mers; a small amount is present at the solvent front and with the high molecular weight protein at the beginning of the gel.

BCNU, CCNU and MeCCNU caused cell-cycle-related sensitivity differences in the human lymphoma cell line, T_1 [10]. The increased sensitivity to BCNU and MeCCNU in G2 and to CCNU and MeCCNU in the early S phase of the cell cycle could reflect, in part, disturbance by the drugs of the synthesis or polymerization and degradation of tubulin which occurs at these two times, respectively [11].

In summary, several nitrosoureas (CCNU, BCNU, MeCCNU, CHI and *trans*-4-OH CCNU) which degrade to form isocyanates have been shown to inhibit the polymerization of purified brain tubulin in a dose-dependent manner. Failure to yield appreciable isocyanate due to intramolecular carbamylation (chlorozotocin and *cis*-2-OH CCNU) resulted in a lack of inhibition of polymerization. The CHI inhibition of tubulin polymerization is stoichiometric. The [^{14}C]-cyclohexyl moiety derived from CCNU appears to bind covalently to the α and β tubulin monomers.

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Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, U.S.A.

ANN E. BRODIE
JOHN R. BABSON
DONALD J. REED

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Effects of lithium chloride on the cholinergic system in different brain regions in mice

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Lithium is a potent therapeutic and prophylactic agent in the treatment of affective disorders, although its mode of action is still unknown [1]. In comparison with a number of reports on the effects of lithium on the brain monoaminergic system, relatively little work has been carried out on the *in vivo* actions of lithium on the central cholinergic system [2,3]. In relation to the role of acetylcholine (ACh) in the control of mood and behavior, it has been hypothesized by Janowsky *et al.* [4] that an imbalance between the central cholinergic and adrenergic systems is the etiology of affective disorders. They considered depression to be a disease of cholinergic dominance and mania to be the converse. Also, an antimuscarinic action of antidepressants in sympathetic ganglia [5] and a correlation between behavioral excitation and decreased brain ACh [6] or between depression and increased ACh level in brain [7] have been reported. Therefore, in the present experiments we examined the effects of a single dose and of chronic administration of lithium chloride (LiCl) on (1) steady state levels of ACh and choline (Ch) and (2) ACh turnover by an indirect method using hemicholinium-3 (HC-3) [8], in different brain regions in mice.

Male ddY mice weighing 20–30 g, were used in all experiments. In the single dose schedule, 4.72 m-equiv./kg of LiCl were injected i.p., and the animals were killed 2 hr later. In the chronic administration schedule, mice were treated twice daily (8:00 a.m. and 8:00 p.m.) for 5 days with 2.36 m-equiv./kg, i.p., of LiCl. On day 6 (7:00 a.m.), mice were given the last dose and killed 2 hr later. LiCl was administered in an isotonic solution. The controls consisted of animals that received an equivalent amount of isotonic sodium chloride.

For the experiments using an intraventricular injection of HC-3, mice were anesthetized with ether the day before they were killed. The skin above the skull was dissected away and the skull around the bregma was exposed; then a point 1 mm lateral from the bregma was marked. On the next day, immediately before the intraventricular injection, the mouse was grasped firmly and a transparent rubber disc (*ca.* 3 mm in diameter, 0.6 mm thick) was stuck on the marked point on the skull to prevent leakage of the drug when the intraventricular injection was made. After this a hypodermic needle (28 gauge, 1.7 cm, cut off from the hub), which was attached to the holder and connected to the microsyringe (50 μl) with polyethylene tubing, was inserted perpedicularly, through the rubber disc, 3 mm into the skull, with the help of a stop attached to the needle. Intraventricular injection of saline caused no apparent behavioral change except for 10–20 sec of immobility immediately after the injection. Saline solution (5 μl) containing 7 μg of HC-3 was injected intraventricularly 30 min before the animals were killed. The NaCl and LiCl groups were always treated concurrently and killed between 9:00 and 11:00 a.m.

For the assays of ACh and Ch, mice were killed by immersion into liquid nitrogen for 2.5–3.0 sec according to their weights. The brains were dissected in a cold box (−2°) into five regions (cortex, hippocampus, striatum, diencephalon and midbrain), basically according to the method of Schubert and Sedvall [9], wrapped individually

Table 1. Effects of a single injection of LiCl on steady state levels of ACh and Ch and HC-3 depleted ACh levels in various brain regions in mice*

