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[Met^5]Enkephalin content in brain regions of rats treated with lithium

(neostriatum/globus pallidus/mania/neuropeptides/psychobiology)

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ABSTRACT **In rats, chronic treatment with lithium elicits a dose-dependent increase in the [Met^5]enkephalin content of nucleus caudatus and globus pallidus. A single injection of lithium fails to change the striatal [Met^5]enkephalin content. The increase in [Met^5]enkephalin caused by chronic lithium is proportional to the serum lithium level. The extent of the increase in striatal [Met^5]enkephalin content levels off at a value of about 250% that of untreated rats. This increase has a time latency of 2–3 days and reaches a plateau at 5 days. The increase that was present at 5 days was no longer evident if the treatment was continued for 2 weeks. Lithium also increases striatal [Leu^5]enkephalin content by an extent equal to the increase of [Met^5]enkephalin. Based on the characteristics of the lithium-induced increase in [Met^5]enkephalin content, it is proposed that lithium may reduce the rate of release of [Met^5]enkephalin from the small enkephalinergic neurons that are intrinsic to the striatum; this action may be related to a change in the regulation of striatal neurons.**

Lithium administration relieves mania and some depressions and prevents the recurrence of manic depressive illness by unknown mechanisms (1–3). A better understanding of the molecular mechanism involved in this therapeutic action would increase our present knowledge of the pathophysiology of manic depressive illness and would help to develop better therapeutic agents.

Like sodium, lithium influences the high-affinity binding of agonists and antagonists to opiate receptors (4). Therefore, lithium could modify opiate receptor affinity by changing receptor conformation, thereby reducing or increasing the activity of endogenous enkephalinergic neuronal systems. Consistent with this prediction, lithium administration has been reported to antagonize codeine analgesia (5). Other reports have shown that lithium increases morphine analgesia but fails to change development of tolerance to the opioid (6).

Modifications of the sensitivity of the brain receptor for catecholamines have been proposed to underlie a number of psychopathologies, including depressive disorders (7). That lithium may affect catecholamine receptors was suggested by reports that it antagonizes the norepinephrine-induced inhibition of the stimulation of platelet adenylate cyclase and cyclic AMP production by prostaglandin E_1 (8). In addition, chronic administration of lithium (9), but not short-term administration (10), has been reported to decrease dopamine synthesis from [^{3}H]tyrosine in rats. Perhaps the action of lithium on catecholamine metabolism reflects a modification in the responsiveness of regulatory presynaptic receptors to endogenous ligands such as enkephalins or endorphins. Recent evidence has indicated that small enkephalinergic neurons intrinsic to the striatum (11) form axo-axonic synapses with striatal dopaminergic axons (12); therefore, endogenous ligands of opiate receptors may control dopaminergic function by acting at presynaptic sites of regulation. In the present study, we investigated the effects of lithium on the [Met^5]enkephalin content of a number of brain structures in rates.

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MATERIALS AND METHODS

Uniformly tritiated [Met^5]enkephalin (16 Ci/mmol) was purchased from New England Nuclear Corporation (Boston, MA) and purified by thin-layer chromatography; [Met^5]enkephalin was purchased from Peninsula Labs., Inc. (San Carlos, CA). Polylysine and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide was purchased from Sigma Co. (St. Louis, MO); hemocyanin and Freund's adjuvant were purchased from Calbiochem (San Diego, CA). Male Sprague–Dawley rats (Zivic Miller, Allison Park, PA), weighing 150–200 g, were used. Upon arrival in the laboratory, the animals were kept at a constant temperature of 23–25° and were housed six animals per cage in quarters illuminated with alternating light and dark periods of 14–10 hr, respectively.

