Cloning and Characterization of the Promoter for a Potassium Channel Expressed in High Frequency Firing Neurons*

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The Kv3.1 potassium channel is expressed in neurons that generate trains of high frequency action potentials in response to synaptic inputs. To understand the mechanisms underlying the regulation and restricted expression pattern of the Kv3.1 gene, we have cloned and characterized its promoter. We first isolated a 5.3-kilobase pair fragment of the Kv3.1 5′-flanking region. When linked to the chloramphenicol acetyltransferase reporter gene, this fragment was found to be active in the undifferentiated PC12 cell line, a neuron-like cell line, but not in a fibroblast cell line. By carrying out a series of deletion analyses in undifferentiated PC12 cells, we have localized the essential promoter region to a highly GC-rich region containing four Sp-1 binding sites. Similar deletion analysis in NIH3T3 cells suggests that multiple silencing elements and enhancing element(s) are involved in the cell type-specific expression of this gene. Further regulatory elements, including one cyclic AMP/calcium response element (CRE) and one Ap-1 element were found in the upstream region of the promoter. Using a stable undifferentiated PC12 cell line transfected with the Kv3.1 5′-flanking region, we determined that promoter activity is enhanced by a cAMP analog and a calcium ionophore. Deletion of the CRE-like element at position −252 eliminated the enhancement of promoter activity by cAMP, and mobility shift assays confirmed that the Kv3.1 CRE sequence binds both a nuclear factor in undifferentiated PC12 cells and recombinant CRE binding protein. Our results suggest that the transcription of the Kv3.1 channel may be regulated by neurotransmitters that elevate cAMP levels in neurons.

The Kv3.1 gene encodes a mammalian voltage-dependent potassium channel that is related to the Drosophila Shaw gene (1, 2). In contrast to many other potassium channels that are expressed in a variety of tissues, the Kv3.1 channel appears to be expressed only in brain and in a subpopulation of lymphocytes (1, 3, 4). In situ hybridization and immunocytochemistry have shown that within the nervous system, the Kv3.1 K⁺ channel is found only in a subset of neurons (4–8). In particular, it is expressed at particularly high levels in neurons that are capable of firing action potentials at high frequencies with little or no adaptation during maintained trains of synaptic inputs (5). Examples of such cells include neurons in auditory brainstem that phase-lock their action potentials to stimulus frequencies of up to several kHz (see Refs. 9–11) and hippocampal and cortical interneurons, which are capable of generating trains of action potentials at several hundred Hz (see Refs. 12 and 13).

The biophysical characteristics of the Kv3.1 channel also differ from those of many other voltage-dependent potassium channels. For example, when the Kv3.1 channel is expressed in a heterologous expression system such as Xenopus oocytes or a mammalian cell line, it activates at relatively positive potentials and has very rapid deactivation kinetics when compared with other members of the Shaker superfamily of channels (1, 14–16). Computer simulation studies suggest that a channel with these characteristics minimizes the relative refractory period that follows individual action potentials, and that changes in the amplitude of such a current alter the ability of a neuron to follow high frequency synaptic inputs (17). The precise factors that determine the level of expression of Kv3.1 channels in neurons are not known. However, it has been shown that the levels of the Kv3.1 message are developmentally regulated (4, 5). Furthermore, experiments in cell lines indicate that Kv3.1 transcription may be regulated by Ca²⁺, cAMP, and growth factors (18). In the present study, we have identified and characterized the promoter region for the Kv3.1 gene to investigate the mechanisms that regulate transcription of the Kv3.1 channel.

**EXPERIMENTAL PROCEDURES**

Library Screening—The 732 bp HindIII-EcoRI fragment derived from the published 5′ noncoding region of the rat cDNA clone D273 (1) was random primer-labeled to a specificity of >10⁶ cpm/μg (19) and used to screen a rat genomic library (Stratogene). We identified five positive clones and determined that they all contained a 6-kb HindII fragment. This 6-kb fragment was subcloned into pBluescript KS (Stratogene) to generate pKS 6000. A 1-kb SmaI-HindIII fragment from pKS 6000 was used as a probe sequence and was also subcloned into pBluescript KS to create the plasmid pKS 1000. Sequencing analysis was conducted using sequenase 2.0 (U. S. Biochemical Corp.) in both strands.

