Pharmacology Biochemistry & Behavior, Vol 10, pp 917–921 Printed in the U.S.A

Inhibition of the Action of Anticonvulsants by Lithium Treatment

KRZYSZTOF KADZIELAWA

Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Box J-267, Gainesville FL 32610

(Received 20 October 1978)

KADZIELAWA, K. *Inhibition of the action of anticonvulsants by lithium treatment* PHARMAC. BIOCHEM BEHAV 10(6): 917–921, 1979.—The present study demonstrates that the effectiveness of anticonvulsants significantly decreases following lithium treatment (3 doses of 50 mg/kg of LiCl given every 12 hr). The anticonvulsant action of phenytoin, phenobarbital and of 3 carbonic anhydrase inhibitors: methazolamide, acetazolamide, ethoxzolamide, was assessed in rats subjected to maximal electroshock. In a chronic study on tolerance development to the anticonvulsant action of acetazolamide, lithium treatment (10 mg/kg per day) has been shown to inhibit gradually the action of acetazolamide. These results are consistent with the hypothesis that norepinephrine and dopamine are involved in the action of anticonvulsants

Lithium Phenytoin Phenobarbital Carbonic anhydrase inhibitors Maximal electroshock

A NUMBER of substances decreasing the level of brain catecholamines or blocking catecholamine receptors have been found to inhibit the activity of anticonvulsants [2, 5, 14–16, 27, 31, 32, 37, 39, 44, 48] and there is substantial evidence indicating that the level of catecholamines correlates inversely with the seizure susceptibility (see [29,49]). It has been suggested that norepinephrine is involved in the anticonvulsant action of carbonic anhydrase inhibitors [14, 27, 44, 48] and phenobarbital [44], while dopamine has been considered as a mediator of phenytoin action [2]. Agonists of dopamine [1, 11, 30, 45, 47] and norepinephrine [21] receptors produce anticonvulsant effect in certain types of seizures. There is an indication of the inhibitory role of specific noradrenergic pathway in control of seizure susceptibility: bilateral destruction of locus coeruleus decreases seizure threshold [4], while electrical stimulation of neurons in this center attenuates the appearance of epileptiform like cortical activity produced by leptazol [25].

The present study was undertaken in order to find possible interaction between lithium treatment and the effect of anticonvulsants. Increased turnover of norepinephrine has been found after acute or short term treatment with lithium [8, 17, 46] and it has been confirmed in several studies [38, 40, 41] that acute or short term treatment with lithium increases intraneuronal deamination of norepinephrine and dopamine. Lithium applied by microiontophoresis antagonizes norepinephrine inhibition of the firing of single neurons in the cerebral cortex and hippocampus [34,43]. Lithium inhibits also sodium fluoride and norepinephrine activated formation of cyclic-AMP in cerebral cortical slices [13]. Thus, lithium increases turnover of catecholamines and attenuates their action at the postsynaptic membrane.

METHOD

Animals

Male albino Sprague-Dawley rats (ARS) weighing 100–125 g were kept for about a week in standard laboratory conditions until they weighed 150–160 g. For chronic study on tolerance development to the anticonvulsant action of acetazolamide, female rats (150–160 g) were used.

Experimental Procedure

Maximal electroshock seizures were produced with 225 mA sine wave current applied for 0.25 sec. Platinum corneal electrodes were used with 0.25% solution of dibucaine hydrochloride as a local anesthetic applied 10 min prior to electroshock. The responding animals were selected and their reaction was checked again after 48 hr. Only animals with tonic extensor phase lasting not less than 4 sec were used for further study. The average duration of this phase was about 6 sec.

The anticonvulsant action was assessed on a quantal basis, as the abolition of the tonic extensor phase. Six to ten groups of rats, each of 10, were used to estimate the ED-50 values, according to the method of Litchfield and Wilcoxon [26].

Drugs

Twenty-four hours after a second electroshock, animals were given the first IP injection of lithium chloride solution, followed by another in 12 hr and a final one 12 hr later—2 hr before electroshock. Thus 3 consecutive doses of 50 mg/kg of LiCl (2.5% sol: 0.2 ml/100 g) were injected every 12 hr.

