Mechanisms Regulating Proenkephalin Gene Expression: Contributions of Transgenic Models

Steven E. Hyman and David Borsook

INTRODUCTION

Transgenic mouse models can contribute significantly to substance abuse research in several ways. In this monograph, the use of gene knockout mice is discussed separately (Hen, this volume). With the caveat that developmental anomalies and gene compensation may distort physiology or mask an interesting phenotype, gene knockout mice are currently one of the most powerful tools available to study the role of particular proteins in neural function. Transgenic mice may also be engineered to express dominant negative mutations of certain proteins; one possible advantage of this approach, where possible, is that gene inactivation can be targeted to a particular brain region. Transgenes can be inserted in a normal or a gene knockout background to study the effects of a mutated gene or of overexpression or ectopic expression of a particular gene. Additionally, transgenic strategies can be used to deliver a foreign protein to a site of interest.

In the research to be described here, a transgenic strategy to study gene regulation in the brain was used. The authors have followed up on previous studies of the human proenkephalin promoter that had utilized transformed cell lines in order to facilitate studies of transcriptional regulation by physiologically relevant stimuli in neuronal cell types of interest.

PROENKEPHALIN GENE REGULATION

The proenkephalin gene encodes the precursor of the endogenous opioid peptides met- and leu-enkephalin. Enkephalin peptides appear to play roles in a variety of physiological processes including descending analgesia, regulation of hormone release, the stress response, and brain reward. Expression of the proenkephalin gene is highly regulated by synaptic activity, cyclic adenosine monophosphate (cAMP), and protein kinase C (PKC), with the

predominant effect on synthesis being transcriptional. For example, in bovine adrenal chromaffin cells in primary culture, proenke-phalin messenger ribonucleic acid (mRNA) levels have been shown to be increased by nicotinic stimulation (Eiden et al. 1984), cAMP (Schwartz et al. 1984; Kley et al. 1987), K+- or veratridine-induced depolarization, calcium ionophores (Kley 1988; Kley et al. 1987), and PKC (Kley 1988). In C6-glioma cells, proenkephalin mRNA levels have been shown to be increased by ß-adrenergic receptor stimulation, which increases intra-cellular cAMP (Yoshikawa and Sabol 1986). In the brain, chronic treatment with dopamine type 2 (D2) receptor antagonists, including haloperidol, increases enkephalin peptides and proenkephalin mRNA in rat striatum (Romano et al. 1987; Sabol et al. 1983; Sivam et al. 1986; Tang et al. 1983). Lesions of the nigrostriatal dopamine pathway, which block transmission via all dopamine receptor types, also induce proenkephalin mRNA (Gerfen et al. 1990, 1991; Jiang et al. 1990; Normand et al. 1988; Voorn et al. 1987; Young et al. 1986). Other neural stimuli that induce proenkephalin mRNA include splanchnic nerve stimulation in the adrenal gland (Fischer-Colbrie et al. 1988; Kanamatsu et al. 1986), electrical stimulation and seizures in the hippocampus (Hong et al. 1980; Morris et al. 1988; White and Gall 1987; Xie et al. 1989; Yoshikawa et al. 1985), nociceptive stimuli in the dorsal horn (Draisci and Iadorola 1989), and stressors such as hypertonic saline or naloxone-precipitated opiate withdrawal in the hypothalamus (Lightman and Young 1987).