	ACh (nmoles/g, mean ± S.E.M.)				Ch (nmoles/g, mean ± S.E.M.)	
	Alone		+ HC-3		Alone	
Brain region	NaCl	LiCl	NaCl	LiCl	NaCl	LiCl
Cortex	13.5 ± 0.5 (6)	16.4 ± 1.6 (6)	7.5 ± 1.4 (5)	4.6 ± 0.5 (6)	49.5 ± 3.1 (5)	47.6 ± 2.7 (5)
Hippocampus	19.0 ± 0.9 (6)	18.7 ± 1.7 (5)	7.6 ± 2.5 (6)	5.6 ± 1.6 (5)	47.8 ± 2.8 (5)	60.4 ± 6.2† (6)
Striatum	49.8 ± 3.1 (6)	50.0 ± 5.3 (6)	7.6 ± 0.9 (5)	10.3 ± 1.4 (6)	105.2 ± 11.5 (6)	119.2 ± 10.8 (5)
Diencephalon	25.4 ± 3.6 (5)	22.0 ± 2.6 (6)	22.1 ± 4.3 (5)	16.9 ± 2.2 (5)	68.2 ± 11.7 (6)	65.2 ± 7.4 (5)
Midbrain	30.0 ± 2.6 (5)	29.2 ± 3.0 (5)	16.5 ± 0.5 (4)	17.2 ± 1.6 (5)	78.8 ± 2.5 (5)	94.8 ± 14.1 (4)

* Mice were treated with 4.72 m-equiv./kg (i.p.) of LiCl or NaCl and killed 2 hr later. HC-3 (7 μg) was injected intraventricularly 30 min before death. The assays for ACh and Ch were performed on two pooled brain regions. The number of experiments is shown in parentheses.

† Statistical significance of difference from NaCl control, $P < 0.025$.

Table 2. Effects of chronic administration of LiCl on steady state levels of ACh and Ch and HC-3 depleted ACh levels in various brain regions in mice*

	ACh (nmoles/g, mean ± S.E.M.)				Ch (nmoles/g, mean ± S.E.M.)	
	Alone		+ HC-3		Alone	
Brain region	NaCl	LiCl	NaCl	LiCl	NaCl	LiCl
Cortex	17.1 ± 0.6 (6)	19.9 ± 0.6† (6)	7.1 ± 0.5 (6)	11.6 ± 1.2‡ (6)	47.1 ± 8.8 (6)	49.6 ± 7.9 (6)
Hippocampus	24.3 ± 2.3 (6)	24.3 ± 2.1 (5)	5.8 ± 0.6 (5)	6.8 ± 1.2 (6)	46.6 ± 9.5 (6)	44.0 ± 9.4 (5)
Striatum	52.4 ± 4.0 (5)	58.9 ± 2.6 (6)	13.7 ± 0.9 (5)	20.8 ± 4.2 (4)	84.9 ± 19.0 (6)	112.2 ± 10.0 (6)
Diencephalon	25.3 ± 0.6 (6)	28.5 ± 2.0 (6)	20.4 ± 2.8 (6)	20.7 ± 1.0 (5)	83.3 ± 12.6 (6)	83.2 ± 9.7 (6)
Midbrain	29.8 ± 1.1 (6)	35.6 ± 1.8§ (6)	26.1 ± 3.5 (6)	21.1 ± 2.2 (5)	94.4 ± 14.9 (6)	98.1 ± 16.0 (5)

* Mice were treated twice daily (8:00 a.m. and 8:00 p.m.) with 2.36 m-equiv./kg (i.p.) of LiCl or NaCl for 5 days. On day 6 (7:00 a.m.), they received the last dose and were killed 2 hr later. HC-3 (7 μg) was injected intraventricularly 30 min before death. The assays for ACh and Ch were performed on two pooled brain regions. The number of experiments is shown in parentheses.

† Statistical significance of difference from NaCl control, $P < 0.01$.

‡ Statistical significance of difference from NaCl control, $P < 0.005$.

§ Statistical significance of difference from NaCl control, $P < 0.025$.

in aluminium foil, and frozen in liquid nitrogen. The time elapsing from death to freezing the tissues in liquid nitrogen was between 4 min (cortex) and 7 min (midbrain). Two pooled brain regions were pulverised under liquid nitrogen in a stainless steel mortar, and ACh and Ch were assayed radio-enzymically according to the method of Aprison *et al.* [10], with some modifications to detect the substances in smaller quantities of tissue. We used choline kinase (EC 2.3.1.32) and choline acetyltransferase (EC 2.3.1.6), purchased from the Sigma Chemical Co. (St. Louis, MO). The lower limit of sensitivity of both assays was 35 pmoles in our hands.

For the determination of total choline esterase, mice were decapitated and a single brain region was homogenized in 20 vol. of 0.1% (v/v) Triton X-100 solution. Fifty microliters of the homogenates were used for colorimetry of the enzyme [11]. The plasma Li^+ concentration 2 hr after the final administration was determined by flame photometry according to Amdisen [12]. Values for the LiCl group were compared to those for the respective NaCl control group using Student's *t*-test.

