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Cyclic AMP regulates potassium channel expression in C6 glioma by destabilizing Kv1.1 mRNA

(gene expression/translational regulation/glia)

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ABSTRACT **The tissue distributions and physiological properties of a variety of cloned voltage-gated potassium channel genes have been characterized extensively, yet relatively little is known about the mechanisms controlling expression of these genes. Here, we report studies on the regulation of Kv1.1 expressed endogenously in the C6 glioma cell line. We demonstrate that elevation of intracellular cAMP leads to the accelerated degradation of Kv1.1 RNA. The cAMP-induced decrease in Kv1.1 RNA is followed by a decrease in Kv1.1 protein and a decrease in the whole cell sustained K^+ current amplitude. Dendrotoxin-I, a relatively specific blocker of Kv1.1, blocks 96% of the sustained K^+ current in glioma cells, causing a shift in the resting membrane potential from −40 mV to −7 mV. These data suggest that expression of Kv1.1 contributes to setting the resting membrane potential in undifferentiated glioma cells. We therefore suggest that receptor-mediated elevation of cAMP reduces outward K^+ current density by acting at the translational level to destabilize Kv1.1 RNA, an additional mechanism for regulating potassium channel gene expression.**

Regulation of gene expression can occur at the level of transcription, translation, or posttranslationally. Among the genes encoding voltage-gated potassium (Kv) channels, transcriptional regulation has been analyzed for Kv1.5 and Kv3.1 (1–5). For example, Takimoto *et al.* (6) demonstrated that expression levels of Kv1.5 were affected at the level of transcription with dexamethasone treatment but that mRNA half-life and protein turnover were unchanged. Posttranslational regulation has also been convincingly demonstrated for a number of Kv channels. For example, phosphorylation by tyrosine kinase, protein kinase C, and protein kinase A have each been shown to modulate Kv channel function (7–9), and a channel-associated β-subunit (Kvβ2) has been shown to promote translocation of Kv1.2 to the plasma membrane (10). In contrast, relatively little is known about regulation of Kv genes at the translational level. Given the fact that many Kv transcripts are large (6–12 kb) but contain relatively small ORFs (1.5–2.5 kb), it seems likely that the untranslated portions of these transcripts may have a regulatory function.

There exists a variety of forms of translational regulation, including changes in nuclear processing of the transcript (splicing, capping, polyadenylation, and nuclear export) as well as changes in the efficiency of translation (ribosome binding, loading, scanning, and initiation) and changes in transcript stability (reviewed in ref. 11). Well studied examples include *S*-adenosylmethionine decarboxylase, regulated by changing the efficiency of ribosome loading and scanning (12), and iron metabolism, regulated by blockade of ribosome scanning and by regulation of transcript stability in the ferritin and transferrin receptor genes, respectively (13). Among ion channel genes, Wymore *et al.* (14) demonstrated that alternatively spliced 3′-untranslated regions (UTRs) from Kv1.4 cause differing levels of Kv1.3 current to be induced in *Xenopus* oocytes injected with cRNA from the chimeric constructs, suggesting that differences in the sequence of the alternative 3′-UTRs result in different translational efficiencies.

We have used the C6 glioma cell line to study the molecular mechanisms regulating the endogenously expressed Kv1.1 potassium channel. We show that when intracellular cAMP levels are increased, there is a rapid reduction in the steady-state level of Kv1.1 RNA but only a small decrease in the transcription rate of the Kv1.1 gene. The decrease in the steady-state abundance of the Kv1.1 transcript is followed by a decrease in Kv1.1 channel protein levels and a decrease in the whole-cell potassium (K^+) current. Finally, we demonstrate that Kv1.1 is important in setting the resting membrane potential of C6 glioma cells. These data provide an example of ion channel gene expression being regulated by cAMP via a destabilization of the RNA transcript.

METHODS

Reagents. Tissue culture media, penicillin/streptomycin, and L-glutamine were purchased from GIBCO/BRL. Fetal bovine serum and equine serum were purchased from HyClone. Forskolin and 1,9-dideoxyforskolin were purchased from Calbiochem, template for rat cyclophilin riboprobe was purchased from Ambion, radioisotopes were purchased from NEN, and recombinant RNase inhibitor was purchased from Promega. All other reagents were purchased from Sigma.