The authors' group and others have studied the mechanisms by which the human proenkephalin gene is regulated by cAMP and Ca2+ using muta-genesis and transfection into transformed cell lines. It was found that an enhancer comprised of three elements acting in combination conferred second messenger activation upon the gene. Within this enhancer, the deoxyribonucleic acid (DNA) regulatory element that was shown to be absolutely required for regulation of the proenkephalin gene by cAMP, depolarization, and Ca2+ is the proenkephalin cAMP response element 2 (CRE-2) element that contains the sequence TGCGTCA (Comb et al. 1988; Hyman et al. 1988, 1989; Nguyen et al. 1990). This element can bind both activator protein 1 (AP-1) and CRE binding protein (CREB) in vitro (Comb et al. 1988; Kobierski et al. 1991; Konradi et al. 1993; Sonnenberg et al. 1989). To complicate matters, the proenkephalin promoter can also be activated by both AP-1 proteins (Kobierski et al. 1991; Sonnenberg et al. 1989) and by CREB (Huggenvik et al. 1991) in cotransfection studies. Both in vitro binding studies (whether by electro-phoretic mobility shift or by footprinting) and cotransfection

studies provide information as to possible transcription factor-target interactions, but they cannot determine what actually happens in particular cell types in vivo.

Because gene regulation may differ markedly between transformed cell lines and neurons, two approaches have been taken. The authors have begun to study binding of transcription factors to key regulatory elements in extracts made from neuronal cell types, rather than using purified proteins from cell lines, and study of promoter function by transfection into neurons in primary culture and by use of transgenic mice has begun. The importance of transgenic models is underscored by several issues. The proenkephalin gene is expressed in a highly complex pattern in the brain, but appropriate cell culture models for the multiple neuronal cell types expressing the gene are lacking. Moreover, transformed cell lines cannot model the connectivity, neurotransmitter receptors, networks of intracellular signal transduction pathways, or the precise mixtures of transcription factors that characterize the different types of enkepha-linergic neurons in the brain. The use of transgenic animals permits investigation of whether the same regulatory elements that appear to be utilized in cell culture models are also utilized in vivo. The use of a beta-galactosidase reporter gene in transgenic models has the additional advantage of simplifying the approach to colocalization of the target gene of interest with putative regulatory proteins because the histochemical reaction used to detect beta-galactosidase activity is highly compatible with immunohistochemistry (Borsook et al. 1994b). A final advantage is that there is no endogenous beta-galactosidase activity in eukaryotic cells, permitting very sensitive detection of proenkephalin gene expression in this transgenic model.

EXPRESSION AND REGULATION OF AN ENKEPHALIN-BETA-GALACTOSIDASE FUSION GENE IN TRANSGENIC MICE

The three initial transgenic lines that were made contained a fusion construct with 3 kilobases (kb) of human proenkephalin gene 5' flanking sequence, the first exon and intron of the human proenkephalin gene, the E.coli lac-Z transcription unit fused to the second proenkephalin exon, and l.2kb of human proenkephalin 3' flanking sequences (Borsook et al. 1992). All of the independent lines of mice, ENK l.l, l.2, and l.3, expressed the fusion gene to some degree. None of the mice had apparent morpho-logic or behavioral abnormalities. No ectopic expression of the gene product was observed when the beta-galactosidase expression was compared to the

endogenous proenkephalin mRNA by in situ hybridization (Borsook et al. 1992, 1994a). However, none of the lines had complete expression of the gene, suggesting that there might be strong integration effects or that some additional regulatory sequences were missing. All three of the lines had good fidelity of expression in the reproductive system (Borsook et al. 1992), and all had some degree of expression in the brain. The ENK l.2 line had expression only in regions corresponding to the periaqueductal gray matter but nowhere else in the brain, and was not further analyzed. ENK l.l and l.3 had much fuller brain expression including the hypothalamus (Borsook et al. 1994a). None of the lines, however, had the expected expression of the transgene in the striatum.

Because proenkephalin gene expression was known to be highly regulated by pharmacologic and physiologic stimuli in the hypothalamus (Lightman and Young 1987), and because in the ENK l.l and l.3 lines basal expression of the transgene in the hypothalamus recapitulated the pattern of expression of the endogenous gene, a detailed analysis of regulation of the transgene within the hypothalamus was undertaken. All further analyses described here were performed with the ENK l.l line.

