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# Proenkephalin-A Gene Regulation in the Rat Striatum: Influence of Lithium and Haloperidol

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## SUMMARY

This report explores the influence of lithium and haloperidol on (Met$^5$)-enkephalin (ME) biosynthesis in the rat striatum. Male Fischer 344 rats were treated intraperitoneally with lithium chloride (4 mEq/kg/day) for 2, 4, or 6 days and sacrificed 24 hr after the last dose; in addition, the effect of lithium at 2 and 24 hr after a single dose was studied. Serum levels increased in a time-related manner on repeated administration of lithium. Lithium increased the striatal ME content (native ME) in a time-dependent fashion, reaching 160% of control following six doses; no changes in ME were observed in hypothalamus and hippocampus. ME levels recovered to control values 8 days after cessation of a 4-day course of repeated administration (4 mEq/kg/day) of lithium. In an attempt to characterize the nature of this selective increase of ME content in the striatum, the precursor content (cryptic ME) as well as the preproenkephalin mRNA abundance was determined. Lithium increased the precursor content in a time-dependent fashion and this pattern closely paralleled the increase in native ME content. The preproenkephalin mRNA abundance with respect to control was quantitated by blot-hybridization of total RNA with a $^{32}$P-labeled cDNA probe derived from rat brain. Lithium increased the mRNA abundance following repeated doses for 4 or 6 days. Concurrent administration of an opiate antagonist, naltrexone (5 mg/kg/day), for 4 days did not influence the changes induced by lithium. On repeated administration (1 mg/kg/day) for 4 days, the neuroleptic, haloperidol, increased the biosynthesis of ME which was more marked than that of lithium administered for the same period; the combination of a haloperidol and lithium regimen did not lead to an additive or synergistic effect. The results indicate that, like haloperidol, repeated injections of lithium increase the biosynthesis of ME in the basal ganglia by increasing the preproenkephalin mRNA abundance and translation process.

In 1949, the Australian psychiatrist J. F. J. Cade discovered the therapeutic effectiveness of lithium in the treatment of mania (1). Since then, numerous experimental and clinical investigations have attempted to understand the mechanism of action of lithium (see reviews in Refs. 2–6). Extensive studies have been conducted over the years on the involvement of classical neurotransmitters such as 5-hydroxytryptamine, acetylcholine, dopamine, norepinephrine, γ-aminobutyric acid, etc., in mediating lithium's effectiveness (see reviews in Refs. 2, 6, and 7). Studies have also indicated the inhibitory effect of lithium on enzymes such as adenyl cyclase (8) and inositol phosphatase (9–11). Despite such wide-ranging studies, it is not clear which of the many known effects are relevant to the mechanism of action of lithium in the prophylaxis and treatment of manic-depressive disorders. The discovery of neuroactive peptides and their role in the neurotransmission/neuromodulation process offers a new direction to search for clues to understand the nature of the neuropsychiatric disorders and the mode of action of psychotropic drugs. There is clinical and experimental evidence to support the notion that opioid peptides play a role for the regulation of mood and activity and that they could be involved in the pathophysiology of affective illnesses and in the action of antimanic and antidepressant treatment modalities (see reviews in Refs. 12–14). Lithium administration has been reported to increase enkephalin levels in the basal ganglia region of the Sprague-Dawley rat (15, 16). *In vitro* addition of lithium to crude synaptic membranes or *in vivo* administration of the drug to Wistar rats for 3 weeks caused down-regulation in the number of opioid-binding sites using $^3$H-enkephalinamide as a ligand in the basal ganglia (12); however, no changes in [$^3$H]naloxone binding to whole brain membrane was observed following chronic lithium treatment to mice (17). Several studies have reported that the opiate antagonist naloxone produced notable improvement in symptoms in manic patients (18, 19). Clinically, a neuroleptic such as haloperidol is used along with lithium during the initial phase of treatment of mania (20). Neuroleptics have been reported to increase ME biosynthesis (21, 22). These studies suggest a possible role of opioid peptides in the effects of drugs used in the mental disorders. The present investigation was designed to understand the influence of lithium and haloperidol alone or in combination on the ME biosynthesis, using molec-

**ABBREVIATIONS:** ME, Met$^5$-enkephalin; PE-mRNA, preproenkephalin mRNA.

ular hybridization techniques to quantitate the PE-mRNA abundance and radioimmunoassays to measure cryptic and native ME content. This study indicates that both lithium and haloperidol increase the biosynthesis of ME in the striatum of rat.

## Materials and Methods

### Chemicals

Trypsin and carboxypeptidase-B were obtained from Sigma Chemical Co., St. Louis, MO. [$\alpha$-$^{32}$P]dCTP (2800 Ci/mmol) was purchased from New England Nuclear, Boston, MA. Haloperidol was obtained from McNeil Laboratories, Inc., Fort Washington, PA. Naltrexone hydrochloride was a gift from Endo Laboratories, Garden City, NJ. The methionine enkephalin radioimmunoassay kit was obtained from Immunonuclear Corp., Stillwater, MN. *Escherichia coli* 294 cells harboring plasmid pRPE2, which contains a rat preproenkephalin cDNA insert (23), was generously supplied by Dr. Steven Sabol (National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD).

