Systemic Cholinergic Agents Induce Seizures and Brain Damage in Lithium-Treated Rats

Abstract. *Administration of pilocarpine or physostigmine to rats treated with lithium chloride produced sustained limbic seizures, widespread brain damage, and increased concentrations of* D-myo-*inositol-1-phosphate (a metabolite of the phosphoinositides, lipids involved in membrane receptor function) in the brain. The syndrome was preventable with atropine. The physostigmine doses and concentrations of blood lithium that caused the syndrome are similar to those considered appropriate for psychiatric chemotherapy.*

Lithium is widely used in psychiatric chemotherapy and is especially effective in the treatment of manic-depressive illness (*1*). Reported side effects of lithium therapy include organic brain syndrome, tremors, convulsions, and a toxic neurodegenerative syndrome (*1, 2*). Although lithium influences many aspects of brain biochemistry, the mechanisms that underlie its therapeutic properties and side effects remain to be elucidated.

We have been exploring the effects of lithium on the metabolism of phosphoinositides, which are *myo*-inositol–containing membrane phospholipids that may be involved in receptor-mediated processes in the brain (*3*). The concentration of D-*myo*-inositol-1-phosphate (M1P), a product of phosphatidylinositol metabolism, is increased in rat brain after treatment with lithium (*4, 5*). This increase results in part from inhibition by lithium of *myo*-inositol-1-phosphatase, the enzyme that hydrolyzes M1P to *myo*-inositol (*6*). The inhibition of this enzyme by lithium explains the concomitant decrease in the concentration of *myo*-inositol in the brain (*7*). A cholinergic mechanism is implicated in these events because atropine, a muscarinic receptor blocker, inhibits both the increase in M1P and the decrease in *myo*-inositol (*4, 8*). Moreover, both pilocarpine, a muscarinic agonist, and physostigmine, an acetylcholinesterase inhibitor, increase M1P levels in rat brain (*8*). We report that treatment of rats with lithium plus pilocarpine or physostigmine markedly potentiates the increase in brain M1P while also causing prolonged seizures and widespread brain damage.

Male Sprague-Dawley rats (225 to 325 g) were injected subcutaneously first with lithium chloride (3 mEq/kg) and then, 24 hours later, with pilocarpine (30 mg/kg) or physostigmine (0.4 mg/kg). Some animals were not treated with lithium but received pilocarpine (30 or 50 mg/kg) or physostigmine (0.4 or 0.6 mg/kg), and some lithium-treated rats received atropine (150 mg/kg) 30 minutes before an injection of pilocarpine.

Animals used for M1P analysis were decapitated and the heads were immediately frozen in liquid nitrogen. Samples of frontal cortex were dissected free along the midline at −20°C and lyophilized, first at −37°C overnight, then at room temperature for 24 hours. Weighed samples were derivatized by silylation and analyzed, in glass columns packed with 3 percent OV-17 on Gas-Chrom Q, by gas chromatography with a flame photometric detector in the phosphorus mode (*9*). In one experiment the M1P was determined to be the D-enantiomer by capillary gas chromatography (*9*) with a chiral liquid phase. Lithium concentrations were determined by atomic absorption spectroscopy in samples of brain homogenate or serum deproteinized by trichloracetic acid.