REGULATION OF THE TRANSGENE IN THE HYPOTHALAMUS

The paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus are major sites of integration of the stress response. The proenkephalin precursor and enkephalin peptides are found at high levels within these nuclei, where they are thought to play an important role in the control of release of hypothalamic stress hormones including cortico-tropin releasing factor (CRF). It has previously been shown in rat models that certain acute and chronic stressors produce significant induction of proenkephalin gene expression within these nuclei. For example, intra-peritoneal (IP) injection of hypertonic saline and naloxone-precipitated opiate withdrawal have been shown to produce rapid and significant inductions of proenkephalin mRNA in the rat PVN (Lightman and Young 1987). Multiple IP daily injections of hypertonic saline have been shown to induce proenkephalin mRNA in the rat PVN even more strongly (Young and Lightman 1992).

Basal expression of the transgene appears as small blue puncta within cells, which may represent inclusion bodies; with induction, neurons fill out with the blue beta-galactosidase reaction product (Borsook et

al. 1994a). By comparing histochemistry for the beta-galactosidase reaction product with in situ hybridization for the endogenous proenkephalin mRNA, the authors' group found that in the ENK 1.1 line of transgenic mice, basal expression of the transgene and induction by hypertonic saline injection (figure 1) and naloxone-precipitated opiate withdrawal closely paralleled expression of the mouse endogenous proenkephalin gene and the regulation previously reported for the rat (Borsook et al. 1994a, 1994c; Lightman and Young 1987). The transgene was also induced in the PVN by other stressors, such as hypovolemia, cold swim, and lipopolysaccharide injection. Since the endogenous gene and the transgene contain similar genomic regulatory sequences but express entirely different mRNAs, the observation that they are induced in parallel is consistent with the hypothesis that stress activation of proenkephalin gene expression in the hypothalamus occurs at the transcriptional level.

CREB INTERACTS WITH THE PROENKEPHALIN GENE IN THE HYPOTHALAMUS

As described above, both AP-1 proteins and CREB can interact with the proenkephalin gene in vitro. However, in cell extracts from rat striatum (Konradi et al. 1993) and mouse hypothalamus (Borsook et al. 1994b), the authors detected CREB, but not Fos binding to the enkephalin CRE-2 element (TGCGTCA) using electrophoretic mobility shift assays with antibody supershifts. CREB binding is detected even under conditions such as acute stress, which give robust AP-1 binding to a consensus AP-1 site (TGACTCA). The CRE-2 site is contained within the regulatory sequences of the transgene, thus the authors performed combined beta-galactosidase histochemistry with immunocytochemistry for CREB to determine the localization of CREB within the PVN with respect to cells expressing the transgene. CREB protein was found in essentially all neurons within the PVN (Borsook et al. 1994b). Moreover, no differences in total CREB protein levels could be observed by immuno-histochemistry in uninjected, normal saline-injected, or hypertonic saline-injected mice, consistent with the idea that CREB is constitutively expressed in most known cell types and is activated by phosphorylation (Gonzalez and Montminy 1989). The authors then sought to determine whether hypertonic saline stress induced phosphorylation of CREB on Ser133 in transgene-expressing neurons. The authors therefore colocalized beta-galactosidase activity with phosphoCREB using an antiserum that specifically detects CREB phosphorylated on Ser133 (Ginty et al. 1993).