Plasmid Construction—Standard molecular cloning techniques were used in all plasmid construction (20), and the orientation and junction sequence were in all cases verified by sequencing the plasmid. Two series of constructs were made, based on the Promega CAT expression vectors pCATenhancer, which contains the SV40 enhancer, and pCBAT, which lacks the SV40 enhancer. pCAT5300 and pCAT5300R were constructed by subcloning the 6-kb HindII fragment from pKS 6000 into the HindIII site in the pCATenhancer. This fragment con-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U44682.

1 The abbreviations used are: bp, base pair(s); kb, kilobase pair(s); CAT, chloramphenicol acetyltransferase; CRE, cAMP/calcium response element; CRED, CRE binding protein; NRSE, neural restrictive silencing element.
tains about 5.3 kb of the 5′-flanking region and 0.7 kb of the noncoding region of the Kv3.1 gene. For pCATE5300, the HindIII fragment was inserted in the 5′ to 3′ orientation. For pCATE5300R, the orientation of the insert was reversed. pCAT5300 was constructed in a similar way using the pCAT-basic vector.

Constructs pCATE1213 and pCAT1213, which were used for stable transfections, were generated using Exonuclease III (Promega) to delete sequence from the 5′-end of pKS 6000. A plasmid with a deletion of approximately 4 kb at its 5′-end was designated as pKS 2000. Its insert was then subcloned into both the pCATE vector to generate pCATE1213 and the pCAT-basic vector to generate pCAT1213. Similarly, pCAT+22 was constructed by deleting pKS 2000 at its 5′-end using Exonuclease III.

Other clones were constructed by using convenient restriction enzyme sites. pCAT528 and pCAT266 were constructed from pCAT1213. pCAT528 was constructed from a PstI and Xhol fragment of pCAT1213, which was blunt end-treated with Klenow enzyme before ligation. pCAT266 was constructed from the PstI (blunt-ended)-Smal fragment of pCAT1213, pCAT226 and pCAT71 were generated from pKS 1000. For pCAT226, the SpeI (blunt-ended)-SalI fragment was inserted into the pCAT-basic vector cut with PstI (blunt-ended) and SalI. Likewise, the NarI (blunt-ended)-SalI fragment was inserted into the pCAT-basic vector cut with PstI (blunt-ended) and SalI to create pCAT71. pCATE71 was generated by inserting the NarI (blunt-ended)-SalI fragment into the pCATE vector.

Cell Culture, DNA Transfection, and CAT Assays—Cells were grown at 37 °C in humidified air with 5% CO2. All PC12 cells used in experiments were undifferentiated PC12 cells, which were grown in Dulbecco's modified Eagle's medium supplemented with 10% horse serum, 5% fetal bovine serum and 2 mM glutamine. NIH3T3 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Medium and serum were purchased from Life Technologies, Inc.

Plasmid DNAs purified by CsCl banding were transfected using LipofectAmine (Life Technologies, Inc.). 5 μg of the CAT constructs and 5 μg of the preP-1α2z (Invitrogen), a plasmid containing the β-galactosidase gene driven by the Rous sarcoma virus promoter, were cotransfected into the cells. 2 days after the transfection, the cells were harvested for analysis. The cell lysates were obtained by three cycles of freeze-thawing. The efficiencies of transfections were normalized using the activity of β-galactosidase in cell lysates as described by Sambrook et al. (20). CAT assays were performed as described by Gorman et al. (21). The conversion rates from chloramphenicol to acetylated forms were quantitated using a liquid scintillation counter.

