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Differential expression of mRNAs for protein kinase inhibitor isoforms in mouse brain

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ABSTRACT **Many neurotransmitters are known to regulate neuronal cell function by means of activation of cAMP-dependent protein kinase (PKA) and phosphorylation of neuronal substrate proteins, including transcription factors and ion channels. Here, we have characterized the gene expression of two isoforms of a protein kinase inhibitor (PKI) specific for PKA in mouse brain by RNase protection and *in situ* hybridization histochemistry. The studies demonstrate that the PKIα isoform is abundant in many regions of the adult mouse brain but particularly in cerebellum, hypothalamus, hippocampus, and cortex. In contrast, PKIβ is present at much lower levels in most brain regions but is found in significant amounts in the cerebellum, as well as in distinct nuclei within the pons, medulla, and hypothalamus. These results are consistent with a regulatory role of endogenous PKI in PKA-mediated signal transduction in brain and suggest differential functions for the two isoforms of PKI within the central nervous system.**

A wide variety of neurotransmitters regulate neuronal function by altering the intracellular concentration of the second messenger cAMP through modulation of adenylate cyclase activity (1). Variations in cAMP concentration in turn regulate the activity of cAMP-dependent protein kinase (PKA), which at low concentrations of cAMP exists as an inactive tetrameric holoenzyme consisting of two regulatory (R) and two catalytic (C) subunits (2, 3). At submicromolar concentrations of cAMP, each R subunit in the holoenzyme will bind cAMP at two allosteric binding sites and subsequently release catalytically active C subunit from the holoenzyme complex. The C subunit is then able to phosphorylate substrate proteins on serine and threonine residues, which often results in a significant change in the biological function of the substrate. For example, phosphorylation of the cAMP response element binding protein (CREB) transcription factor by the C subunit in the nucleus results in an increased rate of transcription for genes containing a cAMP response element (4), while phosphorylation of a large number of ion channels by the C subunit results in altered neuronal excitability (5). In invertebrates such as *Aplysia* (6, 7) and *Drosophila* (8), experimental evidence suggests that these phosphorylations may form the biochemical basis for learning and memory.

In addition to the inhibition of C subunit by R subunit, the C subunit of PKA can also be inhibited by the protein kinase inhibitor (PKI) protein (9, 10). PKI was first isolated as a 75-amino acid, heat-stable protein from skeletal muscle but has been found in a wide variety of tissues. Like the R and C subunits, PKI has been shown to exist in at least two major isoforms. The 75-amino acid PKIα corresponds to that form originally isolated from skeletal muscle (11, 12), while the 70-amino acid PKIβ was more recently isolated from rat testis (13, 14). Both isoforms of PKI contain homologous amino acid residues at sites shown to be important for specific binding to the C subunit. One striking difference between the two PKI isoforms is that the PKIβ isoform is much less effective in the presence of micromolar concentrations of substrate than the PKIα isoform (13). Although the *in vivo* function of the PKIs has not been clearly defined, they have been postulated to regulate the threshold concentration of cAMP at which protein phosphorylation is first increased (10) and the rate at which C subunit is able to exit the nucleus (15). Such a regulatory role for PKI would have profound effects in neuronal cells, where protein phosphorylation is a major mechanism for regulation of neuronal plasticity. With the recent availability of mouse PKI cDNA clones (11, 14), we sought to determine the pattern of expression of PKIα and PKIβ genes within the mouse brain.

MATERIALS AND METHODS

Plasmid Constructs. The template for the Cα antisense RNA probe was constructed by adding *Bam*HI linkers to the 1.3-kb *Nae* I–*Pvu* II fragment of pMCα (16) and inserting it into the *Bam*HI site of pGEM-4 (Promega) to create pCα2. The template for the Cβ antisense RNA probe (pCβ45) was constructed by inserting a 1.2-kb *Sst* I fragment of Cβ into the *Bam*HI site of pGEM-4 after incubating the *Sst* I fragment with DNA polymerase I to create blunt ends and adding *Bam*HI linkers. pCα2 and pCβ45 were linearized with *Sac* I and *Nhe* I, respectively, to create templates for antisense cRNA probes. The 366-bp *Bam*HI–*Stu* I fragment of PKI-8 (nucleotides 1558–1924 of PKIα; ref. 11) was subcloned into the *Bam*HI/*Sma* I sites of pGEM-7Zf(+) (Promega) to create AR-1. This construct was linearized with *Bam*HI or *Eco*RI and used as a template to synthesize antisense or sense cRNA probes, respectively. A 256-bp fragment of PKIβ was amplified by PCR (nucleotides 501–755; ref. 14) and subcloned into the *Bgl* II/*Eco*RI sites of pSP73 (Promega) to create MtPKI.pcr. This DNA fragment will hybridize with mRNA from both PKIβ1 and PKIβ2 isoforms (14). The MtPKI.pcr construct was linearized with *Bgl* II or *Eco*RI and used as a template to synthesize antisense or sense PKIβ cRNA probes, respectively.