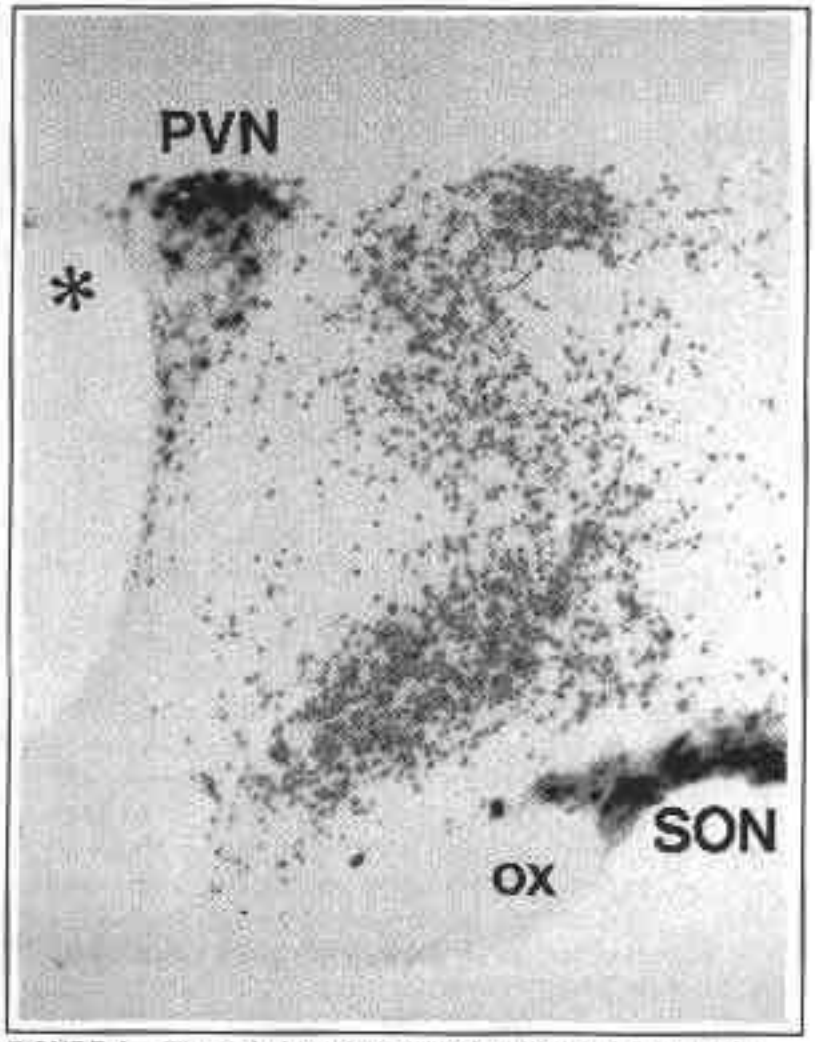

FIGURE 1. *The right hypothalamus of an ENK 1.1 transgenic mouse stained for beta-galactosidase activity after a hypertonic saline stress. The asterisk marks the third ventricle.*

KEY: PVN = paraventricular nucleus; SON = supraoptic nucleus; ox = optic chiasm.

Ten minutes after an injection of 0.15 molar (M) saline, only minimal staining for phosphoCREB was observed in the PVN, indistinguishable from an uninjected animal. Ten minutes after an injection of 1.5 M saline, intense phosphoCREB staining was seen throughout the PVN,

including all cells observed to express the transgene (Borsook et al. 1994b). Mutational analyses are planned to determine whether the CREB binding site is required for stress induction of the transgene within the PVN.

MODULATION OF STRESS-REGULATED PROENKEPHALIN GENE EXPRESSION BY OPIOIDS

In addition to facilitating studies of promoter function, the sensitivity of the transgene also permitted detection of novel forms of regulation of the proenkephalin gene within the hypothalamus. Since the regulatory sequences within the transgene are known, studies of the molecular mechanisms responsible for this regulation will be possible.

Two decades ago, researchers hypothesized that chronic morphine treatment might result in agonist-induced feedback inhibition of endogenous opioid biosynthesis and that this suppression of endogenous opioid peptides might contribute to aspects of opioid dependence and withdrawal (Hughes et al. 1975; Waterfield et al. 1976). Early studies designed to test this hypothesis found little change in steady-state brain enkephalin peptide levels. However, these studies did not address turnover rate or changes within restricted populations of cells. More recently, opioid agonists have been shown to decrease (Uhl et al. 1988) and opioid antagonists to increase (Tempel et al. 1992) expression of proenkephalin mRNA or enkephalin peptides in some cell types, suggesting a direct or indirect opioid receptor-mediated effect on endogenous opioid gene expression.

