Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2345–2349, March 1992
Neurobiology

Preproenkephalin promoter "cassette" confers brain expression and synaptic regulation in transgenic mice

(haloperidol/striatum/dorsal horn/seizure/pain)

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Communicated by Sanford L. Palay, December 16, 1991 (received for review August 9, 1991)

ABSTRACT **The preproenkephalin A gene is a neurotransmitter gene whose expression can be modulated "trans-synaptically" by changes in neuronal activity. DNA sequences lying within 200 base pairs of this gene's transcription start site resemble consensus binding sites for several transcription factor families. In nonneuronal cell cultures, this promoter region is sufficient to mediate gene responses to depolarization, phorbol esters, adenylate cyclase, and calcium fluxes. To assess the role that these cis-acting elements could play in preproenkephalin expression and regulation *in vivo*, the expression of a construct containing this 200-base-pair region fused to the chloramphenicol acetyltransferase gene was examined in transgenic mice. This promoter confers modest expression in brain, adrenal, and small intestine, with substantially higher levels in testis. These elements confer trans-synaptic regulation in two well-studied models of trans-synaptic preproenkephalin upregulation but not in a third system, underscoring the specificity of the regulatory sequence elements implicated in the synaptic regulation of neuronal genes.**

The gene encoding the principal opioid peptide, preproenkephalin, displays exquisite patterns of tissue-specific and regulated expression (e.g., refs. 1–6). When brain activity changes, preproenkephalin mRNA can be "synaptically" or "trans-synaptically" regulated in regions implicated in somatosensory (5, 6), stress (7, 8), epileptogenic (9–14), reproductive/neuroendocrine (15–17), drug administration and withdrawal (7, 18–20), and locomotor (3, 21–23) processes.

Preproenkephalin gene promoter elements that could contribute to this regulation have been studied in cultured cells (24–26). Responses to calcium, adenylate cyclase, and depolarization can be conferred by constructs containing a 200-base-pair 5′ flanking sequence of the preproenkephalin gene; this "cassette" region incorporates sites recognizing the cyclase response element-binding protein (CREB), activator proteins 2, 4, and 1 (AP-2, AP-4, AP-1), and nuclear factor 1 (25–29).

Levels of expression of several of these factors do increase with the trans-synaptic manipulations that augment preproenkephalin expression in the same brain regions. Factors that recognize AP-1 sites (Fos and Jun) increase prior to or parallel with stimulation-induced preproenkephalin upregulation in hippocampus (30) and dorsal horn (31–34) *in vivo*.

Despite *in vitro* data documenting which factors are candidates for mediating trans-synaptic regulation in the brain and *in vivo* studies demonstrating that some of these factors are upregulated in concert with preproenkephalin, little evidence directly documents which DNA elements actually mediate gene upregulation *in vivo*. Transgenic animals provide an approach to understanding the molecular mechanisms that could underlie trans-synaptic regulation *in vivo* (for review, see ref. 35). Studies of mice in which specific segments of the promoter for the preproenkephalin gene are introduced stably into the genome can allow assessment of the transcriptional activity induced by the gene segment *in vivo*. They can provide evidence about which gene regulatory regions are necessary and sufficient to confer tissue- and cell-specific gene expression (36) and gene regulation (37).

In vitro studies of preproenkephalin regulation have focused on pENKAT-12, a plasmid construct containing 193 base pairs of the human preproenkephalin 5′ cassette-region flanking sequence, exon 1, intron 1, part of exon 2, and 1.2 kilobase pairs (kb) of 3′ flanking sequence but no protein coding sequences (24). The current study examines reporter gene expression in transgenic mice directed by these regulatory regions in normal mice and in animals subjected to procedures upregulating the wild-type gene. The results indicate differential utilization of preproenkephalin regulatory elements in different brain circuits and underscore the specificity of mechanisms underlying brain gene regulation *in vivo*.

MATERIALS AND METHODS

DNA Constructs and Transgenic Mice. A 4.5-kb *Pvu* I fragment of pENKAT-12 (24) containing bases −193 to +210 of 5′ flanking region and ≈1 kb of 3′ flanking region of the human preproenkephalin gene fused to the chloramphenicol acetyltransferase (CAT) gene was microinjected into BSJL (C57BL/6J × SJL)F_1 mouse pronuclei to produce transgenic mice, termed HEC, by standard methods (DNX, Princeton, NJ).