Northern Blot Analysis. Total RNA was isolated from mouse brain regions by using an acid guanidinium isothiocyanate/phenol/chloroform protocol (17). Thirty micrograms of total RNA from each region was electrophoresed through a 1% agarose/formaldehyde gel, transferred to a Magna NT nylon membrane (Micron Separations, Westboro, MA), and fixed by baking. Antisense cRNA probes were prepared as described (11) and hybridized to the immobilized RNA at 60°C, as previously described (18). The blots were first washed twice at room temperature for 10 min in 0.5× SET (1× SET = 1.0% SDS/5 mM EDTA/10 mM Tris·HCl, pH 7.5) containing 0.1% sodium pyrophosphate and then washed for 2 h at 70°C.

cRNA Probe and Sense RNA Synthesis. cRNA probes for RNase-protection assays were synthesized for 1 h at 15°C in a 10-μl reaction mixture containing 1× transcription buffer

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Abbreviations: PKA, cAMP-dependent protein kinase; PKI, protein kinase inhibitor; R subunit, regulatory subunit; C subunit, catalytic subunit.

(Promega; 50 mM Tris·HCl, pH 7.5/6 mM $MgCl_2$/10 mM spermidine/10 mM NaCl), 10 mM dithiothreitol, 10 units of RNasin (Promega), 300 μM each of ATP, CTP, and GTP, 4 μM [α-^{32}P]UTP (diluted 4-fold with unlabeled UTP from 3000 Ci/mmol of radiolabeled nucleotide; 1 Ci = 37 GBq; ICN), 500 ng of linearized template, and 1 unit of T7 RNA polymerase (Promega). The specific activity of the synthesized cRNA probe was $>10^9$ cpm/μg. Sense RNA standards were synthesized as above (except for the addition of 500 μM each of ATP, CTP, GTP, and UTP) and quantified spectrophotometrically.

RNase Protection Analysis. Total RNA (4 μg for PKIα or 10 μg for PKIβ) purified from different brain regions was precipitated and resuspended in 40 μl of hybridization buffer [80% (vol/vol) formamide/0.4 M NaCl/10 mM Tris·HCl, pH 7.5/1 mM EDTA/2 × 10^5 cpm of cRNA probe]. For the control reactions, 4 μg (for PKIα) or 10 μg (for PKIβ) of yeast tRNA was mixed with various amounts of the appropriate sense RNA and resuspended in 40 μl of hybridization buffer. All samples were heated at 80°C for 15 min and then hybridized overnight at 50°C. The next morning, 300 μl of RNase solution (10 mM Tris·HCl, pH 7.5/0.3 M NaCl/5 mM EDTA/20 μg of RNase A per ml/200 units of RNase T1 per ml) was added, and samples were incubated at 30°C for 30 min. Proteinase K (10 μl of a 10 mg/ml solution) and Sarkosyl [10 μl of a 10% (wt/vol) solution] were added, and the reaction mixtures were incubated at 30°C for an additional 30 min. To purify RNA, 300 μl of guanidinium isothiocyanate/tRNA solution (4 M guanidinium isothiocyanate/25 mM sodium citrate, pH 7.0/0.5% Sarcosyl/0.1 M 2-mercaptoethanol/33.3 μg of yeast tRNA per ml) and 600 μl of 2-propanol were sequentially added, and RNA was precipitated from the mixture for 10 min at 0°C. After centrifugation, the RNA pellets were washed with 70% ethanol, resuspended in 5 μl of sequencing gel loading buffer, briefly denatured at 80°C, and electrophoresed through a 6% polyacrylamide denaturing gel.

