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Distribution and localization of a G protein-coupled inwardly rectifying K^+ channel in the rat

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Abstract

The cellular distribution of the mRNA of the inwardly rectifying K^+ channel KGA (GIRK1) was investigated in rat tissue by in situ hybridization. KGA was originally cloned from the heart and represents the first G protein-activated K^+ channel identified. It is expressed in peripheral tissue solely in the atrium, but not in the ventricle, skeletal muscle, lung and kidney. In the central nervous system KGA is most prominently expressed in the Ammon's horn and dentate gyrus of the hippocampus, neocortical layers II–VI, cerebellar granular layer, olfactory bulb, anterior pituitary, thalamic nuclei and several distinct nuclei of the lower brainstem. The abundant expression of KGA in many CNS neurons supports its important role as a major target channel for G protein mediated receptor function.

Key words: Potassium channel; Inward rectifier; G-protein gating; mRNA; Hybridization (in situ)

1. Introduction

In recent years it has become clear that cellular signal transduction from seven-helix receptors to ionic channels may occur via G protein/enzyme/ion channel coupling as well as by direct coupling of activated G proteins to the channels in a membrane-delimited process without the involvement of cytoplasmic messengers [1]. The vagal inhibition of atrial myocytes by means of an inwardly rectifying K^+ channel [2,3] represents the best-known form of obligatory channel gating through G proteins. Like heart, many neurons and neuroendocrine cells exhibit inwardly rectifying K^+ channels that are activated by a variety of G protein coupled neurotransmitter receptors. Muscarinic m2, α2 adrenergic, D2 dopamine, histamine, serotonin 1A, A1 adenosine, $GABA_B$, μ and δ opioid, somatostatin and many other receptors converge onto this type of channel probably via their specific coupling to G proteins of the G_i family [4].

A G protein-coupled muscarinic K^+ inward rectifier has recently been cloned from rat heart and expressed in oocytes [5,6]. The channel termed GIRK1/KGA is structurally distinct from the Shaker class of voltage-gated channels: it resembles two other newly cloned voltage-dependent inwardly rectifying channels [7,8] in that each subunit seems to be comprised of only two transmembrane helices surrounding the pore region (H5) and N-and C-terminal cytoplasmic tails. Using in situ hybridization we have performed a systematic study of the gene expression pattern of GIRK1/KGA channels in rat tissue. This pattern may represent expression of a major component of inhibitory process in the CNS, because G protein-gated K^+ channels underlie many receptor-mediated IPSPs in the brain and thus play an important role in central signal processing.

2. Materials and methods

Female adult Wistar rats were anesthetized and decapitated. Tissue was removed and quickly frozen on dry ice. Sections were cut at 12–16 μm on a cryostat, thaw-mounted onto silane-coated slides, fixed with 4% paraformaldehyde in phosphate-buffered saline (pH 7.4), dehydrated and stored under ethanol until hybrizidation. Before hybridization slides were treated with proteinase K (1 μg/ml), acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine and prehybridized for 2 h at 37°C in hybridization buffer (25 mM PIPES, pH 6.8, 0.75 M NaCl, 25 mM EDTA, 1 × Denhardt's solution, 50% deionized formamide, 0.2% sodium dodecyl sulfate, 100 mM dithiothreitol, 250 μg/ml denatured salmon sperm DNA and 250 μg/ml polyadenylic acid). Subsequently sections were incubated in the same hybridization buffer containing dextran sulfate (5%) and the radiolabeled probe (at 0.02–0.2 ng/μl with specific activities of $5–8 \times 10^8$ dpm/μg) overnight at 54°C. The cRNA probes were generated by in vitro transcription using [^{35}S]UTP (DuPont/New England Nuclear, 1200 Ci/mmol). Antisense and sense transcripts were generated with T3 and T7 RNA polymerase, respectively, from two linearized fragments: (i) a ~ 2070 bp *Xho*I/*Eco*RI full-length KGA fragment in pBluescript II KS$^-$ [6], and (ii) a ~ 870 bp *Xho*I/*Pst*I fragment of the same clone including a 291 bp stretch of the KGA 3′ open reading frame. After hybridization sections were washed twice in 4 × SSC plus 50 mM β-mercaptoethanol for 15 min, treated