DNA Isolation and Analysis. DNA was prepared by proteinase K and phenol/chloroform extraction methods from tail tips (38) and was analyzed by slot and/or Southern blot analyses (39) using a CAT cDNA vector, pSV0CAT (40), radiolabeled by random priming.

CAT Assay. CAT activity was determined by using two selective-extraction liquid scintillation assays (41, 42). Background values were obtained from wild-type tissue samples. Extracts were prepared by Polytron (Brinkmann) homogenization in 0.25 M Tris·HCl (pH 7.8) at 4°C, followed by 10 min of microcentrifugation (10,000 × *g*) to pellet cellular debris. Supernatant protein was determined by the Bradford method (Bio-Rad protein assay). Values presented represent

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Abbreviations: CAT, chloramphenicol acetyltransferase; PTZ, pentylenetetrazol.

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at least nine mice and were normalized to the mean control value for each experiment.

RNA Isolation Analysis. RNAs were isolated by CsCl centrifugation and oligo(dT) column methods (ref. 43; Invitrogen, San Diego), electrophoresed, transferred to nylon filters (44), and detected with two hybridization probes: (*i*) a random primer-labeled 1.2-kb *Hin*cII fragment of pENKAT-12 and (*ii*) a 5′-end-labeled oligonucleotide (5′-TGC-CGG-TCC-TCA-GCG-TCT-CTG-CGG-GGT-CAC-GGG-CCA-G-3′) exactly complementary to human but not to mouse mRNA (20 of 37 bases).

Regulation of Gene Expression. Three treatments known to regulate preproenkephalin expression *in vivo* were used. Mice received haloperidol (5 mg/kg, i.p.) for 3 days with the last dose administered 4 hr before sacrifice (45), pentylenetetrazol (PTZ, 40 mg/kg, i.p.) 2 hr before sacrifice (34), complete Freund's adjuvant (30 μl in one hindpaw footpad) 3 days before sacrifice (31), or equal volumes of 0.9% NaCl. Each animal displayed a behaviorally typical convulsion after PTZ injection, each animal injected with haloperidol displayed gross locomotor retardation consistent with neuroleptic effects, and each mouse injected with Freund's adjuvant exhibited footpad redness and edema.

Animals were sacrificed by cervical dislocation and decapitation. Brains were removed, rapidly dissected at 4°C, frozen immediately on dry ice, and stored at −70°C until analysis.

RESULTS

Twenty-two mice developed from injected embryos, 6 animals incorporated the HEC construct in their genomes, and offspring of each of the 5 founders that could be tested were also positive for this construct, suggesting that the gene was maintained in their germ lines (data not shown). Line HEC5 integrated a single copy, HEC2 integrated two or three copies, and HEC1, HEC3, and HEC4 integrated multiple copies. Each line displayed a single insertion site (data not shown).

CAT activity from male and female heterozygous offspring of each founder showed roughly similar patterns of expression with modest and uneven expression of the transgene in brain (Fig. 1) and peripheral tissues (Fig. 2) including spleen, ovary, small intestine, adrenal, and liver. Expression in testis was high in males tested from each line.

Brain CAT activity exceeded background values in striatum, brainstem, hippocampus, cervical spinal cord, and cerebral cortex ($P < 0.05$ in each case). Highest activity was found in the hippocampus and cervical cord (Fig. 1). The patterns of regional expression were similar in each of the five lines. Lines HEC1, HEC3, and HEC5 are described in detail, and line HEC5 was examined for studies of synaptic regulation with confirmatory experiments carried out in HEC2 animals (data not shown).

Northern analyses of RNA extracted from line HEC5 revealed high testis expression of an mRNA of 1.5 kb (arrow, Fig. 3) that hybridized with the random-primed cDNA directed against CAT. An identically sized mRNA, seen in both cortex and testis mRNA, hybridized with the oligonucleotide complementary to the 5′ end of human preproenkephalin mRNA, indicating that transcriptional initiation is unlikely to be 3′ to its normal start site (data not shown).

