Membrane depolarization leads to changes in gene expression that modulate neuronal plasticity. Using representational difference analysis, we have identified a previously undiscovered cDNA, KID-1 (kinase induced by depolarization), that is induced by membrane depolarization or forskolin, but not by neurotrophins or growth factors, in PC12 pheochromocytoma cells. KID-1 is an immediate early gene that shares a high degree of sequence similarity with the family of PIM-1 serine/threonine protein kinases. Recombinant KID-1 fusion protein is able to catalyze both histone phosphorylation and autophosphorylation. KID-1 mRNA is present in a number of unstimulated tissues, including brain. In response to kainic acid and electroconvulsive shock-induced seizures, KID-1 is induced in specific regions of the hippocampus and cortex.

Neurons respond to stimuli through a variety of overlapping signal transduction pathways. Activation of these pathways leads to changes in gene expression that ultimately result in changes in neuronal function. One stimulus, membrane depolarization, leads to a rise in [Ca\textsuperscript{2+}]i, activates Ca\textsuperscript{2+}-calmodulin kinase- and adenyllyl cyclase-dependent (1) signal transduction pathways that are involved in the synaptic plasticity necessary for brain development (2) and for learning and memory (3–6).

We and others are searching for genes induced in neurons by depolarization to identify candidate genes whose expression may mediate neuronal plasticity. Studies using classical differential and subtractive screening procedures have identified many previously known immediate-early genes (IEGs) (7–9), one novel IEG, rheb, enriched in depolarized brain (10), and two novel neuron-specific, depolarization-induced genes, cgpl (8) and synaptotagmin IV (11). Most recently, differential display was used to identify synaptotagmin X, a gene induced in degenerating brain following kainic acid seizures (12). Many of these genes are induced not only by depolarization but also by other stimuli such as nerve growth factor (NGF) (10). The products of genes induced by both types of stimuli presumably function not only during periods of neuronal plasticity or seizure activity but also during neuronal growth and development.

Our goal is to identify genes that are induced in neurons by depolarization and not by neurotrophins and/or other growth factors. We suggest that the products of such genes may have roles specific to neuronal plasticity and/or seizure activity but not to growth and development. To perform this task, we have utilized representational difference analysis (RDA) (13–15) to identify cDNAs for messages induced in PC12 cells by depolarization but not by NGF or epidermal growth factor (EGF).

PC12 cells are widely used as a system for studying both presynaptic events involving synaptic vesicles and neuronal differentiation in response to NGF. PC12 cells respond to membrane depolarization by releasing neurotransmitters from synaptic-like vesicles (16, 17). In contrast, they respond to NGF by differentiating into a more neuronal cell type (18) and to EGF by proliferation (19). We (11, 20, 21) and others (22–24) have previously used PC12 cells to study the induction of IEGs by various stimuli that include both depolarization and NGF. For these reasons, we chose PC12 cells to identify genes induced specifically by depolarization.

RDA is a rapid, sensitive method for identifying differences between two populations of DNA. RDA was originally developed to identify DNA sequences unique to one of two genomic populations (13). The RDA procedure was subsequently modified for comparing cDNA populations (14) and detecting differentially expressed sequences (14, 15, 25, 26). We have recently described the use of RDA to isolate genes preferentially induced in PC12 cells by the neurotransphin NGF versus the mitogen EGF (27).

In this report we again applied RDA to cDNAs from PC12 cells and isolated a number of cDNA fragments that identify messages induced by depolarization but not by NGF or EGF. We describe in detail the identification and characterization of KID-1, a protein kinase induced by depolarization, in PC12 cells and in brain. KID-1 expression is low in unstimulated PC12 cells and is rapidly induced by membrane depolarization. The cDNA sequence of KID-1 predicts a serine/threonine protein kinase with strong homology to the proto-oncogene PIM-1. When expressed as a glutathione S-transferase (GST) fusion protein, KID-1 is able to catalyze both histone phosphorylation and autophosphorylation. KID-1 is an IEG; its expression is induced in PC12 cells in the presence of cycloheximide, a protein synthesis inhibitor. KID-1 is not induced by NGF or EGF. In brain, KID-1 expression is induced after generalized seizures.
and is limited to specific areas of the hippocampus and temporal lobe.