The regulation of the Kv3.1 promoter by different factors was studied in stable cell lines transfected with pCATE1213 and pCAT1213. In some experiments, transfected cells were incubated for 24 h with 1 mM dibutyryl cAMP and 5 μM ionomycin before harvesting and preparation of cell lysates. The amount of protein used in each assay was determined (22) and used for normalization of results.

The pREP-lacZ construct, a plasmid containing lacZ fused to the pCMV vector, was also tested in transiently transfected cells. In these experiments, cells grown in a 60-mm dish were transfected and split 24 h later into four 35-mm dishes. The cells were allowed to attach for several hours and then treated with 1 mM dibutyryl cAMP for 24 h before harvesting and determination of CAT activity.

Primer Extension Analysis and RNase Protection Assay—RNA was isolated by the method of Chomczynski and Sacchi (23). In primer extension analysis (20), a oligonucleotide corresponding to the bases 1123 through 1103 from the Kv3.1 sequence described by Luneau et al. (1), ACCGCCGCCGCTGCTGCCTC, was used as a primer. After being end-labeled with T4 polynucleotide kinase and γ-32P-ATP (6000 mCi/mmol), the primer was annealed with RNA from rat cerebellum and extended using reverse transcriptase. The extension products were visualized by autoradiography on 5% DNA sequencing gels. The size of the cDNA reflects the distance from the primer to the 5′-end of the RNA.

In the RNase protection assay to determine transcription start sites, a [32P]-labeled antisense RNA probe was transcribed using T7 polymerase from pKS 1000 linearized with BamHI. The resulting probe is complementary to the 158 bp of the published 5′ region and extends 286 bp upstream in the genomic clone. Subsequent procedure was similar to that described by Chamberlin and Ryan (24). Briefly, an excess of the 32P-labeled probe was hybridized with 10 μg of total RNA from rat cerebellum or tRNA in 80% formamide at 46 °C. The single-stranded RNAs were then digested with 0.5 μg/ml RNase A and 0.5 μg/ml RNase T1. The protected fragments were run on denaturing polyacrylamide gels and autoradiographed. RNase protection assays were also conducted to analyze the regulation of Kv3.1 mRNA by 1 mM 8-bromo-cAMP. Total RNA from PC12 cells treated for 24–48 h with 1 mM 8-bromo-cAMP and control cells were hybridized with Kv3.1-specific riboprobe as described previously (5). A sense probe from the glyceraldehyde-3-phosphate dehydrogenase gene (Ambion, TX) was used as an internal standard to normalize the amount of total RNA used. Protected bands shown on autoradiography were quantified using an IS-1000 Digital Imaging system (Alpha Innotech Corporation, CA).

Nuclear Extract and Gel Mobility Shift Analysis—Nuclear extract from PC12 cells was prepared using a microscale nuclear extracting method (25). The extract was centrifuged, and the supernatant containing nuclear proteins was collected and stored at −70 °C until use. Recombinant CREB-1 bZIP protein was purchased from Santa Cruz Biotechnology, CA.

For mobility shift assays, a double-stranded oligonucleotide probe (end-labeled with γ-32P-ATP) was made corresponding to the region surrounding the putative CRE site (CCGAGCCCGACGTCCGAGGAG). An oligonucleotide in which the CRE-like sites were mutated, CRM, was also used in the assay as a noncompetitor (CCGAGCCCGACTTGTCGCTGGGAGGAG). In addition, an oligonucleotide probe with the somatostatin CRE core region (26), AGAGATTGCCTGACGTCAAGAGCGTGTCGCTGGGAGGAG, was used as a positive control. The oligonucleotide probes (0.5 ng) were incubated with 2 μg of the extract or 1–2 μg of the recombinant CREB-1 protein and 0.5 μg of poly(dI·dC) in a total volume of 10 μl containing 4% glycerol, 1 mM MgCl2, 0.5 mM EDTA, 0.5 mM dithiothreitol, 50 mM NaCl, 10 mM Tris-HCl, pH 7.5, as described in Ausubel et al. (27). The reaction mixtures were incubated at room temperature for 20 min. Cold double-stranded oligonucleotides in 10- and 100-fold excess were added in the preincubation step (before adding the probe) and used as competitors to show the specificity of the binding. The reaction mixtures were then run on a 4% nondenaturing acrylamide gel at 150 V for more than 3 h, and the products were detected by autoradiography.

