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LITHIUM-INDUCED INCREASE IN THE METABOLISM OF BENZO[a]PYRENE AND DRUGS IN RAT LIVER

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Abstract—Administration of lithium chloride (2.5 mEq/kg/day) to rats for 4 or 12 days increased the rates of hepatic hydroxylation of benzo[a]pyrene, antipyrine and zoxazolamine by 100, 114 and 56 per cent, respectively, and the rate of hepatic conjugation of 4-methylumbelliferone with glucuronic acid by 19 per cent. Liver lithium levels were highly correlated with plasma lithium levels but not with hepatic benzo[a]pyrene hydroxylase activities. *In vitro* addition of lithium to liver 10,000 *g* supernatants did not affect the hydroxylation of benzo[a]pyrene, suggesting that the lithium-induced increase in hepatic drug hydroxylation is not due to enzyme activation and is probably a result of lithium-mediated enzyme induction.

Lithium carbonate is widely used in the treatment and prophylaxis of manic psychosis [1]. Since lithium is often administered together with other drugs, interactions may occur during the processes of absorption, metabolism or excretion. Thus, patients receiving both lithium carbonate and chlorpromazine showed lower plasma levels of chlorpromazine than patients treated with chlorpromazine alone [2]. Parmar *et al.* [3] have reported an increase in hepatic drug metabolizing enzyme activity after treating rats with lithium carbonate for 21 days. Lithium therapy was also shown to alter significantly stress-mediated induction of hepatic tyrosine aminotransferase (EC 2.6.1.5) and tryptophan oxygenase (EC 1.13.11.11) activities [4].

The present report describes the effect of lithium treatment of rats on the *in vitro* hepatic mixed function oxidation of the chemical carcinogen benzo[a]pyrene (BP) and that of zoxazolamine and antipyrine. It was further determined whether lithium treatment affects other hepatic drug metabolizing enzymes such as UDPglucuronosyltransferase (EC 2.4.1.17), in addition to its effect on mixed function oxidases.

MATERIALS AND METHODS

Female rats of the Hebrew University Sabra strain weighing 130–150 g were given lithium or cesium chloride intraperitoneally (1.25 mEq/kg/12 hr). This dose of lithium is relatively high compared to that used in the treatment of manic-depressive subjects (0.5 mEq/kg/day). However, since the plasma half-life of lithium is much shorter in the rat (4–6 hr) than in human subjects (24–36 hr) [5], higher doses of lithium are needed in the rat in order to maintain plasma levels comparable to those of treated patients. Control rats were treated with isoequivalent doses of NaCl. All animals were kept on an *ad lib.* diet. Body weight and water intake were monitored daily. Rats were decapitated 16 hr after the last injection and their livers excised and rinsed in cold 1.15% KCl. 25% (w/v) homogenates were prepared in 1.15% KCl using a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenates were centrifuged at 10,000 *g* for 20 min in a Sorvall refrigerated centrifuge, and enzymatic activities were determined in the resulting supernatants.

Assay of benzo[a]pyrene hydroxylase (EC 1.14.14.2) activity

Liver 10,000 *g* supernatant (equivalent to 10 mg of wet weight tissue) was incubated at 37°C for 10 min in the presence of BP (0.1 μmole in 50 μl acetone), 3 μmoles of $MgCl_2$, 0.1 μmole of EDTA, a NADPH-generating system (1 μmole of NADP, 20 μmoles of glucose-6-phosphate and 1 Kornberg unit of glucose-6-phosphate dehydrogenase), and 0.1M KH_2PO_4–K_2HPO_4 buffer (pH 7.4) in a total volume of 1 ml. The amount of fluorescent phenolic metabolites formed was determined as described by Nebert and Gelboin [6], using 3-hydroxybenzo[a]pyrene as the standard for fluorescence measurements.