### Animals

Male Fischer 344 rats (Charles River, Wilmington, MA) weighing 220–260 g were used for the study. The animals were housed in a colony room with a 12-hr light-dark cycle and maintained at 21± 2° with 50± 10% humidity. Food and water were given *ad libitum*.

### Treatment Regimen

Control animals received normal saline, 0.2 ml/100 g of body weight. Lithium chloride (hereafter referred to as lithium) was dissolved in normal saline and was injected intraperitoneally in a volume of 0.2 ml/100 g of body weight. Rats received lithium at a dose of 4 mEq/kg/day for 2, 4, or 6 days. The control group received six saline injections, whereas the lithium groups received (depending on the number of lithium injections) lithium and/or saline injections for 6 days. This design permitted all groups to receive equal numbers of injections. Twenty-four hr after the last dose, the animals were sacrificed by decapitation with a guillotine. In addition, the effect of a single injection of lithium (4 mEq/kg) at 2 and 24 hr was investigated. Haloperidol was dissolved in glacial acetic acid, diluted with normal saline and adjusted to pH 6.0 with NaOH. Haloperidol (1 mg/kg/day) or naltrexone (5 mg/kg/day) was administered subcutaneously for 4 days. The brain regions (striatum, hypothalamus, and hippocampus) were dissected, and the tissues were frozen immediately on dry ice and subsequently stored at −70°.

**Determination of serum lithium levels.** The determination of serum lithium levels was performed using atomic absorption spectrometry (24).

### Radioimmunoassay of ME-like Immunoreactivity

**Native ME level.** The levels of ME were determined as described earlier (25). Briefly, the frozen tissues were homogenized in 2 N acetic acid and the samples were heated in a boiling water bath for 5 min. The homogenates were centrifuged at 25,000 × *g* for 20 min and an aliquot of the supernatant was lyophilized to dryness. The residue was reconstituted in radioimmunoassay buffer and the supension was centrifuged at 25,000 × *g* for 30 min. An aliquot of the supernatant was used for radioimmunoassay using an antiserum against ME (25). Tritiated ME was used as tracer. Free and bound $^{3}$H-ME were separated by the addition of charcoal slurry and subsequent centrifugation. Cross-reactivities of Leu-enkephalin, ME-Arg-Phe, ME-Arg-Gly-Leu, dynorphin(1-13), and $\beta$-endorphin with ME antiserum were 5, 5.7, 0.1, 0.35, and 0.12%, respectively.

**Cryptic ME level.** The cryptic ME content was determined as described previously (26, 27). In brief, the tissues were homogenized in 0.1 N HCl and the homogenate was boiled for 15 min. An aliquot of the homogenate was subjected to trichloroacetic acid precipitation of proteins. The precipitate was washed three times with diethyl ether and dried and reconstituted in buffer (50 mM Tris-HCl, $CaCl_2$, pH 8.4). An aliquot (containing about 4 mg Eq of the wet weight of tissue) was treated with trypsin to a final concentration of 10 $\mu$g/ml and incubated at 37° for 4 hr. The incubation was terminated by heating the samples on a boiling water bath for 15 min and cooled on an ice bath. Then, carboxypeptidase-B was added to a final concentration of 0.1 $\mu$g/ml and incubated at 37° for 2 hr. At the end of the incubation, the samples were heated on a boiling water bath for 15 min and cooled on an ice bath. The samples were centrifuged at 25,000 × *g* for 30 min. An aliquot of the supernatant was used to determine the ME levels using a radioimmunoassay kit from Immunonuclear Corp. Cross-reactivities of ME-Arg-Phe, ME-Arg-Gly-Leu, Leu-enkephalin, dynorphin(1-13), $\beta$-endorphin, $\alpha$-endorphin, and $\alpha$-neoendorphin with ME antiserum were 1.8, 0.01, 2.8, 0.002, 0.002, 0.10, and 0.002%, respectively.

### Preparation of RNA

Total RNA was isolated from tissues following the method described by Chirgwin *et al.* (28). Briefly, the frozen tissue was homogenized in 4 M guanidinium thiocyanate and centrifuged at 25,000 × *g* for 20 min. The supernatent was treated with acetic acid and ethanol and kept overnight at −20° to precipitate nucleic acids. The precipitate was recovered by centrifugation and treated with guanidine hydrochloride followed by ethanol precipitation. The guanidine hydrochloride extraction step was repeated once. The final pellets were water extracted, and the water extract containing RNA was ethanol precipitated, recovered by centrifugation, dried *in vacuo*, and finally dissolved in water. Absorbance measurements were obtained at 260 and 280 nm using a spectrophotometer. The 260/280 ratio was between 1.77 and 2.0. The RNA samples were routinely stored at −70°.