RESULTS

Cloning of the 5′-Flanking Region of the Kv3.1 Gene—The Kv3.1 gene has two splice variants, Kv3.1a and Kv3.1b (1, 2). The two transcripts diverge at the C terminus, such that the last 10 amino acids in Kv3.1a are replaced in Kv3.1b by 84 amino acids that are encoded by two other exons. It has been shown that Kv3.1a and Kv3.1b are colocalized in the same neurons (5). In our studies, the probe used to screen the genomic library was derived from the EcoRI-Stul fragment (732 bp) of the 5′-noncoding region of Kv3.1b. However, the 5′-flanking region of the Kv3.1b gene will be referred to as that of the Kv3.1 gene for simplicity. About 104 plaques from a rat genomic library were screened, and five positive clones were identified. Using restriction enzyme mapping and Southern blotting, we determined that these overlapping clones span about 25 kb. Three of the overlapping phase clones are shown in Fig. 1. Southern blotting and sequencing analysis demonstrated that a 6-kb HindIII fragment contains approximately 5.3 kb that are upstream of the published 5′-noncoding region (Fig. 1).

Mapping of the Transcriptional Initiation Sites of the Kv3.1 Gene—To characterize the promoter region of the Kv3.1 gene,
we first identified the transcriptional start sites using primer extension analysis and an RNase protection assay (Fig. 2). For primer extension analysis, we designed an antisense oligonucleotide (see "Experimental Procedures") corresponding to the sequence from 38 to 57 bp downstream of the published 5'9-end (1). Extension of this primer yielded two major fragments, 65 and 74 bp in length, and three to four minor bands of larger size (Fig. 2A). This result suggests that the Kv3.1 K+ channel gene has two major transcription start sites and several minor ones further upstream.

To verify the heterogeneous 5'-end of the Kv3.1 mRNA molecule, we also analyzed cerebellar RNA by RNase protection assays (Fig. 2B). A 458-base RNA probe, designed to be complementary to a genomic fragment spanning the putative transcription start sites, was hybridized with total RNA isolated from rat cerebellum (CB) or tRNA and then digested with RNase A and RNase T1 for 30 min (CB lane, left) or 60 min (CB lane, right). No bands were seen with tRNA. A sequence ladder of known sequence was used as a size marker (left four lanes).

Fig. 2. Identification of the transcription start sites of the Kv3.1 gene. A, a primer extension assay. In the right lane (CB), 10 µg of cerebellar total RNA was used as a template in the assay. The primer is located at a position 38–57 bp downstream of the published 5'-end (1). B, RNase protection assay. A labeled riboprobe corresponding to the region spanning the putative transcription start sites was hybridized with 10 µg of total RNA isolated from rat cerebellum (CB) or tRNA and then digested with RNase A and RNase T1 for 30 min (CB lane, left) or 60 min (CB lane, right). No bands were seen with tRNA. A sequence ladder of known sequence was used as a size marker (left four lanes).

Fig. 3. Nucleotide sequence of the rat Kv3.1 promoter region. Numbers represent nucleotide position relative to the most 3' of the major start sites (+1). The other major transcription start site is also labeled with an asterisk. One CRE-like, one Ap-1 consensus element, and five consensus Sp-1 sites are underlined. The junctions of the deletion constructs shown in Fig. 4 are also labeled and underlined.