**Experimental Procedures**

**Cell Culture and Treatment—**PC12 cells, obtained from B. Howard (UCLA), were cultured in RPMI with 10% heat-inactivated horse serum and 5% fetal calf serum in T75 flask. The cells were passaged, at a 1:4 to 1:5 ratio, by trituration when they reached 75–80% confluence. Experiments were performed in cells at 50–60% confluence. KCl (5 mM), forskolin (50 mM), NGF (50 ng/ml), or EGF (10 ng/ml) was added to the cultures for the times indicated in the text or the figures. Growth factors were purchased from Calbiochem, Biomedical Products (Bedford, MA). Forskolin was purchased from Sigma. For each experiment, pooled RNA from three separate experiments (a total of 24 flasks) were pooled. For subsequent experiments, RNAs from the paired flasks treated with each ligand and from three separate experiments (a total of 24 flasks) were pooled. For the synthesis of cDNA to be analyzed by RDA, RNAs from all eight flasks treated with each ligand and from the third round RDA difference product. Fragments that yielded was isolated from individual colonies from among the transformation reaction, cloned into pCRII using a TA Cloning Kit (Invitrogen, Carlsbad, CA). DNAs were amplified using 5 units of Pfu polymerase in a 100- 

**RNA Synthesis and Doping the Driver—**2 μg each of the KCl and forskolin poly(A)+ RNA pools were pooled to make “tester” cDNA. Similarly, 2 μg each of the NGF and EGF poly(A)+ RNA pools were pooled to make “driver” cDNA. Double-stranded cDNA was synthesized from the pooled poly(A)+ RNAs, using a SuperScript Choice cDNA Synthesis Kit (Life Technologies, Inc.), with a combination of 1.0 μg of oligo (dT) and 100 ng of random hexamers.

To evaluate the induction of known genes, Northern blots prepared with the full-length cDNA clone were hybridized with the specific probes to that from GAPDH, and our hybridization signals were quantitated with an Ambis RadioImager (Scanalytics, Billerica, MA). The relative intensities of the signals from the specific probes to that from GAPDH, and our hybridization signals were quantitated with an Ambis RadioImager (Scanalytics, Billerica, MA). The relative intensities of the signals from the specific probes to that from GAPDH, and our hybridization signals were quantitated with an Ambis RadioImager (Scanalytics, Billerica, MA). The relative intensities of the signals from the specific probes to that from GAPDH, and our hybridization signals were quantitated with an Ambis RadioImager (Scanalytics, Billerica, MA). The relative intensities of the signals from the specific probes to that from GAPDH.
acetic acid, pH 4, 1 volume of acid phenol, and 0.2 volume of chloroformiso-amyl alcohol mixture (24:1) were added to the homogenate. After mixing and centrifugation, the aqueous phase was transferred to a new tube, and the extraction was repeated. The aqueous phase was mixed with 1 volume of isopropanol and placed at −20 °C overnight to precipitate RNA. The RNA was pelleted, washed with 80% ethanol, and resuspended in 400 μl of RNA solubilization buffer (0.1% SDS/1 mM EDTA/10 mM Tris, pH 7.5). The solution was frozen, thawed, and extracted twice with equilibrated phenol and twice with chloroform. The aqueous phase was mixed with 40 μl of 3 M sodium acetate, pH 5.2, and extracted once with chloroform-iso-amyl alcohol mixture. This aqueous phase was mixed with 1 ml of ethanol and placed at −20 °C to precipitate RNA. Finally, the RNA was pelleted, washed with 80% ethanol, and resuspended in TE, pH 8.0, at concentrations appropriate for Northern analysis.