### Preparation of cDNA Probe for Hybridization

The plasmids containing the rat brain preproenkephalin cDNA (pRPE2) were isolated from *E. coli* lysates by CsCl-ethidium bromide equilibrium density-gradient centrifugation. A 941-bp Bsp 1286 fragment was isolated from pRPE2 by the endonuclease digestion followed by agarose gel electrophoresis. The fragments were nick-translated with [$\alpha$-$^{32}$P]dCTP (2–5×10$^{8}$ cpm/$\mu$g of DNA). The detailed procedures involved in the above steps have been described by Yoshikawa *et al.* (23). A nick-translation kit from Amersham Corp. was used for the $^{32}$P labeling.

### Northern Blot Analysis

RNA was denatured in a mixture containing 50% formamide and 6% formaldehyde for 15 min at 55°. The denatured RNA was electrophoresed in a 1.2% agarose gel containing 6% formaldehyde. The gel was soaked in 10 mM phosphate buffer (pH 7) and the RNA was transferred to a nylon membrane (Gene Screen, New England Nuclear) by a capillary transfer procedure. The membrane was washed with 10×SSC, air-dried, and baked at 80° for 2 hr.

### Dot-Blot Analysis of RNA

Total RNA was denatured in 7.4% formaldehyde at 60° for 15 min. The denatured RNA was diluted with 100 $\mu$l of 10×SSC and applied to a nylon membrane placed in a filtration manifold (Bethesda Research Laboratories, Rockville, MD). The gravity filtration was allowed to continue for 2 hr; then, the wells were washed with excess of 10×SSC, air-dried, and baked at 80° for 2 hr.

### RNA-cDNA Hybridization Procedures

The RNA blots (Northern blot and dot-blot) were incubated for 16 hr at 42° with 10 ml of hybridization buffer [50% formamide, 10× Denhardt's solution, 0.05 M Tris-HCl (pH 7.5), 1 M NaCl, 3 mM $Na_4P_2O_7$, 1% sodium dodecyl sulfate, 10% dextran sulfate, and 100 $\mu$g/ml of denatured herring sperm DNA] and the $^{32}$P-nick-translated probe was added to a final concentration of 2–5 ng/ml of buffer, and the

incubation was further continued for 19–24 hr. The blots were washed with 2×SSC and 0.1% sodium dodecyl sulfate, air-dried, and subjected to autoradiography using a Kodak film mounted on an intensifying screen. The autoradiograms obtained from dot-blots were scanned with a densitometer (Gilford Instruments, Oberlin OH).

**Statistical analysis of the data.** The data were subjected to one-way analysis of variance (29) to test for overall statistical significance. Post-hoc comparisons between group means were made with Fisher's least significance test. A $p$ value $< 0.05$ was considered significant.

## Results

**Serum levels of lithium following single and multiple doses.** At 2 hr following a single dose (4 mEq/kg) of lithium, serum lithium concentration was 2.7 mEq/liter; this level diminished to 0.4 mEq/liter by 24 hr. On repeated administration, the levels varied as a function of the number of days of drug treatment. Following four daily injections of 4 mEq/kg, the lithium level reached 1 mEq/liter; further administration up to 6 days led to high levels (>5 mEq/liter).

**Influence of lithium on ME levels in discrete regions of rat brain.** Time course studies following lithium treatment revealed a time-related increase in native ME level in the striatum (Fig. 1). A single dose of lithium (4 mEq/kg) did not produce significant changes in ME level at 2 or 24 hr following the administration (89 and 95% of control for 2 and 24 hr, respectively). Repeated injections of lithium for 2, 4, or 6 days produced significant increase in ME in the latter two groups. None of the treatments produced significant alterations in ME levels in hypothalamus or hippocampus. The hypothalamic levels of ME (nmol/g of wet weight, mean ± SE, $n = 4$) for control and one, two, four, and six injections of lithium were: 0.994 ± 0.058, 0.988 ± 0.033, 0.854 ± 0.075, 0.872 ± 0.051, and 0.903 ± 0.062; the hippocampal ME levels ($n = 4$) were: 0.181 ± 0.024, 0.209 ± 0.021, 0.227 ± 0.021, 0.209 ± 0.067 and 0.197 ± 0.082.