LiCl solutions of various concentrations were injected intraperitoneally in a constant volume of 5 ml/kg. Rats (150 g) were killed 16 hr after the last injection by exposing their heads for 3 sec to a focused beam of microwave radiation (2.0 kW, 2.45 GHz, 75 W/cm^2) (13). The brain was sliced into 400 μm sections in a cryostat at −10°. Brain nuclei were punched from the frozen sections with various sizes of stainless steel needles, by the method of Palkovitz (14), as described by Koslow *et al.* (15). Tissue was homogenized with 0.1 M acetic acid and centrifuged at 25,000 × *g* for 20 min. The supernatant fluid neutrialized with 1 M NaOH was then radioimmunoassayed for [Met^5]enkaphalin. For the production of serum directed toward [Met^5]enkaphalin, succinyl-[Met^5]enkaphalin and succinyl-hemocyanin were coupled to polylysine with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. The conjugate, emulsified in complete Freund's adjuvant, was injected intradermally into four sites of the rabbit's back. The injections were repeated at 2-week intervals and the animals were bled after the sixth injection. The assay was carried out in polypropylene tubes. Labeled peptide or brain extract was incubated with antiserum diluted 2000-fold and tritiated [Met^5]enkephalin in 0.5 ml of 0.2 M Tris buffer (pH 7.4) containing 0.1% albumin and 0.06% dextran (wt/vol). The incubation was carried out at 4° for about 24 hr. The tritiated [Met^5]enkephalin bound to the antibody was separated from the free tritiated [Met^5]enkephalin by adding 0.2 ml of 1.5% charcoal slurrey containing 0.15% dextran and 0.9% NaCl (wt/vol), and the radioactivity of an aliquot of the supernatant fluid was measured in a Beckman LS 250 liquid scintillation spectrometer.

Under our conditions, the minimum detection limit was 50 fmol of [Met^5]enkephalin. In the brain, the decline of the im-

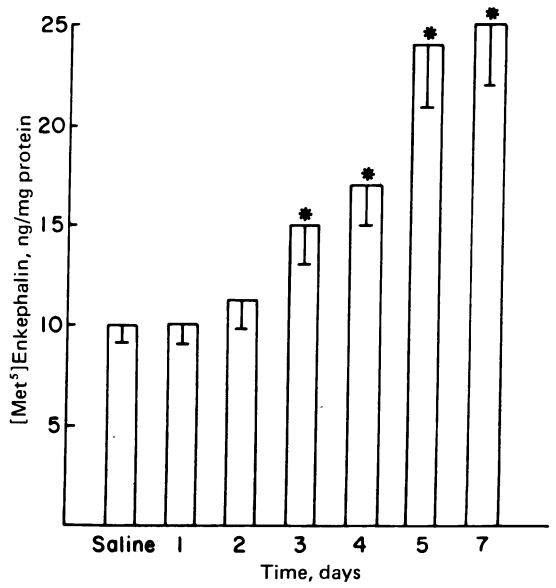

FIG. 1. Time course of the increase in striatal [Met^5]enkephalin content after chronic administration of LiCl (5 meq/kg per day). (*) Results were significantly different from the saline-treated animals ($P < 0.05$). n = 6–10.

munoassayable material due to successive dilution was parallel to that seen after similar dilutions of [Met^5]enkephalin standards. A number of endogenous polypeptides (substance P, neurotensin, somatostatin, angiotensin I, and β-endorphin) failed to compete with [Met^5]enkephalin for binding to the antibody. [Leu^5]Enkephalin has about 10% of the activity of [Met^5]enkephalin in displacing tritiated [Met^5]enkephalin bound to immunoglobulin directed toward [Met^5]enkephalin. Since the brain [Met^5]enkephalin content of both control and lithium-treated animals studied in this report is about 5- to 10-fold greater than that of [Leu^5]enkephalin (16), the values reported here essentially represent [Met^5]enkephalin.

To determine the specificity of the assay we analyzed brain extracts by thin-layer chromatography (butanol/ethylacetate/acetic acid/H_2O in equal parts). We found only one peak of immunoassayable material; this peak has an R_f value of 0.76, equal to that of authentic [Met^5]enkephalin. With this procedure we recovered about 80% of the material. For measurement of [Leu^5]enkephalin, the supernatant fluid of the tissue homogenate was incubated with cyanogen bromide (10 mg in 1 ml of supernatant fluid) at 47° for 60 min. After lyophilization, the residue was dissolved and immunoassayed for [Leu^5]enkephalin with an antibody specific against [Leu^5]enkephalin. Protein was measured by the method of Lowry *et al.* (17), and lithium by the method of Amdisen (18).