TABLE 1

DECREASE IN THE POTENCY OF ANTICONVULSANTS CAUSED BY LITHIUM TREATMENT (MAXIMAL ELECTROSHOCK IN RATS)

Drug	ED50 mg/kg		Decrease in Anticonvulsant Activity	
	Control	LiCl 3×50 mg/kg	PR	fPR
Phenytoin	12.0 (7.7–18.5)	62.0 (43.7–87.9)	5.2 (2.9–9.4)	> 1.8
Phenobarbital	3.2 (2.2–4.6)	7.8 (5.3–11.5)	2.4 (1.6–3.6)	> 1.5
Methazolamide	2.9 (2.3–3.5)	9.8 (8.0–12.0)	3.4 (2.6–4.4)	> 1.3
Acetazolamide	5.2 (4.2–6.4)	20.0 (12.4–32.0)	3.8 (2.2–6.5)	> 1.7
Ethoxzolamide	4.6 (2.8–7.5)	13.0 (8.1–20.9)	2.8 (1.9–4.2)	> 1.5

PR = potency ratio.

Each ED50 value was determined in 6–10 groups of rats, each of 10. The probability limits at $p = 0.05$ are indicated in parentheses.

TABLE 2

BRAIN AND SERUM LITHIUM, SODIUM AND POTASSIUM LEVELS IN THE CONTROL AND LITHIUM TREATED RATS

Treatment	Brain (mEq/kg)			Serum (mEq/L)		
	Li^+	Na^+	K^+	Li^+	Na^+	K^+
Control (8)	0	38.9 (± 1.43)	80.8 (± 1.02)	0	130.6 (± 1.42)	6.4 (± 0.13)
LiCl (17) 3×50 mg/kg	0.34 (± 0.02)	39.5 (± 1.03)	79.2 (± 1.24)	1.15 (± 0.05)	133.4 (± 1.15)	5.86 (± 0.35)

SEM values are given in parentheses.

Control groups received 0.9% NaCl solution (0.2 ml/100 g). Anticonvulsants were given into the stomach 2 hr before maximal electroshock test, together with the third dose of IP saline or LiCl.

The influence of lithium on tolerance development to the anticonvulsant action of acetazolamide was tested in 4 groups (A, B, C, D) each of 10 female rats. Every day rats were given, into the stomach: A—12 mg/kg of acetazolamide: B—12 mg/kg of acetazolamide plus 10 mg/kg of lithium carbonate: C—10 mg/kg of lithium carbonate only and D—control group was given a vehicle solution used to prepare suspensions of acetazolamide and lithium carbonate for groups A, B and C. During the first week, maximal electroshock was applied every day, but only once a week during subsequent 7 weeks.

Phenytoin (Aldrich Chemical Co.) and the 3 carbonic anhydrase inhibitors: methazolamide, acetazolamide (Lederle Lab.) and ethoxzolamide (Upjohn Co.) were prepared in a form of a water suspension, containing 1–4% of Tween-80 and 0.5% of methyl cellulose. The same vehicle was used to prepare lithium carbonate suspension, used in a chronic experiment. Sodium phenobarbital (Eli Lilly and Co.) was given as a solution in water. Control rats received the medium only.

Lithium, Sodium and Potassium Assays

Lithium levels were analyzed in serum and brains of 17 rats treated with lithium chloride (3×50 mg/kg) according to the same schedule as in lithium treated rats tested for interaction with anticonvulsants. Brain and serum sodium and potassium levels were also analyzed. Eight control rats were given IP injections of 0.9% NaCl (0.2 ml/100 g).

A flame photometer (Eppendorf) was used for the analysis of lithium (671 nm filter), sodium and potassium levels in the brain and serum. Each brain (1.6–1.8 g) was homogenized in 10 ml of deionized distilled water, centrifuged at 15.000 G for 10 min: the supernatant was used for lithium, sodium and potassium assays.

RESULTS

Individual ED-50 values were determined in 6–10 groups of rats, each of 10. First column of Table 1 indicates the ED-50 values for the anticonvulsant effect of phenytoin, phenobarbital and 3 carbonic anhydrase inhibitors in control groups of rats. Data in the second and third column indicate the decreased anticonvulsant potency of these drugs in rats treated with lithium chloride (3×50 mg/kg). The most apparent is the interaction of lithium treatment with phenytoin, acetazolamide and methazolamide.