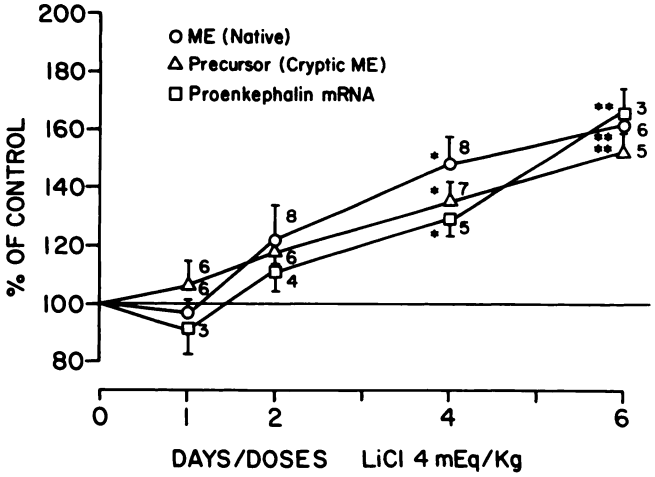


**Fig. 1.** Effects of lithium administration on ME biosynthesis in the rat striatum. Rats received lithium chloride, 4 mEq/kg/day, for 2, 4, or 6 days and were sacrificed 24 hr later. Native and cryptic ME content were determined by radioimmunoassay. The PE-mRNA abundance was quantitated by RNA-cDNA hybridization as described in Materials and Methods. The results are means ± standard error of the indicated number of samples. The results from lithium dosage for 1, 2, 4, and 6 days are presented. The data were normalized into percentage of control. The control value for the native and cryptic ME levels (nmol/g of wet weight, mean ± SE), respectively, were: 2.71 ± 0.164 ($n = 8$) and 0.62 ± 0.029 ($n = 8$). The relative values for mRNA abundance were calculated as described in Results. *, $p < 0.05$; **, $p < 0.01$ compared to control.

**Effect of lithium on ME precursor content.** The precursor content as reflected by the levels of cryptic ME was determined for striatal tissue. The changes expressed as percentage of control following the administration of lithium are shown in Fig. 1. Lithium increased cryptic ME content in a time-related manner, and the trend of response was similar to that observed for native ME. A single dose of lithium did not affect precursor level at 2 or 24 hr after administration.

**Alterations in the abundance of PE-mRNA in the striatum following lithium administration.** Northern blot analysis of total RNA indicted that the $^{32}$P-cDNA probe hybridized with a single species of mRNA of approximately 1450 bases (Fig. 2A), which is similar to that described for rat PE-mRNA (23). The changes in the abundance of PE-mRNA were quantitated by dot-blot analysis. A representative autoradiogram obtained from a dot-blot is shown in Fig. 2B. Quantitation of the density of the autoradiographic dots was done using a scanning densitometer. The scan values of at least three graded concentrations of RNA were pooled; the effect of the treatment was expressed as percentage of control value. Three independent samples (each sample was a pool of two striata) were done for each group and the per cent changes were combined to obtain the mean ± standard error. The changes in the abundance of PE-mRNA in the striatum are shown in Fig. 1. Daily injection of lithium for 2, 4, or 6 days resulted in progressive increase in mRNA abundance; the changes in the latter two groups were statistically significant. A single injection of lithium did not induce significant changes in the PE-mRNA levels at 2 or 24 hr.

**Withdrawal of lithium administration (4 mEq/kg/day for 4 days) on native and cryptic ME levels in the striatum.** After 2 and 4 days of withdrawal from a 4-day regimen of lithium treatment (4 mEq/kg/day), both native and cryptic ME levels were still significantly increased; the levels returned to control values by 8 days post-dose. The ME levels (nmol/ g of wet weight, mean ± SE, $n = 4$–8) for control, 2, 4, and 8 days post-dose were: native ME, 3.24 ± 0.15, 4.94 ± 0.26, 4.80 ± 0.24, and 3.50 ± 0.37; cryptic ME; 0.66 ± 0.031, 0.95 ± 0.047, 1.06 ± 0.108, and 0.90 ± 0.167.

**Effect of naltrexone on lithium-induced changes in ME and precursor level in the striatum.** Daily administration of naltrexone (5 mg/kg) for 4 days did not affect the biosynthesis of ME. Naltrexone failed to alter the lithium-induced increases in native and cryptic ME (Table 1).

**Effect of lithium and haloperidol alone or in combination on ME biosynthesis.** Administration of haloperidol (1 mg/kg/day) for 4 days increased native ME (60%), cryptic ME (50%), and PE-mRNA abundance (74%) in the striatum (Fig. 3). Lithium, as described in the preceding sections, also increased native and cryptic ME content and PE-mRNA abundance, although this abundance was significantly less than that obtained for haloperidol treatment. Concurrent administration of haloperidol and lithium for 4 days produced an increase which was comparable to that observed for haloperidol alone (see Fig. 2, A and B, for a Northern blot and a representative dot-blot, respectively).

