Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6753–6757, July 1995
Neurobiology

Cloning and expression of two brain-specific inwardly rectifying potassium channels

DAVID S. BREDT*†, TIAN-LI WANG‡, NOAM A. COHEN*, WILLIAM B. GUGGINO‡, AND SOLOMON H. SNYDER*§¶‖

Departments of *Neuroscience, §Pharmacology and Molecular Sciences, ‡Physiology, and ¶Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

Contributed by Solomon H. Snyder, March 27, 1995

ABSTRACT **We have cloned two inwardly rectifying K^+ channels that occur selectively in neurons in the brain and are designated BIRK (brain inwardly rectifying K^+) channels. BIRK1 mRNA is extremely abundant and is enriched in specific brainstem nuclei, BIRK1 displays a consensus phosphate-binding loop, and expression in *Xenopus* oocytes has shown that its conductance is inhibited by ATP and adenosine 5′-[γ-thio]triphosphate. BIRK2 is far less abundant and is selectively localized in telencephalic neurons. BIRK2 has a consensus sequence for cAMP-dependent phosphorylation.**

The first K^+ channel genes sequenced were rapidly inactivating A-type, delayed rectifier-type channels identified in *Drosophila*, comprising several gene subfamilies, Shaker, Shaw, Shal, and Shab (1, 2). Later, mammalian homologues for these channels were identified (3–10). Inwardly rectifying K^+ channels are important regulators of cellular functions, such as the resting membrane potential (11) and cholinergic slowing of the heart (12). The four distinct inwardly rectifying K^+ channels which have been molecularly cloned differ markedly in their transmembrane topology from the previously cloned K^+ channels. One of them, ROMK1, is most highly concentrated in the kidney (13); another, IRK1, in heart ventricle, skeletal muscle, and forebrain (14); a third, GIRK1/KGA, in the heart atrium, where it conveys the cholinergic G-protein influences on K^+ conductance (15, 16); and a fourth, HRK1/HIR, is expressed in brain and muscle tissue (17, 18). We now report the cloning and functional expression of two inwardly rectifying K^+ channels that are localized selectively to the brain. We have designated these channels BIRK1 and BIRK2 (brain inwardly rectifying K^+ channels 1 and 2).**

EXPERIMENTAL PROCEDURES

DNA Methods. DNA manipulations were carried out according to standard protocols (19). DNA was sequenced by the dideoxy chain-termination method with a Sequenase kit (United States Biochemical). Fully degenerate oligonucleotide primers were synthesized, corresponding to aa 141–146 and 290–295 in ROMK1 (13). PCR was performed with these primers to amplify first-strand cDNA from rat brain poly(A)$^+$ RNA, at an annealing temperature of 56°C. The PCR products were excised from an agarose gel and cloned into pBluescript (Stratagene), and 12 independent recombinants were sequenced. The two novel sequences obtained, BIRK1 and BIRK2, were used for plaque hybridization under high-stringency conditions [65°C; 0.1× standard saline citrate (SSC)] to isolate cDNA clones from a rat brain library. PCR primers included 9-bp sequences at the 5′ end which allowed subcloning into *Eco*RI and *Bam*HI sites. The primer sequences were 5′-GGGGGATCCACNAT(T/C/A)GGNTA(T/C)GGNTT-3′ and 5′-GCGGAATTCACNACNA(G/T)(T/C)TCA(A/G)TC-3′.

Northern Blot Analysis. Poly(A)$^+$ RNA samples were electrophoresed in 1% formaldehyde/1% agarose gels. The size-separated RNAs were then transferred to nitrocellulose membranes, hybridized (42°C; 50% formamide/6× SSC/0.5% SDS) with random primer-labeled probes (3×10^6 ^{32}P cpm/ng; 10^8 cpm per filter), and washed at high stringency (65°C; 0.2× SSC/0.5% SDS). Blots were exposed to x-ray film for various times as described in Fig. 2. A 0.9-kb 5′ fragment of BIRK1 was used as a probe. Full-length cDNA probes were used for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and BIRK2, 1.4 and 2.9 kb, respectively.