This treatment with lithium resulted in an average concentration of 0.34 mEq/kg of Li^+ in the brain and 1.15 mEq/L of Li^+ in serum (Table 2). No significant changes in Na^+ and K^+ levels in serum and in the brain of lithium

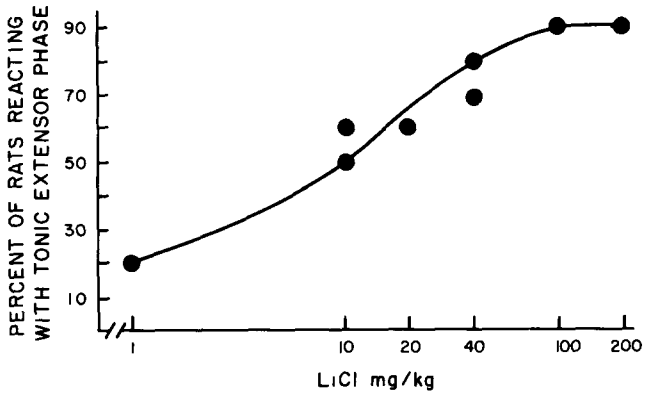

FIG. 1. The influence of a series of single doses of IP LiCl (abscissa, log scale) on the response to 12 mg/kg of acetazolamide. Lithium and acetazolamide were applied 2 hr before the electroshock. Each test was performed in 10 rats. Acetazolamide (12 mg/kg) alone produced 100% abolition of the tonic extensor phase.

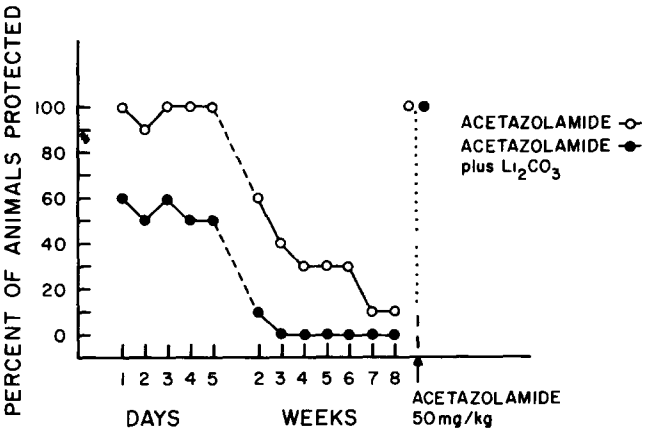

FIG. 2 Tolerance development to the anticonvulsant action of acetazolamide (12 mg/kg)—○—and its modification by lithium carbonate (10 mg/kg)—●—over a period of 8 weeks. Acetazolamide and Li_2CO_3 were given into the stomach 2 hr before electroshock. During the first week the maximal electroshock was applied every day and later only once a week. On the last, 58th day of this chronic experiment, a high dose of 50 mg/kg of acetazolamide protected all animals in both groups.

treated rats were noted (Table 2). The reported values represent the final concentrations of these ions in the supernatant of brain homogenate, but they were calculated in relation to the weight of individual brain.

LiCl given alone at 50 mg/kg every 12 hr did not increase the response to maximal electroshock: however with higher doses of LiCl: 3×100 mg/kg and 3×150 mg/kg the duration of the tonic extensor phase increased by 10–20 and 20–50%, respectively. Therefore, these doses were not chosen for the study on possible interaction with anticonvulsants. However, in one trial, LiCl treatment at 3×150 mg/kg produced a 9-fold increase in the ED-50 value of methazolamide: up to 20 (13.9–28.6) mg/kg.

The effect of LiCl against acetazolamide was dose dependent up to 100 mg/kg, as illustrated in Fig. 1. This was tested against 12 mg/kg of acetazolamide, which at this dose produced 100% protection against maximal electroshock.

Figure 2 illustrates the development of tolerance to the anticonvulsant action of acetazolamide. The anticonvulsant action of acetazolamide was inhibited by lithium, regardless of the level of its anticonvulsant activity. During the third week of treatment with acetazolamide (Group A) only 40% of animals were protected, but in the group with combined lithium treatment (Group B) the anticonvulsant effect of acetazolamide was completely abolished. In a third group of rats receiving 10 mg/kg of lithium carbonate only (Group C) over a period of 8 weeks, the response to maximal electroshock was preserved as in the control group (D) of rat receiving only medium.

