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Comparison of Acute and Chronic Lithium Treatment on ^{3}H-Norepinephrine Uptake by Rat Brain Slices

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Abstract. The effects of lithium chloride, given IP in high (2.5 – 15 mEq/kg) and low (1.25 mEq/kg) doses twice daily, upon ^{3}H-norepinephrine uptake and retention were examined with slices from four regions of the rat brain, namely brain stem, hypothalamus, parietal cortex, and caudate nucleus. Control uptake was significantly higher in slices from the caudate nucleus and lower in brain stem slices than in slices from hypothalamus or parietal cortex. With the higher doses, uptake increased significantly in all regions after 2 days of treatment and returned to normal by 7 days. With the lowest dose, uptake was increased after 7 days but normal at 14 days. Only in caudate slices was there a second elevation of ^{3}H-norepinephrine uptake after 21 days of treatment, which returned to normal by 42 days. No other effects were observed during 70 days of treatment with lithium. The correlation between changes in ^{3}H-norepinephrine uptake and plasma lithium levels was studied.

Key words: Lithium chloride – Norepinephrine – Acute – Chronic – Dose effect – Brain stem – Hypothalamus – Parietal cortex – Caudate nucleus – Rats

The 'biogenic amine hypothesis' of major affective disorders predicts that the neuropharmacologic changes in mania should lead to an excess of norepinephrine (NE) (and other amines) at post-synaptic receptor sites (Schildkraut, 1973; Shopsin et al., 1974). Therefore, a specific antimanic drug, such as lithium, should reduce the post-synaptic availability or effectiveness of NE. Several experimental attempts to verify this prediction in animals have been reported. Acute and subacute studies (10 days or less of lithium treatment) have demonstrated a slight decrease in NE content (Leonard, 1975) and an increase of NE turnover in the rat brain (Corrodi et al., 1967; Sterne et al., 1969; Schildkraut et al., 1969; Greenspan et al., 1970; Poitou and Bohuon, 1975), a decrease in stimulated neuronal release in rat brain slices (Katz et al., 1968), and an increase in labeled NE uptake by rat brain synaptosomes (Colburn et al., 1967; Kuriyama and Speken, 1970). All of these findings are consistent with a lithium-induced decrease in the post-synaptic availability of NE.

There have been no studies of the effects of long-term lithium administration upon the uptake of NE by various regions of the brain. Two weeks or more of lithium treatment did not affect NE turnover (Corrodi et al., 1969; Ho et al., 1970; Bliss and Ailion, 1970; Poitou and Bohuon, 1975) in various rat brain preparations. Nevertheless, the clinical antimanic effect of lithium persists in some patients for months to years (Klerman, 1978). There are significant procedural differences among these previous experiments, such as the system studied (i.e., uptake versus turnover, slices versus synaptosomes), dose level and dosage regimen, use of metabolic inhibitors, and the method and route of administration of both lithium and exogenous NE. Thus, it is difficult to compare these experiments. Also, there is only limited information on regional brain differences (Ho et al., 1970). The purpose of the present study was to compare the effects of short- and long-term lithium administration upon ^{3}H-NE uptake by slices from various regions of the rat brain. Knapp and Mandell (1975) have demonstrated that the effect of lithium on the biosnthesis of 5-hydroxytryptamine (5-HT) is temporally biphasic. If the effect of lithium upon catecholamine-containing neurons in the brain is also biphasic, a parametric study with a consistent procedure would be necessary to demonstrate this effect.

Materials and Methods

Subjects. Male Sprague-Dawley rats, weighing 200 – 250 g at the beginning of the experimental periods, were obtained from Spartan

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Table 1. Doses of lithium chloride, dosage regimens, and time of sacrifice for rats in high- and low-dose groups

Groups	Dose (mEq/kg)	Dosage regimen	Time of sacrifice (after the last dose)
High dose	15	1 dose	2 or 16 h
	5	twice daily for 2 days	16 h
	2.5	twice daily for 7 days	16 h
Low dose	1.25	1 dose	2 or 16 h
	1.25	twice daily for 2, 7, 14 21, 42, or 70 days	16 h

Farms in Lansing, Michigan. All animals were maintained in a small mammal laboratory colony with regulated light-dark cycle, temperature, and humidity. They received rat chow and water ad libitum. Those animals treated with the high-dose regimen tended to show diarrhea, and at the highest dose a 25% mortality rate was observed. The low-dose subjects, however, not only maintained their body weights but continued to grow, weighing up to 400 g by week 10. In these animals, diarrhea was not present after day 7 of treatment.