## Discussion

Repeated administration of lithium to rats leads to time-dependent increase in ME level, precursor content, and PE-mRNA abundance in the rat striatum. These results indicate

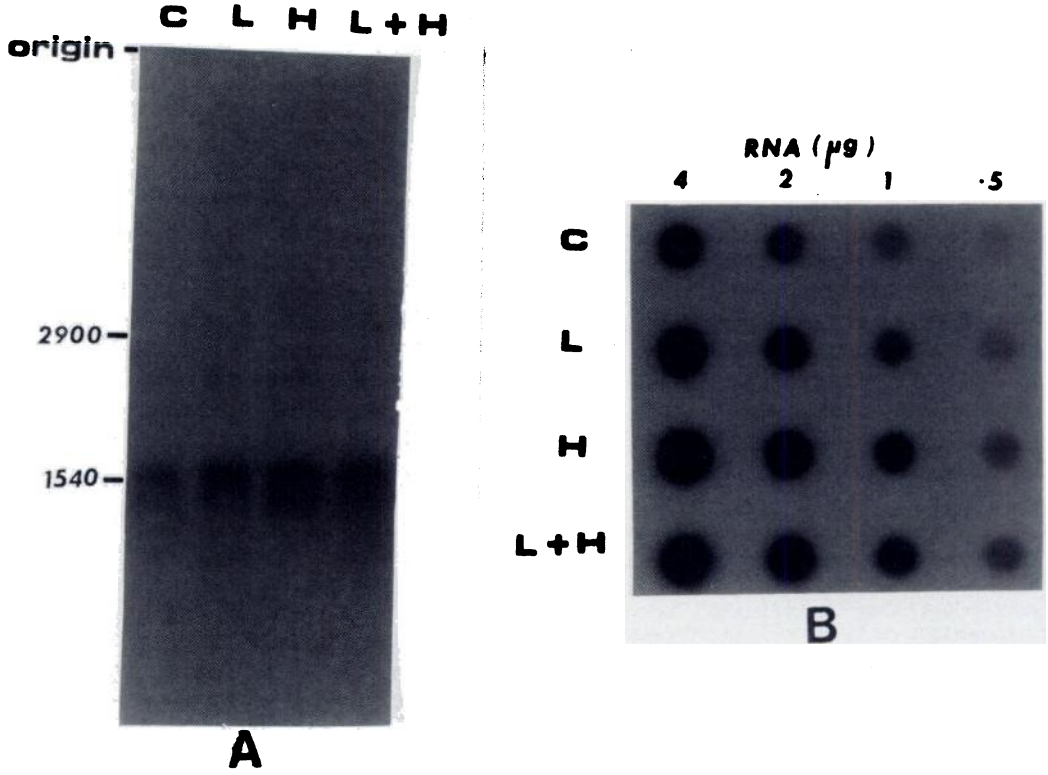


**Fig. 2.** A. Northern blot analysis of PE-mRNA from rat striatum: influence of lithium and haloperidol on the mRNA abundance. Total RNA was denatured and electrophoresed on a 1.2% agarose gel containing 6% formaldehyde, transferred to a nylon membrane, and hybridized with a nick-translated Bsp 1286 fragment of pRPE2 plasmid. An autoradiogram of 48 hr exposure is shown. *C*, control; *L*, lithium (4 mEq/kg/day × 4), *H*, haloperidol (1 mg/kg/day × 4). B. Dot-blot analysis of the effect of lithium and haloperidol on PE-mRNA abundance. The indicated amount of total RNA was denatured and blotted onto nylon membrane and then hybridized with a nick-translated Bsp 1286 fragment of pRPE2 plasmid. An autoradiogram of 48 hr exposure is shown. The groups are the same as in A.

TABLE 1

**Effect of concurrent administration of naltrexone and lithium on native and cryptic ME levels in the rat striatum**

Values are means ± standard error. Numbers in parentheses indicate the number of samples used. Naltrexone: 5 mg/kg/day × 4; lithium: 4 mEq/kg/day × 4.

| Treatment | ME | |
|---|---|---|
| | Native | Cryptic |
| | *nmol/g wet wt.* | |
| Control | 3.24 ± 0.15 (7) | 0.66 ± 0.031 (8) |
| Naltrexone | 3.49 ± 0.21 (5) | 0.78 ± 0.047 (4) |
| Lithium | 4.69 ± 0.11[a] (5) | 1.04 ± 0.124[a] (5) |
| Naltrexone + lithium | 5.02 ± 0.24[a] (5) | 1.15 ± 0.091[a] (4) |

[a] $p < 0.01$ compared to control.

that lithium increases the biosynthesis of ME. The changes were significant when the serum lithium concentration was raised to 1 mEq/liter or higher by a regimen of repeated administration of the drug (4 mEq/kg/day for 4 or 6 days). Repeated administration of lithium has been reported to increase ME content (15) and [Leu$^5$]-enkephalin content (16, 30). The mechanism of this increase was explored in an earlier study using a cell-free translation system and it was found that lithium increased preproenkephalin formation (31) following the administration of lithium for 5 days at a dose of 5 mEq/kg/day. The present study using the molecular approach to quantitate mRNA abundance confirms and extends these observations that lithium increases the biosynthesis of ME. The changes in the ME system are evident on repeated administration which produced a sustained serum lithium level. These factors are reminiscent of those regulating clinical effectiveness of lithium in manic patients who generally begin to respond to therapy after 3–7 days of treatment if the serum lithium concentrations are in the vicinity of 1 mEq/liter. Furthermore, one of the most frequent side-effects of lithium is hand tremors (6, 32, 33), and this has also been observed following chronic administration of lithium to normal human subjects (34). It is tempting to speculate the possibility whether the extrapyramidal side-effects might be related to changes in enkephalin dynamics in the basal ganglia. In the present study, when lithium treatment was discontinued following a 4-day regimen of treatment, the increase in ME content persists for at least 4 days and returns to basal values within 8 days. This suggests the reversible nature of the perturbation process on the ME system.