Kainic Acid and Electroconvulsive Shock Treatment and Preparation of Post-seizure RNA for Northern Analysis—Wistar rats (270 to 300 g) were housed with free access to rat chow and water for at least 24 h before the experiment. KA treatment was carried out as described previously (34). Briefly, rats were injected with KA (10 mg/kg) intraperitoneally and kept in isolated cages under surveillance to classify the seizure class. Animals not developing seizures above class III (35) by 30 min after injection were discarded. Animals were sacrificed at 0 (control), 0.5, 1, 2, and 4 h after injection.

ECS treatment was delivered through two platinum electrodes implanted in the scalp 1 cm apart. A single stimulus was delivered with a S48 Stimulator (Grass Instruments, Quincy, MA) set at 110 V, 150 pulses/s, 0.5 ms in duration, at a train rate of 0.75 trains/s, and a train duration of 250 ms. Each animal developed a tonic-clonic seizure that lasted 12–15 s and recovered in 40–50 s. Control animals had electrodes implanted but did not receive ECS. ECS-treated animals were sacrificed at 0.5, 1, 2, and 4 h after seizure.

KA- and ECS-treated rats were killed by decapitation. The hippocampus and cortex were dissected, and RNA was isolated as described previously (36). Two separate experiments were performed with three animals in each condition analyzed.

Kainic Acid Treatment, Preparation of Brain Sections, and in Situ Hybrdization—Kainic acid treatment, preparation of brain sections, and in situ hybridization were carried out as described previously (11, 37) with the following modifications. Male Sprague-Dawley rats were injected with kainic acid (12 mg/kg) subcutaneously and sacrificed at 1, 4, and 8 h after the initiation of seizure activity. Control animals did not receive any handling. For statistical analysis, four animals were prepared for each treatment group and for control. Following hybridization, the sections were washed for 20 min in 0.2 M SSC for a few hours. The high stringency wash was performed at 55 °C for 30 min in 0.2 × SSC. The antisense (5′-GGGACGCCGTTCCAAGA-GGGACCTCAAGGACAAAGAAGGAAACAC-3′) and the sense (5′-GGGTCGGC-GGGTGGACCCACCTCCCGAGTGAAGTCTACAG-3′) oligonucleotides were selected from nucleotides 227–286 of the KID-1 cDNA sequence.

RESULTS

Enrichment of RDA Driver for Genes Preferentially Induced by Depolarization and Forskolin versus Growth Factors in PC12 Cells—To prepare the starting mRNA populations to be used to identify cDNAs for messages induced by depolarization, but not by growth factors, we employed four populations of PC12 cells: cells treated individually with KCl (55 mM), forskolin (50 mM), NGF (50 ng/ml), or EGF (10 ng/ml). We used forskolin-treated cells as well as KCl-treated cells to prepare our tester mRNA populations (13), from which we planned to clone cDNAs for genes preferentially induced by depolarization, because many genes strongly induced by depolarization in brain are also strongly induced by forskolin in PC12 cells (11, 38, 39). Because we had no prior knowledge of the kinetically of induction of differentially expressed messages, different time points were pooled for each inducer to prepare RNAs for the tester and driver populations to be used for RDA (see “Experimental Procedures” for details).

One of the advantages of the RDA procedure is that the driver population, which in this case was derived from mRNA prepared from NGF- and EGF-treated cells, can be artificially enriched or “doped” with cDNAs for genes known to be more strongly expressed in the tester population. Fragments of genes known to be preferentially expressed in the tester population can be eliminated in the RDA procedure as a consequence of this doping. As a result, restriction fragments from other preferentially induced messages in the tester population can be selected during the amplification steps. Preliminary experiments, using Northern blots to evaluate the induction of several genes in PC12 cells by the four inducers (KCl, forskolin, NGF, and EGF), confirmed that secretogranin I (38, 39), synaptotagmin IV (11), and NGFI-B/nur77/TIS1 (22, 23) were indeed preferentially induced by KCl and forskolin. Moreover, the approximate levels of these messages in the pooled tester mRNA populations could be estimated from these Northern blots. The driver cDNA, prepared from NGF- and EGF-treated cells, was doped with an approximately 10-fold excess of the cDNAs for each of these rat genes prior to preparation of the driver amplicons. Tester amplicons, derived from the cDNA prepared from the pooled KCl- and forskolin-treated cells, and driver amplicons, derived from the doped cDNA prepared from the pooled NGF- and EGF-treated cells, were used to carry out RDA reactions using procedures we described previously (27) with modifications (see “Experimental Procedures” for details).