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with RNase A (50 μg/ml) for 30 min at 37°C, and washed twice with 2 × SSC for 1 h, followed by two 15-min high-stringency washes at 55–65°C in 0.1 × SSC. Specimens were then dehydrated, air-dried and exposed to Kodak X-OMAT XAR5 film for 4–10 days at 4°C. Selected slides were subsequently dipped in photographic emulsion Kodak NTB2, incubated for 4–8 weeks and then developed in Kodak D-19 for 2.5 min. For analysis with bright- and darkfield optics, sections were Nissl counterstained with Cresyl violet to confirm cytoarchitecture. Rat brain structures were identified and confirmed according to Paxinos and Watson [9,10]. Fig. 1F shows hybridization with the 870 bp KGA riboprobe, all other data shown represent labeling patterns of full length KGA transcripts.

The following controls were performed: adjacent sections were (a) hybridized with sense riboprobes, (b) digested with RNase before hybridization, (c) hybridized with a mixed antisense/sense riboprobe containing a 10-fold excess of sense probe, or (d) washed at high temperatures (~100°C) near the melting temperature T_m of the mRNA:cRNA hybrids.

3. Results

In situ hybridization with the specific KGA mRNA transcripts revealed abundant expression of KGA in the rat CNS and heart. Antisense riboprobes from the full-length KGA cDNA and from the 3′ fragment coding for the C-terminal tail of KGA showed identical labeling profiles. On the X-ray film images most intense hybridi-