***In Situ* Hybridization.** *In situ* hybridization was performed (20) with end-labeled-base oligonucleotide probes: BIRK1, 5′-GTAATAGACCTTGGCAACTGATGTCATCTTGGCACAGAGGTGGAG; ACCCTCCCCGCTGCGGAGGGGGAGATCTTGCAA; GGGGGACGCCACTTTCACAACCTGGTCAAAAAGGCTGAAGTCAGC-3′; BIRK2, 5′-CTCCGACTCCAGTTCCTCCTTGCCCATGCCGTAGAGTGGACTGTC; GGGGGACGCCACTTTCACAACCTGGTCAAAAAGGCTGAAGTCAGC; GTAATAGACCTTGGCAACTGATGTCATCTTGGCACAGAGGTGGAG-3′.

Electrophysiology. Oocyte preparations and two-electrode voltage-clamp recordings have been described (21, 22). Except in external K^+ substitution experiments, most of the recordings were collected from the oocytes bathed in 50 mM K^+ perfusion solution containing 50 mM KCl, 48 mM NaCl, 0.2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Hepes (pH 7.4). ATP and adenosine 5′-[γ-thio]triphosphate (ATP[γS]) were purchased from Boehringer Mannheim and were dissolved in normal Ringer's saline. The drugs were injected into oocytes by an automatic pressure injector (IM-200; Narishige, Greenvale, NY). The needle tips for injection were 3–5 μm in diameter and the injection volume was ≈10 nl.

RESULTS

Molecular Cloning and Sequencing of BIRK1 and BIRK2 Channels. Degenerate oligonucleotide primers were constructed to conserved regions shared by the inwardly rectifying K^+ channels IRK1 and ROMK1 and used in reverse transcription–PCR to amplify homologous genes from rat brain RNA preparations. Of 12 sequenced products, 5 corresponded to IRK1 whereas 4 and 3 corresponded to two distinct sequences that we have designated BIRK1 and BIRK2, respectively (Fig. 1). These products were employed to screen rat brain cDNA libraries. Of 300,000 clones screened, 24 hybridized to the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ATP[γS], adenosine 5′-[γ-thio]triphosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

†Present address: Department of Physiology, University of California, San Francisco, School of Medicine, Room S-859, 513 Parnassus Avenue, San Francisco, CA 94143-0444.
‖To whom reprint requests should be addressed.