Animals were treated for 3 days with haloperidol, a classic upregulator of preproenkephalin (45). Treated HEC5 mice showed 4- to 6-fold increases in striatal CAT activity ($P \leq 0.005$; Fig. 4), marginally significant increases in cerebral cortex (nucleus accumbens included) specimens, and unchanged CAT activity in the other brain regions (Fig. 4) or peripheral tissues (data not shown).

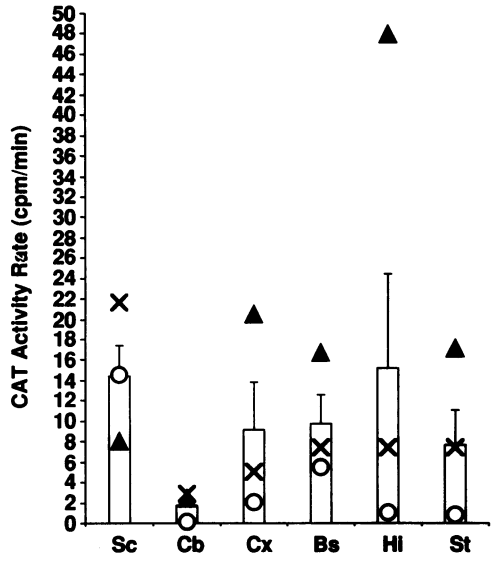

FIG. 1. CAT activity in neural tissue extracts from HEC transgenic mice. Rates for HEC5 (▲), HEC3 (○), and HEC1 (×), with background values from nontransgenic littermate controls subtracted, were averaged to yield bar height ± SEM. Cervical spinal cord (Sc), cerebellum (Cb), cerebral cortex (Cx), brainstem (Bs), hippocampus (Hi), and striatum (St) CAT activities are the rate of [^{3}H]acetyl-CoA (NEN) converted (cpm/min) from aqueous tissue extract phase to organic scintillant phase by 100 μg of extract protein per 250-μl assay mixture.

The lumbar spinal cords of animals sacrificed 3 days after unilateral Freund's adjuvant injection revealed a lateralized

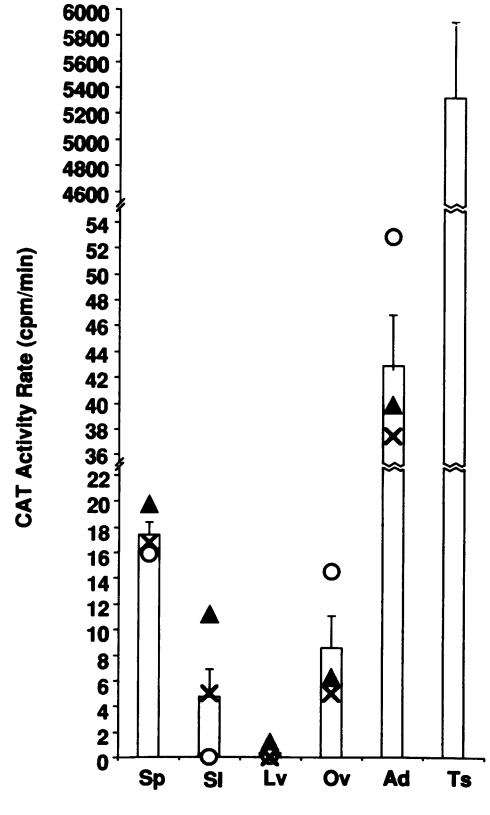

FIG. 2. CAT activity in peripheral tissues from HEC transgenic mice. Spleen (Sp), small intestine (SI), liver (Lv), ovary (Ov), adrenal gland (Ad), and testis (Ts) values from HEC5 (▲), HEC3 (○), and HEC1 (×) were obtained, corrected for background activity, and averaged as noted in Fig. 1. Testis values for lines 1, 3, and 5 were 5523, 4020, and 6478 cpm/min, respectively.