After three rounds of RDA reactions, the initial tester cDNA population becomes greatly simplified (Fig. 1A). Ethidium bromide staining of the initial tester and driver cDNAs demonstrates a homogenous distribution of DNA throughout the
The predicted open reading frame of 326 amino acids. The 5'-kilobase size of the mRNA. The sequence contains a predicted nucleotides (Fig. 2), which agrees well with the estimated 2.1-kb cDNA clones from a PC12 cDNA library. The nucleotide sequence is 79% G and C. The PIM-1 cDNA (40) (Fig. 3). The two predicted proteins are 76% identical. The predicted open reading frame of the depolarization-induced clone shares strongest sequence similarity among proteins from other mammalian species with the PIM-1 oncoprotein products, a family of serine/threonine protein kinases (Fig. 3). The three mammalian PIM-1s are 94–97% similar to each other. Analysis of the amino acid sequences demonstrates that the depolarization-induced gene and Xenopus PIM-1 are more closely related to each other than they are to the mammalian PIM-1 cDNAs. Moreover, the depolarization-induced gene is more closely related to the mammalian PIM-1s than is Xenopus PIM-1. For these reasons, we conclude that (i) the depolarization-induced gene we have isolated and Xenopus PIM-1 are likely to be species orthologues and (ii) Xenopus PIM-1 is not the Xenopus orthologue of the mammalian PIM-1s.

We named this new depolarization-induced gene KID-1, for kinase induced by depolarization. Pair distances analysis shows that KID-1 and Xenopus PIM-1 are 76% similar to each other. In contrast, KID-1 is 65% similar to the mammalian PIM-1s, and Xenopus PIM-1 is 62–63% similar to the mammalian PIM-1s. These relationships are illustrated by phylogenetic analysis (Fig. 3B). We suggest that (i) KID-1 is a previously unidentified member of the PIM-1 gene family and (ii) Xenopus PIM-1 is likely to be the Xenopus orthologue of KID-1 rather than PIM-1.

**KID-1 Is a Protein Kinase**—We expressed recombinant KID-1 protein in bacteria as a GST fusion protein (Fig. 4). The fusion protein has a predicted molecular mass of 66 kDa. A band of this size is seen in the purified GST-KID-1 fusion protein preparation (Fig. 4, lanes 1 and 2). Western blotting with anti-GST antibody confirmed that the 66-kDa band is a GST fusion protein (data not shown). An *in vitro* protein kinase assay demonstrated that the GST-KID-1 fusion protein is able to catalyze both phosphorylation of histone H1 protein and autophosphorylation (Fig. 4, lane 2). There was no protein kinase activity in the lane containing the control, GST-PRMT1 fusion protein (Fig. 4, lane 4).

KID-1 contains sequence similarities to other protein kinases at many crucial residues and shares similarities with both the serine/threonine and tyrosine kinases. However, KID-1 contains residues in two catalytic subdomains, *Lys*172 and *Glu*174 in subdomain VI and *Gly*206, *Thr*207, and *Tyr*219 in subdomain VIII, that are specifically conserved in the family of serine/threonine kinases and that are thought to determine serine/threonine specificity (43). In addition, the human and murine PIM-1 proteins and the presumed Xenopus KID-1 orthologue, Xenopus PIM-1, have serine/threonine kinase activity (32, 40, 44, 45). It is likely that KID-1 is also a serine/threonine protein kinase.