Using the authors' mouse model, it was found that chronic morphine administration dramatically suppresses transgene expression compared with animals that received either placebo pellets or no pellets. The chronic morphine regimen consisted of 8 milligram (mg) morphine pellets inserted subcutaneously (SC) for 3 days, followed by 25 mg pellets for 4 days. Control animals received either placebo pellets SC for 7 days or were unoperated. The suppression of transgene expression by chronic morphine administration was to almost unobservable levels, and was seen throughout the hypothalamus (Borsook et al. 1994c). Animals injected with morphine (10 mg/kg) or normal saline IP every 6 hours for 5 days displayed a level of suppression of transgene expression that was similar to the animals that received morphine pellets. There were no differences between the placebo pellet and the unoperated conditions.

Chronic (7 days) administration of naltrexone pellets (10 mg or 25 mg) SC had minimal effects on transgene expression (Borsook et al. 1994c). While there was some slight increase in the intensity of puncta, there was no filling of cells with the beta-galactosidase reaction product, the indicator of substantial induction of the transgene.

Surprisingly, administration of morphine (10 mg/kg IP) 4 hours prior to a hypertonic saline stressor (acute condition) or administration of a morphine pellet (8 mg) for 24 hours prior to a hypertonic saline stressor (subacute condition) produced a marked enhancement of the already substantial stress-induced increase in transgene expression within the PVN and SON (Borsook et al. 1994c). In addition, regions of the hypo-thalamus, in which transgene expression is not induced by hypertonic saline stress alone, showed marked induction of the transgene when stress followed morphine pretreatment. These areas include the preoptic region, the nucleus circularis, the lateral hypothalamus, the ventromedial nucleus, and the retrochiasmatic SON (Borsook et al. 1994c). Thus, acute or sub-acute morphine administration markedly sensitizes restricted populations of proenkephalin-expressing hypothalamic neurons to stress. Chronic opioid pretreatment (7 days) prior to hypertonic saline stress inhibits this response, suggesting that desensitization has occurred. Pretreatment with naltrexone inhibits stress induction of transgene expression below the expected level observed in mice receiving only hypertonic saline injections (Borsook et al. 1994c).

In this series of experiments, the authors' transgenic model was used to examine the effects of acute, subacute, and chronic opioid administration on stress regulation of proenkephalin gene expression within the hypo-thalamus. The advantages of the transgenic model are that it is more sensitive and more readily quantified than in situ hybridization and that genomic sequences responsible for the observed regulation are known to be contained within the transgene, making subsequent analysis of transcriptional mechanisms possible.

These results, which were confirmed by in situ hybridization for the endogenous mRNA (Borsook et al. 1994c), are consistent with the hypothesis that acute or subacute administration of morphine sensitizes enkephalinergic hypothalamic neurons to stress. The use of a morphine pellet in the subacute paradigm makes it unlikely that the superinduction is due to mild, behaviorally unobservable withdrawal acting along with the saline stress. Moreover, administration of

opioids chronically, using a paradigm that produced marked dependence (as demonstrated by the possibility of producing naloxone-precipitated withdrawal) not only suppressed basal expression, but also inhibited stress-induced expression of the transgene.

It was initially surprising that acute or subacute morphine pretreatment prior to a hypertonic saline stressor superinduces transgene expression. Morphine has principally inhibitory effects on target neurons mediated by G-protein inhibitory (Gi)-linked opiate receptors, especially mu receptors. As noted by Akil (this volume), there are relatively few mu receptors expressed on neurons in the PVN, although there are relatively high levels of kappa opioid receptors. These observations make it unlikely that the effects of morphine on PVN neurons are direct or cell autonomous, but the effects likely involve intermediary circuitry.