DISCUSSION

The present study shows that the effectiveness of several anticonvulsants classified in different chemical and pharmacological groups significantly decreases following lithium treatment. An increased intraneuronal deamination of norepinephrine and dopamine [17, 18, 24, 38, 40, 41] and the antagonism to norepinephrine at the postsynaptic membrane [13, 34, 43] are the possible factors responsible for the observed reduction in the potency of these anticonvulsants produced by lithium. The factor by which their ED-50 value increases after lithium treatment varies from 2.4 to 5.2. It would be important to explain why the anticonvulsant action of carbonic anhydrase inhibitors [14, 15, 16, 48] is much more affected by reserpine treatment than that of phenytoin and phenobarbital [2,16].

The control ED-50 values of the anticonvulsants reported here are close to the estimates given by others for phenytoin and phenobarbital [6] and for acetazolamide and methazolamide [15].

The 3 consecutive doses of 50 mg/kg of lithium chloride given every 12 hr resulted in an average Li serum concentration of 1.15 mEq/L, which is close to the therapeutic serum levels of Li usually achieved in patients with mania (0.8–1.2 mEq/L). Thus this should be considered as a possible clinically important interaction. Higher doses of lithium were found to increase the duration of the tonic phase and in patients with evident lithium intoxication there have been reported several cases of gand mal seizures [42,50]. Other cases have been reviewed [20] and discussed [9,12]. Combined administration of lithium and α-methyl-p-tyrosine potentiates the intensity of audiogenic seizures in rats [19] and this could be related to the depletion of brain norepinephrine [7,8].

Acetazolamide has been found to be effective in both petit mal and grand mal epilepsy, unfortunately tolerance develops within 2–3 weeks of treatment with this drug [33]. Tolerance development to the repeated administration of acetazolamide has been studied in rats [22,23]. Tolerance develops also to the anticonvulsant action of CO_2 and there is a cross tolerance between CO_2 and acetazolamide [23]. This supports the idea of Maren [28] that the anticonvulsant action of carbonic anhydrase inhibitors is related to the secondary retention of CO_2 in the brain, following elevated pCO_2 on the venous side of the circulation. It is possible that the mechanism of this tolerance mediated by brain CO_2 retention involves changes in neuronal norepinephrine and dopamine turnover. Such changes have been reported recently following CO_2 exposure [10]: an increase in

norepinephrine and serotonin utilization and decrease in dopamine turnover.

There is very little information concerning possible fine changes in the kinetics of brain monamines produced by antiepileptic drugs: an increase in brain serotonin has been reported in rats treated with high doses of anticonvulsants [3]: a pronounced anticonvulsant effect of monoamine oxidase inhibitors has been related to the elevation of brain serotonin and norepinephrine [37]: it has been shown that phenytoin inhibits potassium stimulated and calcium dependent norepinephrine release from cortical slices [35].

Further studies will indicate whether the mechanism of action of anticonvulsant drugs involves some fine changes in the turnover and release of norepinephrine and dopamine in the brain.

ACKNOWLEDGEMENT

The advice and encouragement of Dr. T H Maren is gratefully acknowledged.