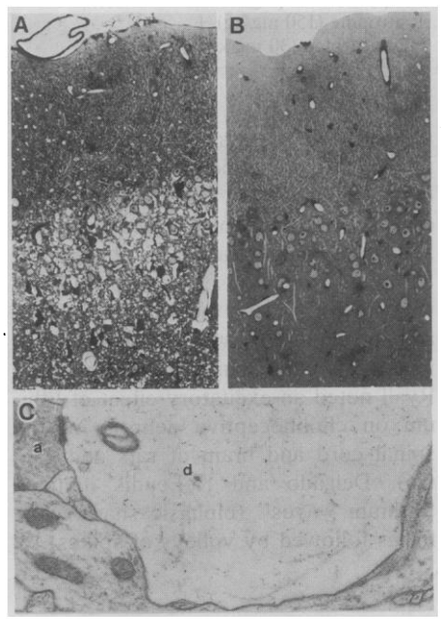

Fig. 1. (A and B) Micrographs of the olfactory (pyriform) cortex of an experimental rat (A) and a control rat (B) (both ×180). Both rats received lithium chloride (3 mEq/kg, subcutaneously) and, 24 hours later, pilocarpine (30 mg/kg, subcutaneously) and were killed 4 hours thereafter. The control rat was given atropine (150 mg/kg, subcutaneously) 30 minutes before its injection of pilocarpine. Seizure activity was absent in the control animal but was present for more than 3 hours in the experimental rat. The pyriform cortex from the control rat appears normal, whereas that from the experimental animal exhibits swelling of both glial and neuronal structures and dark cell degeneration of neuronal cell bodies. The tissue changes in (A) resemble those in several other regions of the brain of this animal and those that result from repetitive limbic seizures induced by other methods (*10*). (C) Electron micrograph showing a swollen dendrite (*d*) in the cingulate cortex of the experimental rat (×26,000). Note the normal appearance of the presynaptic axonal bouton (*a*). Edematous degeneration of dendrites, with axonal sparing, is a cardinal feature of the excitotoxic type of damage that glutamate or aspartate cause (*10, 11*).

Behavioral effects were scored for 4 hours after the administration of pilocarpine or physostigmine to lithium-treated rats. They were then anesthetized and perfused transcardially with aldehyde fixatives. Their brains were prepared for histopathological evaluation by light and electron microscopy (*10*).

Animals treated with lithium alone appeared mildly sedated after 24 hours but were otherwise asymptomatic. Rats receiving pilocarpine or physostigmine alone showed piloerection, salivation, and chromodacryorrhea within the first hour—all signs of peripheral cholinergic stimulation. Animals given both lithium and a cholinomimetic displayed these signs as well as a syndrome that began with staring, mouth automatisms, head bobbing, blinking, and wet-dog shakes and culminated in seizures in which the rats reared up on their hind limbs, with the forepaws and head in clonus. The seizures (*11*) commenced after 30 minutes (mean interval, 31.9 ± 3.6 minutes, $N = 21$), lasted 30 to 45 seconds, and recurred every 2 to 5 minutes throughout the observation period.

The cytopathological reaction of the brain after 4 hours of such seizure activity was conspicuous (Fig. 1). Ultrastructural observations revealed massive swelling of neuronal dendrites and swelling or dark cell degeneration of neuronal cell bodies. Astroglia in the vicinity of affected neuronal cell bodies also exhibited swelling. Axonal components were largely unaffected. The brain regions that were affected most severely and consistently included the pyriform and entorhinal cortices, several thalamic and amygdaloid nuclei, the hippocampus, the septum, and the cerebral cortex. This morphological reaction closely resembles that resulting from sustained limbic seizures induced by other methods (*11*).

The mean concentration of lithium in serum was 0.69 ± 0.03 mEq/liter 10 hours after injection ($N = 5$) and 0.16 ± 0.01 mEq/liter ($N = 5$) after 24 hours (*12*). After injection of lithium (3 mEq/kg) or pilocarpine (30 mg/kg) alone, M1P levels in the cortex increased twofold and fourfold, respectively (Table 1). Combined administration of lithium and

pilocarpine (same doses) resulted in a 40-fold elevation in M1P, as did treatment with a substantially higher dose of lithium alone (10 mEq/kg). Neither seizures nor brain damage occurred in rats that received only lithium (3 or 10 mEq/kg), pilocarpine (30 or 50 mg/kg), or physostigmine (0.4 or 0.6 mg/kg), whereas both effects occurred in all rats given lithium and pilocarpine and in 67 percent of the animals given lithium and physostigmine (Table 2). When atropine was given to lithium-treated rats 30 minutes before pilocarpine, seizures and brain damage were prevented and the elevation in M1P was less than fourfold.

