Pharmacological and Biochemical Comparison of Lithium and Reference Antidepressants (33953)

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Although lithium is well established as a psychoactive agent, investigations of its therapeutic and prophylactic efficacy in both phases of manic-depressive psychoses have been limited primarily to those of a clinical nature (1–3). Biochemical and pharmacological studies which have been conducted tend to emphasize the effects of lithium on electrolyte balance (4, 5) and especially membrane transport (6–8) with little emphasis on other possible biochemical and pharmacological aspects. More importantly, few attempts have been made to compare or contrast these aspects with those of other known psychoactive agents (9, 10). Especially interesting was the reported effectiveness of lithium in depressive phases, but no comparisons with antidepressants used in other forms of depression were found. Evolving from this deficiency were the following studies comparing the action of various lithium salts with those of representatives of two distinct types of antidepressants; the tricyclic compounds, generally employed in endogenous depressions, and amphetamine, generally employed in exogenous or mild reactive depressions.

One area of comparison is the effect of lithium, the tricyclics and amphetamine on cholinesterases of dog blood and rat brain. Pulver *et al.* (11) reported that imipramine strongly inhibited butyrylcholintesterase (BuChE) of human plasma at concentrations which only mildly inhibited acetylcholinesterase (AcChE) of human erythrocytes. This observation and those of Desmedt and La Grutta (12), suggesting a possible correlation between EEG arousal in the cat and selective inhibition of BuChE, stimulated an investigation of a number of psychoactive agents to determine if they, too, selectively inhibited BuChE. This is not to suggest that specific inhibition of BuChE is involved in the mechanism of action of antidepressants. However, the correlation does appear to exist and may warrant consideration as a possible new approach to understanding the action of antidepressants.

Pharmacologically, lithium, the tricyclics, and amphetamine are compared in a profile of relatively specific tests which have been developed to distinguish the tricyclics from other types of antidepressants. Those tests which are most frequently utilized for general screening purposes and employed in the following studies, are those involving the antagonism of reserpine- (13–15) and tetrabenazine- (16–18) induced depressions.

Methods and Materials. Cholinesterase activity of dog blood was determined by Ammon's manometric technique as modified by Desmedt and La Grutta (12, 19). Acetylcholine chloride (Matheson, Coleman and Bell) was used as substrate for AcChE obtained from canine erythrocytes. Butyrylcholine iodide (Aldrich Chemical Co.) was used as substrate for BuChE obtained from canine plasma. The incubation mixture contained 1.0 ml of 0.05 *M* $NaHCO_3$; 0.2 ml of 0.05 *M* substrate; 0.02 ml of erythrocytes; or 0.05 ml plasma and sufficient distilled water to bring the final volume to 2.0 ml.

Cholinesterase activity of rat brain was also assayed manometrically using the method of Orzel and Weiss (20). Acetylmethylcholine bromide (Calbiochem) and butyrylcholine iodide were used as substrates for AcChE and BuChE, respectively. Female Sprague-Dawley (Dublin) rats (120–150 g) were decapitated and brains were removed and frozen rapidly for use as source of cholinesterase enzymes.

Lineweaver-Burk plots were used for analysis of kinetic data. Reversibility studies followed the method proposed by Ackermann and Potter (21).

The following drugs were the generous gifts of their respective laboratories: amitriptyline HCl and cyproheptadine from Merck

and Co., Inc.; nortriptyline HCl from Eli Lilly and Co.; imipramine from Geigy Pharmaceuticals; desipramine from Lakeside Laboratories; amphetamine sulfate, chlorpromazine HCl and diphenylpyraline from Smith, Kline and French Lab.; diphenhydramine from Parke, Davis and Co.; and tetrabenazine from Roche Labs. All other compounds utilized in these studies were obtained commerically.

Female, ICR (Dublin) mice (20–25 g) were used in all the pharmacological tests. Reversal of reserpine hypothermia was determined by the method of Askew (22) except that animals were caged individually instead of aggregated after receiving reserpine. Tetrabenazine antagonism was tested as cited by Vernier *et al.* (23) with one exception. A dose of 50 mg/kg, i.v., was found to give more consistent results than the recommended 32 mg/kg, ip. Anticonvusant activity was determined with a number of agonists. Drugs were administered prior to administration of agonists and results were evaluated by the method of Litchfield and Wilcoxon (24). The agonists and doses employed were as follows (mg/kg): eserine salicylate, 0.5, iv, 30 min after test drug; nicotine alkaloid, 1.25, iv, 15 min after test drug; caffeine, 125, iv, 1 hr after test drug; pentylenetetrazol, 75, iv, 1 hr after test drug (25); strychnine sulfate 3.0, ip, 1 hr after test drug; and thiosemicarbazide, 20, ip, 1 hr after test drug. The end point of drug protection in each of the above tests was prevention of death in the animal within 24 hr following administration of the agonist.