Opiate antagonists such as naloxone have been tried in manic patients with varying improvement in symptoms (18). However, a lack of effect of opiate antagonists in alleviating manic symptoms has also been reported (35–37). In the present study, naltrexone administration did not affect the ME system nor did it alter the lithium-induced changes. Chronic administration of naloxone or naltrexone to rats via osmotic minipumps for 8 days at a dose of 9 mg/kg/day did not affect ME levels in

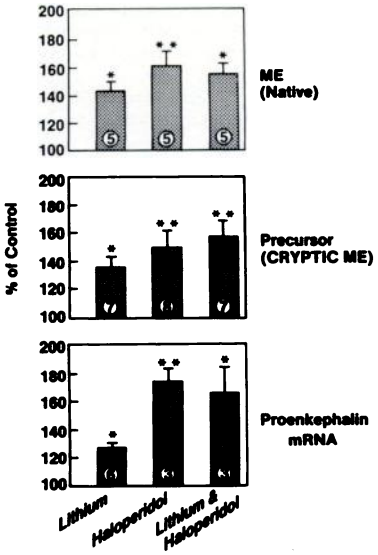


**Fig. 3.** Effect of concurrent administration of lithium and haloperidol on ME biosynthesis in the rat striatum. Lithium (4 mEq/kg/day, intraperitoneally) and haloperidol (1 mg/kg/day, subcutaneously) alone or in combination were administered for 4 days. The levels of ME, precursor, and the abundance of PE-mRNA were determined as described in Materials and Methods. The data are normalized into percentage of control. Each value is the mean ± standard error of the indicated number of samples inside the *bar*.

the striatum.[1] Thus, the blockade of opiate receptors does not appear to influence enkephalin dynamics. However, the $\mu$-receptor antagonists such as naloxone and naltrexone may not be the most appropriate antagonists for evaluation of regulation of ME, a peptide with activity at $\delta$-type opiate receptors. Studies investigating the opiate receptor changes following chronic lithium administration to mouse indicate that opiate binding sites ($^3$H-naloxone) to whole brain were not altered (17); others have reported decreased binding ($^3$H-enkephalinamide) in the rat basal ganglia (12, 38). It is not clear whether the decrease in binding sites is a consequence of the increase in striatal enkephalin levels.

Neuroleptics such as haloperidol, alone or in combination with lithium, are used in the initial stages of the treatment of mania (20). Haloperidol has been shown to increase ME levels (21, 25) and PE-mRNA abundance in rat striatum (21, 22, 31). It has been proposed that striatal ME neurons are under tonic inhibitory control by nigrostriatal dopaminergic neurons and that the blockade of dopamine receptors by haloperidol produces a disinhibition of the ME system leading to increased ME biosynthesis (21). Since lithium also induces ME biosynthesis in the striatum, it may be asked whether the effects of lithium are modulated via the dopamine system. In the present study, the combined treatment with lithium and haloperidol failed to produce any additive or synergistic effect; it is not clear whether they act by different mechanisms. However, if haloperidol elicited its maximal effect, lithium might not be able to produce an additive effect even if these agents act by similar mechanisms. At this juncture it is pertinent to note some of the conflicting reports concerning the interaction between lithium and haloperidol. Lithium has been reported to prevent haloperidol-induced supersensitivity of dopamine receptors (39); however, other work has failed to substantiate this effect (40, 41). In behavioral studies, concurrent administration of lithium and haloperidol has been reported to inhibit the neuroleptic-induced increases in apomorphine-precipitated locomotion, aggressiveness, and stereotypy (42, 43). Chronic administration of lithium reduced $^3$H-spiroperidol binding in rat striatum (44) but this could not be confirmed by others (40). Furthermore, Reches *et al.* (40) were unable to confirm another report (45) in which chronic lithium suppressed the neuroleptic-induced supersensitivity of electrophysiological response in substantia nigra neurons. Thus, the data are equivocal with regard to the interaction between lithium and haloperidol on the dopamine system.

The advent of measuring specific mRNA by molecular hybridization techniques combined with radioimmunoassays to quantitate peptide content has facilitated the study of peptide gene regulation by pharmacological agents. Using this approach, the present study reveals that both lithium and haloperidol increased the biosynthesis of ME; this increase appears to be due to increased mRNA abundance, precursor synthesis, and processing. Thus, lithium and haloperidol might indeed prove to be valuable tools to study the pharmacological regulation of enkephalin biosynthesis.