Lithium-treated rats given the cholinergic agonist arecoline (40 mg/kg, subcutaneously) manifested the full seizure and brain damage syndrome, indicating that these toxic effects characterize the response to combinations of lithium and cholinomimetics in general. [Arecoline has been proposed for cholinergic therapy of mental disorders (*13*) because it penetrates the blood-brain barrier.] To ascertain whether cholinomimetics precipitate a neurotoxic syndrome when administered to animals maintained on lithium on a long-term basis, we treated four rats subcutaneously with lithium chloride (3 mEq/kg) daily for 8 days and then injected pilocarpine (30 mg/kg) on day 9. The full seizure and brain damage syndrome occurred in each animal.

Our findings do not necessarily imply that lithium or cholinomimetic agents or even their combination exert a direct neurodestructive action. The cytopathological reaction observed is a reproducible consequence of sustained limbic seizures induced by any of several methods (*11*); moreover, it appears to be identical to the excitotoxic type of damage caused by the putative transmitters glutamate and aspartate (*10*), which may be released at many synaptic loci in the course of persistent seizure activity.

The neurotoxic syndrome we describe is precipitated in lithium-treated rats by pilocarpine, which acts directly as an agonist at acetylcholine (ACh) receptors, or by physostigmine, which inhibits ACh breakdown and prolongs the action of ACh at its receptors. Atropine, which blocks muscarinic ACh receptors, antagonizes all components of the syndrome: seizures, brain damage, and most of the increase in M1P. Thus the seizure activity probably stems, at least in part, from increased activity of ACh receptors.

The brain damage is probably a consequence of the prolonged seizure activity (*11*), but the role of lithium and M1P changes in ACh receptor–mediated seizure activity is unresolved. Antagonism by atropine of the lithium-induced elevation in M1P (*4, 8*) signifies that lithium influences cholinergic function through a central mechanism. Jope reported increased ACh metabolism after short- and long-term administration of lithium (*14*). Samples *et al.* observed that lithium treatment increased the mortality rate in rats subsequently given physostigmine (1 mg/kg, subcutaneously) (*15*). Haas and Ryall noted an excitatory effect of lithium on cholinoceptive neurons in the spinal cord and brain of rats and cats (*16*). Delgado and DeFeudis induced "lithium waves" (biphasic sharp wave forms followed by volleys of spikes) by microinjecting lithium into monkey amygdala (*17*). Injection of ACh into the same site after decay of the lithium waves caused a return of this wave form, intermixed with ACh-type bursting activity. This effect of lithium may be specific for the amygdala, as the response was not obtained from other brain regions. Since microinjection of cholinergic agonists or cholinesterase inhibitors into the rat amygdala induces a seizure and brain damage syndrome similar to that described here (*18*), a role for the amygdala in the pathogenesis of such effects warrants consideration. A centrally mediated effect of lithium on seizure susceptibility is indicated by evidence that lithium (6 mEq/kg) reduces the threshold for electrically induced convulsions in rats (*19*) and may prolong electroshock-induced seizure activity in humans (*20*). Muscarinic mechanisms may or may not be involved in the altered responsiveness to electrically induced seizures. It should be determined whether lithium, which alters the permeability of the blood-brain barrier to choline (*21*), similarly alters permeability to cholinomimetic substances.

Lithium is widely used in psychiatry, and its potential uses include treatment of conditions (mania and senile dementia) for which cholinomimetic treatment is also sometimes advocated (*22, 23*). Cholinomimetics are used in other fields of medicine (*24*) and are commonly encountered in the form of insecticides. The reported adverse effects of lithium in humans include lowering of the seizure threshold and precipitation of temporal lobe seizures (*25*), a form of epilepsy that resembles "limbic" seizures in animals. Also, severe neurodegenerative reactions have been reported in patients receiving both lithium and neuroleptics (*26*).