***In Situ* Hybridization Histochemistry.** Mouse brains were removed, frozen, sectioned (15-μm sections), and processed for *in situ* hybridization histochemistry, as previously described (19). Antisense and sense cRNA probes to PKIα and PKIβ were generated by using linearized AR-1 and MtPki.pcr constructs, [^{35}S]UTP (Amersham), and SP6 or T7 RNA polymerase according to the manufacturer's specifications. The cRNA probe was diluted in hybridization buffer to yield 2 × 10^6 dpm per 30 μl of buffer, and sections were hybridized at 54°C for 16–20 h. The final wash after RNase A digestion was in 0.5× SSC at 58°C for 60 min. Sections were dipped in Kodak NTB-2 nuclear emulsion (1- to 3-week exposure).

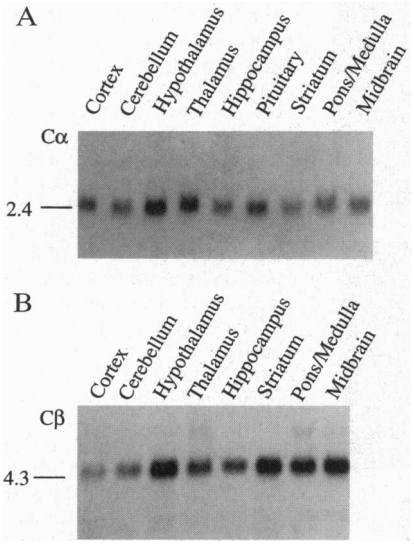

FIG. 1. Northern blot analysis of Cα and Cβ mRNA in mouse brain. Thirty micrograms of total RNA isolated from the indicated regions of mouse brain was loaded in each lane, electrophoresed through two 1% agarose/formaldehyde gels, and transferred to nylon filter membranes. One filter was hybridized to an antisense Cα probe (*A*), while the second filter was hybridized to an antisense Cβ probe (*B*). The sizes of the hybridization-positive bands, as determined by comparison with RNA size standards, are shown on the left.

RESULTS AND DISCUSSION

Expression of PKA C Subunit Isoform (Cα and Cβ) mRNA in Mouse Brain. To compare directly the relative expression of the C subunit isoforms with PKI isoform expression in dissected regions of mouse brain, total RNA was prepared from dissected mouse brain regions and utilized for both Northern blot analysis of Cα and Cβ expression and RNase protection analysis of PKIα and PKIβ expression as described below. The Northern blot analysis using antisense Cα probe (Fig. 1*A*) detected a 2.4-kb transcript in pituitary and all mouse brain regions examined, with the highest levels of expression observed in hypothalamic and thalamic regions. The Cβ probe detected a 4.3-kb transcript in all mouse brain regions examined (Fig. 1*B*). The highest levels of Cβ expression were observed in hypothalamus, striatum, pons/medulla, and midbrain. The autoradiograms shown in Fig. 1 were exposed for different times to attain comparable hybridization signals (0.5 h for Cα and 3 h for Cβ). Since hybridization probes of similar

