M., Gaymard, F. and Grignan, C. (1992) Science 256, 663–665.
[7] Aldrich, R. (1993) Nature 362, 107–108.
[8] Nichols, C.G. (1993) Trends Physiol. Sci. 14, 320–323.
[9] Ho, K., Nichols, C.G., Lederer, W.J., Lytton J., Vassilev, P.M., Kanazirska, M.V. and Hebert, S.C. (1993) Nature 362, 31–38.
[10] Kubo, Y., Baldwin, T.J., Jan, Y.N. and Jan, L.Y. (1993) Nature 362, 127–133.
[11] Kubo, Y., Reuveny, E., Slesinger, P.A., Jan, Y.N. and Jan, L.Y. (1993) Nature 364, 802–806.
[12] Dascal, N., Schreibmayer, W., Lim, N.F., Wang, W., Chavkin, C., DiMagno, L., Labarca, C., Kieffer, B.L., Gaveriaux-Ruff, C., Trollinger, D., Lester, H.A. and Davidson, N. (1993) Proc. Natl. Acad. Sci. USA 90, 10235–10239.
[13] Isacoff, E., Jan, Y.N. and Jan, L.Y. (1990) Nature 345, 530–534.
[14] Honoré, E., Attali, B., Romey, G., Lesage, F., Barhanin, J. and Lazdunski, M. (1992) EMBO J. 11, 2465–2471.
[15] Rettig, J., Heinemann, S.H., Wunder, F., Lorra, C., Parcej, D.N., Dolly, J.O. and Pongs, O. (1994) Nature 369, 289–294.
[16] Lesage, F., Attali, B., Lazdunski, M. and Barhanin, J. (1992) FEBS Lett. 310, 162–166.
[17] Guillemare, E., Honoré, E., Pradier, L., Lesage, F., Schweitz, H., Attali, B., Barhanin, J. and Lazdunski, M. (1992) Biochemistry 31, 12463–12468.
[18] Kieffer, B.L., Befort, K., Gaveriaux-Ruff, C. and Hirth, C.G. (1992) Proc. Natl. Acad. Sci. USA 89, 12048–12052.
[19] Morishige, K.I., Takahashi, N., Jahangir, A., Yamada, M., Koyama, H., Zanelli, J.S. and Kurachi, Y. (1994) FEBS Lett. 346, 251–256.
[20] Koyama, H., Morishige, K., Takahashi, N., Zanelli, J.S., Fass, D.N. and Kurachi, Y. (1994) FEBS Lett. 341, 303–307.