When the levels of ACh and Ch in the mouse brain regions, which were obtained in this study using a near-freezing method [10] for sacrifice, were compared with those obtained by a microwave irradiation method, the values for ACh were identical to those in the same strain of mice after 1.3 kW microwave radiation for 2 sec [13], although Ch levels showed higher values than those reported by Nordberg [14] because of the postmortem change (Table 1).

In the single injection experiments, LiCl had no effect on either the steady state levels of ACh and Ch or the decreased ACh levels seen after HC-3 in the five regions, except for the Ch level in hippocampus, which was increased by LiCl (Table 1) in spite of the high plasma concentration of Li^+ (2.3 ± 0.07 m-equiv./l, N = 5), which exceeded the therapeutic level (0.5–1.5 m-equiv./l). Krell and Goldberg [2] have reported that acute administration of LiCl decreased the ACh level in whole brain of the mouse. This discrepancy might come from the difference in the doses used in the two experiments. Krell and Goldberg administered a total of 10 m-equiv./kg which is more than double the dose of LiCl used in the present experiments.

Chronic administration of LiCl produced a significant increase in the steady state levels of ACh in cortex and midbrain without affecting those of Ch in the given regions (Table 2). The plasma concentration of Li^+ at this time was 0.7 ± 0.03 m.equiv./l (N = 5). The decreased ACh levels seen after HC-3 in cortex were significantly higher in the LiCl group than in the NaCl control group. A similar trend was observed in striatum; however, the change was not significant because of the high variation (Table 2). Since the activity of choline esterase in cortex was not affected by chronic LiCl (Table 3), the increase in steady state and HC-3 depleted levels of ACh in cortex from LiCl-treated mice may be a result of a reduced ACh release in this area. This is consistent with the findings of Bjegović and Randić [15] that the evoked release of ACh from cat somatosensory cortex into the superfusate was inhibited by Li^+ (totally replacing Na^+ in the medium). In midbrain, LiCl also elevated the steady state level of ACh (Table 2) and slightly increased the activity of choline esterase (Table 3). In contrast to the effect in cortex, the rate of depletion of ACh after HC-3 was greater in the LiCl group (40 percent) than in the control group (12 percent). This suggests that the turnover of ACh was increased by LiCl in midbrain. It is not clear how LiCl increased both the steady state level and the turnover of ACh. It has been reported that when rat brain cortex and midbrain slices were incubated in high K^+ medium, although similar amounts of ACh were released from both regions, there was no change in ACh content in midbrain, while the ACh content in cortex was decreased [16]. This unique property of the midbrain cholinergic system might have some relation to the unusual effect of LiCl in midbrain. However, further work is required to clarify the mechanism of the effect of LiCl on the cholinergic system in midbrain.

Table 3. Effects of chronic administration of LiCl on choline esterase activities in cortex and midbrain in mice*

Brain region	Activity (μmoles/g/min, mean ± S.E.M.) NaCl	LiCl
Cortex	7.18 ± 0.60 (6)	8.06 ± 0.40 (6)
Midbrain	11.72 ± 0.22 (6)	12.45 ± 0.15† (6)

* Mice were treated with LiCl or NaCl in the same manner as described in the legend of Table 2. The number of experiments is shown in parentheses.

† Statistical significance of difference from NaCl control, $P < 0.05$.

It has been reported previously that chronic administration of LiCl had no effect on the ACh level in the whole brain in mice [2], but the present experiments have demonstrated that the action of chronic LiCl differs in various regions of the brain. The effect of chronic LiCl on the steady state level of ACh is the opposite of that reported by Rónai and Vizi [3], who showed that LiCl caused a decrease in ACh in the medulla oblongta in rats. However, not only did they administer a higher dose (4.7 m-equiv./kg, twice daily, five doses), but also the ACh levels were too low (e.g. 4.82 nmoles/g in cortex).

In these experiments we have shown that a single administration of a high dose of LiCl has no measurable effect on the steady state level and the HC-3-induced depletion of ACh in the five brain regions tested, and that chronic lithium administration reduces the activity of cholinergic neurons in cortex and increases both the ACh level and the rate of HC-3-induced depletion of ACh in midbrain tissue at a plasma concentration of Li^+ within the therapeutic range.

Department of Pharmacology
Pharmaceutical Institute,
Tohoku University,
Aobayama, Sendai 980, *Japan*

TATSUO MIYAUCHI
SUMIKO OIKAWA
YOSHIMI KITADA

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Half-lives of salsolinol and tetrahydropapaveroline hydrobromide following intracerebroventricular injection

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Although interest in the physiological action of tetrahydroisoquinolines (TIQs) was sparked by the hypothesis that these compounds might be formed following the ingestion of alcohol [1, 2], progress in testing this theory stalled due to failure to detect the formation of these amine–aldehyde condensation products after the administration of alcohol [3]. However, behavioral tests in rodents support the notion that the presence of TIQs may underlie some of the actions of alcohol. TIQs can exacerbate the effects of ethanol on sleeping time [4] or enhance the severity of withdrawal from ethanol [5]. Infusion of various TIQs into a cerebral ventricle of the rat induces the animal to ingest voluntarily large quantities of alcohol [6–8]. In the infusion experiments, the minute quantities of TIQ employed to produce behavioral alterations suggest that only exceedingly small amounts of these compounds would need to be formed following alcohol ingestion in order to influence behavior.