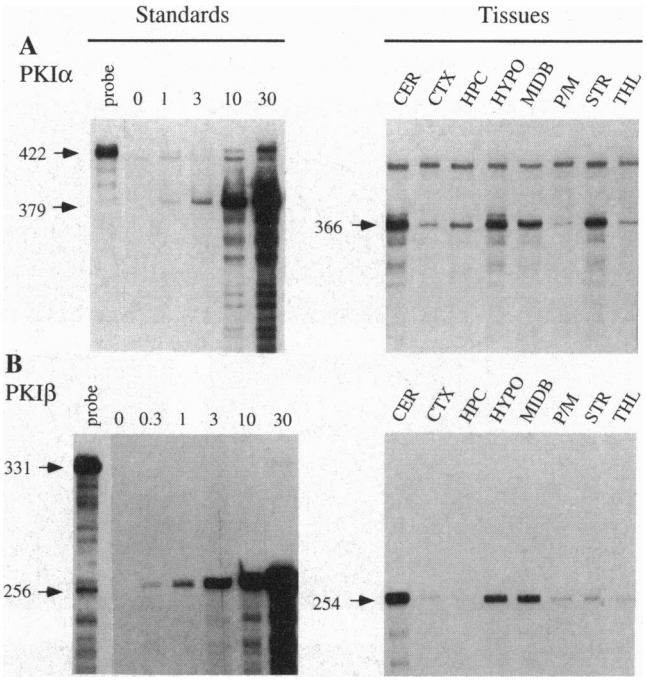

FIG. 2. RNase protection analysis of PKIα and PKIβ mRNA in mouse brain. (*A*) Antisense PKIα cRNA probe (422 nt) was hybridized to PKIα sense RNA standards (0–30 pg) or to 4 μg of total RNA isolated from dissected mouse brain regions. The 366-nt fragment in mouse brain tissue corresponds to protected PKIα mRNA transcripts. The upper band in these tissue samples represents undigested cRNA probe (422 nt). (*B*) Antisense PKIβ cRNA probe (331 nt) was hybridized to PKIβ sense RNA standards (0–30 pg) or to 10 μg of total RNA isolated from dissected mouse brain regions. The 254-nt fragment in mouse brain tissue corresponds to protected PKIβ mRNA transcripts. The slight differences in size (13 nt and 2 nt) for protected mouse brain PKIα and PKIβ RNA vs. sense RNA standards, respectively, are due to 13 or 2 nucleotides of linker DNA included in the RNA sense and complementary antisense cRNA probes. The sizes indicated were determined by comparison with DNA size standards. Autoradiograms were exposed for 14 h (*A*) or 48 h (*B*) at −80°C with intensifying screens. CER, cerebellum; CTX, cortex; HPC, hippocampus; HYPO, hypothalamus; MIDB, midbrain; P/M, pons/medulla; STR, striatum; THL, thalamus.

Table 1. Quantitation of PKI isoform mRNA by RNase protection

Tissue	PKIα	PKIβ
Cerebellum	++++	++
Cortex	+/++*	+
Hippocampus	++	+/−
Hypothalamus	+++	++
Midbrain	+++	++
Pons/medulla	+	+
Striatum	+++	+
Thalamus	+	+/−

Quantitation of PKI mRNA levels was determined by densitometric analysis of autoradiograms shown in Fig. 2. Relative abundance is indicated as follows: ++++ = 3–10 pg of PKI mRNA per μg of total RNA; +++ = 1–3 pg of PKI mRNA per μg of total RNA; ++ = 0.3–1 pg of PKI mRNA per μg of total RNA; + = 0.1–0.3 pg of PKI mRNA per μg of total RNA; − = <0.1 pg of PKI mRNA per μg of total RNA. *Abundance of PKIα mRNA in cortex varied significantly in different RNA preparations, most likely due to variation in cortical regions included in the dissection.

specific activity were used, these results demonstrate that Cα mRNA is expressed at higher levels than Cβ mRNA in several regions of the mouse brain including cortex, cerebellum, thalamus, hippocampus, and hypothalamus. These results are consistent with previous *in situ* hybridization histochemical studies (20), which demonstrated expression of both Cα and Cβ isoforms throughout the brain.

RNase Protection Analysis of PKI Isoform (PKIα and PKIβ) Expression in Mouse Brain. RNase protection assays were performed to quantitate expression of PKI isoforms in mouse brain. For PKIα (Fig. 2*A*), a protected fragment of 366 nucleotides was apparent in all regions of the brain examined, with the highest levels of PKIα expression in cerebellum, hypothalamus, and striatum. For PKIβ (Fig. 2*B*), a protected fragment of 254 nucleotides was observed in all isolated brain regions after hybridization with a PKIβ cRNA probe. The highest levels of PKIβ expression were observed in cerebellum, hypothalamus, and midbrain. Densitometric analysis of protected fragments and sense RNA copy standards from several experiments permitted quantitation of PKI isoform mRNA levels, as shown in Table 1.

The results from the RNase protection experiment are consistent with previous Northern blot analyses that examined PKIα and PKIβ mRNA expression in rat cortex and cerebellum (12). Both transcripts were present in rat cortex and cerebellum, with PKIβ expression in cortex significantly lower than PKIα (12). The results described here extend those findings to demonstrate PKIα and PKIβ expression in all mouse brain regions examined, with significantly elevated levels of PKIα compared with levels of PKIβ in almost all regions of the brain.

***In Situ* Hybridization Histochemical Localization of PKIα and PKIβ in Mouse Brain.** *In situ* hybridization histochemistry was utilized to localize PKIα and PKIβ mRNAs within the various regions of the adult mouse brain. The specificity of