Results and Discussion. Several tricyclic antidepressants, amitriptyline, nortriptyline, imipramine, and desipramine, specifically inhibit BuChE from both enzyme sources Table I). This pattern of inhibition was also observed with lithium carbonate and several antihistamines. Generally, the tricyclics, and especially lithium carbonate, appeared to inhibit brain BuChE more specifically than plasma BuChE. The antihistamines were not as effective as the structurally related tricyclics and appear to inhibit both sources similarly. Interestingly chlorpromazine, although it is structurally similar to the tricyclics, does not appear to selectively inhibit BuChE. Amphetamine, however, moderately inhibits both AcChE and BuChE.

TABLE I. Inhibition of Cholinesterases by Various Agents.

Compounds (10^{-3} *M*)	Percentage inhibition			
	Blood cholinesterase		Brain cholinesterase	
	BuChE	AcChE	BuChE	AcChE
Amitriptyline	90	27	90	2
Nortriptyline	92	25	90	2
Imipramine	88	29	84	3
Desipramine	92	26	90	0
Lithium carbonate	53	23[a]	98	20
Physostigmine	93	90	97	97
Amphetamine	30	30	32	33
Chlorpromazine	13	34	3	31
Cyproheptadine	70	9	68	6
Diphenhydramine	78	20	70	11
Diphenylpyraline	51	4	58	5

[a] Stimulation.

To determine if the enzymatic inhibition noted with lithium carbonate was a true function of the lithium ion, various salts of lithium, as well as other mono and divalent ions, were tested in the ChE systems. Those included were the bromide, chloride, and carbonate salts of lithium and the corresponding salts of sodium, potassium, calcium, and magnesium. No salts other than those of lithium exhibited selective inhibition of BuChE. This is especially noteworthy since lithium and several of these ions have been interchangeable in both stimulating and inhibiting other enzyme systems (26–28).

Kinetic studies of BuChE inhibition by amitriptyline, desipramine, and lithium carbonate suggest competitive inhibition for each compound with respective K_i values of 4.0×10^{-5}, 2.0×10^{-5}, and 5.3×10^{-5}. Figure 1 represents the incorporation of these studies into one Lineweaver-Burk plot.

Further comparison of the inhibition by the tricyclics and lithium led to reversibility studies. Both were found to be reversible inhibitors of BuChE (Fig. 2).

Pharmacologically, lithium does not appear to share the same degree of similarity of

TABLE II. Anticonvulsant Effect on Various Antidepressants (ED_{50}, mg/kg, ip).

	Anti-caffeine	Anti-eserine	Anti-nicotine	Antipentyl-enetetrazol	Anti-strychnine	Anti-thiosemi-carbazide
Desipramine	61.5[a]	26.3	16.5	I[b]	I	I
Amitriptyline	I[b]	2.7	4.3	20.0[a]	I	I
Amphetamine (r)	I	I	18.6	I	I	I
Lithium	I	I	I	I	I	I

[a] Estimate only.
[b] I = Inactive.

action with the tricyclics as demonstrated in our enzyme studies. In some test situations, however, lithium appears to be similar to the tricyclics. For example, in reversal of reserpine hypothermia, commonly employed for detecting potential antidepressants, lithium carbonate mimicked the action of both the reference antidepressants and amphetamine

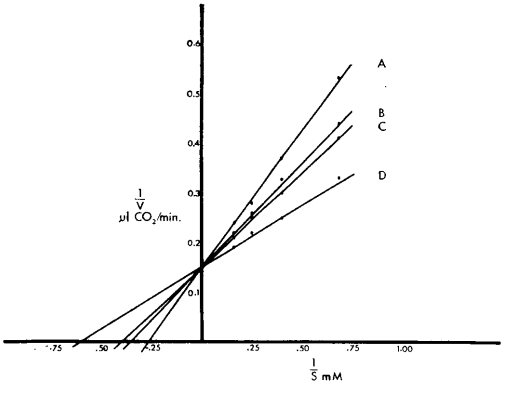

FIG. 1. Lineweaver-Burk plot of inhibition of canine plasma BuChE by desipramine (4×10^{-6} *M*) (A); lithium carbonate (5×10^{-4} *M*) (B); and amitriptyline (4×10^{-5} *M*) (C); D represents control. Substrate (*S*) was butyrylcholine iodide; Velocity (*V*) is expressed in microliters of µl CO_2 per minute.