RESULTS

In rats, a single injection of 5 meq of LiCl per kg failed to change the striatal [Met^5]enkephalin content at 2, 12, and 24 hr after treatment. In contrast, when this dose was repeated once daily for at least 3 days, the striatal [Met^5]enkephalin content was increased significantly 16 hr after the last injection (Fig. 1). The extent of the increase was proportional to the duration of the treatment up to 5 days; thereafter, it reached a plateau which was maintained up to 7 days of treatment at a concentration 2.5 times that of normal rats (Fig. 1). Such treatment also increased the striatal concentration of [Leu^5]enkephalin from 1.5 ± 0.3 ng/mg of protein in control rats to 2.8 ± 0.3 ng/mg of protein in rats treated with LiCl (5 meq/kg) for 7 days. When the injections of LiCl were continued for 14 days, the increase in striatal [Met^5]enkephalin content subsided and the increase that was present at 7 days was no longer evident after 2 weeks of treatment with 2 meq/kg per day (Table 1). To establish whether the increase of [Met^5]enkephalin content caused by LiC was confined to the striatum, we measured this peptide in seven brain regions of rats injected with 5 meq of LiCl once daily for 7 days. The rats were killed 16 hr after the last injection. The data reported in Table 2 show that the nucleus caudatus and globus pallidus were the only two brain regions in which LiCl caused a very marked increase in [Met^5]enkephalin content. It is important to note that an increase in [Met^5]enkephalin content failed to occur in septum and amygdala, two brain areas in which enkephalinergic neurons appear to be involved in the elaboration of emotional behavior.

Table 1. [Met^5]Enkephalin content in striatum of rats injected with LiCl (2 meq/kg per day)

Days of treatment	[Met^5]Enkephalin, ng/mg protein		% of control	P
	Saline	Lithium		
7	9.8 ± 1.0	15 ± 1.8	153	<0.01
14	9.5 ± 0.8	9.9 ± 0.9	104	>0.05

Each value is the mean ± SEM of at least six determinations.

Table 3 lists the [Met^5]enkephalin content after 7 days of treatment with various doses of LiCl. The increment in the [Met^5]enkephalin content was maximal with 5 meq/kg per day, but a significant increment was recorded even with one-fifth of this dose. Only 1 meq/kg per day was sufficient to increase the striatal [Met^5]enkephalin content significantly. The data of Table 3 show that the time course of the [Met^5]enkephalin increment, in striatum of rats injected with various dose regimens of LiCl is correlated with the serum concentration of lithium.

The lithium-induced elevation of the striatal [Met^5]enkephalin content subsided gradually after lithium was discontinued. When LiCl was injected for 7 days (5 meq/day intraperitoneally) and then discontinued, the striatal [Met^5]enkephalin content was 50% higher than the control value on the third day after the drug and had returned to control levels by the seventh day.

DISCUSSION

The present study indicates that, in rats, repeated doses of lithium produce a time- and dose-dependent increase in the [Met^5]enkephalin content of striatum and globus pallidus. During the first few days of treatment, the extent of the [Met^5]enkephalin increase is related to the blood lithium con-

Table 2. [Met^5]Enkephalin content in brain regions of rats injected once daily for 7 days with LiCl (5 meq/kg per day)

Region	[Met^5]Enkephalin, ng/mg protein		% of control	P
	Saline	LiCl		
Nucleus caudatus	10 ± 1.2	25 ± 2.2	250	<0.001
Globus pallidus	76 ± 16	136 ± 20	180	<0.001
Septum	3.0 ± 0.27	3.0 ± 0.40	100	NS*
Amygdala	5.8 ± 0.80	5.6 ± 0.70	96	NS
Medulla oblongata + pons	2.5 ± 0.40	2.7 ± 0.30	108	NS
Hypothalamus	5.2 ± 0.70	5.5 ± 0.80	106	NS
Frontal cortex	0.70 ± 0.10	0.66 ± 0.10	95	NS

*Not significant.

Table 3. Striatal [Met^5]enkephalin content in rats injected daily for 7 days with various doses of LiCl

Dose, meq/kg/day	[Met^5]Enkephalin,* ng/mg protein	Serum Li^+ conc.,† meq/liter
Saline	10 ± 0.8	0
1	14 ± 1.2	0.06 ± 0.01
2	15 ± 1.5	0.1 ± 0.02
3	17 ± 2.1	0.37 ± 0.08
4	20 ± 3.0	0.6 ± 0.1
5	26 ± 5.2	0.8 ± 0.2

* Each value is the mean ± SEM of at least six determinations performed 16 hr after the last lithium injection. All the values are significantly different from the control value ($P < 0.05$).
† $n = 3$.