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**References**

1. Cade, J. F. J. Lithium salts in the treatment of psychotic excitement. *Med. J. Aust.* **36**:349–352 (1949).
2. Schou, M. Pharmacology and toxicology of lithium. *Annu. Rev. Pharmacol. Toxicol.* **16**:231–243 (1976).
3. Schou, M. Bibliography on the biology and pharmacology of lithium. *Neuropsychobiology* **5**:241–265 (1979).
4. Johnson, F. N. The psychopharmacology of lithium. *Neurosci. Behav. Rev.* **3**:15–30 (1979).
5. Rosenthal, N. E., and F. K. Goodwin. The role of the lithium ion in medicine. *Annu. Rev. Med.* **33**:555–568 (1982).
6. Murray, J. B. Lithium therapy for mania and depression. *J. Gen. Psychol.* **112**:5–33 (1985).
7. Lerer, B. Studies on the role of brain cholinergic systems in the therapeutic mechanisms and adverse effects of ECT and lithium. *Biol. Psychiatry* **20**:20–40 (1985).
8. Belmaker, R. H. Receptors, adenylate cyclase, depression and lithium. *Biol. Psychiatry* **16**:333–350 (1981).
9. Allison, J. H., and M. A. Stewart. Reduced brain inositol in lithium-treated rats. *Nature New Biol.* **233**:267–268 (1971).
10. Sherman, W. R., A. L. Leavitt, M. P. Honchar, L. M. Hallcher, and B. E. Phillips. Evidence that lithium alters phosphoinositide metabolism: chronic administration elevates primarily D-myo-inositol-1-phosphate in cerebral cortex of the rat. *J. Neurochem.* **36**:1947–1951 (1981).
11. Sherman, W. R., L. Y. Munsell, B. G. Gish, and M. P. Honchar. Effects of systemically administered lithium on phosphoinositide metabolism in rat brain, kidney and testis. *J. Neurochem.* **44**:798–807 (1985).
12. Stengaard-Pederson, K., and M. Schou. Opioid peptides and receptors in relation to affective illness: effects of desipramine and lithium on opioid receptors in rat brain. *Acta Pharmacol. Toxicol.* **56**(Suppl. 1):170–179 (1985).
13. Vereby, K. Opioids in mental illness: theories, clinical observations, and treatment possibilities. *Ann. N. Y. Acad. Sci.* **398**:1–512 (1982).
14. Gold, S. M., A. C. Pottash, D. Sweeney, D. Martin, and I. Extein. Antimanic, antidepressant, and antipanic effects of opiates: clinical, neuroanatomical and biochemical evidence. *Ann. N. Y. Acad. Sci.* **398**:140–150 (1982).
15. Gillin, J. C., J. C. Hong, H. Y. T. Yang, and E. Costa. (Met$^5$)-Enkephalin content in brain regions of rats treated with lithium. *Proc. Natl. Acad. Sci. USA* **75**:2991–2993 (1978).
16. Bloom, F. E., G. Baetge, S. E. Deyo, A. Henberg, L. Koda, P. J. Magistrelti, W. J. Shoemaker, and D. A. Staunton. Chemical and physiological aspects of the action of lithium and antidepressant drugs. *Neuropharmacology* **22**:359–365 (1983).
17. Amir, S., and R. Simatov. Chronic lithium administration alters the interaction between opiate antagonists and opiate receptors *in vivo*. *Neuropharmacology* **20**:587–591 (1981).
18. Janowsky, D., L. L. Judd, L. Huey, N. Roitman, D. Parker, and D. Segal. Naloxone effects on manic symptoms and growth hormone levels. *Lancet* **11**:320 (1978).

[1] S. P. Sivam and J.-S. Hong, unpublished observations.