(Fig. 3). An oral dose of 10 mg/kg of lithium approximated amitriptyline in elevating body temperature. When given ip, however, lithium was ineffective at the same dose as the tricyclics and amphetamine. In fact, a dose 10 times that of the reference compounds was required to elicit a response.

In normal rats, housed at room temperature (25°), lithium again mimicked the tricyclic drugs in producing slight hypothermia, whereas amphetamine produced a hyperthermia (Fig. 4). Again, lithium was not as effective as the reference compounds employed in this study, but the overall pattern of response was similar. Both the tricyclics and lithium caused an initial drop of two degrees in the first 0.5 hr. The temperature of the lithium-treated animals slowly returned to normal; however, the tricyclics continued to lower the temperature reaching a maximum effect after 2–3 hr before returning to normal.

Antagonism of tetrabenazine depression is another commonly employed test for study-

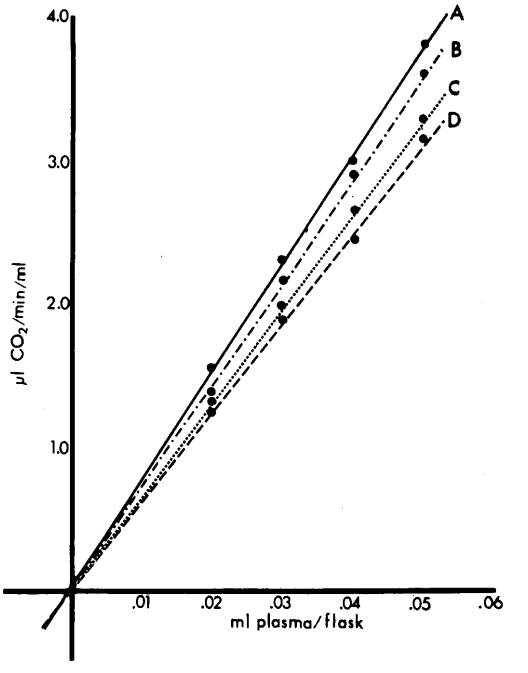

FIG. 2. Ackermann, Potter reversibility determination for inhibition of canine plasma BuChE: A = control, B = lithium carbonate (10^{-4} *M*); C = amitriptyline (10^{-4} *M*); and D = desipramine (10^{-4}*M*).

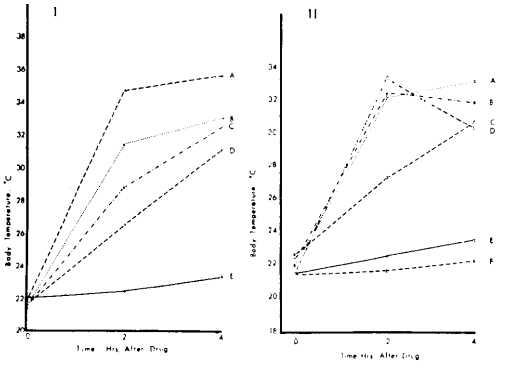

FIG. 3. Reversal of reserpine hypothermia: (I) 10 mg/kg, po, of drug. (II) 2.0 mg/kg, ip, of drug. A = amphetamine; B = desipramine; C = amitriptyline; F = lithium, and E = control; D in (II) represents lithium at a dose of 20 mg/kg, ip, the dose required to elicit a response via an ip route of administration.