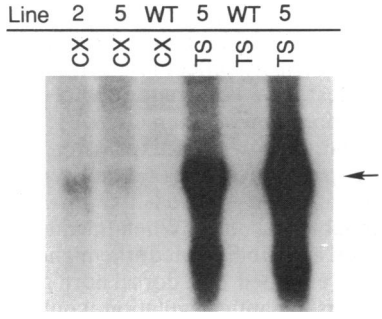

FIG. 3. CAT mRNA levels in HEC2 and HEC5 transgenic mice and in wild-type (WT) mice. Ten micrograms of total mRNA from cerebral cortex (CX) or testis (TS) was electrophoresed in each lane, and the blot was probed with a radiolabeled 1.1-kb CAT DNA fragment. Note comigration of the ≈1.5-kb band (arrow) from cerebral cortex and testis; the smaller band is likely to represent a degradation product.

increase in CAT activity that paralleled findings for the endogenous gene (5, 6). These studies revealed a 4- to 7-fold enhancement in CAT activity ipsilateral to the adjuvant injection ($P \leq 0.025$; Fig. 5).

In mice sacrificed 2 hr after PTZ-induced seizures, no significant changes in CAT activity were detected in three experiments (Fig. 6 *Upper*). RNA extracted from BSJL6 wild-type mice subjected to identical seizures and sacrificed at the same time points did show significantly (≈2-fold) enhanced expression of the endogenous preproenkephalin gene (Fig. 6 *Lower*), detected by a radiolabeled rat preproenkephalin cDNA (46) in Northern analyses normalized for loading by washing and reprobing with radiolabeled human β-actin cDNA (47).

DISCUSSION

The present work documents the patterns of expression conferred by a cassette-region construct of the preproenkephalin gene promoter and the regulation that these sequences can mediate.

Parallels with wild-type gene expression are consistent with necessary and sufficient roles for the sequences studied here in some aspects of gene regulation. The brain, testis, adrenal, spleen, and small intestine expression in all HEC lines examined is consistent with wild-type gene expression in each of these tissues. Values for CAT indistinguishable from background values are obtained in liver, which is devoid

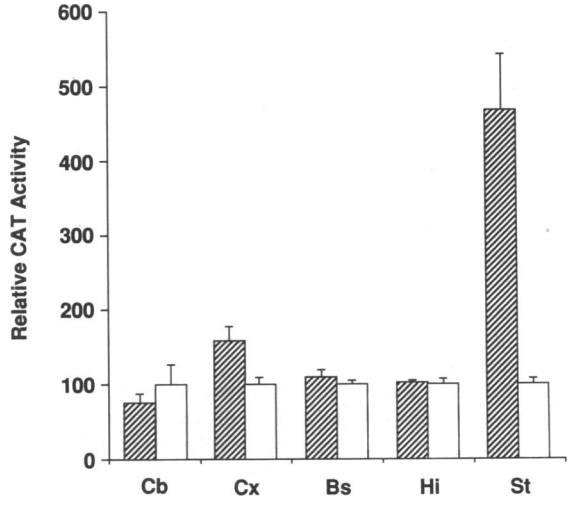

FIG. 4. Effect of haloperidol treatment on CAT activity levels in cerebellum (Cb), cerebral cortex (Cx), brainstem (Bs), hippocampus (Hi), and striatum (St). Hatched bars, haloperidol-treated mice; open bars, saline-injected controls (control CAT activity was set at 100). CAT assay was as in Fig. 1 legend. Bars show mean ± SEM for three experiments with three HEC5 mice each.

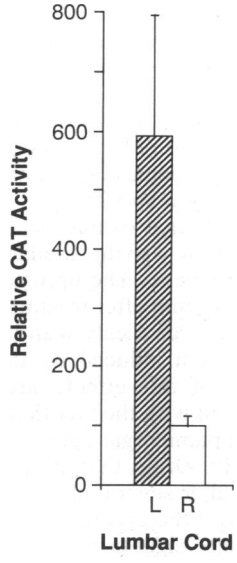

FIG. 5. Effect of primary afferent stimulation on CAT activity levels in the left (L) and right (R) lumbar spinal cord. Left footpad injections of complete Freund's adjuvant were made in HEC5 mice 3 days before sacrifice. CAT assay was as in Fig. 1 legend.