**The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U27558 and U27582).

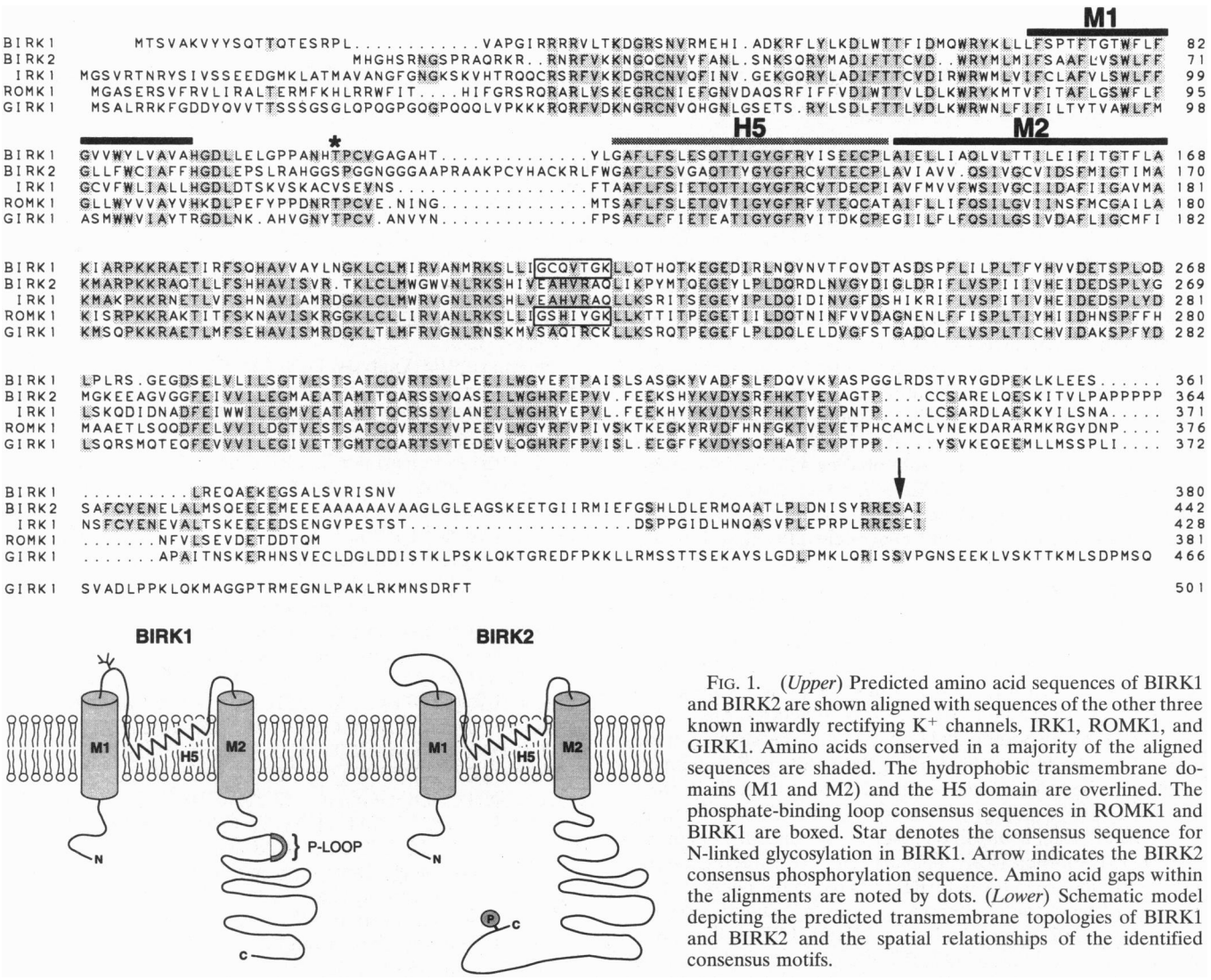

FIG. 1. (*Upper*) Predicted amino acid sequences of BIRK1 and BIRK2 are shown aligned with sequences of the other three known inwardly rectifying K^+ channels, IRK1, ROMK1, and GIRK1. Amino acids conserved in a majority of the aligned sequences are shaded. The hydrophobic transmembrane domains (M1 and M2) and the H5 domain are overlined. The phosphate-binding loop consensus sequences in ROMK1 and BIRK1 are boxed. Star denotes the consensus sequence for N-linked glycosylation in BIRK1. Arrow indicates the BIRK2 consensus phosphorylation sequence. Amino acid gaps within the alignments are noted by dots. (*Lower*) Schematic model depicting the predicted transmembrane topologies of BIRK1 and BIRK2 and the spatial relationships of the identified consensus motifs.

BIRK1 probe and 2 to the BIRK2 probe. The largest BIRK1 clone, 2.8 kb, and the largest BIRK2 clone, 2.9 kb, were sequenced. These sequences correspond to those of two channels reported while this work was in preparation (23).

Both BIRK1 and BIRK2 are clearly members of the inwardly rectifying K^+ channel family, with negligible resemblance to outward rectifiers. Like the other inward rectifiers, BIRK1 and BIRK2 display only two membrane-spanning domains (M1 and M2). They also possess an H5 consensus sequence, a component of the presumed ion permeation pore, which is conserved in a superfamily of voltage- and second messenger-gated cation channels (24). Within H5 both BIRK1 and BIRK2 display the Tyr-Gly motif which is characteristic of K^+ channels. The M2 region of each channel possesses an acidic amino acid at a position corresponding to amino acid 171 of the ROMK1 channel. This residue was recently demonstrated to gate the voltage-dependent intracellular Mg^{2+} block resulting in inward rectification (25–29). Alignment of BIRK1 and BIRK2 with the other cloned inwardly rectifying K^+ channels reveals that these genes fall into subfamilies. BIRK1 displays 47% amino acid sequence identity to the previously cloned inward rectifier K^+ channel ROMK1, and only 35% and 37% identity to IRK1 and GIRK1, respectively. BIRK2 evinces 91% amino acid sequence identity to HRK1/HIR, 56% identity to IRK1, and only 32% identity to ROMK1 and GIRK1. BIRK1 and BIRK2 display 32% amino acid sequence identity to each other. Thus BIRK1 and ROMK1 constitute two members of one subfamily and BIRK2, HRK1/HIR, and IRK1 belong to a second.

Inward rectifiers within these subfamilies share certain important sequence motifs. BIRK1 possesses a phosphate-loop binding consensus site (Gly-Xaa_4-Gly-Lys) at the same location as a comparable site in ROMK1 (Fig. 1). This motif is absent in BIRK2. A consensus sequence for N-linked glycosylation in the extracellular region bridging M1 and H5 is shared by BIRK1 and ROMK1 and is absent in BIRK2 (Fig. 1). At the carboxyl terminus, BIRK2 has the cAMP-dependent phosphorylation consensus sequence Arg-Arg-Xaa-Ser, which is not present in BIRK1. Interestingly, IRK1 contains the identical consensus sequence at the carboxyl terminus, despite the fact that there is no other sequence homology between IRK1 and BIRK2 in this region of the channels.