Drugs. Lithium chloride dissolved in sterile water was administered IP to experimental subjects twice daily at 8 a.m. and 8 p.m.; control rats received IP saline injctions instead of lithium chloride. Frazer et al. (1973) have shown that brain levels of lithium are stable for 16 h when given for 10 days on a twice-daily basis. Indeed, there was only a one-and-a-half-fold difference over 16 h after a single injection. ^{3}H-NE was obtaind from New England Nuclear (> 99% purity).

Dosage Regimens. For the lithium-treated subjects, two dosage regimens were used, a high dose and a low dose (Table 1). The high-dose subjects received the maximally tolerated dose for each specified treatment duration (twice the specified dose produced mortality rates above 50%). These animals received 15 mEq/kg once only, 5 mEq/kg twice daily for 2 days, or 2.5 mEq/kg twice daily for 7 days. Some of the single-dose (15 mEq/kg) subjects were killed 2 h post-injection; all others were killed 16 h after the last injection. At 14 days and thereafter, the maximally tolerated dose was 1.25 mEq/kg (equivalent to the low dose). Therefore, the high-dose regimen ended at 7 days.

In the low-dose regimen, all rats received 1.25 mEq/kg once only or twice daily for 2, 7, 14, 21, 42, or 70 days. Some of the single-dose subjects were killed 2 h post-injection; all others were killed 16 h after the last injection.

Measurement of ^{3}H-NE Uptake. ^{3}H-NE uptake was determined as discussed previously (Steinberg and Smith, 1970). Animals were killed by decapitation. Brains were immediately removed and placed in ice-cold Krebs-Heinzleit buffer solution. Two 0.5-mm thick slices were cut, from each half of the brain, from four different brain regions: brain stem, hypothalamus, parietal cortex, and caudate nucleus.

Brain slices were incubated for 10 min in the presence of $10^{-7}M$ ^{3}H-NE. Preliminary studies showed that the uptake of ^{3}H-NE is linear over a 20-min period. The slices were then washed twice with fresh buffer and incubated for 6 min further. The slices were then homogenized in ice-cold 5% trichloroacetic acid, centrifuged, and the supernatant was collected. NE was extracted on alumina columns and eluted with 0.2 N acetic acid into scintillation vials.

Samples were counted in a model 2003 Packard Tri Carb liquid scintillation spectrometer for a period of time sufficient to give a standard error of counting less than 2%. Recovery of NE was 75 ± 3%. All values represent the means of at least six determinations. Analysis of variance was used to determine the significance of differences (Myers, 1966).

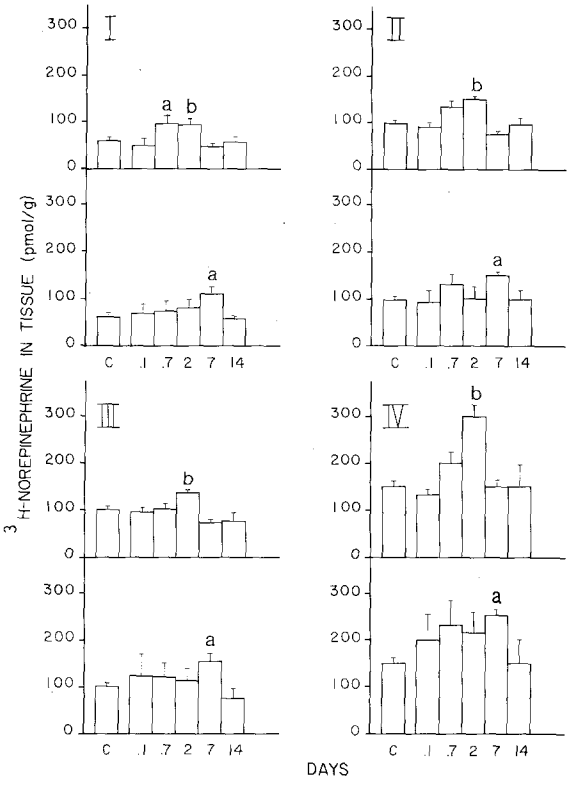

Fig. 1. Uptake of ^{3}H-norepinephrine by rat brain slices. *Abscissa: C* control. Arabic numbers represent number of days of treatment with lithium before sacrifice: 0.1 days, six animals killed 2 h after a single dose; and 0.7 days, six animals killed 16 h after a single dose. All other times represent six to ten animals killed 16 h after 2, 7, or 14 days, respectively, of twice-daily injections. *Ordinate:* ^{3}H-norepinephrine expressed as pmol/g of tissue. Each bar represents the mean value. Vertical lines represent SEM: [a] $P < 0.05$; [b] $P < 0.005$; *I* brainstem, *II* hypothalamus, *III* parietal cortex, and *IV* caudate nucleus. In each quandrant, the *upper graph* represents observations made with the high-dose regimen; *lower graph* represents observations made with the low-dose regimen. In each brain region the data at 0.1, 0.7, 2, and 7 days are from different animals for the high- and low-dose regimens; the control and 14-day data, however, are from the same animals. They are represented twice, under both regimens, for ease of comparison. See text for details