tent. The persistence of the [Met^5]enkephalin increase was transient, and after 14 days of treatment it could no longer be seen. Since the antibody used had a certain degree of cross-reactivity with [Leu^5]enkephalin, we carried out a number of tests to ascertain the validity of our measurements. That the increase of [Met^5]enkephalin elicited by lithium was due to an accumulation of authentic [Met^5]enkephalin was ascertained by three independent methods: (*i*) disappearance of the [Met^5]enkephalin from striatum in control and lithium-treated rats after incubation with cyanogen bromide; (*ii*) characterization by thin-layer chromatography of the immunoassayable material; and (*iii*) the affinity of the striatal immunoassayable material for the [Met^5]enkephalin antibody in saline- or lithium-treated rats was similar to that of authentic [Met^5]enkephalin, as shown by the immunoassay of various dilutions of [Met^5]enkephalin standards and striatal extracts.

The present experiments indicate that in lithium-treated rats an increase of [Met^5]enkephalin can be observed only in striatum and globus pallidus but not in septum amygdala, medulla oblongata and pons, hypothalamus, and frontal cortex. Since the normal concentration of [Met^5]enkephalin in these areas is $1/10$–$1/2$ that in globus pallidus or striatum and since probably the cellular location may differ in different brain areas, it is conceivable that the lack of accumulation reported here may be due to insufficient precision of our present methods. Alternatively, from the highly localized action of lithium on striatal [Met^5]enkephalin we can infer that lithium may not act on generalized enzymes that metabolize [Met^5]enkephalin but that its action depends on some specific regulatory mechanism related to the synaptic organization of striatum. Evidence reported in this paper also indicates that lithium administered for 7 days increases the [Leu^5]enkaphalin content to 190% of control values. It is important to note that lithium causes a comparable increase in percent of striatal [Leu^5]enkaphalin and [Met^5]enkephalin contents. This suggests that if lithium is acting on a regulatory mechanism for striatal [Met^5]enkephalin, the same or a similar mechanism is operative for the control of striatal [Leu^5]enkaphalin.

The increase in striatal [Met^5]enkephalin content occurs only after a few days of lithium injection; it appears to be dependent on serum lithium levels and independent of the route of lithium administration. When high doses of lithium are used, the increase in striatal [Met^5]enkephalin content reaches a plateau in about 5 days. These time constants for the [Met^5]enkephalin changes elicited by lithium are reminiscent of those regulating its clinical effects in manic patients, who typically show little clinical improvement during the first few days of lithium treatment but begin to improve after 3–7 days of treatment if the serum concentrations of lithium are high enough.

When lithium treatment is discontinued, the striatal [Met^5]enkephalin content returns to basal values within 3–5 days. When lithium treatment is continued for 14 days, the striatal [Met^5]enkephalin content returns to basal values in 14 days despite the continuation of lithium administration. A previous report from this laboratory has shown that chronic administration of haloperidol and other antischizophrenic drugs also increases the striatal [Met^5]enkephalin content selectively (19). The increase in striatal [Met^5]enkephalin induced by this neuroleptic occurs after a latency period of about 1 week. The increase persists throughout the duration of the haloperidol treatment, and an increase can still be observed for about 2 weeks after haloperiodol is discontinued (19). In contrast, the increase in the [Met^5]enkephalin content caused by chronic lithium administration is transitory, as if it were a reflection of an adaptation to a compensatory mechanism. One might speculate that lithium, through an indirect mechanism, perhaps a change in the postsynaptic receptor sensitivity, slows down the rate of [Met^5]enkephalin release from striatal enkephalinergic neurons. As in other neuronal systems, and perhaps also in the enkephalinergic system, if the rate of transmitter utilization is decreased, the steady state of the transmitter increases and remains high until the rate of synthesis is slowed down and brought into balance with the new reduced rates of utilization. The increase in striatal [Met^5]enkephalin content elicited by lithium is transient, suggesting that this drug might slow down the release of [Met^5]enkephalin from the small intrinsic neurons of striatum by an indirect mechanism which has not yet been characterized.

In conclusion, the present experiments add credence to the involvement of [Met^5]enkephalin in affective disorders by showing that treatment with LiCl, which reduces manic behavior and is advantageously used as a prophylactic in treating bipolar psychosis (1–3), can change the [Met^5]enkephalin content in striatum.

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