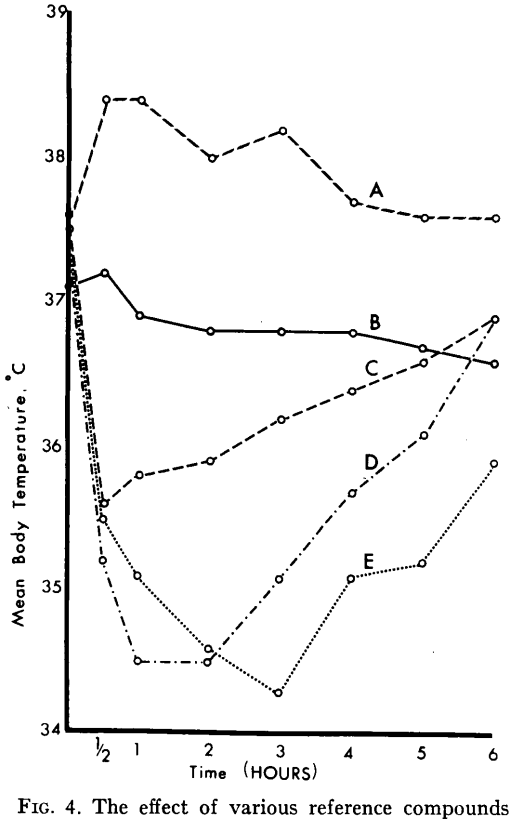

FIG. 4. The effect of various reference compounds and lithium (20 mg/kg, ip) on body temperature in normal rats: A = amphetamine; B = control; C = lithium; D = amitriptyline; and E = desipramine.

ing antidepressant drugs. Appropriate doses of lithium tended to reverse tetrabenazine depression (Fig. 5). However, as the dose was increased, it appeared to reinforce the tetrabenazine action. Therefore, an ED_{50} could not be determined for lithium as it was for the reference compounds.

Benesova (29) reported that antidepressants of various classes can be differentiated on the basis of their effects on the toxicity of various anticonvulsants. In this respect, the tricyclics are known to have anticonvulsant effects against eserine and nicotine. Therefore, lithium was tested against a number of agonists (Table II), but failed to protect against any of the convulsants employed.

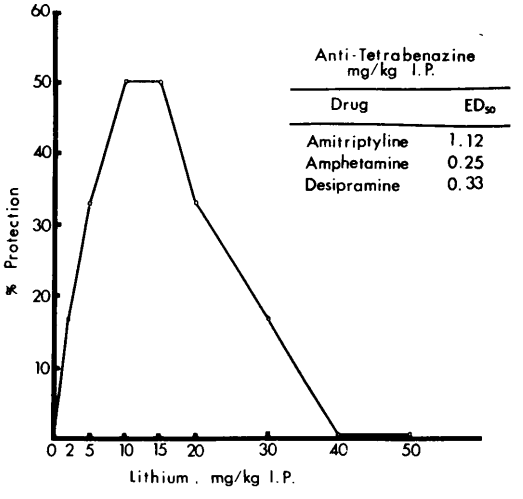

FIG. 5. Antagonism of tetrabenzaine depression in mice ED_{50} could not be determined for lithium.

Summary. Lithium, like amitriptyline and desipramine, was a competitive, reversible inhibitor of BuChE. Pharmacologically, lithium appeared to parallel the action of the reference compounds in antagonizing the hypothermic action of reserpine and reversing tetrabenazine depression. Lithium, again like the reference tricyclics, produced hypothermia in normal animals. However, lithium was unlike the reference tricyclics in its apparent lack of antagonism against eserine or nicotine.

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Vol. 130 (1969), in the article "A Host Mediated Microbial Assay for the Detection of Mutagenic Compounds," by M. G. Gabridge and M. S. Legator, pp. 831-834:

Page 832, column 2, the footnote to Table II should read:

[a] Compounds 1, 4, 5, and 6 were obtained from Calbiochem, Los Angeles, Calif.; 2 and 17 from Nutritional Biochemical Corp., Cleveland, Ohio; 3 from Matheson, Coleman and Bell, East Rutherford, N. J.; 7 from Hoffmann-La Roche, Nutley, N. J.; 8 from California Chemical Co., Richmond, Calif.; 9 and 12 from Bristol Laboratories, Syracuse, N. Y.; 10 from Parke-Davis Co., Detroit, Mich.; 11 and 16 from The Upjohn Co., Kalamazoo, Mich.; 13 from Aldrich Chem. Co., Milwaukee, Wis.; 14 from Lederle Laboratories, Pearl River, N. Y.; 15 from the Bureau of Drug Abuse Control, FDA; 18 from Fisher Scientific Co., Pittsburgh, Pa.; and 19 from Eastman Organic Chemicals, Rochester, N. Y.