Cell Culture. The C6 glioma cell line was purchased from the American Type Culture Collection and maintained at less than 80% confluence in F10 medium supplemented with 15% equine serum, 2.5% fetal bovine serum, 2 mM glutamine, and 10 units/ml penicillin G, 10 μg/ml streptomycin sulfate. Cells between passages P41 and P50 were seeded at 2.0×10^4 cells per cm^2 except that a lower density was used for seeding cells used for electrophysiology (see below). Single-dose pharmacological treatments were initiated 24 h after seeding; cells were harvested at the time periods indicated in the figure

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Abbreviations: RPA, ribonuclease protection assay; UTR, untranslated region; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; IBMX, 3-isobutyl-1-methylxanthine; Iso, (−)-isoproterenol; Kv, voltage-gated potassium; LDH, lactate dehydrogenase; PKA, protein kinase A; DTX-I, dendrotoxin-I; CHO, Chinese hamster ovary; RMP, resting membrane potential; CRE, cAMP response element; GRE, glucocorticoid response element.

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legends. For all experiments, vehicle was added to untreated control cells. All experiments were repeated at least twice.

RNA Analysis. RNA was extracted from the cells by using guanidinium as described (15). For RNase protection assay (RPA), 5 μg of total RNA per sample was incubated with two ^{32}P-radiolabeled riboprobes, one for mouse Kv1.1 (16) and one for rat cyclophilin. The RPA protocol is that of Bordonara *et al.* (17) except that Kv1.1 riboprobe was synthesized with 0.4 μM UTP in the reaction mixture and cyclophilin with 20 μM UTP in the reaction mixture. In the hybridization mixture, 5×10^5 cpm of Kv1.1 and 1×10^5 cpm of cyclophilin were included per sample. The dried gels were exposed to a PhosphorImager screen (Molecular Dynamics) for quantitative analysis. The results are represented as the ratio of Kv1.1 protected fragment/cyclophilin protected fragment. Statistics were calculated using a two-tailed *t* test.

Nuclear Run-On. Nuclei were prepared from 5×10^7 cells as described (18) except that 18 strokes of the homogenizer were required to obtain clean nuclei. Nuclei were resuspended in 200 μl of glycerol storage buffer, and nuclear run-on transcription reactions were started immediately by addition of 250 μl of 2× buffer with NTPs, 250 μCi (25 μl) of [^{32}P]UTP, and 5 μl of RNase inhibitor (20–40 units/μl) (18). The nascent RNA transcripts were extracted using guanidinium as described (19). The final pellet was resuspended in 2 ml of hybridization solution (10 mM Tes, pH 7.4/10 mM EDTA/0.3 M NaCl/5% SDS), and denatured salmon sperm DNA was added to a final concentration of 0.2 mg/ml. This was hybridized to membrane-bound probes at 60°C for approximately 42 h and washed (18). PhosphorImager analysis was used for quantitation.

Slot Blot Preparation. Probes were subcloned into M13 mp18 and M13 mp19, and single-stranded DNA was prepared. The following regions were subcloned: for EF-1alpha, the *Eco*RI fragment encompassing the entire ORF; for glyceraldehyde-3-phosphate dehydrogenase, the *Pst*I fragment encompassing 1.2 kb of the ORF; for lactate dehydrogenase (LDH), the *Bbs*I to *Xba*I fragment from the pLDHA-5 plasmid (gift of R. Jungmann); for Kv1.1, a 370-bp region of unconserved 3′ sequence including 160 bp of 3′-UTR or the same 5′-Kv1.1 sequence used for riboprobe synthesis subcloned into the M13 vectors. Using a slot-blot apparatus (Bio-Rad), 5 μg of single-stranded DNA was bound per slot in 6× SSC and then UV cross-linked (Stratagene).

Protein. Following treatments, cells were washed twice with PBS, and a crude membrane fraction was prepared (20). For analysis, 25 μg of protein per lane was separated on SDS/8% polyacrylamide gels and subjected to immunoblotting as described (21), except that primary antibody was used at a 1/400 dilution, and the chemiluminescent detection system was the Pierce Super Signal chemiluminescent substrate.

Protein Kinase A (PKA) Assay. PKA activity was assayed as described (22). This assay quantitated the kinase activity in the extracts that resulted in incorporation of ^{32}P onto the peptide substrate, Kemptide, and could be inhibited by the PKA-specific peptide inhibitor, PKI.

Electrophysiological Measurements. Cells were seeded at a low density ($<1.2 \times 10^4$ cells per ml) on small glass chips, as cells may be connected by gap junction after proliferation and contact between cells. Cells were washed with Ringer's solution before the measurement of K^+ currents. Single isolated cells were used for patch–clamp measurement (23, 24). Pipette capacitance was compensated before the formation of gigaseal. Membrane capacitance was measured by reading the value for slow capacitance after optimal compensation of capacitance currents in whole-cell configuration. Voltage-dependent K^+ currents were activated by depolarizing voltage steps. Current was amplified by an EPC-7 (List, Germany). Leak and capacitance currents were subtracted by using current recordings with negative voltage step. Current density was calculated by dividing current amplitude by the cell membrane capacitance. Resting membrane potential was measured in current-clamp mode ($I = 0$).