BIRK1 and BIRK2 mRNAs Are Differentially Localized in the Brain. Northern blot analysis of rat brain BIRK1 mRNA revealed a single species of 5.5 kb (Fig. 2). No expression was detected in kidney, spleen, liver, heart, and skeletal muscle. Substantial levels occur in all brain regions, with highest amounts in the brainstem. This distribution contrasts strikingly with that of BIRK2. Two distinct sizes of BIRK2 mRNA were observed, 6 and 3 kb. These two species must likely derive from alternative splicing, as they have essentially identical regional distributions. Like the BIRK1 message, BIRK2 mRNAs were not detected in kidney, spleen, liver, heart, and skeletal muscle. BIRK2 is most prominently expressed in the cerebral cortex,

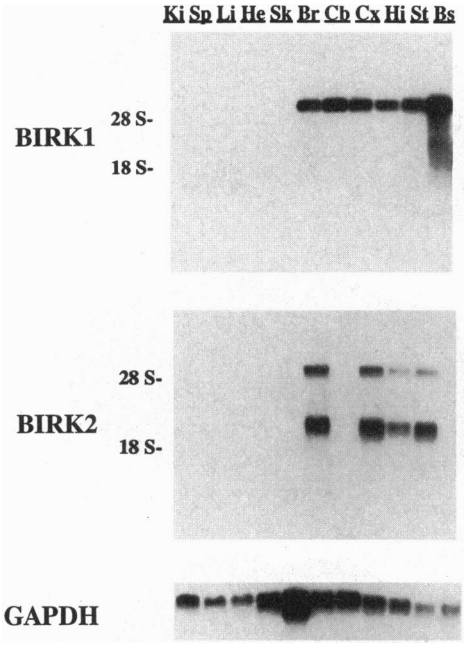

FIG. 2. BIRK mRNA expression in brain and peripheral tissues. Northern blot was carried out with poly(A)$^+$ RNA (10 μg per lane) from kidney (Ki), spleen (Sp), liver (Li), heart (He), skeletal muscle (Sk), whole brain (Br), cerebellum (Cb), cerebral cortex (Cx), hippocampus (Hi), corpus striatum (St), and brainstem (Bs). BIRK1 and BIRK2 mRNAs were present only in brain tissue. The BIRK1 probe hybridized to a 5.5-kb band ubiquitous in the brain but most enriched in the brainstem. BIRK2 recognized bands of 5.6 and 3 kb which were present only in telencephalic brain regions. Hybridization of an identical blot with GAPDH cDNA indicated the integrity of the RNA in all tissues. All three blots were hybridized under similar conditions. Exposure times were adjusted relative to signal strengths: BIRK1, 24 hr; BIRK2, 14 days; GAPDH, 12 hr. Positions of 18S and 28S rRNAs are indicated.

with somewhat lower levels in the corpus striatum, very modest amounts in the hippocampus, and no expression in the cerebellum or brainstem. The most striking difference between BIRK1 and BIRK2 involves relative amounts of mRNA. Comparable intensities in Northern blot analysis were obtained for the most enriched brain regions with exposure times of 24 hr for BIRK1 and 2 weeks for BIRK2. By Northern blot analysis, we compared the abundance of BIRK1 mRNA to that of GAPDH mRNA. The autoradiographic intensity for BIRK1 was about half that for GAPDH when parallel blots were hybridized under identical conditions with probes specific for these two genes (Fig. 2).

In situ hybridization for BIRK1 mRNA (Fig. 3 *A–C*) in brain revealed a neuronal distribution which paralleled the regional distribution shown by Northern blot analysis. In a parasagittal section of adult rat brain, high levels of expression were apparent in specific brainstem nuclei, including the trigeminal nucleus and the pontine nucleus. BIRK1 expression was also apparent in superior and inferior colliculi. Ammon's horn and dentate gyrus of the hippocampus, anterior pretectal nucleus of thalamus, and cerebral cortex. Horizontal sections of brain allowed further definition of the sites of BIRK1 expression. The highest densities of BIRK1 mRNA in the brain were observed in the area postrema. Other brainstem nuclei of high-density hybridization included the vestibular nuclei, the motor and spinal trigeminal nuclei, the ventral cochlear nucleus, and the ventral nucleus of the lateral lemniscus. In the forebrain, high levels of BIRK1 message were apparent in the subthalamic nuclei and in linear stripes lining the lateral, third, and fourth ventricles.