**KID-1 Is an Immediate Early Gene Preferentially Induced by Depolarization and Forskolin in PC12 Cells**—There is little basal expression of KID-1 in untreated PC12 cells (Fig. 5A). Upon treatment with depolarizing levels of KCl or with forskolin, KID-1 expression is induced by 0.75 h, peaks by 1.5 h, and persists, albeit at diminished levels, for at least 4 h. There is no demonstrable induction of KID-1 by NGF or by EGF. In contrast, NGF and EGF are able to induce the expression of two other IEGs (c-fos and TIS8), demonstrating the efficacy of these growth factors for induction of gene expression in this experiment. Thus, KID-1 is induced specifically by membrane depolarization and/or activation of adenyl cyclase and *not* by neurotrophins or growth factors in PC12 cells.

**KID-1 Is Present in Brain and Other Tissues**—We detected KID-1 mRNA in a variety of tissues from unstimulated rats,
including a number of brain regions (Fig. 6). We performed Northern analysis of total RNA from these tissues, using the Clustal method and MegAlign software. Residues identical to KID-1 are highlighted in black. Protein kinase catalytic subdomains are indicated by Roman numerals beneath the aligned sequences. Individual residues conserved among serine/threonine protein kinases (+), tyrosine protein kinases (#), and both types of protein kinases (*) are indicated above the aligned sequences (43).

**Fig. 3.** KID-1 shares homology with PIM-1 and other protein kinases. A, the predicted amino acid sequences of KID-1, Xenopus PIM-1, and the various mammalian PIM-1s were aligned using the Clustal method and MegAlign software. Individual residues conserved among serine/threonine protein kinases (+), tyrosine protein kinases (#), and both types of protein kinases (*) are indicated above the aligned sequences (43). B, phylogenetic analysis of the amino acid sequence of KID-1 and the members of the PIM-1 family was performed using MegAlign software. The KID-1 and Xenopus PIM-1 amino acid sequences are more closely related to each other than to the mammalian PIM-1 amino acid sequences.

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is most prevalent in heart, kidney, lung, brainstem, and pituitary.

**KID-1 Is Induced in Brain by Seizure Activity**—Although we demonstrated differential induction of KID-1 by depolarization in cultured PC12 cells, our major objective is to identify genes...
that may serve as mediators of plasticity in response to neuronal stimulation in vitro. We used seizure induction by both KA (11) and ECS (36) to determine whether KID-1 expression occurs in brain in response to depolarization. KID-1 message levels are elevated in the hippocampus and/or cerebral cortex following seizures induced either by administration of systemic KA or by ECS (Fig. 7). However, this Northern analysis shows that the patterns of KID-1 induction by ECS and KA are different. KA leads to maximum induction at 2 h in the hippocampus and little or no induction in the cortex. ECS leads to maximum induction at 1 h in both the hippocampus and in the cortex. In contrast to KA, the peak levels of induction in response to ECS are similar in the hippocampus and the cortex.

We used in situ hybridization to more specifically define the brain regions expressing KID-1 mRNA following KA-induced seizures. Coronal brain sections (10 μm) were hybridized with an oligonucleotide probe for KID-1 (Fig. 8). Hybridization of the KID-1 antisense oligonucleotide was eliminated when a 100-fold excess of unlabeled probe was added to the hybridization solution, when sections were pretreated with RNase A, and when a sense strand oligonucleotide was used as probe (data not shown).

As demonstrated by Northern hybridization, seizure activity increased KID-1 mRNA levels in both the hippocampus and the cortex. Pyramidal cells in the CA3 region of the hippocampus exhibited strong induction (210% of control) as early as 1 h after seizure onset. The levels of KID-1 expression remained increased in this region up to 8 h after seizure onset. In contrast, the granule cells of the dentate gyrus and the piriform cortex exhibited peak expression at 4 h (240 and 210% of control, respectively), with the levels of expression decreasing by 8 h. The cortex overlying the hippocampus (especially the superficial layers) showed a progressive increase in KID-1 mRNA levels beginning at 1 h and reaching statistical significance at 8 h after seizure onset. The pyramidal cells of the CA1 region of the hippocampus and the dorsal thalamic nuclei showed no statistically significant increases in KID-1 mRNA expression.