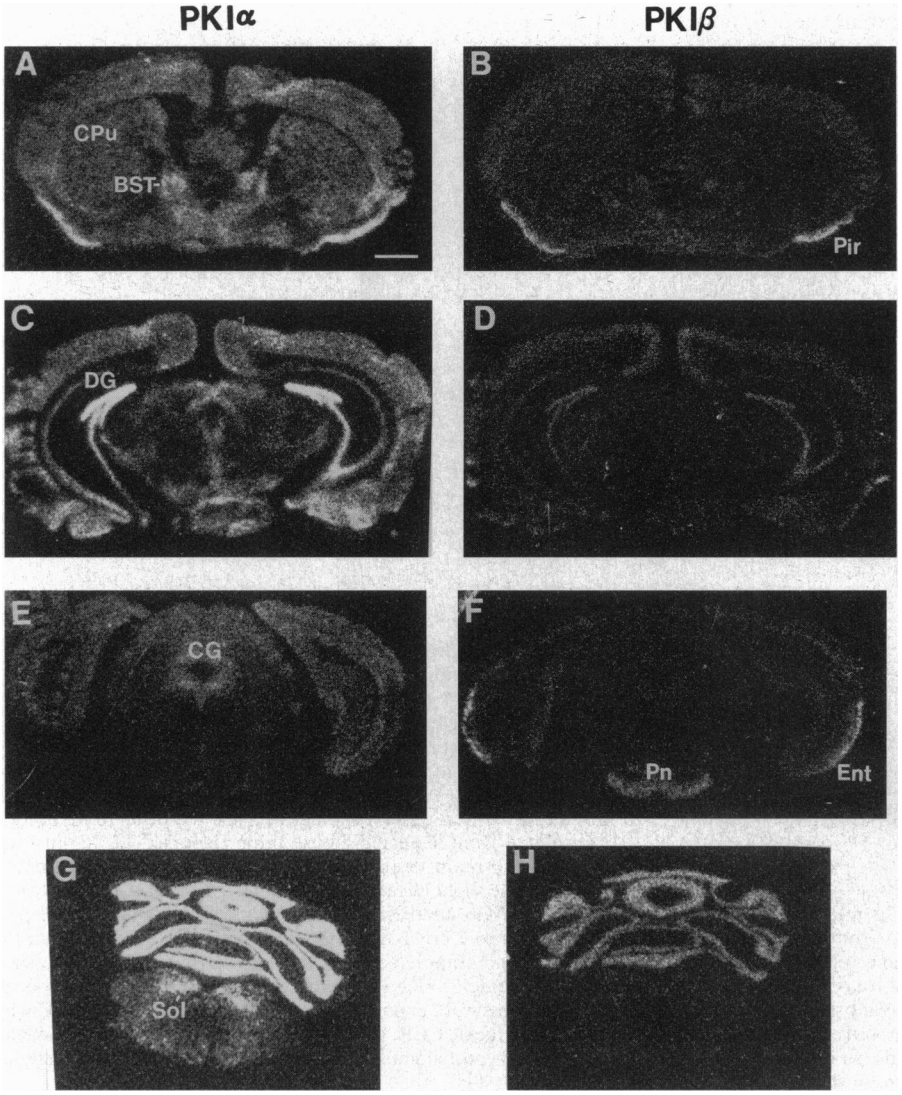

FIG. 3. Comparative hybridization histochemical localization of PKIα and PKIβ mRNA in mouse brain. Dark-field autoradiograms (48-h exposure) of coronal sections of mouse brain oriented rostrocaudally demonstrate regional distribution of neurons that hybridize with ^{35}S-labeled cRNA probes for PKIα (*A*, *C*, *E*, and *G*) or PKIβ (*B*, *D*, *F*, and *H*). High levels of PKIα expression are observed in caudate putamen (CPu), bed nucleus of the stria terminalis (BST), medial preoptic area, and cortical layers II–VI, including the piriform cortex (Pir) (*A*), while PKIβ mRNA is most abundant in the piriform cortex at this level of the brain (*B*). PKIα and β mRNA expression in hippocampus (DG, dentate gyrus) is shown in *C* and *D*, respectively. In coronal sections through the pons (*E* and *F*), PKIβ is observed at high levels in the pontine nucleus (Pn) and entorhinal cortex (Ent) (*F*), and PKIα expression is observed predominantly in the cerebral cortex, central gray (CG), and the superior colliculus (*E*). (*G* and *H*) PKI mRNA expression in the cerebellum and medulla. Both PKIα and -β mRNA are detected in the granular layer of the cerebellum (*G* and *H*), while PKIα mRNA is also observed in the nucleus of the solitary tract (Sol) and reticular nuclei (*G*). (Bar = 1 mm.)