In situ hybridization patterns of BIRK2 mRNA (Fig. 3 *D–G*) resembled results of Northern blot analysis. Highest levels occurred in the forebrain, with negligible levels in the brainstem and diencephalon. The dentate gyrus of the hippocampus was prominently labeled, more than the CA1–CA3 regions. High levels in gray matter of the cerebral cortex were most prominent in superficial layers, whereas the corpus callosum and other white-matter areas were unlabeled. The corpus striatum displayed substantial BIRK2 hybridization, as did the olfactory tubercle. The very low mRNA levels in the granule cell layer of the cerebellum were probably nonspecific, being retained following RNase treatment.

Contrasting Electrophysiological Properties of BIRK1 and BIRK2. Injection of *in vitro* transcribed RNA for BIRK1 and BIRK2 channels into *Xenopus* oocytes produced inwardly rectifying currents with slight voltage-dependent inactivation at negative potentials (Fig. 4). For both BIRK1 and BIRK2, inward current increased linearly with the extent of hyperpolarization. The slope conductance at negative potentials for BIRK1 was 10.3 $\pm$ 2.4 μS, and for BIRK2 was 20.3 $\pm$ 0.9 μS. BIRK1 had a larger outward current at voltages above the K^+ equilibrium potential than BIRK2. Inwardly rectifying currents expressed from both clones were sensitive to external Ba^{2+} (Fig. 4). Ba^{2+} (50 μM) blocked the currents in a voltage-dependent manner and was most effective at negative voltages. To determine the ionic specificity of the channels we monitored the reversal potential in the presence of various external K^+ concentrations. The slope of the plot of the reversal potential as a function of the logarithm of the external K^+ concentration followed a Nernstian relationship, demonstrating that both BIRK1 and BIRK2 are selectively permeable to K^+ (data not shown).

BIRK1 displays a consensus sequence for ATP binding which is absent in BIRK2. Therefore, we evaluated effects of ATP[γS] and ATP injections in oocytes (Fig. 5). ATP[γS] (5 nmol), a nonmetabolizable analogue of ATP, almost completely abolished the inward conductance of BIRK1 but barely affected BIRK2. ATP (25 nmol) inhibited BIRK1 currents, but less so than ATP[γS]. Maximal inhibition of BIRK1 inward current occurred 15 min after injection of ATP and gradually recovered to preinjection values over 40 min, probably due to ATP metabolism. The average suppression by ATP[γS] of BIRK1 currents at 15 min was 90% $\pm$ 7%, compared with 44% $\pm$ 10% by ATP.

DISCUSSION

One of our most striking findings is the extraordinarily high density of BIRK1 mRNA. What brain function might be served by so abundant a channel? Regulation of neuronal resting membrane potential is one possibility, as the resting membrane potential is primarily determined by the K^+ conductance and would not be affected by outwardly rectifying channels, which are active only in depolarized cells. Because of its high density and significant outward current at depolarizing potentials, BIRK1 may be the most likely determinant of resting membrane potential, though the other inward rectifiers, ROMK1, IRK1, GIRK1, and BIRK2, may also participate.

The sensitivity of BIRK1 to ATP suggests a role in regulating neuronal responses to glucose. In the pancreas, inhibition of ATP-sensitive K^+ channels following glucose metabolism to ATP depolarizes β cells, triggering insulin release (30–33). Conceivably, BIRK1 similarly regulates neuronal firing in response to varying glucose levels.

In BIRK2 the carboxyl-terminal protein kinase A consensus sequence, shared with IRK1 and HRK1/HIR, may mediate neurotransmitter modulation of channel activity by cAMP. Thus, glutamate inhibits an inwardly rectifying K^+ conductance by stimulating adenylyl cyclase and activating protein kinase A (34). Norepinephrine, serotonin, and histamine also