REFERENCES

1 Anlezark, G. M. and B. S. Meldrum Effects of apomorphine, ergocornine and piribedil on audiogenic seizures in DBA/2 mice. *Br. J Pharmac* **53:** 419–421, 1975.
2. Bhattacharya, S. K., P. K. S. P. Reddy and P K. Das. Studies on the role of brain monoamines in the anticonvulsant action of diphenylhydantoin and nialalmide-induced potentiation of diphenylhydantoin in albino rats. In *Drugs and Central Synaptic Transmission*, edited by P B Bradley and B. N. Dhawan. Baltimore· University Park Press, 1976, pp. 156–164.
3 Bonnycastle, D D , N. J Giarman and M. K. Paasonen Anticonvulsant compounds and 5-hydroxytryptamine in rat brain *Br J Pharmac.* **12:** 228–231, 1957
4. Bourn, W. M. Modulation of audiogenic seizures by cortical norepinephrine in the rat. *Neurosci Abstr.* **1:** 710, 1975
5 Browning, R. A. and R L. Simonton Antagonism of the anticonvulsant action of phenytoin, phenobarbital and acetazolamide by 6-hydroxydopamine. *Life Sci* **22:** 1921–1930, 1978.
6 Consroe, P. and A. Wolkin Cannabidiol-antiepileptic drug comparisons and interactions in experimentally induced seizures in rats *J Pharmac exp Ther* **201:** 26–32, 1977
7 Corrodi, H , K Fuxe and M. Schou. The effect of prolonged lithium administration on cerebral monoamine neurons in the rat *Life Sci* **8:** 643–651, 1969
8. Corrodi, H , K. Fuxe, T. Hokfelt and M. Schou. The effect of lithium on cerebral monoamine neurons. *Psychopharmacologia* **11:** 345–353, 1967.
9 Demers, R., R. Lukesh and J Prichard. Convulsion during lithium therapy *Lancet* **2:** 315–316, 1970.
10 De Yebenes Prous, J. G , A. Carlsson and M. A Mena Gomez The effect of CO_2 on monoamine metabolism in rat brain *Naunyn-Schmiedeberg's Arch Pharmac* **301:** 11–15, 1977.
11. Dow, R C , A G Hill and J. K. McQueen Effects of some dopamine receptor stimulants on cobalt-induced epilepsy in the rat. *Br J Pharmac* **52:** 135, 1974
12 Erwin, C. W , C. J Gerber, S D. Morrison, J. F. James and N. C Goldsboro. Lithium carbonate and convulsive disorders. *Archs gen. Psychiat Chicago* **28:** 646–648, 1973
13 Forn, J. and F. G Valdecasas Effects of lithium on brain adenyl cyclase activity *Biochem Pharmac.* **20:** 2773–2779, 1971
14 Gray, W. D and C E Rauh The anticonvulsant action of inhibitors of carbonic anhydrase· Relation to endogenous amines in brain. *J Pharmac exp Ther* **155:** 128–133, 1967.
15 Gray, W D , C E. Rauh, A. C Osterberg and L. M. Lipchuch The anticonvulsant actions of methazolamide (a carbonic anhydrase inhibitor) and diphenylhydantoin *J Pharmac exp Ther* **124:** 149–160, 1958.
16 Gray, W D., C E Rauh and R W Shanahan The mechanism of the antagonistic action of reserpine on the anticonvulsant effect of inhibitors of carbonic anhydrase. *J Pharmac exp Ther* **139:** 350–360, 1963
17 Greenspan, K , M. S. Aronoff and D. F Bogdanski Effects of lithium carbonate on turnover and metabolism of norepinephrine in the rat brain-correlation to gross behavioral effects *Pharmacology* **3:** 129–136, 1970
18 Hesketh, J. E., N M. Nicolaou, G W Abruthnott and A. K Wright. The effect of chronic lithium administration on dopamine metabolism in rat striatum. *Psychopharmacology* **56:** 163–166, 1978
19 Jobe, P. C., A L Picchioni and L Chin. Effect of lithium carbonate and α-methyl-p-tyrosine on audiogenic seizure intensity. *J. Pharm Pharmac* **25:** 830–831, 1973.
20. Jus, A., A. Villeneuve, J. Gautier, A. Pires, J M Cote, K Jus, R Villeneuve and D Perron. Some remarks on the influence of lithium carbonate on patients with temporal epilepsy *Int. J clin Pharmac* **7:** 67–74, 1973.
21. Kellog, C Audiogenic seizures relation to age and mechanisms of monoamine neurotransmission *Brain Res.* **106:** 87–103, 1976
22. Koch, A. and D M Woodbury. Production of tolerance to anticonvulsant effect of acetazolamide *Fed Proc.* **15:** 447–448, 1956.
23 Koch, A and D M. Woodbury. Effects of carbonic anhydrase inhibition on brain excitability *J Pharmac exp Ther* **122:** 335, 1958.
24. Komiskey, H. and C K Buckner Effects of lithium on adrenergic amine uptake in rat brain synaptosomes. *Neuropharmacology* **13:** 159–164, 1974.
25 Libet, B., C. A. Gleason, E. W. Wright and B Feinstein Suppression of an epileptiform type of electrocortical activity in the rat by stimulation in the vincity of locus coeruleus. *Epilepsia* **18:** 451–462, 1977.
26 Litchfield, J T and F. Wilcoxon. A simplified method of evaluating dose-effect experiments. *J Pharmac. exp Ther* **96:** 99–113, 1949.
27 Lotti, V. H , M L Torchiana and C. A Stone The anticonvulsant action of methazolamide in mice· antagonism by various inhibitors of dopamine β-hydroxylase *Eur J Pharmac* **44:** 387–390, 1977
28. Maren, T H Carbonic anhydrase: chemistry, physiology, and inhibition *Physiol Rev* **47:** 595–781, 1967.
29 Maynert, E. W , T J Marczynski and R. A Browning The role of the neurotransmitters in the epilepsies *Adv Neurol* **13:** 79–147, 1975
30 McKenzie, G. M and F. E Soroko The effects of apomorphine (+)-amphetamine and L-Dopa on maximal electroshock convulsions—a comparative study in rat and mouse. *J. Pharm. Pharmacol* **24:** 696–701, 1972
31 Mennear, J. H and A. D. Rudzik. Potentiation of the anticonvulsant action of acetazolamide *J Pharm Pharmacol* **18:** 833–834, 1966
32 Meyer, H and H. H Frey. Dependence of anticonvulsant drug action on central monoamines. *Neuropharmacology* **12:** 939–947, 1973.