SUMMARY

This chapter gives an overview of the utility of one type of transgenic mouse model for substance abuse research, the use of transgenes in which genomic regulatory sequences of interest are coupled to a reporter gene. Using this mouse model, exploration of the mechanisms regulating expression of the proenkephalin gene in the mouse hypothalamus have begun. The resulting information supplements experiments on gene regulation performed in cell lines with information about regulation by pharmacologic and physiologic stimuli of interest within the brain. With special reference to mechanisms of opioid dependence, the particular model that has been examined appears to be a useful tool to investigate mechanistic aspects of the regulation of endogenous opioid genes by exogenous opioids.

REFERENCES

Borsook, D.; Falkowski, O.; Burstein, R; Strassman, A.; Konradi, C.; Dauber, A.; Comb, M.; and Hyman, S.E. Stress-induced regulation of a human proenkephalin-ß-galactosidase fusion gene in the hypothalamus of transgenic mice. Mol Endocrinol 8:116-125, 1994a.

Borsook, D.; Falkowski, O.; Rosen, H.; Comb, M.; and Hyman, S.E. Opioids modulate stress-induced proenkephalin gene expression in the hypothalamus of transgenic mice: A model of endogenous opioid gene regulation by exogenous opioids. J Neurosci 14:7261-7271, 1994c.

Borsook, D.; Konradi, C.; Falkowski, O.; Comb, M.; and Hyman, S.E. Molecular mechanisms of stress-induced proenkephalin gene regulation: CREB interacts with the proenkephalin gene in the mouse hypothalamus and is phosphorylated in response to hyperosmolar stress. Mol Endocrinol 8:140-148, 1994b.

Borsook, D.; Rosen, H.; Collard, M.; Herrup, K.; Comb, M.; and Hyman,S.E. Expression and regulation of a proenkephalin ß-galactosidase fusion gene in the reproductive system of transgenic mice. Mol Endocrinol 6:1502-1510, 1992.

Comb, M.; Mermod, N.; Hyman, S.E.; Pearlberg, J.; Ross, M.; and Goodman, H.M. Proteins bound at adjacent DNA elements act synergistically to regulate human proenkephalin cAMP inducible transcription. EMBO J 7:3793-3805, 1988.

Draisci, G., and Iadarola, M.J. Temporal analysis of increases in c-fos, preprodynorphin and preproenkephalin mRNAs in rat spinal cord. Mol Brain Res 6:31-37, 1989.

Eiden, L.E.; Giraud, P.; Dave, J.R.; Hotchkiss, A.J.; and Affolter, H.U. Nicotinic receptor stimulation activates enkephalin release and biosynthesis in adrenal chromaffin cells. Nature 312:661-663, 1984.

Fischer-Colbrie, R.; Iacangelo, A.; and Eiden, L.E. Neural and humoral factors separately regulate neuropeptide Y, enkephalin, and chromogranin A and B mRNA levels in rat adrenal medulla. Proc Natl Acad Sci U S A 85:3240-3244, 1988.

Gerfen, C.R.; Engber, T.M.; Mahan, L.C.; Susel, Z.; Chase, T.N.; Monsma, F.J., Jr.; and Sibley, D.R. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429-1432, 1990.

Gerfen, C.R.; McGinty, J.F.; and Young, W.S. Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: In situ hybridization histochemical analysis. J Neurosci 11:1016-1031, 1991.

Ginty, D.D.; Kornhauser, J.M.; Thompson, M.A.; Bading, H.; Mayo,K.E.; Takahashi, J.S.; and Greenberg, M.E. Regulation of CREB phosphorylation in the suprachiasmatic nucleus by light and circadian clock. Science 260:238-241, 1993.

Gonzalez, G.A., and Montminy, M.R. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59:675-680, 1989.

Hong, J.S.; Wood, P.L.; Gillin, J.C.; Yang, H.Y.; and Costa, E. Changes of hippocampal Met-enkephalin content after recurrent motor seizures. Nature 285:231-232, 1980.