Cells were perfused with Ringer's solution of the following composition: 135 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, and 10 mM Hepes, adjusted to pH 7.4 with NaOH. Pipette resistance was 2–3 MΩ when filled with intracellular solution: 140 mM KCl, 2 mM $MgCl_2$, 5 mM EGTA, and 10 mM Hepes, adjusted to pH 7.2 with KOH. For solutions containing dendrotoxin-I (DTX-I), 0.01% BSA was added to reduce nonspecific binding. A multibarreled solution exchange system applied the toxin to cells. All data are expressed as the mean ± SEM.

RESULTS

To study the mechanism of the endogenous regulation of expression of Kv1.1 potassium channel, we screened neuronal (Neuro-2-A, NB41A3, N4, N18, SH-SY-5Y, IMR-32) and non-neuronal (CHO, AtT-20, MDCK, COS-7, C6 glioma) cell lines by RPA to find ones that expressed the Kv1.1 channel. We found that only C6 glioma expressed Kv1.1 in the unstimulated state, as previously reported by Wang *et al.* (24). C6 glioma were treated with a single, continuous application of various pharmacological agents known to stimulate second-messenger systems, and changes in the steady-state levels of Kv1.1 RNA were measured relative to cyclophilin RNA. When the cells were treated with the phorbol ester, phorbol 12-myristate 13-acetate (100 nM), or elevated extracellular KCl (50 mM), the steady-state level of Kv1.1 RNA was reduced to less than 50% of control levels when measured at either 6 or 24 h (Table 1). When the cells were treated with the calcium ionophore A23187 (1 μM), there was a biphasic change in the Kv1.1 steady-state RNA level with about 40% of control remaining after 6 h of treatment; this was followed by a rebound to ≈130% of control after 24 h of treatment (Table 1).

Table 1. Effect of pharmacological treatments on Kv1.1 mRNA steady-state levels

	Kv1.1/cyclophilin mRNA level, % of control			
	2 h	4 h	6 h	24 h
KC1	—	—	50.2 ± 1.1***	48.0 ± 2.8***
PMA	—	—	41.7 ± 1.6***	43.4 ± 4.0***
A23187	—	—	41.3 ± 3.6***	129.2 ± 9.6***
Forskolin	54.7 ± 0.1*	47.2 ± 3.4*	38.7 ± 1.4*	22.5 ± 0.1***
1,9-dideoxyforskolin	—	—	—	93.6 ± 0.5
8-cpt-cAMP	42.6 ± 1.3*	—	17.5 ± 3.6*	9.4 ± 0.3***
Iso/IBMX	49.7 ± 0.3**	23.8 ± 0.4***	19.8 ± 0.4***	18.5 ± 2.9***
Iso	—	—	47.2 ± 0.7**	—
IBMX	—	—	58.2 ± 0.1*	—

PMA, phorbol 12-myristate 13-acetate; 8-cpt-cAMP, 8-(4-chlorphenylthio)-adenosine 3′,5′-cyclic monophosphate. *, $P \leq 0.05$; **, $P \leq 0.005$; ***, $P \leq 0.001$.

When the C6 glioma were treated with 10 μM (−)-isoproterenol + 500 μM 3-isobutyl-1-methylxanthene (Iso/IBMX) to elevate intracellular cAMP concentrations, there was a 5-fold decrease in the steady-state level of Kv1.1 RNA (Fig. 1). Focusing on this robust change, we determined the time course of the decrease in control or Iso/IBMX-treated cultures taking time points out to 72 h of treatment. Kv1.1 RNA level was ≈50% of control by 2 h and showed maximal effect after 4–6 h of treatment (Fig. 1*B*). Similar decreases were seen when cAMP levels were raised by use of forskolin (10 μM) or the nonhydrolyzable cAMP analog 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (8-cpt-cAMP, 100 μM) (Table 1). Combined treatment with Iso/IBMX was necessary to attain the full decrease as Iso or IBMX alone after 6 h showed levels of Kv1.1 RNA at 50% of control compared with 20% for Iso/IBMX (Table 1 and Fig. 2*B*). Garber *et al.* (25) and Zerr and Feltz (26) have demonstrated that forskolin can act as a direct blocker of K^+ currents. In light of these observations, we tested the inactive forskolin analog, 1,9-dideoxy forskolin (10 μM), and determined that it did not affect Kv1.1 RNA levels (Table 1). Therefore, we conclude that the effect of forskolin on decreasing Kv1.1 transcript levels was via activation of adenylyl cyclase.