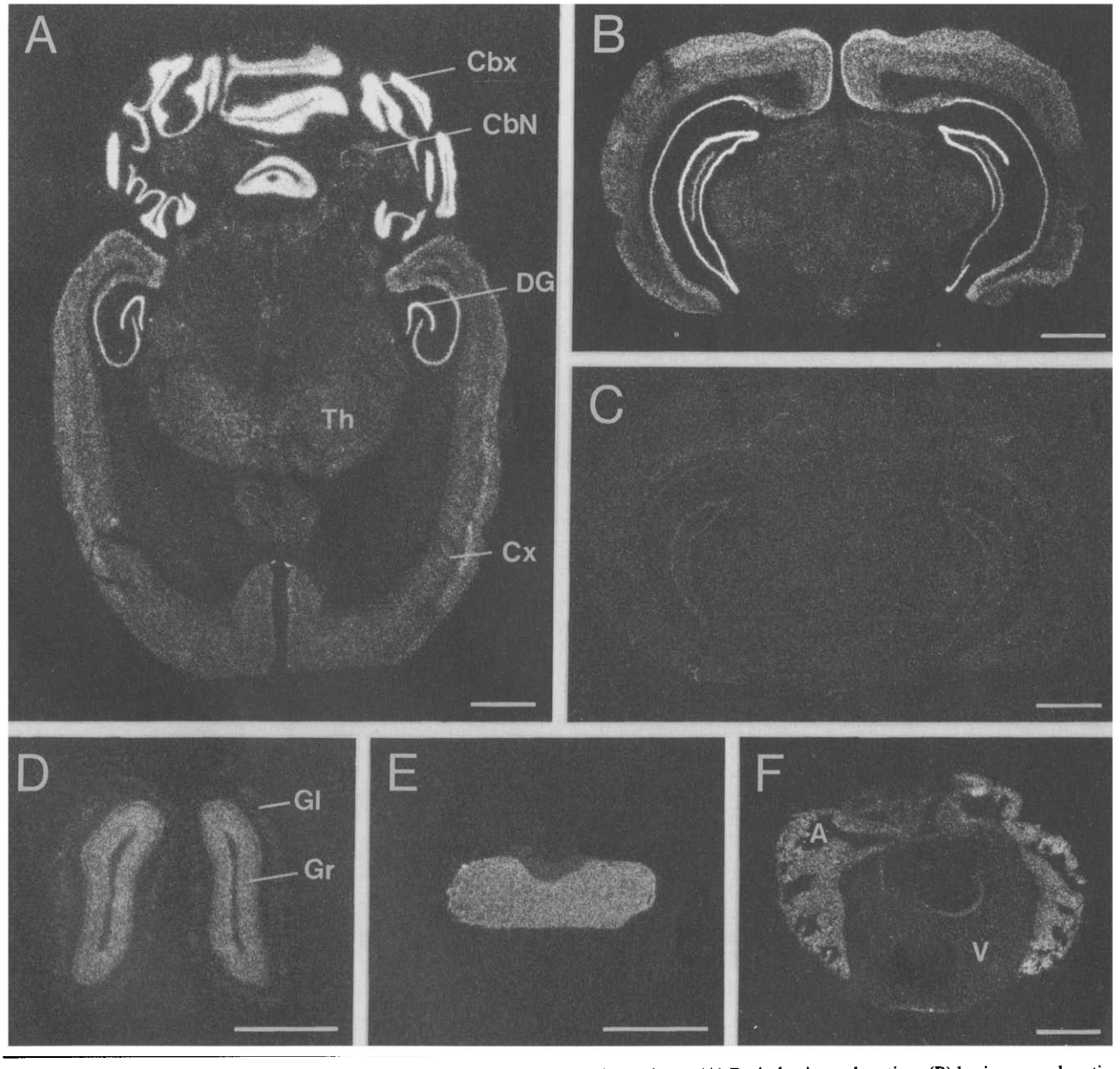

Fig. 1. Negative X-ray film images showing distribution of KGA mRNA in adult rat tissue. (A) Brain horizontal section; (B) brain coronal section; (C) adjacent coronal section hybridized with sense probe; (D) olfactory bulb; (E) pituitary gland; (F) heart horizontal section. A, atrium; Cx, cortex; Cbx, cerebellar cortex; CbN, cerebellar nuclei; DG, dentate gyrus; Gl, Glomerular layer; Gr, granule cell layer; Th, thalamus; V, ventricle. Scale bars represent 2 mm. Exposure time 5 days.

zation signals were found in the atrium of the heart and in the hippocampal region, cortex, cerebellum, the anterior pituitary and distinct nuclei in the lower brainstem of the CNS (Fig. 1). Moderate to strong labeling of KGA was seen in the olfactory bulb, thalamus and the amygdala nuclei; low level expression was observed in the basal ganglia, raphe nuclei, retina and hypothalamus. In peripheral tissues no specific signal was observed in the lung and no expression could be detected in the cardiac ventricle, skeletal muscle and kidney. In contrast, we found highly abundant expression of ROMK1 mRNA [7] in the inner medulla of the rat kidney, while it was basically absent from nervous tissue (not shown).

No hybridization signal could be detected in parallel sections that had been hybridized with either of the sense RNA probes (e.g. Fig. 1C) or treated with RNase A before hybridization. In addition, only very low levels of specific signal were seen in the sections hybridized with the mixed sense/antisense riboprobe (not shown).

Analysis of emultion-dipped parasagittal, coronal and horizontal brain sections revealed more details of the hybridization pattern on the cellular level. Table 1 summarizes the results with the abundance of label expressed as the relative density of silver grains. We now describe some regions with more remarkable signals. In the olfactory system both the main and accessory olfactory bulb show labeled neurons in the granule cell layer. In the main olfactory bulb mitral cells and some tufted cells are positive also; periglomerular cells are only weakly labeled. The most intense labeling is seen in neuronal cell bodies in the anterior olfactory nucleus.