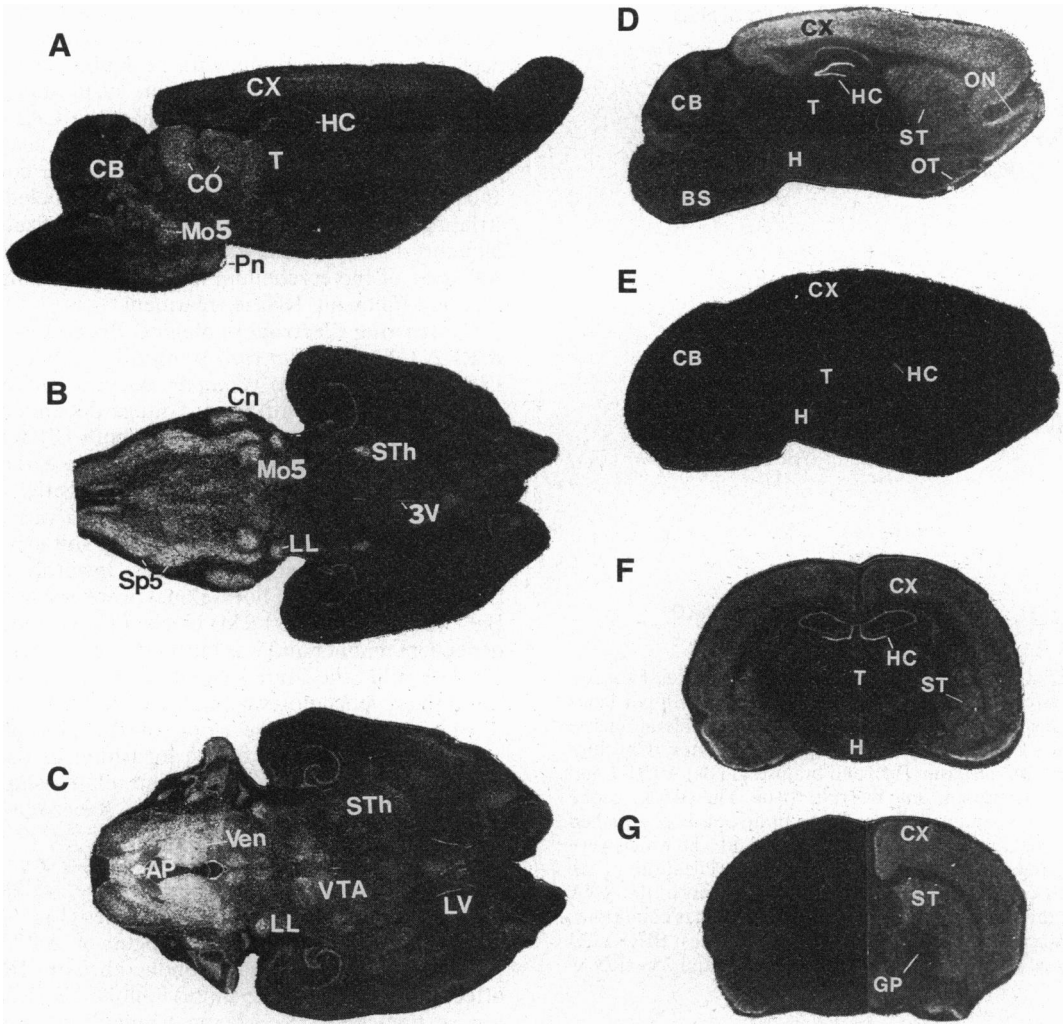

FIG. 3. Localization of BIRK1 and BIRK2 mRNAs in brain. (*A–C*) *In situ* hybridization to BIRK1 mRNA shows high levels of expression in discrete brain regions. In parasagittal sections (*A*) BIRK1 hybridization is apparent in certain forebrain structures, including the cerebral cortex (CX), hippocampus (HC), and thalamus (T). In the mid- and hindbrain BIRK1 is enriched in the superior and inferior colliculi (CO), the pontine nucleus (Pn), the motor nucleus of the trigeminal nerve (Mo5), and the deep cerebellar nuclei and granule cell layer of the cerebellum (CB). Horizontal sections (*B* and *C*) reveal extremely high levels of BIRK1 expression in specific brainstem nuclei. Densest levels are present in the area postrema (AP), with high levels occurring in the vestibular nucleus (Ven), cochlear nucleus (Cn), spinal trigeminal nucleus (Sp5), and the lateral lemniscus (LL). High levels also are apparent in the ventral tegmental area, the subthalamic nuclei (STh), and linear stripes lining the ventricles (3V). (*D–G*) *In situ* hybridization to BIRK2 mRNA shows the channel to be exclusively expressed in the forebrain. Highest levels are found in the cerebral cortex (CX) and dentate gyrus of the hippocampus (HC). High densities of silver grains are also present in the corpus striatum (ST), olfactory tubercle (OT), and anterior olfactory nucleus (ON). Essentially no hybridization is seen in the midbrain or hindbrain. H, hypothalamus; GP, globus pallidus. All hybridization is eliminated by RNase treatment of the sagittal brain section (*E*) or the left "split-brain" coronal section (*G*).

suppress K^+ currents via protein kinase A (35). Certain opiate receptors and muscarinic cholinergic receptors inhibit adenylyl cyclase to open K^+ channels (36).