To demonstrate that treatments with Iso/IBMX and forskolin were indeed elevating cAMP concentrations in the cells, we assayed Kv1.1 RNA levels and protein kinase A activity in paired cultures treated with the various agents for 6 h. RNA was prepared from half of the cultures for RPA assays; protein was extracted from the remaining cultures for PKA activity assays. Fig. 2 shows that there was an inverse correlation between the PKA activity and the Kv1.1 steady-state RNA level, suggesting that the decrease of the Kv1.1 transcript is linked to activation of PKA.

To examine the mechanism of regulation of Kv1.1 transcript levels by cAMP, we measured the Kv1.1 message half-life by treating parallel cultures with vehicle, Iso/IBMX, or the transcription inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (Fig. 3*A*). Cultures were harvested at several time points, and RNA level was measured by RPA. Half-life was determined from semilogarithmic plots of the data (Fig. 3*A*). In untreated control cells, there was no change in the Kv1.1 transcript level, whereas with the transcription inhibitor DRB the Kv1.1 RNA half-life was ≥6 h, and with Iso/IBMX half-life was <3 h. Essentially identical results were obtained when transcription was inhibited with actinomycin D (data not shown). These data indicate that increasing cAMP leads to an increased rate of degradation of Kv1.1 RNA, one that is more rapid than can be accounted for by turning off gene transcription with either of the classic transcription inhibitors DRB or actinomycin D.

Besides increased degradation, an alternative explanation for the decrease in Kv1.1 RNA with increasing cAMP might be that newly synthesized Kv1.1 RNA was blocked from nuclear export. This type of regulation has been observed for host transcripts in viral infected cells (27, 28). To determine whether increases in cAMP lead to a block of nuclear export of Kv1.1 RNA, we performed RPAs on the cytoplasmic RNA versus nuclear RNA prepared from C6 glioma treated for 6 h with vehicle or Iso/IBMX. The Kv1.1 transcript level for Iso/IBMX compared with control-treated cells was 19% in cytoplasmic RNA and 23% in nuclear RNA. These decreases are of the same magnitude as seen in total cellular RNA (Table 1; Figs. 1*B* and 2), suggesting that the decrease in Kv1.1 RNA was not caused by a block of nuclear export.

To determine the effect of increased cAMP on transcription of the Kv1.1 gene, we conducted nuclear run-on transcription assays. Transcription measured from nuclei prepared from cells treated for 6 h demonstrated that there was a 35% decrease in Kv1.1 signal relative to that detected in untreated cells (Fig. 3*B*). As a positive control for the assay, measurements of transcription of LDH made with the same nuclei showed an average 3.5-fold increase in transcription with Iso/IBMX treatment, as previously reported by Jungmann *et al.* (29). These data suggest that increased cAMP causes an apparent decrease in Kv1.1 transcription. A caveat to this interpretation is that, because newly synthesized RNA is measured in the assay, nuclear run-on assays assume that the stability of the newly transcribed RNA is unaffected by the