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Different Cell Lines—To determine whether the 5.3 kb of the 5'-flanking region of the Kv3.1 gene contains promoter activity, we ligated this fragment to the CAT reporter gene. The SV40 enhancer was also included in some of the constructs (see "Experimental Procedures"). The activity of this fragment was monitored by transiently transfecting the constructs into undifferentiated PC12 cells, which express the Kv3.1 channel, and into NIH3T3 cells, which do not express the Kv3.1 gene. In each case, CAT activity driven by the Kv3.1 promoter was compared with that driven by the SV40 promoter, which is believed to give nonselective activity in most mammalian cell lines.

In PC12 cells, in the absence of the SV40 enhancer, CAT expression driven by the 5.3-kb fragment was 2-3 times higher than that produced by the SV40 promoter (Table I). In contrast, this fragment exhibited little if any activity compared with the SV40 promoter in NIH3T3 fibroblasts, suggesting that the tissue-specific expression of the Kv3.1 gene may, at least in part, be dependent on the upstream regulatory region of the Kv3.1 gene. In control experiments, constructs that comprised the vector without the 5.3-kb fragment or that contained the enhancer, a nonspecific enhancer, the activity of the 5.3-kb fragment in NIH3T3 cells was increased and the cell type specificity of this upstream regulatory region was quenched (Table I).

Functional analysis of Kv3.1 promoter activity in undifferentiated PC12 cells and in NIH3T3 cells. Left, the extent of the 5' deletions of the promotor region in different constructs are represented qualitatively by the lengths of the thin lines, which are coupled to the CAT reporter gene at the right end of each construct. The approximate positions of Ap-1, CRE, and Sp-1 sites are indicated in the constructs. Right, CAT activities of the different deletion constructs are expressed relative to the vector pCATSV40, which contains the SV40 promoter. All of the values are normalized to the activities of β-galactosidase gene, which was cotransfected with CAT constructs. pCAT+22 was only transfected into undifferentiated PC12 cells, not into NIH3T3 cells. Results represent the mean relative activities ± S.E. from three to seven independent experiments with two different plasmid preparations.

**Table I**

| Constructs | Relative CAT activitya |
|------------|------------------------|
|            | PC12 Cells | NIH3T3 Cells |
| A          |            |              |
| pCATSV40   | 1          | 1            |
| pCAT3900   | 2.63 ± 0.34 (n = 4) | 0.043 ± 0.014 (n = 4) |
| B          |            |              |
| pCAT enhancer | 0.022 (n = 1) | NDb         |
| pCATSV40E  | 1          | 1            |
| pCAT5300E  | 1.703 ± 0.230 (n = 5) | 0.432 ± 0.210 (n = 3) |
| pCAT6300RE | 0.053 (n = 1) | NDb         |

a Values represent relative CAT activities of the Kv3.1 promoter compared with that of the SV40 promoter. Each value is mean ± S.E. from 3–5 independent experiments except for pCATenhancer and pCAT5300RE and is normalized with the expression of the co-transfected β-galactosidase plasmid.

b ND, not determined.
Cloning and Characterization of the Kv3.1 Promoter

Regulation of the Kv3.1 Gene Promoter by cAMP—Previous work has suggested that the levels of Kv3.1 mRNA in wild-type and ras-transfected AtT20 cells may be regulated by cAMP (18). In undifferentiated PC12 cells, using an RNAase protection assay, we have found that the levels of Kv3.1a mRNA increased by 50–100% after 24–48 h of treatment with 1 mM 8-bromo-cAMP (n = 3, data not shown). The presence of a CRE-like element in the promoter region suggests that the effect of cAMP on the level of Kv3.1 mRNA may be mediated through the binding of cyclic AMP/CREB or related transcriptional factors to the Kv3.1 promoter. To investigate whether cAMP regulates Kv3.1 promoter activity, we made a stable PC12 cell line transfected with pCATE1213. The pCATE1213 plasmid contains 1213 bp of the upstream region, which includes the CRE-like element. Cells were treated with 1 mM dibutyryl cAMP for 24 h and harvested for CAT assays together with control untreated cells. As shown in Fig. 5, 1 mM dibutyryl cAMP increased CAT activity by approximately 4-fold (p < 0.02). We also made a stable PC12 cell line transfected with pCATE1213, which lacks the SV40 enhancer. 24 h of treatment with 1 mM dibutyryl cAMP increased CAT activity by 296.4 ± 31.7 (n = 3, p < 0.05).