Since the pattern of brain damage described here resembles that associated with human temporal lobe epilepsy (*27*), treatment of animals with lithium and cholinomimetics may provide a valuable model for studying mechanisms of epilepsy and epilepsy-related brain damage.

Table 1. Concentrations of M1P in rat cerebral cortex after subcutaneous injection of lithium chloride, pilocarpine, both agents, or both agents plus atropine. Animals given lithium or pilocarpine alone were decapitated after 24 hours and 1 hour, respectively. Animals treated with both lithium and pilocarpine received the pilocarpine 24 hours after the lithium and were killed 1 hour later. Animals receiving all three agents were given atropine 23.5 hours after the lithium injection, were given pilocarpine 30 minutes later, and were killed 1 hour thereafter. Values are means ± standard errors (N = 5 per group).

Treatment	M1P (mmole/kg, dry weight)
Control	0.22 ± 0.01
Lithium (3 mEq/kg)	0.85 ± 0.15
Pilocarpine (30 mg/kg)	0.53 ± 0.06
Lithium (30 mEq/kg) + pilocarpine (30 mg/kg)	8.95 ± 1.34
Lithium (10 mEq/kg)	8.85 ± 0.61
Lithium (3 mEq/kg) + atropine (150 mg/kg) + pilocarpine (30 mg/kg)	0.77 ± 0.18

Table 2. Incidence of the seizure and brain damage (SBD) syndrome in rats treated with lithium or cholinergic agents or combinations thereof. The animals were scored as positive for SBD only if they displayed repetitive seizures and brain damage. Of the 55 animals studied, all but two had either no seizures and no brain damage or repetitive (status) seizures for more than 3 hours and clear-cut brain damage. The exceptions were two of the six rats given lithium and physostigmine. Both displayed an occasional seizure and had no detectable brain damage.

Treatment	N	SBD (%)
Lithium (3 mEq/kg)	8	0
Lithium (10 mEq/kg)	6	0
Pilocarpine (30 mg/kg)	6	0
Pilocarpine (50 mg/kg)	4	0
Physostigmine (0.6 mg/kg)	7	0
Lithium (3 mEq/kg) + pilocarpine (30 mg/kg)	12	100
Lithium (3 mEq/kg) + physostigmine (0.4 mg/kg)	6	67
Lithium (3 mEq/kg) + atropine (150 mg/kg) + pilocarpine (30 mg/kg)	6	0

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11. A syndrome of status limbic seizures and disseminated brain damage can be induced in rats by subcutaneous treatment with kainic acid [T. A. Fuller and J. W. Olney, *Life Sci.* **24**, 1793 (1979)] or dipiperidinoethane [J. W. Olney, T. A. Fuller, R. C. Collins, T. deGubareff, *Brain Res.* **200**, 231 (1980)] or by injection into the amygdala of kainic acid [Y. Ben-Ari, E. Tremblay, O. P. Ottersen, R. Naquet, *ibid.* **165**, 362 (1979)], folic acid [J. W. Olney, T. A. Fuller, T. deGubareff, *Nature (London)* **292**, 165 (1981)], or various cholinergic agents, including pilocarpine and physostigmine [J. W. Olney, T. deGubareff, J. Labruyere, *Nature (London)* **301**, 520 (1983)]. These and related findings [R. S. Sloviter and B. P. Damiano, *Neurosci. Lett.* **24**, 279 (1981); J. W. Olney, T. Fuller, T. deGubareff, *Brain Res.* **176**, 91 (1979); R. C. Collins and J. W. Olney, *Science* **218**, 177 (1982)] support the hypothesis that much of the cytopathology associated with status seizure activity may stem from excessive release and intrasynaptic accumulation of the endogenous transmitters glutamate and aspartate.
12. In psychiatric chemotherapy lithium is administered daily for months or years. The dose schedule is adjusted to achieve serum concentrations in the therapeutic range, as determined in blood samples drawn 10 hours after the most recent dose. Serum lithium levels in our rats were below the range (0.8 to 1.5 mEq/liter) considered necessary for effective chemotherapy in humans.
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23. In chemotherapy physostigmine is used intravenously in doses up to 0.03 mg/kg. This dose may be roughly comparable, in terms of the drug's concentration in the blood, to the subcutaneous dose we used (0.4 mg/kg).
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28. Supported by PHS grants MH-14677, NS-05159, and RSA MH-38894 to J.W.O. and by the Epilepsy Foundation of America. We thank T. DeGubareff, J. Labruyere, and L. Y. Munsell for excellent technical assistance. A preliminary version of this report was presented at the annual meeting of the Society for Neuroscience, Minneapolis (1982).