19. Volavka, J., B. Anderson, and G. Koz. Naloxone and naltrexone in mental illness and tardive dyskinesia. *Ann. N. Y. Acad. Sci.* **398**:97–102 (1982).
20. Campbell, H. D., and H. S. Moffic. Guidelines for combination of lithium and neuroleptics. *Tex. Med.* **79**:60–62 (1983).
21. Tang, F., E. Costa, and J. P. Schwartz. Increase of poenkephalin mRNA and enkephalin content of rat striatum after daily injections of haloperidol for 2 to 3 weks. *Proc. Natl. Acad. Sci. USA* **80**:3841–3844 (1983).
22. Hong, J. S., K. Yoshikawa, T. Kanamatsu, and S. L. Sabol. Modulation of striatal enkephalinergic neurons by antipsychotic drugs. *Fed. Proc.* **44**:2535–2539 (1985).
23. Yoshikawa, K., C. Williams, and S. L. Sabol. Rat brain preproenkephalin mRNA: cDNA cloning primary structure and distribution on the central nervous system. *J. Biol. Chem.* **259**:14301–14308 (1984).
24. Pybus, J., and G. N. Bowers, Jr. Measurement of serum lithium by atomic absorption spectroscopy. *Clin. Chem.* **16**:139–143 (1970).
25. Hong, J. S., H.-Y. T. Yang, W. Fratta, and E. Costa. Rat striatal ($Met^5$)-enkephalin content after chronic treatment with cataleptogenic and non-cataleptogenic antischizophrenic drugs. *J. Pharmacol. Exp. Ther.* **205**:141–147 (1978).
26. Cupo, A., P. A. Pontarotti, Th. Jarry, and M. Dellaage. A new immunological approach to the detection and the quantitation of the $Met^5$-enkephalin precursors in rat brain. *Neuropeptides* **4**:375–387 (1984).
27. Sivam, S. P., and J. S. Hong. GABAergic regulation of enkephalin in rat striatum: alterations in $Met^5$-enkephalin level, precursor content and preproenkephalin mRNA abundance. *J. Pharmacol. Exp. Ther.* **237**:326–331 (1986).
28. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rulter. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294–5299 (1979).
29. Winer, B. J. *Statistical Principles in Experimental Design*. McGraw-Hill, New York (1962).
30. Gillin, J. C., J. S. Hong, H.-Y. T. Yang, and E. Costa. Does lithium act by selectively affecting brain enkephalin system? in *Lithium: Controversies and Unresolved Issues* T. B. Cooper, S. Giershon, N. S. Kline, and M. Shou, eds.). Excerpta Medica, Amsterdam, 781–788 (1979).
31. Sabol, S. L., K. Yoshikawa, and J. S. Hong. Regulation of methionine-enkephalin precursor messenger RNA in rat striatum by haloperidol and lithium. *Biochem. Biophys. Res. Commun.* **113**:391–399 (1983).
32. Kane, J., A. Rifkin, P. Quitkin, and D. F. Klein. Extrapyramidal side-effects with lithium treatment. *Am. J. Psychiatry* **135**:851–853 (1978).
33. Tyrer, P., M. S. Alexander, A. Regan, and I. Lee. An extrapyramidal syndrome after lithium therapy. *Br. J. Psychiatry* **136**:191–194 (1980).
34. Bech, P., J. Thomsen, S. Prytz, P. B. Vendsborg, K. Zilstorff, and O. J. Rafaelsen. The profile and severity of lithium-induced side effects in mentally healthy subjects. *Neuropsychobiology* **5**:160–166 (1979).
35. Gold, M. S., R. K. Donabedian, and M. Dielard. Antipsychotic effect of opiate agonists. *Lancet* **2**:398–399 (1977).
36. Davis, G. C., I. Extein, V. Reus, W. Hamilton, R. M. Post, and F. K. Goodwin. Failure of naloxone to reduce manic symptoms. *Am. J. Psychiatry* **137**:1583–1585 (1980).
37. Pickar, D., F. Vartanian, W. E. Bunney, Jr., H. D. Meier, M. T. Gastpar, R. Prakash, B. B. Setthi, B. Belgaer, M. Tsultulkousa, G. Junghunz, T. N. Nedopil, W. Verhoeven, and H. Van Praag. Short-term naloxone administration in schizophrenic and manic patients. A WHO collaborative study. *Arch. Gen. Psychiatry* **39**:313–319 (1982).
38. Stengaard-Pederson, K., and M. Schou. *In vitro* and *in vivo* inhibition by lithium of enkephalin binding to opiate receptors in rat brain. *Neuropharmacology* **21**:817–823 (1982).
39. Pert, A., J. E. Rosenblatt, C. Sivitt, C. B. Pert, and W. E. Bunney. Long-term treatment with lithium prevents the development of dopamine receptor supersensitivity. *Science* (*Wash. D. C.*) **201**:171–173 (1978).
40. Reches, A., H. R. Wagner, V. Jackson, and S. Fahn. Chronic lithium administration has no effect on haloperidol-induced supersensitivity of pre- and post-synaptic dopamine receptors in rat brain. *Brain Res.* **246**:172–177 (1982).
41. Staunton, D. A., P. J. Magistretti, W. J. Shoemaker, S. N. Deyo, and F. E. Bloom. Effects of chronic lithium treatment on dopamine receptors in the rat corpus striatum. II. No effect on denervation or neuroleptic-induced supersensitivity. *Brain Res.* **232**:401–412 (1982).
42. Allikmets, L. H., M. Stanley, and S. Gershon. The effect of lithium on chronic haloperidol enhanced apomorphine aggression in rats. *Life Sci.* **25**:165–170 (1979).
43. Verimer, T., D. B. Goodale, J. P. Long, and J. R. Flynn. Lithium effects on haloperidol-induced pre- and post-synaptic dopamine receptor supersensitivity. *J. Pharm. Pharmacol.* **32**:665–670 (1980).
44. Rosenblatt, J. E., C. A. Pert, B. Layton, and W. E. Bunney. Chronic lithium reduces $^3$H-spiroperidol binding in rat striatum. *Eur. J. Pharmacol.* **67**:321–322 (1980).
45. Gallagher, D. W., A. Pert, and W. E. Bunney. Haloperidol-induced presynaptic dopamine supersensitivity is blocked by chronic lithium. *Nature* (*Lond.*) **273**:309–312 (1978).

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