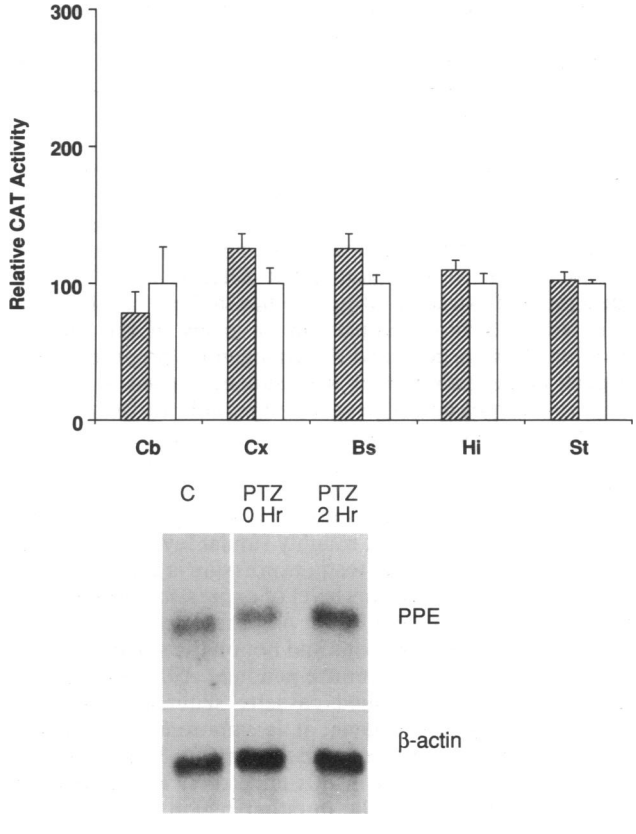

FIG. 6. Effect of PTZ-induced seizures on CAT activity and endogenous preproenkephalin (PPE) A mRNA levels. (*Upper*) HEC5 CAT activity measurements. Hatched bars, PTZ-injected HEC mice; open bars, saline-injected HEC controls. CAT assay and abbreviations are as in Fig. 1 legend. (*Lower*) Northern analysis of total RNA (8 μg per lane) isolated from mouse hippocampi. Wild-type mice were saline (control, C)- or PTZ-injected and hippocampi were isolated 0 or 2 hr after injection.

of significant preproenkephalin mRNA (48). Several central nervous system regions that display significant preproenkephalin mRNA levels also display CAT activity above background levels in HEC mice, including cerebral cortex, hippocampus, and striatum (48). These findings provide some contrast to work in cultured cells (49) and highlight the importance of using transgenic mice for analysis of regulatory elements conferring tissue specificity.

Two of the three studied examples of preproenkephalin synaptic regulation show striking similarities with the regulation of the wild-type gene. The upregulation of preproenkephalin mRNA in striatum after treatment with haloperidol (21, 23, 44, 50, 51) accords nicely with our findings of 4- to 6-fold CAT upregulation induced by the same drug. The regional distribution of this effect, largely limited to the striatum, again fits well with the fact that the wild-type gene is upregulated by dopaminergic agents in striatum and accumbens only (45, 50). Drugs that influence striatal dopaminergic function can alter several of the transcription factors that could interact with the preproenkephalin promoter cassette (52). These results suggest that the gene regions studied here, while insufficient for full basal striatal expression, are nevertheless sufficient for prominent preproenkephalin upregulation upon dopamine receptor blockade.

Primary afferent stimulation provides trans-synaptic regulation in the spinal cord. Injection of adjuvant into the footpad induces an inflammatory response that is likely to stimulate both large- and small-fiber inputs to dorsal horn enkephalinergic neurons, where preproenkephalin expression is enhanced 1.5- to 2-fold (6). The 4- to 7-fold enhancement of CAT activity observed in the present study suggests that the promoter/enhancer regions studied here suffice to yield upregulation. Primary afferent stimulation has also been noted to upregulate dorsal horn expression of a number of transcription factors with possible activities at this promoter region (31–33, 53).