hybridization was assessed by hybridizing adjacent sections throughout the brain with a corresponding sense cRNA probe. No specific signal was detected with sense PKIα or PKIβ hybridization probes. Fig. 3 provides a comparative overview of the distribution of mRNAs coding for PKIα and PKIβ in several brain regions. The specific activities of the hybridization probes were similar, allowing a qualitative comparison between mRNA levels observed after equal autoradiographic exposure times. PKIα mRNA is more abundant and shows a broad distribution throughout the brain, while PKIβ is more restricted in its distribution pattern but with high levels of expression in some localized regions. High levels of PKIα expression are seen in cortical layers II–VI, including the piriform cortex and entorhinal cortex, while PKIβ expression in cortex is observed most readily in piriform, entorhinal, and cingulate cortex. PKIα mRNA is quite abundant in the dentate gyrus and hippocampal layers CA1, CA2, and CA3 (Figs. 3*C* and 4*A* and *C*). PKIβ mRNA is expressed in the dentate gyrus, CA3, and CA2 regions (Figs. 3*D* and 4 *B* and *D*), but at significantly lower levels than PKIα mRNA. At more caudal levels, the hilar cells of the dentate gyrus express significant levels of PKIβ (Fig. 4*D*).

In the cerebellum, both PKIα and PKIβ mRNA are localized to the granular layer, while PKIα mRNA is also expressed weakly in the molecular layer of the cerebellum. Examination of emulsion-dipped slides has also demonstrated that while both PKIα and PKIβ are expressed in Purkinje cells, much higher levels of PKIα expression are observed in these cells (data not shown).

Analysis of emulsion-dipped slides demonstrated many other major sites of expression of PKIα and PKIβ mRNA (Tables 2 and 3). Of particular interest is the localized PKIβ expression in the hypothalamus. PKIα exhibits high levels of expression throughout the hypothalamus, while PKIβ is expressed specifically in the parvocellular division of the paraventricular nucleus, the periventricular nucleus, the suprachiasmatic nucleus, and the supraoptic nucleus of the hypothalamus (Fig. 4*E* and data not shown). The overall distribution of PKI expression does not strictly correlate with either isoform of the C subunit; however, previous studies (20) have shown that Cα is the mostly widely distributed C subunit isoform, and the current studies indicate that PKIα is the most widely expressed PKI isoform.

The high levels of PKA catalytic subunit in the brain are thought to reflect its importance as a second messenger for many neurotransmitters. The broad distribution of PKI isoform expression throughout the brain, as demonstrated in this study, suggests that PKI may also serve an important modulatory role for this second messenger system in the nervous system. Previous studies have suggested that total PKI levels in brain would be sufficient to block ≈20% of the total PKA (10). Quantitation of PKIα *in situ* hybridization autoradiographs has demonstrated a 10-fold difference in expression of PKIα across various brain regions (data not shown), suggesting that PKIα may be able to inhibit even higher amounts of PKA activity in localized brain nuclei. Finally, the distinct patterns of PKIα and PKIβ mRNA expression suggest differential functions for the two isoforms of PKI in the central nervous system.