BIRK2 and HRK1/HIR are 91% identical, suggesting that BIRK2 is the rat homologue of the human HRK1/HIR. Northern analysis reveals that some differences do exist in

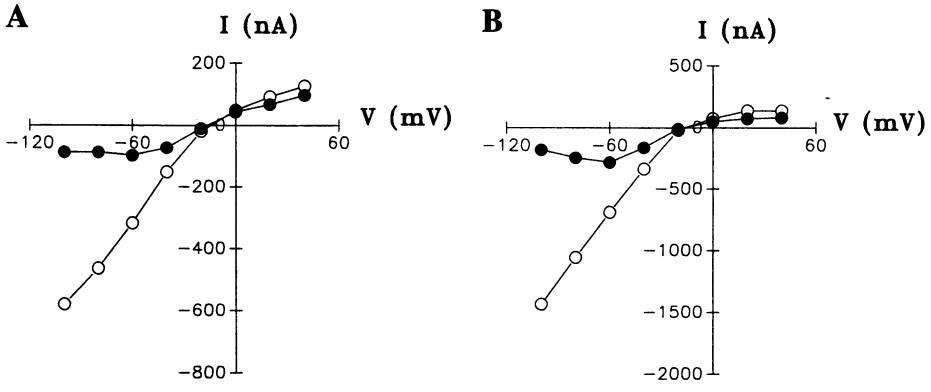

FIG. 4. Inwardly rectifying currents from *Xenopus* oocytes injected with cRNA transcribed from BIRK1 (A) and BIRK2 (B) cDNA clones. The membrane potential was held at −20 mV and stepped to +40, +20, 0, −20, −40, −60, −80, and −100 mV as shown. During the recordings, the oocytes were perfused in 50 mM K^+ solution without (○) or with (●) 50 μM Ba^{2+}. External Ba^{2+} inhibited the expressed currents of both clones in a voltage-dependent manner. Current–voltage relationships of BIRK1 (*A*) and BIRK2 (*B*) are shown after 500 ms of voltage stimulus.

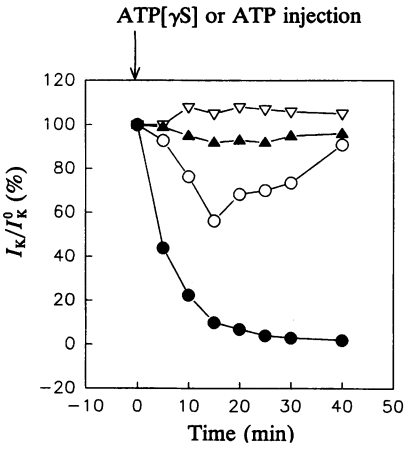

FIG. 5. ATP[γS] and ATP inhibit BIRK1 (●, ○) but not BIRK2 (▲, ▽) current. K^+ currents (I_K) generated by both clones at −80 mV were measured at various times following ATP[γS] (●, ▲) or ATP (○, ▽) exposure and were expressed as a ratio to the original currents before ATP[γS] or ATP addition (I_K^0).

localization, with HIR abundant in cardiac tissue, where BIRK2 is absent (18). HIR but not BIRK2 occurs in the amygdala (18).

This work was supported by U.S. Public Health Service Grants MH18501 (to S.H.S.) and DK32753 (to W.B.G.), Research Scientist Award DA00074 (to S.H.S.), Training Grant GM07309 (to D.S.B.), and Predoctoral Fellowship SF 30MH1034102 (to N.A.C.) and by grants of the W. M. Keck and Stanley Foundations.