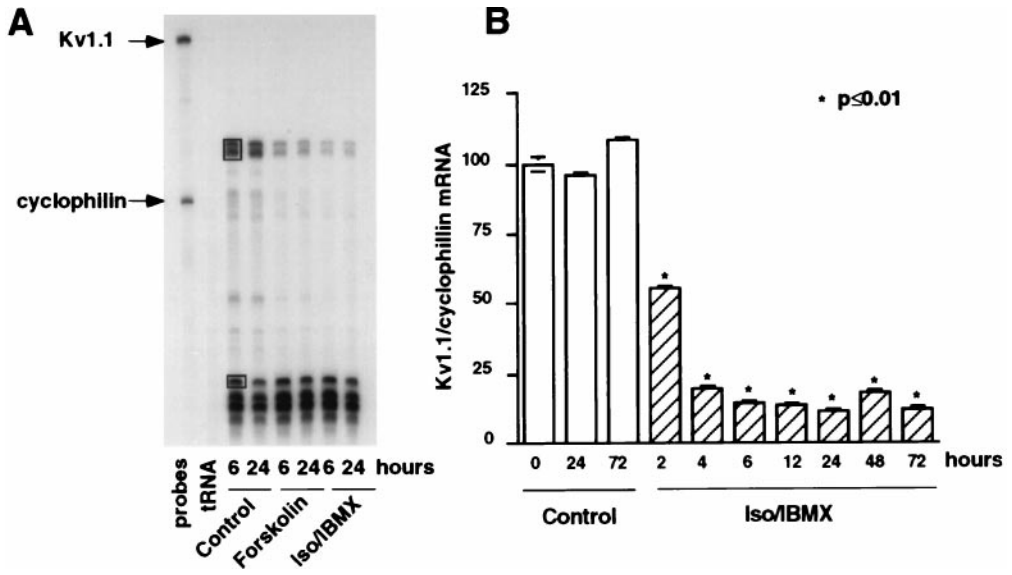

FIG. 1. Kv1.1 RNA level decreases with elevation of intracellular cAMP. (*A*) A representative RPA gel showing single samples at 6 and 24 h for control, forskolin, and Iso/IBMX-treated cultures. Undigested, full-length probes (Kv1.1 and cyclophilin) are indicated. Protected bands that were quantitated are *boxed* in the third lane. The mouse Kv1.1 probe protected two bands in rat-derived C6 glioma. Cyclophilin consistently gave a single band with a smear below. The largest cyclophilin band was used to quantify changes in Kv1.1 RNA levels. (*B*) Kv1.1 RNA levels in C6 glioma were treated with vehicle (control) or Iso/IBMX for up to 72 h. Triplicate plates were collected for each treatment group. Cyclophilin levels were unchanged over 12 h but declined in both treated and control samples collected at 24, 48, and 72 h. Kv1.1 levels did not change significantly over 72 h in control samples. Therefore, the data have been corrected to the cyclophilin levels at 0 h. Statistical analysis included a one-way ANOVA followed by a Tukey's multiple comparison test.

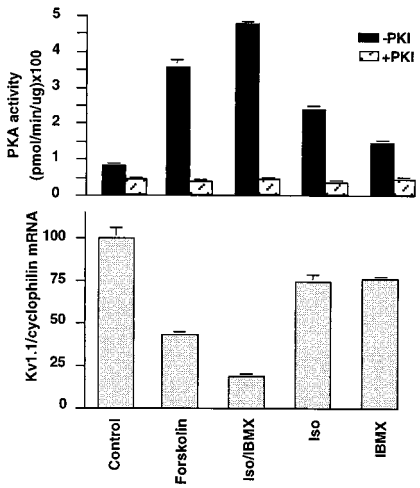

FIG. 2. PKA activity and Kv1.1 RNA levels are inversely correlated. Paired sets of C6 glioma cultures were treated with the indicated pharmacological agents for 6 h and then harvested for PKA activity assays (*Upper*) and RPA assays (*Lower*). See methods for details.

pharmacological treatments. Given that we know Iso/IBMX treatment affects RNA stability (Fig. 3*A*), the apparent 35% reduction in newly synthesized Kv1.1 transcript measured by nuclear run-on assay may overestimate the actual change in Kv1.1 transcription rate. Alternative assays of transcription rate, like approach to steady state (30, 31), give similar results for Kv1.1 in C6 glioma (data not shown) but suffer from the same inherent caveat.

Schimke (32) noted that, in general, tightly regulated proteins are encoded by RNAs that turn over rapidly. Having determined that the Kv1.1 transcript was rapidly decreased when cAMP levels were elevated, we performed Western blot analysis on membrane fractions prepared from control or Iso/IBMX-treated cells (21). As seen in Fig. 4, Kv1.1 protein levels in Iso/IBMX-treated cells first show a noticeable decrease at 9 h, with barely detectable levels at 48 h.

To measure changes in density of voltage-dependent K^+ channels in the plasma membrane, we conducted whole-cell patch–clamp experiments on control and Iso/IBMX-treated cells. Potassium currents were activated by voltage steps from a holding potential of −70 mV to different levels of depolarization. The currents started to activate around −40 mV, near the activation threshold for Kv1.1 (16, 33). To compare channel density, K^+ current was elicited by a voltage step from −70 mV to 0 mV (Fig. 5*A*). As some cells showed fast-inactivating currents at the beginning of depolarizing pulse (see also ref. 24), the amplitude of sustained K^+ current was measured as the mean of current during the last 10 ms of a 100-ms-long pulse [Kv1.1 expressed in CHO cells shows <5% inactivation after a 500-ms pulse (16)]. Because relatively high variability was observed in current density between cells whether treated or control, we performed a population study (Fig. 5*B*). Current density of control and treated groups were significantly different ($P \leq 0.01$, two-tailed t test) with prolonged Iso/IBMX treatment, 24 and 48 h. These differences were consistently observed in four different sets of cells.