Huggenvik, J.I.; Collard, M.W.; Stofko, R.E.; Seasholtz, A.F.; and Uhler,M.D. Regulation of the human enkephalin promotor by two isoforms of the catalytic subunit of cyclic adenosin 3',5'-monophosphate-dependent protein kinase. Mol Endocrinol 5:921-930, 1991.

Hughes, J.; Smith, T.W.; Kosterlitz, H.W.; Fothergill, L.A.; Morgan,M.A.; and Morris, H.R. Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258:577-580, 1975.

Hyman, S.E.; Comb, M.; Lin, Y.S.; Pearlberg, J.; Green, M.R.; and Goodman, H.M. A common trans-acting factor is required for cyclic AMP-induction of multiple neurotransmitter-related genes. Mol Cell Biol 8:4225-4233, 1988.

Hyman, S.E.; Comb, M.; Pearlberg, J.; and Goodman, H.M. Transcription factor AP-2 acts synergistically with the cyclic AMP and phorbol ester-inducible enhancer of the human proenkephalin gene. Mol Cell Biol 9:321-324, 1989.

Iadarola, M.J.; Shin, C.; McNamara, J.O.; and Yang, H.Y. Changes in dynorphin, enkephalin and cholecystokinin content of hippocampus and substantia nigra after amygdala kindling. Brain Res 365:185-191, 1986.

Jiang, H.K.; McGinty, J.F.; and Hong, J.S. Differential modulation of striatonigral dynorphin and enkephalin by dopamine receptor subtypes. Brain Res 507:57-64, 1990.

Kanamatsu, T.; Unsworth, C.D.; Diliberto, E.J., Jr.; Viveros, O.H.; and Hong, J.S. Reflex splanchnic nerve stimulation increases levels of proenkephalin A mRNA and proenkephalin A-related peptides in the rat adrenal medulla. Proc Natl Acad Sci U S A 83:9245-9249, 1986.

Kley, N. Multiple regulation of proenkephalin gene expression by protein kinase C. J Biol Chem 263:2003-2008, 1988.

Kley, N.; Loeffler, J.P.; Pittius, C.W.; and Hollt, V. Involvement of ion channels in the induction of proenkephalin A gene expression by nicotine and cAMP in bovine chromaffin cells. J Biol Chem 262:4083-4089, 1987.

Kobierski, L.A.; Chu, H.M.; Tan, Y.; and Comb, M.J. cAMP-dependent regulation of proenkephalin by JunD and JunB: Positive and negative effects of AP-1 proteins. Proc Natl Acad Sci U S A 88:10222-10226, 1991.

Konradi, C.; Kobierski, L.A.; Nguyen, T.V.; Heckers, S.H.; and Hyman,S.E. The cAMP-response-element-binding protein interacts, but Fos protein does not interact, with the proenkephalin enhancer in rat striatum. Proc Natl Acad Sci U S A 90:7005-7009, 1993.

Lightman, S.L., and Young, W.S. Changes in hypothalamic preproenkephalin A mRNA following stress and opiate withdrawal. Nature 328:643-645, 1987.

Morris, B.J.; Feasey, K.J.; ten Bruggencate, G.; Herz, A.; and Hollt, V. Electrical stimulation in vivo increases the expression of proenkephalin mRNA and decreases the expression of prodynorphin mRNA in rat hippocampal granule cells. Proc Natl Acad Sci U S A 85:3226-3230, 1988.

Nguyen, T.V.; Kobierski, L.; Comb, M.J.; and Hyman, S.E. The effect of depolarization on expression of the human proenkephalin

gene is synergistic with cAMP and dependent upon cAMP-inducible enhancer. J Neurosci 10:2825-2833, 1990.

Normand, E.; Popovici, T.; Onteniente, B.; Fellmann, D.; Piatier-Tonneau, D.; Auffray, C.; and Bloch, B. Dopaminergic neurons of the substantia nigra modulate preproenkephalin A gene expression in rat striatal neurons. Brain Res 439:39-46, 1988.