Measurement of Plasma Lithium. Lithium levels were determined with a model 305B Perkins-Elmer atomic absorption spectrometer. Levels were determined at 2 and 16 h after a single dose of either 1.25 or 15 mEq/kg, at 2 and 16 h after the last dose of either 1.25 or 5 mEq/kg given twice daily for 2 days, and at 2 and 16 h after the last dose of 1.25 mEq/kg given twice daily for 14 days.

Results

Control slices from the four brain regions differed in the amount of ^{3}H-NE which was taken up (Fig. 1). The largest amount was taken up by caudate slices, and the smallest by brain stem slices. Slices from the hypothalamus and parietal cortex took up intermediate amounts.

Table 2. Uptake of ^{3}H-norepinephrine by slices (pmol/g of tissue) from four regions of the rat brain after various periods of treatment with lithium chloride[a]

	Control	21 days	42 days	70 days
Brain stem	61 ± 4.7	71 ±10.5	51 ± 7.8	79 ± 5.5
Hypothalamus	94 ± 7.7	147 ±27.2	84 ± 17.4	110 ± 9.5
Parietal cortex	101 ± 6.9	138 ±26.1	88 ± 18.9	182 ± 10.1
Caudate nucleus	149 ± 15.9	317[b] ± 51.2	153 ± 37.5	182 ± 27.8

[a] Each value represents the mean of at least six determinations ± SEM
[b] $P < 0.025$

Table 3. Plasma lithium levels (mEq/l)[a] obtained at 2 and 16 h after the designated doses

	15 mEq/kg 1 Dose	5 mEq/kg Twice daily 2 Days	1.25 mEq/kg 1 Dose	1.25 mEq/kg Twice daily	
				2 Days	14 Days
2 h	10.7 ± 1.3	2.9 ± 1.3	0.97 ± 0.10	0.42 ± 0.08	0.53 ± 0.09
16 h	3.9 ± 1.0	2.0 ± 0.5	0.07 ± 0.04	0.08 ± 0.04	0.14 ± 0.05

[a] Mean of six determinations ± SEM

In slices from all four brain regions, the uptake of ^{3}H-NE was increased significantly ($P < 0.05$) after 7 days of administration of the lowest dose of lithium (1.25 mEq/kg). At no other time was the uptake after this dose significantly different from control in any brain region, and in all four regions uptake returned to or below control values by day 14 of treatment (Fig. 1).

The increase in ^{3}H-NE uptake occurred earlier with the administration of higher doses of lithium (Fig. 1). Although no brain areas showed a change from control values at 2 h after a single dose of lithium (15 mEq/kg), brain stem slices showed a significant increase at 16 h after a single dose of 15 mEq/kg ($P < 0.05$). In slices from all regions, a significant increase was observed at 2 days ($P < 0.005$); these animals received 5 mEq/kg twice daily. At 7 days, with a twice-daily dose of 2.5 mEq/kg, all subjects had returned to control levels or below. In comparing the low to the high-dose groups at 2 days, the uptake by high-dose subjects was significantly greater than low-dose subjects ($P < 0.005$) in each brain region. At 7 days the reverse was true: uptake by brain slices from low-dose animals was higher than for brain slices from high-dose subjects ($P < 0.001$).

Uptake of ^{3}H-NE was also determined after 21, 42, and 70 days of treatment with 1.25 mEq/kg twice daily (Table 2). Only in caudate slices was there a significant increase in uptake ($P < 0.025$), which occurred at 21 days and returned to normal by 42 days.

Plasma lithium levels were determined after various doses and durations of treatment (Table 3). At 2 h post-injection, the plasma concentrations which were achieved after a single dose of 15 mEq/kg were approximately four-times greater than the concentrations achieved during the 2-day regimen with an intermediate dose of 5 mEq/kg twice daily, and approximately 20-times greater than the concentrations achieved after 14 days or more at a dose of 1.25 mEq/kg twice daily. At 16 h post-injection, when plasma levels were stable, the concentrations achieved after a single dose of 15 mEq/kg were only twice that achieved after the 2-day regimen with the intermediate dose of 5 mEq/kg, but still more than 20-times greater than the concentration achieved with the chronic regimen. Plasma levels 2 h after a single dose of 1.25 mEq/kg were twice as great as those after 2 days of treatment, which suggests that renal excretion of lithium takes approximately 2 days to stabilize.