The finding of similar patterns of expression in several lines of mice incorporating the preproenkephalin promoter/CAT construct (HEC) suggests that regions within this construct are responsible for the patterns of expression noted. Each line of HEC transgenic animals shares patterns of expression that are similar to one another but different from the wild-type gene. One striking difference lies in the level of expression. Northern blot comparison of levels of the mRNAs encoding the transgene and the endogenous preproenkephalin gene demonstrates that the sequences used here allow transgene expression at levels comparable to those of the wild-type gene only in testis (data not shown). CAT assays also indicate that HEC animals robustly express this gene construct only in testis, while brain levels of expression are a small fraction of testis values. The wild-type gene, on the other hand, is expressed at roughly similar levels in brain and testis (54). That high-level testis expression is conferred in all HEC lines suggests that this level of expression is dependent on the specific sequences in the preproenkephalin promoter cassette or associated regions and not on the site of insertion of the transgene into the mouse genome. Although we have focused on the well-studied cassette sequences in the 5′ flanking regions of the gene, it is important to note that limited intronic sequences and extensive 3′ flanking sequences are also present in this construct. Regulatory elements could conceivably lie in either region, although they have not been well characterized.

In animals whose basal brain expression of the transgene is significantly lower than that of the endogenous preproenkephalin gene, several cautions must be observed. A severalfold change in CAT levels is likely to reflect the synthesis of fewer additional CAT molecules than would be required to induce the same fold change over a higher baseline. This feature necessitates care in directly comparing the magnitude of inductions in CAT and preproenkephalin mRNA levels. For example, the haloperidol-induced 6-fold CAT induction in striatum could actually reflect less synthesis of new CAT mRNA molecules than that required to induce a 2-fold enhancement of preproenkephalin mRNA levels in this same paradigm. Second, the low brain expression makes efforts to localize CAT expression to specific cell types difficult, despite application of otherwise sensitive *in situ* hybridization techniques (unpublished data). When the different quadrants of the spinal cord are subdissected, the highest CAT activity lies in the sample enriched with dorsal horn gray matter (data not shown). Trans-synaptic regulation would be unlikely to resemble the regulation of the wild-type gene if expression were principally glial. These results argue that at least much of the activity measured here results from neuronal expression.

Differences from wild-type gene expression imply that the promoter cassette sequences are insufficient to confer all of the expression patterns characteristic of the wild-type gene, although they could still prove necessary in combination with other elements missing from the constructs. Brain region-to-region differences in CAT activity display significant differences from preproenkephalin expression. Striatum displays CAT levels no higher than those of the hippocampus, despite striatal preproenkephalin mRNA levels as much as 6–12 times those in hippocampus (48). Both the differences in regional rank order of expression and the absolutely lower levels of expression in brain as a whole, when compared to expression of the endogenous gene, argue that additional regulatory elements are necessary to achieve wild-type expression levels in nontesticular tissues. Additional regulatory elements could lie further 5′ to the transcriptional start site. Achieving full wild-type expression levels and patterns could also require proper intron/exon structure (55).

One major and well-documented example of synaptic regulation is absent from HEC mice. The upregulation of preproenkephalin mRNA levels in hippocampus following seizures has been extensively studied in several laboratories (9, 12, 34). Northern analyses (Fig. 6) document upregulation of the wild-type gene in the mouse strains used here. Each animal examined displayed a behaviorally unmistakable seizure. Nevertheless, CAT activities extracted from the hippocampi and striata of mice sacrificed after seizures in each of three experiments showed no significant differences from saline-injected control values. There appears to have been sufficient time for the modestly expressed transgene to significantly accumulate product; recent experiments using electrical peripheral nerve stimulation in HEC5 mice have documented 1.5- to 2-fold increases in dorsal horn CAT activity in the nucleus caudalis in animals sacrificed well within 2 hr of treatment onset (56). Although transcription factors that recognize sequences in the preproenkephalin promoter cassette region are upregulated in hippocampus following seizures, the HEC construct appears to be insufficient to recognize all of the intracellular mediators required for seizure-induced preproenkephalin upregulation.

A number of studies in transgenic animals have characterized the ability of specific regulatory sequences, usually those found in 5′ flanking regions, to confer cell type-, development- and tissue-specific expression (57, 58). Fewer studies have examined the ability of specific promoter regions to confer *in vivo* regulation with hormonal activity changes (37). Little work documents which gene regulatory elements are necessary for synaptic and trans-synaptic gene regulation in neurons *in vivo*. Synaptic regulation in each circuit does not appear to utilize the same constellation of DNA-binding domains. Study of regulatory processes in specific neurons within specific circuits is thus crucial for full understanding of mechanisms of synaptic gene regulation in the brain.