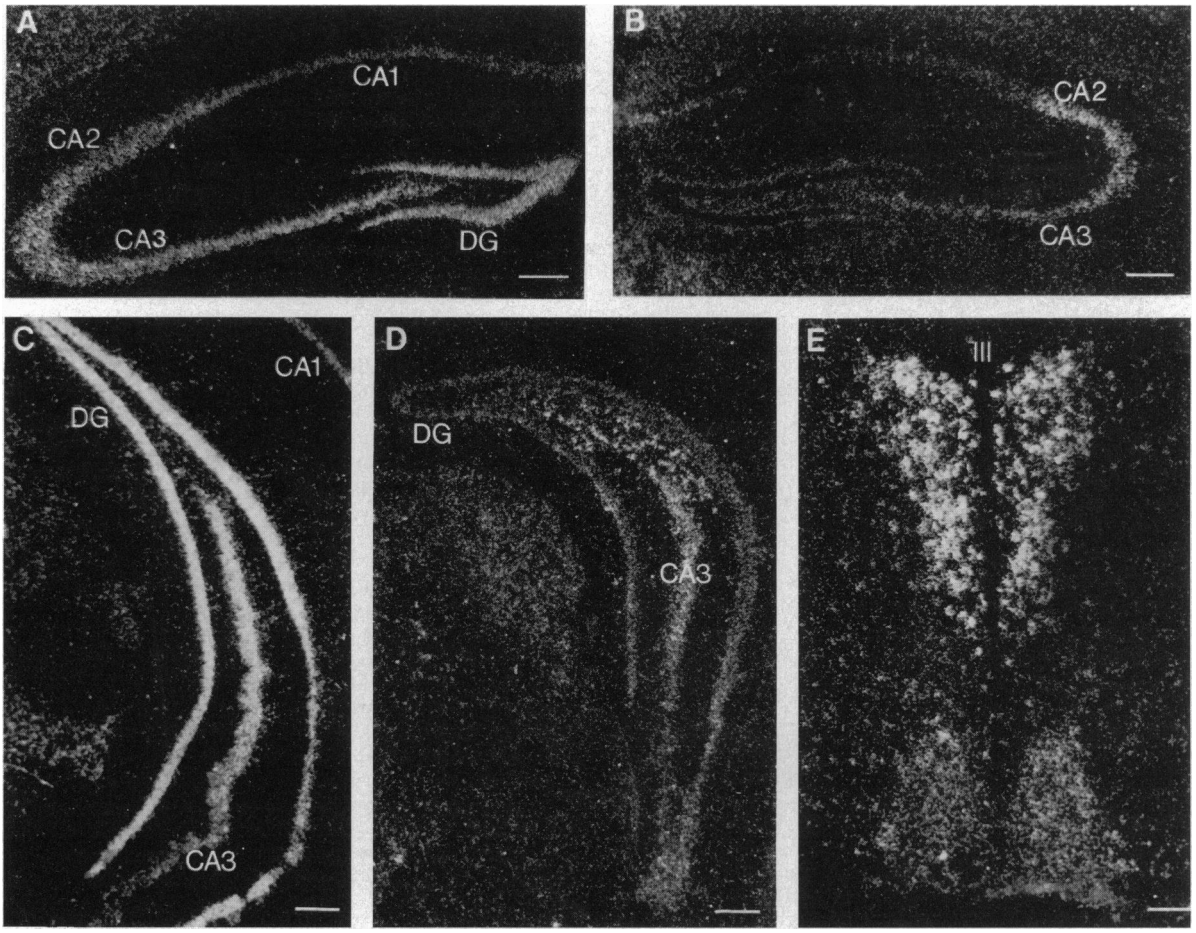

FIG. 4. Darkfield photomicrographic localization of PKIα and PKIβ mRNA in mouse hippocampus and hypothalamus. (*A*) PKIα mRNA is present in hippocampal cell layers CA1, CA2, and CA3 and the dentate gyrus (DG). (*B*) PKIβ mRNA is most abundant in layers CA2 and CA3 in this region of the hippocampus. (*C*) PKIα mRNA is highly expressed in dentate gyrus and CA3, with lower levels of expression in CA1 as observed in *A*. (*D*) PKIβ mRNA is expressed in dentate gyrus and hippocampal layer CA3, with scattered hilar cells also expressing PKIβ mRNA. (*E*) PKIβ mRNA is highly expressed in localized regions of the hypothalamus, including the parvocellular division of the paraventricular nucleus, the periventricular nucleus, and the suprachiasmatic nucleus. III, third ventricle. (Bar = 200 μm in *A–D* and 100 μm in *E*.)

Table 2. Brain regions expressing the highest levels of PKIα mRNA

Region	Abundance*
Telencephalon	
Piriform cortex	++++
Entorhinal cortex	+++
Cerebral cortex, layers II–VI	+++
Hippocampal formation	
CA1	+++
CA2, CA3	++++
Dentate gyrus	++++
Hilar cells (scattered)	++/+++
Nucleus accumbens	++++
Caudate putamen	+++
Lateral septal nucleus	+++
Bed nucleus of stria terminalis	++++
Amygdaloid complex	++++
Preoptic area (medial, anterior, lateral)	++/+++
Diencephalon	
Thalamus	
Anterodorsal	+++
Centromedial	++
Paratenial	+++
Paraventricular	++++
Medial geniculate	++
Habenula, medial	+++
Zona incerta	++++
Hypothalamus	
Lateral hypothalamus	+++
Posterior hypothalamus	++/+++
Anterior hypothalamus	+++
Dorsomedial hypothalamus	+++
Ventromedial hypothalamus	+++
Paraventricular nucleus	+++
Suprachiasmatic nucleus	++/+++
Arcuate nucleus	
Supramammillary nucleus	+++
Mesencephalon	
Central gray	+++
Interpeduncular complex	++
Superior colliculus	++/+++
Inferior colliculus	+++
Dorsal raphe and raphe complex	++
Pons and medulla	
Laterodorsal tegmental nucleus	++
Parabrachial nucleus	++/+++
Reticular nucleus (parvocellular, intermediate, lateral, and gigantocellular)	++/+++
Spinal trigeminal	++/+++
Medial vestibular	++/+++
Prepositus hypoglossal	++/+++
Nucleus of solitary tract	++++
Inferior olivary complex	++
Cerebellar cortex	
Granular layer	+++
Purkinje cell layer	++++