Discussion

The results of the present study clarify the findings of previous investigations of the effect of lithium on noradrenergic mechanisms: an increase in NE uptake within the first 2 weeks of administration, which could result in decreased post-synaptic availability of NE, followed by a return to control levels ('no effect') at later times. In this study, this pattern was consistent in all brain regions despite differences in control levels of uptake.

The time of peak effects was dose dependent: the effects of the higher doses occurred earlier and returned to control levels earlier than the low dose. This dose effect was not due purely to a delay in attainment of effective brain lithium levels. Frazer et al. (1973) demonstrated that brain lithium levels after 10 days of twice-daily injections are approximately the same as after only one injection. In this study, only in the brain stem was there an effect of a single lithium injection, which occurred after 16 h when the plasma, and presumably brain lithium levels, were lower than at 2 h post-injection. This observation indicates that even though a higher drug level had been obtained at 2 h, a delay occurred before the change in uptake was observed. Since it appears that the attainment of a high brain lithium level does not explain the initial increase in NE uptake, some further change, still unidentified, presumably must occur. Nevertheless, different brain lithium levels do apparently determine when the effects occur. Estimations of brain lithium levels in this study,

using comparisons of the plasma levels in this study to those of Frazer et al. (1973), and then extrapolating from the comparisons of brain to plasma levels in that study (Figs. 1 and 2 from Frazer et al., 1973) indicate that: 1) lithium (5 mEq/kg) twice daily for 2 days produced brain levels considerably greater than those at 0.5 mEq/kg; 2) 2.5 mEq/kg twice daily for 7 days produced levels approximately equal to those at 0.5 mEq/kg; and 3) 1.25 mEq/kg twice daily for 2 days or more produced levels less than those at 0.5 mEq/kg. Therefore, higher tissue concentrations led to earlier effects. And, although there are acute differences in lithium concentrations between the brain regions used in this study (Ebadi et al., 1974), at 2 days and thereafter concentrations in these brain regions become nearly equal (Bond et al., 1975). Therefore, differences in tissue lithium concentrations do not explain absolute differences between brain regions.

The present findings are partially consistent with the biogenic amine hypothesis of affective disorders which suggests that antimanic drugs act in part upon noradrenergic mechanisms. That is, lithium increases tissue uptake of NE, therefore, making it less available to stimulate post-synaptic receptors. Two problems are evident, however. First, within 2 weeks, uptake returned to control levels. Although lithium is clinically effective in reducing or preventing manic behavior for extended periods of time, the effect on NE uptake is brief. Possible explanations are 1) that over-availability of NE alone does not account for mania, 2) that further mechanisms as well as uptake, or regional effects either in regions not studied or in more localized brain areas, are also involved in the antimanic effect, or 3) that the subjects are rats instead of humans, and presumably are not manic. Second, lithium seems to be effective in at least some depressions (Mendels, 1976). To preserve consistency between the biogenic amine hypothesis and the demonstrated effect of lithium on depression, it will be necessary to explain how an increase in uptake would functionally lead to increased post-synaptic availability of NE. Maletzky and Blachly (1971) have suggested that lithium's major effect may be one of stabilization of neuronal function; such a mechanism may explain how lithium could have both antimanic and antidepressant properties.

This study shows that the effect of lithium on ^{3}H-NE uptake by brain slices is temporally biphasic, just as it is on the metabolic conversion of tryptophan to serotonin (5-HT) (Knapp and Mandell, 1975). This biphasic response (initial perturbation followed by a compensatory return to control levels) was demonstrated despite numerous differences in the systems studied: 1) NE uptake versus serotonin synthesis; 2) brain slices versus synaptosomes and biosynthetic enzymes; 3) four brain regions including striatum (caudate nucleus) versus striatum only; 4) different doses of lithium used. Despite these differences, not only was a similar compensatory response observed, but the time courses observed were similar, i.e., an initial response within the first week, followed by a return to control levels by 14–21 days. Interestingly, the second increase in uptake observed in this study in the caudate nucleus at 21 days suggests the need to look for, at later times in other systems, late effects which have been missed in previous time-limited studies. The findings of analogous biphasic responses in different amine systems suggests that such biphasic compensatory mechanisms may be very common in a variety of neuropharmacological systems.

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