Since the importance of the involvement of TIQs in the action of alcohol is dependent on demonstrating that they are formed following the ingestion of alcohol alone, estimating the brain levels achieved during chronic infusion experiments would indicate the level of sensitivity required for an assay to detect levels of these compounds capable of influencing behavior. Until now, there has been no indication of the minimum level of sensitivity required. In order to make estimates of the level of assay sensitivity required, the half-lives of salsolinol and tetrahydropapaveroline hydrobromide (THP) are needed. This paper describes the determination of these values.

Female rats of the Long–Evans strain, weighing 200–300 g, were anesthetized with an intraperitoneal injection of 30 mg/kg of sodium pentobarbital. The animal was then placed in a stereotaxic instrument in the DeGroot orientation. The scalp was incised and a burr hole was drilled in the skull at AP 5.8, 1.5 mm lateral to the midline. After lowering a 23 gauge injection needle 3.0 mm into the brain so that the tip rested in the ventricle, 1.0 μg salsolinol hydrobromide or 10.0 μg THP in 10.0 μl of artificial cerbrospinal fluid [9] were injected. The animals were killed by cervical dislocation at intervals after the injection. The whole brain was rapidly removed, frozen in liquid nitrogen, and weighed. The brains were kept frozen at − 70° until assayed later the same day.

Both salsolinol and THP have been shown to be metabolized *in vitro* via *O*-methylation [10]. Therefore, in a second experiment, 250 mg/kg of pyrogallol, an inhibitor of catechol-*o*-methyltransferase, was administered intraperitoneally 30 min prior to the intraventricular injection of the TIQ.

Brains were homogenized at room temperature in 6 ml of a solution of 0.5 M HCl, 0.1 M $HClO_4$, and 1% sodium metabisulfite with 100 ng of 3,4-dihydroxybenzylamine added to each sample as an internal standard. The homogenate was centrifuged at 2000 *g* for 10 min at 0°. The supernatant fraction was transferred to a 10 ml beaker containing 100 μl each of 5% sodium metabisulfate and 10% EDTA disodium salt. The pH was then adjusted to 8.5 with NaOH. The sample was immediately placed in a conical vial containing 80 mg of alumina which had been washed previously with a 1:1 mixture of $CHCl_3$ and CH_3OH. The suspension was shaken for 12 min at room temperature. The liquid was then aspirated and the alumina was washed three times with distilled water. After the final aspiration, 400 μl of 1 N acetic acid were added to the alumina and shaken for 10 min. This eluate was then assayed using high-pressure liquid chromatography with an electrochemical detector [11]. A pellicular Vydac SCX stationary phase was dry packed in a 50 cm × 2 mm i.d. glass column. The mobile phase consisted of a mixture of 380 ml of 0.1 M citric acid, 320 ml of 0.2 M Na_2HPO_4, and 200 ml of distilled deionized water with 1 drop of toluene added to each liter to prevent microbial growth. The detector potential was set at 0.7 V. The flow rate was 0.4 ml/min, generating a column pressure averaging 500–600 psi.

The amount of salsolinol recovered as a percentage of that injected at different time points is given in Fig. 1. The half-life of this compound in the brain was determined to be 12.5 min. As shown in Fig. 1, administration of 250

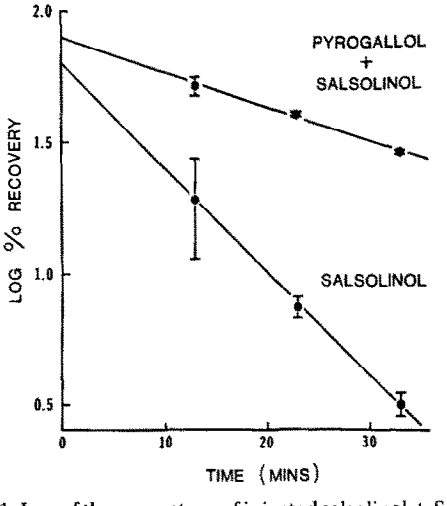

Fig. 1. Log of the percentage of injected salsolinol ± S.E.M. recovered at different times after the intraventricular injection of 1.0 μg salsolinol, with (N = 9) and without (N = 44) prior intraperitoneal administration of 250 mg/kg of pyrogallol.