1. Tempel, B. L., Papazian, D. M., Schwarz, T. L., Jan, Y. N. & Jan, L. Y. (1987) *Science* **237,** 770–775.
2. Butler, A., Wei, A., Baker, K. & Lawrence, S. (1989) *Science* **243,** 943–947.
3. Tempel, B. L., Jan, N. Y. & Jan, L. Y. (1988) *Nature (London)* **332,** 837–839.
4. Baumann, A., Grupe, A., Ackermann, A. & Pongs, O. (1988) *EMBO J.* **7,** 2457–2463.
5. Frech, G. C., VanDongen, A. M. J., Schuster, G., Brown, A. M. & Joho, R. H. (1989) *Nature (London)* **340,** 642–645.
6. McKinnon, D. (1989) *J. Biol. Chem.* **264,** 8230–8236.
7. McCormack, T., De Miera, V. S. & Rudy, B. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5227–5231.
8. Baldwin, T. J., Tsaur, M. L., Lopez, G. A., Jan, Y. N. & Jan, L. Y. (1991) *Neuron* **7,** 471–483.
9. Pak, M. D., Baker, K., Covarrubias, M., Butler, A., Ratcliffe, A. & Salkoff, L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4386–4390.
10. Roberds, S. L. & Tamkun, M. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1798–1802.
11. Hille, B. (1989) *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA), pp. 127–130.
12. Sakmann, B., Noma, A. & Trautwein, W. (1983) *Nature (London)* **303,** 250–253.
13. Ho, K., Nichols, C. G., Lederer, W. J., Lytton, J., Vassilev, P. M., Kanazirska, M. V. & Hebert, S. C. (1993) *Nature (London)* **362,** 31–38.
14. Kubo, Y., Baldwin, T. J., Jan, Y. N. & Jan, L. Y. (1993) *Nature (London)* **362,** 127–132.
15. Kubo, Y., Reuveny, E., Slesinger, P. A., Jan, Y. N. & Yan, L. Y. (1993) *Nature (London)* **364,** 802–806.
16. 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. & Davidson, N. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10235–10239.
17. Makhina, E. N., Kelly, A. J., Lopatin, A. N., Mercer, R. W. & Nichols, C. G. (1994) *J. Biol. Chem.* **269,** 20468–20474.
18. Perier, F., Radeke, C. M. & Vandenberg, C. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6240–6244.
19. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
20. Jaffre, S. R., Cohen, N. A., Rousault, T. A., Klausner, R. D. & Snyder, S. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12994–12998.
21. Wang, T. L., Guggino, W. B. & Cutting, G. R. (1994) *J. Neurosci.* **14,** 6524–6536.
22. Lu, L., Motrose-Rafizadeh, C. & Guggino, W. B. (1990) *J. Biol. Chem.* **265,** 16190–16194.
23. Bond, C. T., Pessia, M., Xia, X.-M., Lagrutta, A., Kavanaugh, M. P. & Adelman, J. P. (1994) *Recept. Channels* **2,** 183–191.
24. Jan, L. Y. & Jan, Y. N. (1990) *Trends Neurosci.* **13,** 415–419.
25. Lu, Z. & MacKinnon, R. (1994) *Nature (London)* **371,** 243–246.
26. Lopatin, A. N., Makhina, E. N. & Nichols, C. G. (1994) *Nature (London)* **372,** 366–379.
27. Wible, B. A., Taglialatela, M., Ficker, E. & Brown, A. M. (1994) *Nature (London)* **371,** 246–249.
28. Stanfield, P. R., Davies, N. W., Shelton, P. A., Sutcliffe, M. J., Khan, I. A., Brammar, W. J. & Conley, E. C. (1994) *J. Physiol. (London)* **478,** 1–6.
29. Fakler, B., Brandle, U., Glowatzki, E., Weidemann, S., Zenner, H.-P. & Ruppersberg, J. P. (1995) *Cell* **80,** 149–154.
30. Ashcroft, F. M., Harrison, D. E. & Ashcroft, S. J. H. (1984) *Nature (London)* **312,** 446–448.
31. Cook, D. L. & Hales, C. N. (1984) *Nature (London)* **311,** 271–273.
32. Rorsman, P. & Trube, G. (1985) *Pflügers Arch.* **405,** 305–309.
33. Sturgess, N. C., Ashford, M. J. L., Cook, D. L. & Hales, C. N. (1985) *Lancet* **ii,** 474–475.
34. Schwarz, E. A. (1993) *Neuron* **10,** 1141–1149.
35. Pedarzaini, P. & Storm, J. F. (1993) *Neuron* **11,** 1023–1035.
36. North, R. A., Williams, J. T., Suprenant, A. & Christie, M. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5487–5491.