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Digoxin-Inactivating Bacteria: Identification in Human Gut Flora

Abstract. *Digoxin, the most widely used cardiac glycoside, undergoes significant metabolic conversion in many patients to cardioinactive metabolites in which the lactone ring is reduced. This appears to occur within the gastrointestinal tract. An attempt was made to isolate and identify the organisms capable of reducing digoxin from stool cultures obtained from human volunteers. Of hundreds of isolates studied, only* Eubacterium lentum, *a common anaerobe of the human colonic flora, converted digoxin to reduced derivatives. Such organisms were also isolated in high concentrations from the stools of individuals who did not excrete these metabolites when given digoxin in vivo. When the growth of* E. lentum *was stimulated by arginine, inactivation of digoxin was inhibited. Neither the presence of these organisms alone nor their concentration within the gut flora appeared to determine whether digoxin would be inactivated by this pathway in vivo.*

The cardiac glycoside digoxin (Fig. 1) is the most widely used drug in the treatment of heart disease and the seventh most commonly prescribed medication in the United States (*1*). Although earlier studies suggested that digoxin escaped metabolic degradation and was excreted from the body largely unaltered, recent work indicates that the drug is metabolized in a substantial minority of patients (*2, 3*). Approximately one in ten individuals taking digoxin excretes large amounts of reduced metabolites, such as dihydrodigoxin (Fig. 1), in which the lactone ring on the molecule is saturated (*3, 4*). Such digoxin reduction products (or DRP), which bind poorly to the cardiac receptor site (membrane-associated Na^+,K^+–dependent adenosinetriphosphatase), are only minimally concentrated by cardiac tissue, undergo rapid excretion, and possess much less cardiac activity than digoxin (*5–7*). Patients who make massive amounts of DRP may have strikingly increased requirements for the parent drug (*4, 8*). It has not been determined why most patients treated with digoxin consistently make no reduced metabolites or only trivial amounts (so-called DRP nonexcretors), whereas others consistently form moderate to marked quantities of DRP (excretors) (*3*).

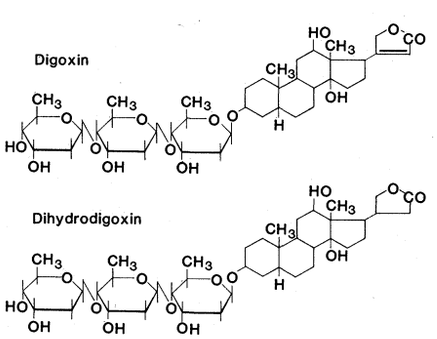

Fig. 1. The single double bond in the lactone ring of digoxin (above) is reduced in dihydrodigoxin (below).

We recently demonstrated that DRP were formed in the gastrointestinal tract of excretor subjects apparently exclusively as the result of the action of enteric bacteria (*7*). The organisms responsible for the conversion of digoxin to DRP have not previously been identified. We report here the isolation and identification of anaerobic organisms present in the human gut flora capable of reducing digoxin, as well as experiments designed to test the hypothesis that variation in the concentration of such organisms accounts for differences in the metabolic behavior of excretor and nonexcretor subjects.

Fresh stool samples from two human volunteers who were known to be heavy DRP excretors were cultured anaerobically in chopped meat glucose broth (Scott Laboratories) containing digoxin