We are grateful to Dr. Michael Comb for thoughtful discussions and the vector pENKAT-12 and to Ms. Carol Sneeringer for help with the manuscript. This work was supported by the intramural program of the National Institute on Drug Abuse, the National Institute of Neurological Disorders and Stroke, the National Institute of Mental Health, the McKnight Foundation, the Sloan Foundation, the American Parkinson's Disease Association, and the Juvenile Diabetes Association.

1. Harlan, R. E., Shivers, B. D., Romano, G. J., Howells, R. D. & Pfaff, D. W. (1987) *J. Comp. Neurol.* **258,** 159–184.
2. Tecott, L. H., Rubenstein, J. L. R., Paxinos, G., Evans, C. J., Eberwine, J. H. & Valentino, K. L. (1989) *Dev. Brain Res.* **49,** 75–86.
3. Tang, F., Costa, E. & Schwartz, J. P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3841–3844.
4. Douglass, J., Grimes, L., Shook, J., Lee, P. H. & Hong, J.-S. (1991) *Mol. Brain Res.* **9,** 79–86.
5. Nishimori, T., Buzzi, M. G., Moskowitz, M. A. & Uhl, G. R. (1989) *Mol. Brain Res.* **6,** 203–210.
6. Iadarola, M. J., Douglass, J., Civelli, O. & Naranjo, J. R. (1988) *Brain Res.* **455,** 205–212.
7. Lightman, S. L. & Young, W. S. (1987) *Nature (London)* **328,** 643–645.
8. Lightman, S. L. & Young, W. S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4306–4310.
9. Yoshikawa, K., Hong, J.-S. & Sabol, S. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 589–593.
10. Morris, B. J., Feasey, K. J., Bruggencate, G. T., Herz, A. & Hollt, V. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3226–3230.
11. Lee, P. H. K., Zhao, D., Xie, C. W., McGinty, J. F., Mitchell, C. L. & Hong, J. S. (1989) *Mol. Brain Res.* **6,** 263–273.
12. Xie, C. W., Lee, P. H. K., Takeuchi, K., Owyang, V., Li, S. J., Douglass, J. & Hong, J. S. (1989) *Mol. Brain Res.* **6,** 11–19.
13. Moneta, M. E. & Hollt, V. (1990) *Neurosci. Lett.* **110,** 273–278.
14. Gall, C. (1988) *J. Neurosci.* **8,** 1852–1862.
15. Kew, D. & Kilpatrick, D. L. (1989) *Mol. Endocrinol.* **3,** 179–184.
16. Muffly, K. E., Jin, D. F., Okulicz, W. C. & Kilpatrick, D. L. (1988) *Mol. Endocrinol.* **2,** 979–985.
17. Lauber, A. H., Romano, G. J., Mobbs, C. V., Howells, R. D. & Pfaff, D. W. (1990) *Mol. Brain Res.* **8,** 47–54.
18. Normand, E., Popovici, T., Fellmann, D. & Bloch, B. (1987) *Neurosci. Lett.* **83,** 232–236.
19. Li, S. J., Jiang, H. K., Stachowiak, M. S., Hudson, P. M., Owyang, V., Nanry, K., Tilson, H. A. & Hong, J. S. (1990) *Mol. Brain Res.* **8,** 219–225.
20. Uhl, G. R., Ryan, J. P. & Schwartz, J. P. (1988) *Brain Res.* **459,** 391–397.
21. Schwartz, J. P., Simantov, R., Ryan, J. P. & Uhl, G. R. (1988) in *Progress in Endocrinology*, eds. Imura, H., Shizume, K. & Yoshida, S. (Elsevier, New York), pp. 1125–1129.
22. Uhl, G. R., Navia, B. & Douglas, J. (1988) *J. Neurosci.* **8,** 4755–4764.
23. Petrack, B., Emmett, M. R., Rao, T. S., Kim, H. S. & Wood, P. L. (1990) *Life Sci.* **46,** 687–691.
24. Comb, M., Birnberg, N. C., Seasholtz, A., Herbert, E. & Goodman, H. M. (1986) *Nature (London)* **323,** 353–356.
25. Comb, M., Mermod, N., Hyman, S. E., Pearlberg, J., Ross, M. E. & Goodman, H. M. (1988) *EMBO J.* **7,** 3793–3805.