*PKIα mRNA abundance was evaluated subjectively from *in situ* hybridization slides by several investigators. The region(s) of highest intensity was defined as ++++ (very dense); lower levels of intensity were defined as +++ (dense) or ++ (moderate). Numerous other brain regions exhibit low levels of PKIα mRNA or scattered hybridization-positive cells; those regions have not been included in the above list. The levels of expression of PKIα and PKIβ (Table 3) were determined independently and should not be directly compared.

Table 3. Brain regions expressing the highest levels of PKIβ expression

Region	Abundance*
Telencephalon	
Piriform cortex	++++
Cingulate cortex	++
Entorhinal cortex	++++
Cerebral cortex-superficial layer of V	++
Hippocampal formation	
CA2, CA3	+++
Dentate gyrus	++
Hilar cells (at caudal levels)	+++
Globus pallidus	+++
Diencephalon	
Thalamus	
Centromedial nucleus	++/+++
Anterior	++
Geniculate, medial and lateral	++
Habenula, medial	++
Hypothalamus	
Paraventricular nucleus, parvocellular	++++
Periventricular nucleus	++++
Supraoptic nucleus	++
Suprachiasmatic nucleus	++
Pons and medulla	
Pontine nucleus	+++
Dorsal cochlear nucleus	++/+++
Cerebellar cortex	
Granular layer	+++

*Abundance is determined as described for Table 2. The levels of expression of PKIα (Table 2) and PKIβ mRNA were determined independently and should not be directly compared.

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1. Walaas, S. I. & Greengard, P. (1991) *Pharmacol. Rev.* **43,** 299–349.
2. McKnight, G. S. (1991) *Curr. Opin. Cell Biol.* **3,** 213–217.
3. Scott, J. D. (1991) *Pharmacol. Ther.* **50,** 123–145.
4. Lee, K. (1991) *Curr. Opin. Cell Biol.* **3,** 953–959.
5. Swope, S. L., Moss, S. J., Blackstone, C. D. & Huganir, R. L. (1992) *FASEB J.* **6,** 2514–2523.
6. Hawkins, R. D., Kandel, E. R. & Siegelbaum, S. A. (1993) *Annu. Rev. Neurosci.* **16,** 625–665.
7. Bailey, C. H. & Kandel, E. R. (1993) *Annu. Rev. Physiol.* **55,** 397–426.
8. Davis, R. L. (1993) *Neuron* **11,** 1–14.
9. Walsh, D. A., Glass, D. B. & Mitchell, R. D. (1992) *Curr. Opin. Cell Biol.* **4,** 241–251.
10. Walsh, D. A., Angelos, K. L., Van Patten, S. M., Glass, D. B. & Garetto, L. P. (1990) in *Peptides and Protein Phosphorylation*, ed. Kemp, B. E. (CRC, Boca Raton, FL), pp. 43–84.
11. Olsen, S. R. & Uhler, M. D. (1991) *J. Biol. Chem.* **266,** 11158–11162.
12. Van Patten, S. M., Howard, P., Walsh, D. A. & Maurer, R. (1992) *Mol. Endocrinol.* **6,** 2114–2122.
13. Van Patten, S. M., Ng, D. C., Th'ng, J. P. H., Angelos, K. L., Smith, A. J. & Walsh, D. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5383–5387.
14. Scarpetta, M. A. & Uhler, M. D. (1993) *J. Biol. Chem.* **268,** 10927–10931.
15. Fantozzi, D. A., Harootunian, A. T., Wen, W., Taylor, S. S., Feramisco, J. R., Tsien, R. Y. & Meinkoth, J. L. (1994) *J. Biol. Chem.* **269,** 2676–2686.
16. Uhler, M. D. & McKnight, G. S. (1987) *J. Biol. Chem.* **262,** 15202–15207.
17. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–169.
18. Day, R., Schafer, M. K. H., Collard, M. W., Watson, S. J. & Akil, H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1320–1324.
19. Seasholtz, A. F., Bourbonais, F. J., Harnden, C. E. & Camper, S. A. (1991) *Mol. Cell. Neurosci.* **2,** 266–273.
20. Cadd, G. & McKnight, G. S. (1989) *Neuron* **3,** 71–79.