To determine if the CRE-like element at −252 participates in the cAMP response of the Kv3.1 promoter, we carried out transient transfections using the series deletion constructs. Fig. 6 shows that promoter activity was increased by a 24-h treatment with 1 mM dibutyryl cAMP in cells transfected with constructs containing the CRE-like element (pCATE1213, pCAT528, and pCAT266), but not in cells transfected with pCAT226, in which the CRE-like element is deleted. These data suggest that the region from −266 to −226 surrounding the CRE-like element at position −252 is crucial for the cAMP response of the Kv3.1 promoter.

Mobility Shift Assay—To investigate further the possible role of the CRE-like element, we carried out a mobility shift assay to determine if this element recognizes and binds to CREB or related proteins. An oligonucleotide corresponding to the region surrounding the CRE-like element was radiolabeled and tested for its ability to bind nuclear extracts from PC12 cells. A somatostatin CRE oligonucleotide known to bind to CREB proteins (26), was used as a positive control. We found that the mobility of the putative CRE element from the Kv3.1 promoter region is retarded in the presence of PC12 cell nuclear extract to generate a band that is of similar size to that observed with the somatostatin CRE sequence (Fig. 7A). In both cases, the formation of the bands could be reduced or eliminated by an excess of the corresponding nonradioactive oligonucleotides, indicating that binding was specific. This result suggests that the putative CRE in the Kv3.1 promoter region binds to nuclear protein(s) in a similar fashion to the somatostatin CRE. We further found that 10-fold molar excess of the somatostatin CRE was able to eliminate the protein binding to the Kv3.1 CRE oligonucleotide, strongly suggesting that the protein that binds to the Kv3.1 CRE-like element is likely to be CREB protein (Fig. 7B). To test this hypothesis, we used recombinant CREB protein to conduct a mobility shift analysis and found that recombinant CREB protein was able to bind to the Kv3.1 CRE oligonucleotide. This binding could not be competed with CRM, an oligonucleotide in which the CRE-like sites have been mutated (Fig. 7C), supporting the idea that the Kv3.1 CRE binds to CREB itself or a closely related protein. Taken together, our results from transient transfections and gel mobility shift assays strongly suggest that the CRE-like element at position −252 is responsible for the cAMP response of the Kv3.1 gene promoter.

Regulation of the Kv3.1 Promoter by Ca2+—Besides being regulated by cAMP, levels of Kv3.1 mRNA have been shown to be regulated by elevation of intracellular calcium in wild-type and ras-transfected AtT20 cells (18). Here we report that Kv3.1 promoter activity can be up-regulated by treatment with the calcium ionophore, ionomycin (5 μM) in PC12 cells. Using the stable PC12 cell line transfected with pCATE1213 described above, we observed an increase in CAT activity induced by ionomycin of more than 50% (p < 0.05) (Fig. 5). When both dibutyryl-cAMP and ionomycin treatments were combined, there was also a marked increase in CAT activity (p < 0.001), although this was not significantly different from that of dibutyryl-cAMP alone.

To exclude the possibility that the SV40 enhancer, which is incorporated in the pCATE1213 vector (see "Experimental Procedures"), contributes to the effects of ionomycin effects, we...
conducted experiments using pCATE71, which also contains SV40 enhancer but lacks putative transcriptional factor binding elements. We found that the CAT activities in cells transfected with pCATE71 were not up-regulated by 5 μM ionomycin (5.7% ± 0.61, n = 4). These data suggest that the effects of cAMP and Ca²⁺ are mediated directly by the Kv3.1 promoter region from 21213 bp to 271 bp.