When cells were acutely treated (3 min) with Iso/IBMX, the amplitude of the K^+ current was not changed (100 ± 4% of that before the treatment, $n = 5$). A similar lack of acute effect on K^+ current was observed when either PKI or PKA catalytic subunit was included in the patch pipet during whole-cell recordings of Kv1.1-transfected CHO cells (16).

Kv1.1 is one of three Shaker family potassium channels (Kv1.1, Kv1.2, and Kv1.6) sensitive to DTX-I found in the venom of black mamba snakes (34). C6 glioma were treated with DTX-I to determine whether the K^+ currents recorded in

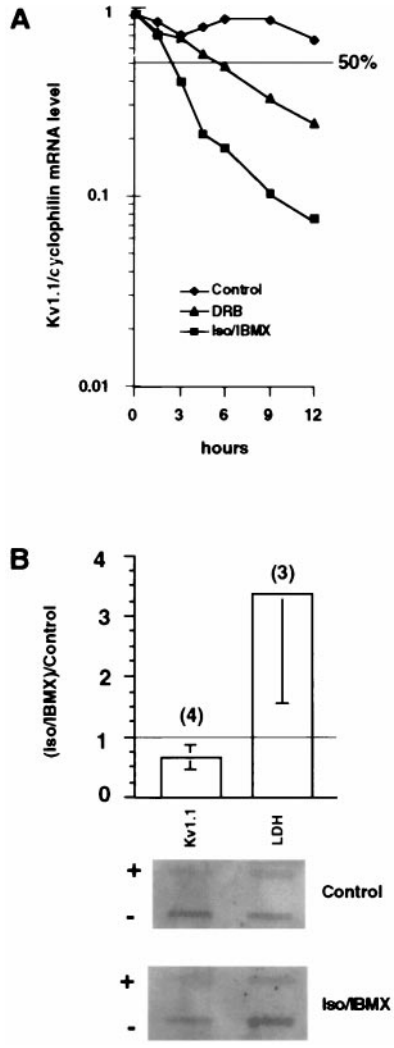

FIG. 3. Elevation of cAMP affects the rate of Kv1.1 RNA degradation with only a small affect on transcription of Kv1.1. (*A*) Kv1.1 RNA half-life was determined by initiating pharmacological treatments on duplicate cultures/treatment/time point and harvesting RNA at the indicated times. RPA analysis was used to follow Kv1.1 RNA levels relative to cyclophilin. Cyclophilin levels did not change significantly with any of these treatments at any of these time points. Error bars are not shown because error was ≤2% at each time point. Half-life measurements were made by plotting results on a semi-log plot (50% level indicated by the thin horizontal line.) Three independent experiments, with $n = 2$ for each treatment at each time point, were conducted with the same results. (*B*) For nuclear run-on transcription assays, nuclei were prepared from cells treated for 6 h with vehicle (control) or Iso/IBMX. Quantitation represents the average of the ratio of Iso/IBMX over control for the antisense strand with the number of measurements indicated above the bar. Standard deviation for Kv1.1 = 0.23 and for LDH = 1.82. No change would be equal to 1.0 as indicated by the horizontal line on the graph. The radiographic image is one representative experiment. The (+) indicates the sense strand probe, and the (−) indicates the antisense strand probe.

these cells were sensitive to this relatively specific Kv channel blocker. DTX-I (100 nM) reduced K^+ currents significantly (6 ± 1% of control, $n = 14$; Fig. 6*A*). Recovery from block by DTX-I was complete in some but not in all cells. Because resting membrane potential (RMP) of untreated C6 glioma cells was −40.4 ± 1.3 mV ($n = 10$, Fig. 6*B*) and was sensitive to DTX-I (−7.9 ± 2.5 mV, $n = 10$), we suspected that DTX-I sensitive K^+ currents, probably containing Kv1.1 subunits, were responsible for generation of the RMP. Indeed, Northern blot analysis of poly(A)$^+$ RNA prepared from unstimulated

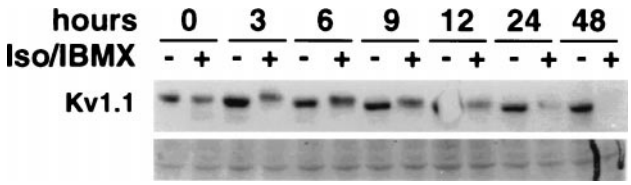

FIG. 4. Elevation of cAMP decreases Kv1.1 protein. Cells were treated with vehicle (control) or Iso/IBMX for the indicated times, and a crude membrane fraction was prepared. Western blot analysis (*Upper*) was performed with a polyclonal antibody that specifically recognizes Kv1.1 protein (21). Equal amounts of protein were loaded per lane as evidenced by a prominent 51-kDa band of a Coomassie-stained paired gel (*Lower*).

and Iso/IBMX-stimulated C6 glioma showed that only Kv1.1 was detectable and not Kv1.2, Kv1.5, or Kv1.6 (data not shown). The depolarizing effect of DTX-I was reversible (after wash out -37.4 ± 3.2 mV, $n = 7$) (Fig. 6*B*).