Throughout neocortex and limbic cortex, all large somata throughout cortical layers II-VI that appear lightly stained in the Nissl preparations are heavily labeled (Fig. 2B); these are likely to be pyramidal neurons. The high-power micrograph in Fig. 2C demonstrates that silver grains are localized only above the large neuronal cell bodies, but not in the smaller-sized somata which may represent non-pyramidal neurons and glia.

All pyramidal cells in the CA1, CA2 and CA3 field of Ammon's horn in the *hippocampus* are strongly labeled (Fig. 2A), and the level of expression is even higher in the granule cells of the dentate gyrus (Fig. 2A,D). A few neurons in the hilus of the dentate gyrus and the molecular layer of Ammon's horn are also labeled (Fig. 2A). Strong signals are also observed in the tenia tecta and indusium griseum of the less differentiated precommissural hippocampus, which are relay structures in the olfactory system.

In the subcortical regions of the forebrain, KGA and mRNA expression was generally weak, except for the

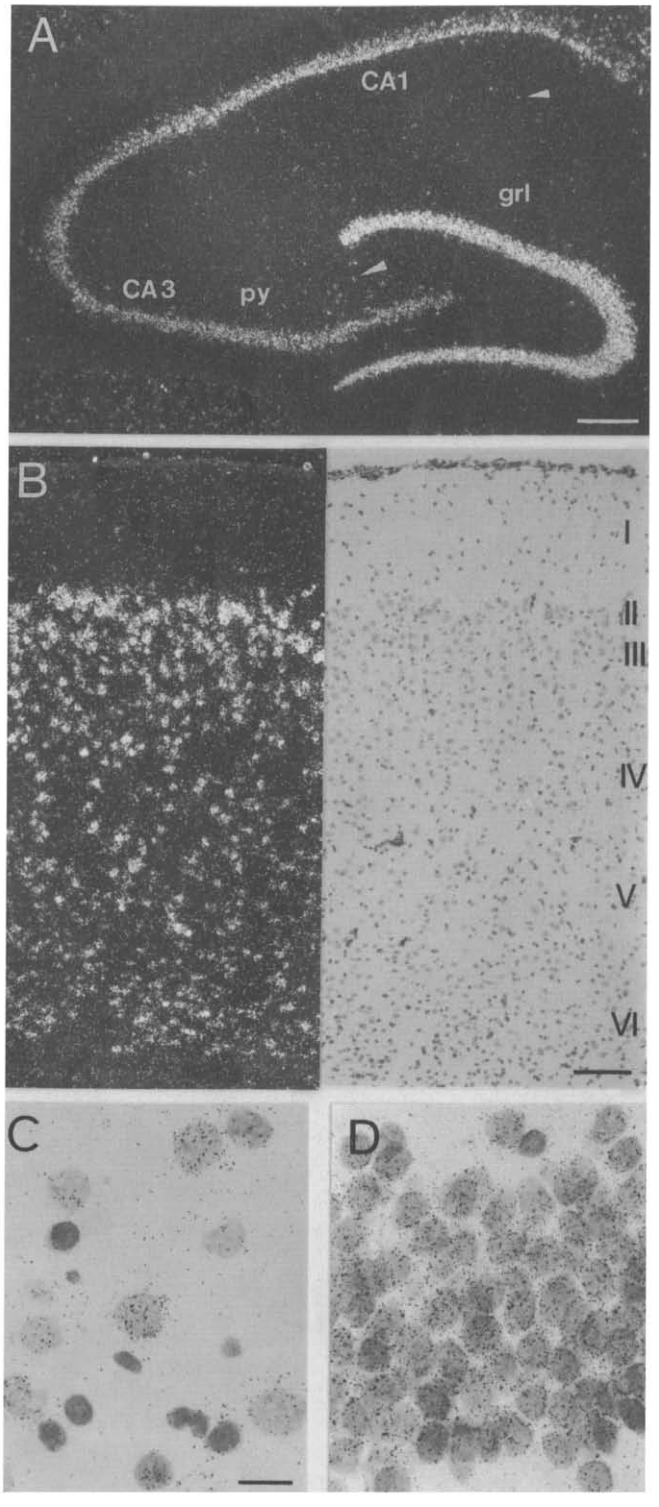

Fig. 2. (A) Dark-field photomicrograph of a horizontal section through the hippocampal dentate gyrus region. KGA mRNA labeling is located in the pyramidal layer (py) of the CA1-CA3 fields of the Ammon's horn and in the dentate granule cell layer (grl). Some few cells in the molecular layer are also labeled (arrowheads). (B) Dark-field and bright field view of identical coronal sections through the entorhinal cortex. Strong KGA expression is shown through layers II–VI. Scale bars represent 250 μm in (A) and 125 μm in (B). (C,D) High power bright-field micrographs illustrating KGA labeled pyramidal-type neurons in neocortical layer III (C) and labeled granule cells in the dentate gyrus (D). Smaller cells, possibly corresponding to non-pyramidal neurons and glia, have not accumulated silver grains. Scale bar represents 25 μm. Emulsion dipped sections exposed for 53 days.

geniculate nucleus, the oculomotor nuclei, the thalamic reticular nucleus and the lateral septal nucleus. Low to moderate labeling was scattered in all nuclei of the amygdaloid complex and the hypothalamus. In general, neither the caudate-putamen, globus pallidus nor the substantia nigra of the basal ganglia seem to contain detectable levels of KGA mRNA, but analysis of dipped brain sections reveals a very few strongly labeled scattered cells.