26. Nguyen, T. V., Kobierski, L., Comb, M. & Hyman, S. E. (1990) *J. Neurosci.* **10,** 2825–2833.
27. Hyman, S. E., Comb, M., Lin, Y.-S., Pearlberg, J., Green, M. R. & Goodman, H. M. (1988) *Mol. Cell. Biol.* **8,** 4224–4233.
28. Hyman, S. E., Comb, M., Pearlberg, J. & Goodman, H. M. (1989) *Mol. Cell. Biol.* **9,** 321–324.
29. Chu, H. M., Fischer, W. H., Osborne, T. F. & Comb, M. (1991) *Nucleic Acids Res.* **19,** 2721–2728.
30. Sonnenberg, J. L., Macgregor-Leon, P. F., Curran, T. & Morgan, J. I. (1989) *Neuron* **3,** 359–365.
31. Draisci, G. & Iadarola, M. J. (1989) *Mol. Brain Res.* **6,** 31–37.
32. Menetrey, D., Gannon, A., Levine, J. D. & Basbaum, A. I. (1989) *J. Comp. Neurol.* **285,** 177–195.
33. Williams, S., Evan, G. I. & Hunt, S. P. (1990) *Neuroscience* **36,** 73–81.
34. Sonnenberg, J. L., Rauscher, F. J., III, Morgan, J. I. & Curran, T. (1989) *Science* **246,** 1622–1625.
35. Rosenfeld, M. G., Crenshaw, E. B. & Lira, S. A. (1988) *Annu. Rev. Neurosci.* **11,** 353–372.
36. Lira, S. A., Brenshaw, E. B., Glass, C. K., Swanson, L. W. & Rosenfeld, M. G. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4755–4759.
37. Tremblay, Y., Tretjakoff, I., Peterson, A., Antakly, T., Zhang, C. X. & Drouin, J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8890–8894.
38. Hogan, B., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
39. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
40. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
41. Neumann, J. R., Morency, C. A. & Russian, K. O. (1987) *Biotechniques* **5,** 444–447.
42. Seed, B. & Sheet, J.-Y. (1988) *Gene* **67,** 271–277.
43. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
44. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
45. Abood, M. E., Eberwine, J. H., Erdelyi, E. & Evans, C. J. (1990) *Mol. Brain Res.* **8,** 243–248.
46. Yoshikawa, K., Williams, C. & Sabol, S. L. (1984) *J. Biol. Chem.* **259,** 14301–14308.
47. Hanukoglu, I., Tanese, N. & Fuchs, E. (1983) *J. Mol. Biol.* **163,** 673–678.
48. Iadarola, M. J., Naranjo, J. R., Duchemin, A. M. & Quach, T. T. (1989) *Peptides* **10,** 687–692.
49. Seasholtz, A., Comb, M., Histon, D., Martin, M., Thomas, G. & Herbert, E. (1987) in *Progress in Brain Research*, eds. Seil, F. J., Herbert, E. & Carlson, B. M. (Elsevier, New York) Vol. 71, pp. 13–22.
50. Mochetti, I., Schwartz, J. P. & Costa, E. (1985) *Mol. Pharmacol.* **28,** 86–91.
51. Morris, B. J., Hollt, V. & Herz, A. (1988) *Neuroscience* **25,** 525–532.
52. Young, S. T., Porrino, L. J. & Iadarola, M. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1291–1295.
53. Uhl, G. R., Walther, D., Nishimori, T., Buzzi, G. M. & Moskowitz, A. (1991) *Mol. Brain Res.* **11,** 133–141.
54. Yoshikawa, K., Maruyama, K., Aizawa, T. & Yamamoto, A. (1989) *FEBS Lett.* **246,** 193–196.
55. Brinster, R. L., Allen, J. M., Behringer, R. R. & Gelinas, R. E. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 836–840.
56. Takemura, M., Donovan, D. M. & Uhl, G. R., *Mol. Brain Res.*, in press.
57. Swift, G. H., Hammer, R. E., MacDonald, R. J. & Brinster, R. L. (1984) *Cell* **38,** 39–46.
58. Wawrousek, E. F., Chepelinsky, A. B., McDermott, J. B. & Piatigorsky, J. (1990) *Dev. Biol.* **137,** 8–76.