DISCUSSION

Kv channels are involved in determining excitability in a variety of cell types (35). In neurons, Kv channels are likely to be involved in repolarizing the membrane following action potentials and in regulating neurotransmitter release. In lymphocytes, Kv1.3 has been shown to set the RMP, which in turn is permissive for proliferation (36). Thus, because of their critical functions, expression as well as activity of Kv channels must be carefully regulated. We show here that intracellular cAMP levels regulate expression of Kv1.1 by inducing a destabilization of the Kv1.1 transcript.

Previous work has shown that cAMP regulates transcription of Kv1.5, a Kv channel expressed in cardiac and pituitary cells (1–4). Takimoto *et al.* (37) have clearly demonstrated that PKA activity is required for basal transcription of Kv1.5 and that Kv1.5 RNA stability is unchanged when PKA activity is inhibited. Promoter elements identified in the 5′-flanking region of the Kv1.5 gene include a cAMP response element (CRE), a glucocorticoid response element (GRE), and a newly identified Kv1.5 repressor element (KRE). The GRE is likely to be involved in mediating the dexamethasone-induced increase in transcription in cells of both pituitary and cardiac origin. Interestingly, the CRE is likely to be involved in the cAMP-stimulated increase in transcription in cells of cardiac origin and decrease in transcription in cells of pituitary origin.

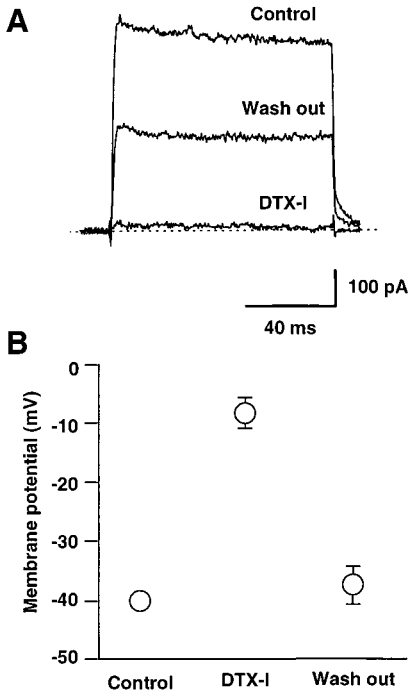

FIG. 6. Potassium currents and the resting membrane potential in C6 glioma cells are sensitive to DTX-I. (*A*) Currents were recorded in Ringer's solution (control) after 2 min of perfusion with 100 nM DTX-I containing Ringer's (DTX-I) and after 3.5 min of wash out with Ringer's (wash out). K^+ currents were activated by the same voltage protocol as Fig. 5*A*. (*B*) Membrane potential in current-clamp mode ($I = 0$) was measured under the same conditions as *A*.

These opposing effects on transcription coupled to elevation of cAMP might be explained by the large family of CRE binding proteins, including CREB/CREM, that have been shown to have both stimulatory and inhibitory effects on transcription (38). Analysis of the proximal 1.5 kb of promoter region 5′ of the Kv1.1 transcriptional start site reveals that it does not contain CRE or GRE elements and does not respond to cAMP in luciferase reporter gene constructs (M.L.A. and B.L.T., unpublished results).

Levin *et al.* (39, 40) reported that elevation of cAMP in *Xenopus* oocytes expressing cRNA encoding the Kv1.1 ORF