DISCUSSION

The Shaw-type K⁺ channel, Kv3.1, is expressed primarily in neurons (4–8), and levels of Kv3.1 mRNA can be altered by seizures (18), as well as by second messengers (18). To understand the mechanisms underlying transcriptional control of the Kv3.1 gene, we have cloned the promoter region of the Kv3.1 K⁺ channel gene. We find that the Kv3.1 gene contains two principal transcription start sites and that the proximal promoter region is extremely GC-rich. The essential promoter activity of the Kv3.1 gene appears to reside in the region 226 bp upstream of the start sites.

When 5.3 kb of the 5'-flanking region was transfected into undifferentiated PC12 cells, a neuron-like cell line, it produced high transcriptional activity, about 2-3-fold greater than that of the SV40 promoter. In contrast, this fragment was essentially inactive in NIH3T3 fibroblast cells, indicating that the 5'-regulatory region plays a crucial role in the tissue-specific expression of this gene. Similar neuronal-restrictive promoter activity has been previously demonstrated for the neuron-specific rat brain type II sodium channel (30). Within this region, a silencing element was found to be responsible for restricting the expression of this gene to neurons (31). This neural restrictive silencing element has also been shown to be present in other neuronal genes, like the SCG10 gene (32, 33) and the human synapsin I gene (34). Interestingly, we have found an element in the Kv3.1 5'-flanking region from 2743 to 2717 bp bearing 75% identity to the NRSE in the SCG10 gene. As we deleted the Kv3.1 5'-flanking region from 21213 to 271 bp, promoter activity increased, suggesting this NRSE-like element may also play a silencing role here, although more detailed study will be needed to confirm this hypothesis.

A CRE-like element was found at position –252, 5' to the five putative Sp-1 binding sites. This element is known to mediate the response of transcription rate to an elevated level of cAMP through CREB. When phosphorylated by either the cAMP-dependent protein kinase or by a calcium/calmodulin-dependent protein kinase, the CREB protein binds to CRE elements en-
hancement transcription. Further upstream of the CRE-like element, there exist two consensus Ap-1 elements, which may be activated by products of immediate early genes. Previous studies have shown that, besides being regulated by seizure activity (18), the expression levels of the Kv3.1 gene can also be regulated by extrinsic factors. Studies in the AtT20 cell line have shown that basic fibroblast growth factor, Ca\(^{2+}\) activated by extrinsic factors. Additional studies have shown that, besides being regulated by seizure activity and cAMP up-regulate the level of Kv3.1 transcripts. Our findings strongly suggest that the CRE-like element at –252 mediates the response of the Kv3.1 gene to elevations of cAMP levels, since we found that deletion of the CRE-like element eliminated the response of the promoter to the cAMP analog. Moreover, an oligonucleotide with the Kv3.1 CRE sequence binds a nuclear protein to generate a band that matches with that obtained with a somatostatin CRE oligonucleotide, which is known to bind the CREB protein. Using recombinant CREB protein, we have further demonstrated that the protein that binds to the Kv3.1 CRE oligonucleotide is probably CREB itself or a closely related protein.

Both in situ hybridization and immunocytochemistry studies have indicated that the Kv3.1 gene is highly enriched in neurons along auditory pathways, which are capable of firing action potentials at high frequencies (5, 7, 8). It has been suggested that the presence of a channel with the biophysical characteristics of the Kv3.1 channel allows neurons to generate trains of action potentials that follow high frequency synaptic stimulation with little adaptation (16, 17), and in the case of neurons in auditory brainstem nuclei, aids in the precise temporal locking of the phase of action potentials to the phase of auditory inputs (17). The finding that the activity of the Kv3.1 promoter is strongly enhanced by a cAMP analog suggests that neurotransmitters or hormones that elevate cAMP levels may produce changes in the excitability of neurons that express this channel.

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