Romano, G.J.; Shivers, B.D.; Harlan, R.E.; Howells, R.D.; and Pfaff,D.W. Haloperidol increases proenkephalin mRNA levels in the caudate-putamen of the rat: A quantitative study at the cellular level using in situ hybridization. Brain Res 388:33-41, 1987.

Sabol, S.L.; Yoshikawa, K.; and Hong, J.S. Regulation of methionine-enkephalin precursor messenger RNA in rat striatum by haloperidol and lithium. Biochem Biophys Res Commun 113:391-399, 1983.

Schwartz, J.P.; Quach, T.T.; Tang, F.; Kageyama, H.; Guidotti, A.; and Costa, E. Cyclic-AMP-mediated modulation of gene expression in adrenal chromaffin cell cultures. Adv Cyclic Nucleotide Res 17:529-534, 1984.

Sivam, S.P.; Strunk, C.; Smith, D.R.; and Hong, J.S. Proenkephalin-A gene regulation in the rat striatum: Influence of lithium and haloperidol. Mol Pharmacol 30:186-191, 1986.

Sonnenberg, J.L.; Rauscher, F.J., III; Morgan, J.I.; and Curran, T. Regulation of proenkephalin by Fos and Jun. Science 246:1622-1625, 1989.

Tang, F.; Costa, E.; and Schwartz, J.P. Increase of proenkephalin mRNA and enkephalin content of rat striatum after daily injection of haloperidol for 2 to 3 weeks. Proc Natl Acad Sci U S A 80:3841-3844, 1983.

Tempel, A.; Kessler, J.A.; and Zukin, R.S. Chronic naltrexone increases expression of preproenkephalin and preprotachykinin mRNA in discrete brain regions. J Neurosci 10:741-747, 1992.

Uhl, G.R.; Ryan, J.P.; and Schwartz, J.P. Morphine alters preproenkephalin gene expression. Brain Res 459:391-397, 1988.

Voorn, P.; Roest, G.; and Groenewegen, H.J. Increase of enkephalin and decrease of substance P immunoreactivity in the dorsal and ventral striatum of the rat after midbrain 6-hydroxydopamine lesions. Brain Res 412:391-396, 1987.

Waterfield, A.A.; Hughes, J.; and Kosterlitz, H.W. Cross tolerance between morphine and methionine-enkephalin. Nature 260:624-625, 1976.

White, J.D., and Gall, C.M. Differential regulation of neuropeptide and proto-oncogene mRNA content in the hippocampus following recurrent seizures. Brain Res 427:21-29, 1987.

Xie, C.W.; Lee, P.H.; Takeuchi, K.; Owyang, V.; Li, S.J.; Douglass, J.; and Hong, J.S. Single or repeated electroconvulsive shocks alter the levels of prodynorphin and proenkephalin mRNAs in rat brain. Mol Brain Res 6:11-19, 1989.

Yoshikawa, K., and Sabol, S.L. Expression of the enkephalin precursor gene in C6 rat glioma cells: Regulation by beta-adrenergic agonists and glucocorticoids. Mol Brain Res 1:75-83, 1986.

Yoshikawa, K.; Hong, J.S.; and Sabol, S.L. Electroconvulsive shock increases preproenkephalin messenger RNA abundance in rat hypothalamus. Proc Natl Acad Sci U S A 82:589-593, 1985.

Young, W.S., and Lightman, S.L. Chronic stress elevates enkephalin expression in the rat paraventricular and supraoptic nuclei. Mol Brain Res 13:111-117, 1992.

Young, W.S.; Bonner, T.I.; and Brann, M.R. Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. Proc Natl Acad Sci U S A 83:9827-9831, 1986.

AUTHORS

Steven E. Hyman, M.D.

David Borsook, Ph.D., M.D.

Harvard Medical School

and

Laboratory of Molecular and Developmental Neuroscience
Massachusetts General Hospital, CNY-2
149 13th Street
Charlestown, MA 02129

Click here to go to page 72