**DISCUSSION**

Using RDA, we identified KID-1, a previously undiscovered protein kinase induced by depolarization both in PC12 pheochromocytoma cells and in brain. KID-1 shares a high degree of homology with the PIM-1 proto-oncogenes, a family of serine/threonine protein kinases. Importantly, KID-1 mRNA is induced by depolarization but not by neurotrophins or growth factors. KID-1 is the first protein kinase demonstrated to be specifically induced by membrane depolarization in neurons.

The KID-1 cDNA predicts a 326-amino acid protein with a high degree of sequence similarity to the PIM-1 proto-oncogenes. This family of genes has been well studied for its role in leukemoid oncogenesis (46, 47). The mouse and human PIM-1 genes have both auto- and exogenous serine/threonine protein kinase activity (32, 44, 45). In murine hematopoietic cells, PIM-1 is an IEG induced by growth factors (48) and encodes a cytoplasmic protein (44). Mast cells derived from the bone marrow of PIM-1-deficient mice demonstrate an impaired growth response to interleukin-3 (49). Therefore, PIM-1 is believed to be a cytoplasmic modulator of the interleukin-3 signal transduction pathway.

KID-1 and Xenopus PIM-1 (40) are more closely related to each other than either is to the mammalian PIM-1s. Thus, the gene referred to as Xenopus PIM-1 seems likely to be the Xenopus orthologue of KID-1 rather than PIM-1. Like PIM-1 and Xenopus PIM-1, KID-1 is able to catalyze both autophosphorylation and phosphorylation of other proteins (Fig. 4). Xenopus PIM-1 has autophosphorylating activity for both serine and threonine residues (40). KID-1 and Xenopus PIM-1 are identical at all residues conserved in protein kinases, except for the conservative substitution at position 46 of Leu in KID-1 for Val in the Xenopus protein (Fig. 3). In addition, KID-1 and Xenopus PIM-1 are virtually identical throughout subdomains VIII and IX, which play major roles in forming protein/peptide binding pockets (50). Therefore, it seems likely that KID-1 and Xenopus PIM-1 will have similar protein kinase substrates.

We recently found that rat PIM-1, like KID-1, is induced in specific regions of the brain following generalized seizure activity. Because of their shared sequence homology and response to seizure activity, we are planning to perform detailed comparisons of the substrate specificities and biological activities of PIM-1 and KID-1.

Protein phosphorylation is a crucial part of many neuronal signal transduction systems (51). Depolarization-induced IEGs are likely to be important mediators of the neuronal plasticity that follows synaptic activity (1, 52). As a putative cytoplasmic protein kinase and an IEG induced specifically by depolarization, KID-1 is a strong candidate for such a mediator of synaptic plasticity. KID-1 is unique in that it is the only protein kinase currently known to be induced by membrane depolarization, without significant induction by NGF or EGF, in PC12 cells. The only other genes known to share this pattern of preferential induction by depolarization versus growth factors in PC12 cells are MKP-1/3CH 134 (22), a dual specificity protein phosphatase, and a pair of closely related nuclear orphan receptors, NGFI-B/nur77/TIS1 (22) and NURR1/HzF-3 (53, 54).

The differential induction of KID-1 by both membrane depolarization and forskolin versus growth factors implicates a cAMP response element/cAMP response element-binding protein-mediated signal transduction pathway (6). Two possible explanations for the greater maximal induction of KID-1 observed following forskolin treatment, as compared with the induction observed following KCl treatment (Fig. 5A) are: (i) differences in the activation of adenyl cyclase and (ii) differences in the activation of CREB. We plan to address these mechanisms in future studies.
The differences in the pattern of KID-1 expression in response to KA and ECS (Fig. 7) are consistent with the mechanisms of seizure induction by these two agents. KA generates multiple seizures that originate in the limbic system and may last for hours. ECS causes a single, massive cortical depolarization. Therefore, the induction of KID-1 by KA is greater in the hippocampus than in the cortex and lasts longer than the induction of KID-1 by ECS.