33 Millichap, J. G. Anticonvulsant action of diamox in children. *Neurology* **6:** 552–559, 1956.
34. Phillis, J. W. and J. J. Limacher. Effects of some metallic cations on cerebral cortical neurons and their interactions with biogenic amines. *Can J Physiol Pharmacol.* **52:** 566–574, 1974.
35. Pincus, J H. and S Lee. Diphenylhydantoin and calcium *Archs Neurol , Chicago* **29:** 239–244, 1973.
36. Prockop, D. J., P. A. Shore and B B. Brodie. Anticonvulsant properties of monoamine oxidase inhibitors. *Ann N.Y Acad. Sci* **80:** 643–651, 1959.
37. Quatrone, A and R. Samanin. Decreased anticonvulsant activity of carbamazepine in 6-hydroxydopamine-treated rats. *Eur J. Pharmac.* **41:** 333–336, 1977
38 Rastogi, R. B. and R. L. Singhal. Lithium: modification of behavioral activity and brain biogenic amines in developing hyperthyroid rats. *J Pharmac. exp. Ther* **201:** 92–119, 1977.
39. Rudzik, A. D. and J. H. Mennear. The mechanism of action of anticonvulsants II. Acetazolamide. *Life Sci.* **5:** 747–756, 1966.
40 Schildkraut, J. J. The effects of lithium on norepinephrine turnover and metabolism: basic and clinical studies. *J nerv. ment Dis* **158:** 348–360, 1974
41. Schildkraut, J., M. Logue and G. A Dodge. The effects of lithium salts on the turnover and metabolism of norepinephrine in rat brain. *Psychopharmacologia* **14:** 135–141, 1969.
42. Schou, M., A. Amdisen and J. Trap-Jensen. Lithium poisoning. *Am. J. Psychiat.* **4:** 520–527, 1968.
43 Segal, M. Lithium and the monoamine neurotransmitters in the rat hippocampus. *Nature* **250:** 71–72, 1974.
44. Simonton, R. L. and R. A. Browning. Increased sensitivity to maximal electroshock seizures following selective destruction of noradrenergic neurons with 6-hydroxydopamine. *Neurosci. Abstr* No. 453, VII Annual Meeting, Anaheim, California, Nov. 6-10, 1977.
45. Stach, R. and D. Kacz. Effect of combined dopaminergic and GABA-ergic stimulation on ouabain-induced epileptiform activity. *Epilepsia* **18:** 417–423, 1977
46 Stern, D. N., R. R. Fieve, N. H. Neff and E Costa. The effect of lithium chloride administration on brain and heart norepinephrine turnover rates. *Psychopharmacologia* **14:** 315–322, 1969.
47. Stull, R. E., P. C. Jobe, P. F. Geiger and G. G Ferguson. Effects of dopamine receptor stimulation and blockade on Ro 4-1284-induced enhancement of electroshock seizure. *J Pharm Pharmacol.* **25:** 842–844, 1973.
48. Torchiana, M. L., V. J. Lotti and C A. Stone. The anticonvulsant effect of carbonic anhydrase inhibitors in mice—a noradrenergic mechanism of action. *Eur. J. Pharmac* **21:** 343–349, 1973.
49. Tower, D B. The neurochemistry of convulsive states. In. *Chemical Pathology of the Nervous System,* edited by P. J. Folch London: Pergamon, 1961, pp. 307–344.
50. Wharton, R. N. Grand mal seizures with lithium treatment *Am. J Psychiat* **126:** 1446, 1969.