Remarkable expression levels were detected in selected relay nuclei of the lower brainstem, such as the red nucleus and the precerebellar pontine nuclei, which receive input or project to the cortex or cerebellum (Fig. 3A,B). The interpeduncular nucleus, the cochlear and vestibular nuclei and the small locus coeruleus in the pontine tegmentum with its massive noradrenergic output also show strong signals. The superior colliculus, which is the major target of retinal ganglion cells represents another sensory relay system with high levels of KGA expression.

On X-ray film images, the granular layer of the cerebellar cortex as a whole gives the most intense signal; however, each medium-sized granule cell is only moderately labeled. In contrast to IRK1 [8,20], KGA mRNA cannot be detected in Purkinje cells, but in the molecular layer a few cells which may be stellate or basket cells are positive. Prominent signals are shown in all fastigial, interposed, and dentate central cerebellar nuclei (Fig. 3C).

KGA mRNA is expressed weakly by cells in the proximal inner nuclei layer (INL) of the retina and by neurons in the ganglion cell layer (data not shown). No labeling was observed in the outer nuclear layer and over the distal end of the INL.

4. Discussion

In this report we describe the overall and cellular localization of mRNA expression for the rat heart/brain G protein-activated K^+ inward rectifier KGA (GIRK1). Strong expression of KGA in heart atria together with physiological data on KGA expression in Xenopus oocytes [5,6] indicate that KGA indeed represents the well-known target channel for muscarinic m2 receptor

Table 1
Distribution of KGA mRNA in the CNS of the adult rat

Brain region	Relative expression	Brain region	Relative expression
Olfactory system		Lower brainstem	
Main olfactory bulb	++	Red nucleus	++++
Accesory olfactory bulb	++	Superior colliculus	++
Olfactory tubercle	++	Inferior colliculus	++
Anterior olfactory nucl.	++	Interpeduncular nucleus	++
Neocortex	+++	Locus coeruleus	++
Limbic cortex	+++	Superior olivary nuclei	+
Hippocampus		Pontine nuclei	+++
Dentate gyrus granule cells	++++	Raphe nuclei	+/−
CA1 pyramidal	++++	Pontine reticular formation	+
Ammon's horn (CA1-CA3)		Central gray	++
pyramidal cells	++++	Cerebellum	
tenia tecta	+++	Deep nuclei	+++
indusium griseum	++++	Molecular layer	+
Septum		Granule cell layer	++
Stria terminalis	−	Purkinje cells	−
Lateral septal nucleus	++	Corpus callosum	−
Basal ganglia		Spinal cord	
Caudate putamen	−/+	Spinal trigeminal nuclei	+++
Ventral pallidum	−	Retina	
Globus pallidus	−/+	ONL	−
Claustrum	+	INL	+
Substantia nigra	−/+	GCL	+
Amygdala	+/++	Pineal gland	−
Hypothalamic region	+	Pituitary, anterior	++++
Thalamus	++		
Thalamic retiocular nucleus	+++		
Geniculate nucleus	+++		
Habenular nuclei	+		
Ventroposterior nuclei	+		
Lateroposterior nuclei	++/+++		