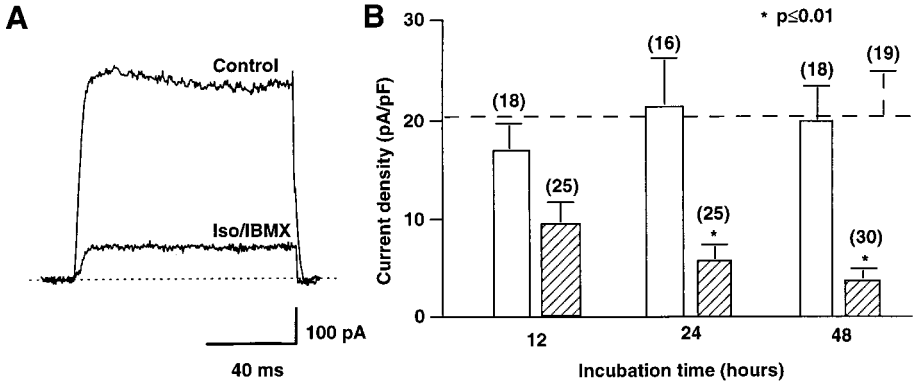

FIG. 5. Elevation of intracellular cAMP level decreases K^+ current density. (*A*) Representative K^+ currents in a cell treated with vehicle (control) and in another cell treated with Iso/IBMX for 48 h. Currents were elicited by a voltage step from -70 mV to 0 mV in whole-cell configuration. Recordings with typical level of currents were selected for illustration. Membrane capacitance was 20 pF for the control and 15 pF for the treated cell. (*B*) Summary of current density in control cells (open bar) or in cells treated with Iso/IBMX (hatched bar) for various incubation times. For the group labeled 12 h, cells were patch-clamped between 10 and 14 h after the start of treatments. Cells were measured between 21 and 27 h for the group labeled 24 h and between 45 and 51 h for the group labeled 48 h. Current density was calculated as current at 0 mV divided by membrane capacitance. Dashed line indicates the current density of control cells at 0 h. Number of cells measured for each condition is given in parentheses.

results in an increase in the levels of Kv1.1 RNA and protein and an increase in amplitude of the resulting delayed rectifier type K^+ current. The relevance of these experiments to endogenous regulation of Kv1.1 is unclear, however, because only the Kv1.1 ORF—absent the Kv1.1 promoter, 5′-UTR region, or 3′-UTR region—was expressed in the amphibian oocyte system. Using a eukaryotic system, Bosma *et al.* (16) made stable transfections with a construct expressing the Kv1.1 ORF in normal CHO cells and in CHO cells stably transfected with a dominant negative mutation of the PKA regulatory subunit. In contrast to the oocyte system, these studies demonstrated that in cells with a chronic reduction of PKA activity, there is a 3-fold increase in Kv1.1 protein and a 3.4-fold increase in whole cell current density. As with the Levin *et al.* (39, 40) studies, the study by Bosma *et al.* (16) was done without the Kv1.1 promoter, 5′-UTR region, or 3′-UTR region in the Kv1.1 expression constructs. The most likely explanation for the opposing results seen in these studies is the difference in expression systems.

To avoid the limitations of the above studies, we investigated the molecular mechanism of regulation of expression of the endogenously expressed Kv1.1 gene in C6 glioma cells. The results reported here are consistent with the observations of Bosma *et al.* (16), namely, that when cAMP levels are elevated, Kv1.1 protein is decreased as is the peak current amplitude of a fast-activating, sustained delayed rectifier type of K^+ current (50% reduction by 12 h). These decreases were preceded by a 5-fold decrease in the steady-state level of Kv1.1 RNA. The rapid reduction in Kv1.1 RNA ($t_{1/2} < 3$ h) results from post-transcriptional destabilization of the transcript because we can demonstrate at most only a 35% reduction in Kv1.1 gene transcription. Similar observations have been reported for the β_2-adrenergic receptor (41, 42) and for type 1 angiotensin II receptor (43), both of which respond to increased cAMP by decreasing the stability of their RNA without measurable effect on their rates of transcription.

Kv1.1 is expressed strongly in certain neurons in adult rodents (44–46). During development, Kv1.1 is expressed in undifferentiated glia in the mouse embryo (47) and in isolated sciatic nerve in early postnatal rats (48). At the electrophysiological level, a sustained outward K^+ current is down-regulated during Schwann cell differentiation (49). In addition, 24–72 h of incubation of cultured oligodendrocytes with tumor necrosis factor-α has been shown to elevate intracellular cAMP levels, leading to a subsequent down-regulation of an outwardly rectifying K^+ current and a decrease in the RMP (50). Our findings show that unstimulated C6 glioma express Kv1.1 and that the resting membrane potential in these cells is affected by DTX-I blockade of K^+ currents. It is possible, therefore, that translational regulation of Kv1.1 expression plays a role in glial proliferation or differentiation.

Why might glia respond to cAMP at the translational level? Given that Kv1.1 has a large genomic unit and long 5′- and 3′-UTRs (M.L.A. and B.L.T., unpublished results), regulating expression of Kv1.1 at the translational level may allow for a more rapid or a more localized response to changes in cellular cAMP levels. The fact that many other ion channel genes have large untranslated regions suggests that this form of gene regulation (translational regulation) may be an efficient mechanism for regulating cellular excitability.

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