The anatomic distribution and time course of KID-1 expression, as demonstrated by in situ hybridization (Fig. 8), is unique among known depolarization-induced genes. Starting

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**Fig. 5.** KID-1 is an immediate early gene induced by KCl and forskolin but not growth factors. A, time course of KID-1 induction. Total RNA (10 μg/lane) from PC12 cells was subjected to electrophoresis on agarose and transferred to nylon membranes. Specific treatments and durations are indicated in the figure. Samples from untreated cells are indicated by 0. Exposure time for KID-1 was 24 h with two intensifying screens. Hybridization with probes for c-fos (48-h exposure time with two screens) and TIS8 (3-h exposure time with one screen) shows that the cells responded appropriately to the growth factor treatments. GAPDH signal (30-h exposure time with one screen) is shown to evaluate mRNA loading. B, KID-1 is an immediate early gene. Induction of KID-1 in the presence of cycloheximide (CHX). Total RNA (8 μg/lane) from PC12 cells was subjected to electrophoresis on agarose and transferred to nylon membranes. Specific treatments are indicated. All treatments were for 1.5 h. Exposure time for KID-1 was 24 h with two intensifying screens. Hybridization with probes for c-fos (5-h exposure time with two screens) and TIS8 (1-h exposure time with one screen) shows that the cells responded appropriately to the treatments. GAPDH signal (30-h exposure time with one screen) is shown to evaluate mRNA loading.

**Fig. 6.** KID-1 mRNA is present in unstimulated tissues, including brain. Total RNA (10 μg/lane) from tissues of untreated rats was subjected to electrophoresis on agarose, transferred to a nylon membrane, and probed with the 718-bp fragment representing KID-1 (7-day exposure time with two intensifying screens). Total RNA (10 μg) from PC12 cells treated with forskolin + cycloheximide was included as a positive control. GAPDH signal (15-h exposure time with one screen) is shown to verify that the RNA is intact in all samples. Ethidium bromide staining of the 18 S ribosomal RNA is shown to evaluate total RNA loading.

**Fig. 7.** KID-1 mRNA is induced in brain by seizures. Time course of KID-1 induction in response to seizures. Total RNA (10 μg/lane) from rat hippocampus or cortex was subjected to electrophoresis on agarose and transferred to nylon membranes. Specific treatments and duration are indicated in the figure. Northern blots show RNA pooled from two to four representative individuals for each condition. Exposure time for KID-1 was 5 days with two intensifying screens. GAPDH signal (10-h exposure time with one screen) is shown to evaluate RNA loading.
from 1 h after KA-induced seizures, KID-1 expression was significantly increased in the piriform cortex, the pyramidal cells of the CA3 region, and the granule cells of the dentate gyrus of the hippocampus. Interestingly, the pyramidal cells of the CA1 region of the hippocampus never exhibited a significant increase of KID-1 expression after KA-induced seizures. Other depolarization-induced genes show different patterns of induction. cpgI and sytX are expressed in only part of the limbic system (8, 12), rheb is expressed simultaneously throughout the limbic system (10), and sytIV exhibited maximal induction at 4 h after seizures in the dentate gyrus and only at 8 h in the CA1 and CA3 regions of the hippocampus (37). The relative increase in KID-1 expression is also quite different from that of other IEGs, such as c-fos, which increased more than 10-fold within 1 h of seizure onset (55). KID-1 is more similar to genes such as sytIV (11, 37) or SG2 (56), which increase 2–3-fold within 4 h of seizure onset.

This study demonstrates several advantages of RDA as a method to identify differentially induced genes. RDA is rapid; the procedure allows performance of a search for differentially induced sequences without the labor intensive construction of subtracted cDNA libraries. For the RDA experiment described in this report the procedure, from preparation of tester and driver cDNAs to electrophoretic analysis of the third round difference product, was completed in approximately 4 weeks. RDA also allows the doping out of previously identified genes, so that only fragments of previously unknown genes are isolated. In principle, by sequentially doping a driver cDNA population with the difference product of the previous RDA experiment, one should be able to iteratively isolate new, differentially induced species until the population is exhausted.

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