In situ hybridization signals obtained for ^{35}S-labeled KGA probes on brain sections (dipped sections and X-ray film) were rated according to the relative grain density:
++++, very abundant; +++, abundant; ++, moderate; +, low; −, background level = no expression.

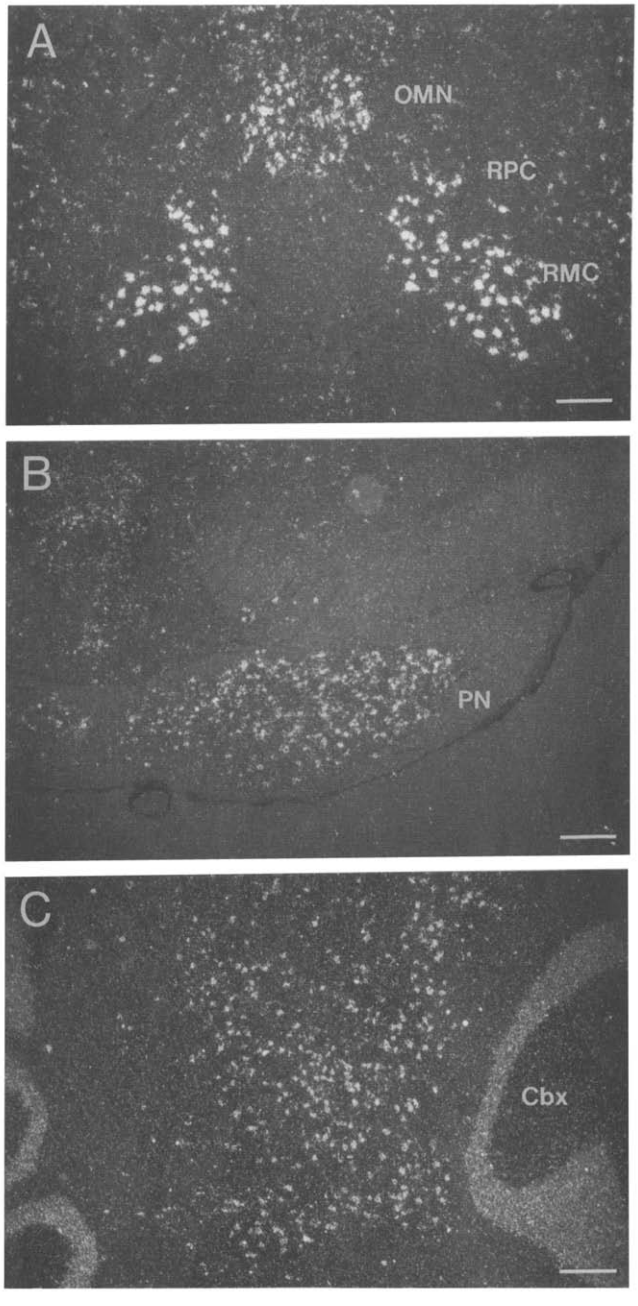

Fig. 3. Expression pattern of KGA transcripts in selected brain nuclei. Very abundant expression is seen in the parvocellular (RPC) and magnocellular (RMC) regions of the bilateral red nuclei in the lower brainstem as well as the paired oculomotor nuclei (OMN) of the midbrain tegmentum (A). Strong label is also found in the precerebellar pontine nuclei (PN) of the lower brainstem (B) and multiple deep cerebellar nuclei (C); Cbx, cerebellar cortex. Scale bars represent 250 μm. Emulsion dipped sections exposed for 37 days.

mediated inhibition, the mechanism that underlies the slowing of the heart rate by vagal stimulation. KGA single-channel recordings identify a 38–42 pS conductance channel both in oocytes [5,6] and native atrial tissue [6,11,12] activated by receptor stimulation or by internal application of G_i-subunits. Several lines of evidence suggest the presence of homologues of the ~40 pS atrial channel in the brain, A cDNA clone (KGB1) almost identical to KGA with only 5 different bases at the 5′ untranslated region (UTR) and an additional 41 bp in the 5′ UTR has been isolated from a rat brain cDNA library and functionally expressed in oocytes [6]. This gene may code for the inwardly rectifying K^+ conductance, that serves as a target for the convergent action of many inhibitory neurotransmitters in hippocampal CA1 pyramidal cells [13], in thalamic neurons [14] and in the striatum [15]. For the former two regions, our in situ hybridization data match the physiological observations. Interestingly, G protein gating of an inwardly rectifying K^+ channel in hippocampal neurons is reported to be mediated bu $G\alpha_o$ subunits [16], which are ineffective in activating the native channel in atrial myocytes. In light of the recent finding that $G\beta\gamma$ subunits are likely to be the primary regulators of KGA activity [17] these earlier reports may have to be reevaluated.

Northern blot hybridization of rat brain RNA with KGA cDNA indicates the presence of several RNA species; these may represent splice variants, isoforms of KGA or homologous genes for other K^+ inward rectifiers [6]. In GH_3 and AtT20 clonal rat anterior pituitary cells ACh and somatostatin activate a similar inwardly rectifying conductance, however with a slightly larger 55 pS conductance [18]. An 80 pS channel represents an effector for the action of opiates and possibly norepinephrine and somatostatin in the locus coeruleus [19]; multiple conductance states can be activated by serotonin in the dorsal raphe [20]. The widespread hybridization pattern with KGA antisense probes, combined with the diversity of conductances measured for inwardly rectifying K^+ channels, suggests that some of this diversity may occur because hetero-oligomeric channels form that contain KGA subunit(s) along with other, yet unknown subunits.

Our in situ hybridization data agree with previous Northern blot results showing that KGA is expressed in the brain and atrium, but not in ventricle, kidney, and skeletal muscle. The expression pattern of KGA contrasts with the distribution of the voltage- and ATP dependent ROMK1 type of K^+ inward rectifiers which is not detectable in the brain and shows a distinct expression pattern in the outer medulla of rat kidney. In situ hybridization in the mouse brain for another voltage-dependent K^+ inward rectifier, IRK1, also shows significant label in the olfactory bulb and cortex, but distinct differences from the KGA expression pattern in other brain regions, e.g. positive staining in the striatum, caudate putamen, molecular layer and Purkinje cells in the cerebellum [21].

Several observations suggest that our data represent specific hybridization to KGA and/or closely homologous channels. (1) Control experiments are described in section 2. (2) Nearly identical distribution patterns are revealed with full length hybridization probes (~2070 bp)

and a 291 bp C-terminal region (plus 535 bp 3′ UTR) which are identical between KGA and KGB and show no homology to either IRK1 or ROMK1. (3) Distinct hybridization patterns are obtained with KGA, ROMK1, and IRK1. Further details about specificity can be obtained only when and if additional KGA homologs are cloned and sequenced so that differential hybridization probes can be designed.

G protein activated K^+ inward rectifiers are the target for multiple signaling pathways and underlie receptor-stimulated inhibitory postsynaptic potentials in many neurons. Our results demonstrate that one or several KGA homologues due to the wide and highly selective expression in many brain regions may significantly contribute to neuronal signal processing in the CNS.

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