Assay of zoxazolamine hydroxylase activity

Liver 10,000 *g* supernatant (equivalent to 25 mg of wet weight tissue) was incubated at 37°C for 15 min with 4,6-^{3}H-zoxazolamine (0.25 μmole; 500,000 d.p.m., kindly provided by Dr. A. H. Conney, Hoffmann LaRoche Inc., U.S.A.), the NADPH-generating system described for the BP hydroxylase assay, and 0.1 M phosphate buffer (pH 7.4) in a total volume of 1 ml. The hydroxylation of zoxazolamine to 6-hydroxyzoxazolamine was measured by determining ^3H_2O, as described by Tomaszewski *et al.* [7].

Assay of antipyrine hydroxylase activity

The reaction mixture containing 10,000 *g* supernatant (equivalent to 10 mg of wet weight liver), *N*-methyl-C^{14}-antipyrine (1 μmole; 260,000 d.p.m.), and the NADPH-generating system described for the BP hydroxylase assay, in a final volume of 1 ml of 0.1 M phosphate buffer (pH 7.4), was incubated at 37°C for 30 min. The formation of 4-hydroxyantipyrine was estimated by the method of Kapitulnik *et al.* (manuscript in preparation). The reaction was stopped by the

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addition of 5 ml of ethyl acetate followed by vigorous shaking for 5 min. Four milliliters of the ethyl acetate extract was evaporated to dryness under nitrogen, and the residue was dissolved in 100 μl of acetone. Fifty microliters of the acetone solution was cochromatographed with non-radioactive 4-hydroxyantipyrine on a Gelman I.T.L.C.-SA chromatographic sheet using a benzene–ethyl acetate–ethanol (4:5:1) solvent system [8]. After chromatography, the area of the chromatogram containing 4-hydroxyantipyrine was cut out, transferred to scintillation vials and extracted with Insta-gel.® (Packard Instrument Co., Downers Grove, IL), and the radioactivity was determined in a Packard Tricarb Scintillation Spectrometer.

Assay of 4-methylumbelliferone–UDPglucuronosyltransferase activity [9]

One volume of 10,000 *g* supernatant obtained from the 25% (w/v) liver homogenate was mixed with 3 vol of digitonin suspension (12 mg/ml) and kept at 0°C for 30 min before preparation of the reaction mixture. The reaction mixture containing untreated or digitonin-activated 10,000 *g* supernatant (equivalent to 0.5 mg of wet weight liver), 70 nmoles of 4-methylumbelliferone (4-MU), 0.85 μmole of UDPGA, 3 μmoles of $MgCl_2$, in a final volume of 0.2 ml of 0.06 M Tris–HCl buffer (pH 7.5), was incubated at 37°C for 15 min. The reaction was stopped by the addition of 0.8 ml of ice-cold H_2O and 5 ml of $CHCl_3$ followed by vigorous shaking for 1 min. The tubes were centrifugated for 5 min and the $CHCl_3$ layer was discarded. Two additional $CHCl_3$ extractions were performed for the complete removal of unreacted substrate. One hundred microliters of the aqueous phase containing 4-MU glucuronide were incubated at 37°C for 60 min with 100 units of β-glucuronidase and 0.2 ml of 0.1 M acetate buffer (pH 5.0). Three milliliters of 0.5 M glycine buffer (pH 10.3) were then added to the samples and the fluorescence of 4-MU was measured (excitation: 363 nm; emission: 448 nm).

Protein was measured in liver 10,000 *g* supernatants by the method of Lowry *et al.* [10].

Lithium concentrations were determined at 670 nm with an atomic absorption spectrophotometer (Perkin Elmer model 403) in plasma samples obtained from rats before they had been killed (14–16 hr after the last LiCl injection) and in the 10,000 *g* supernatants of rat liver used for the enzymatic assays.

Statistical analysis of the data was carried out by Student's *t* test.

RESULTS

Administration of LiCl (2.5 mEq/kg/day) to rats for 4 days increased the hepatic hydroxylation of BP, zoxazolamine and antipyrine by 32, 56 and 114 per cent, respectively (Table 1), while there was no change in the conjugation of 4-MU with UDPGA both with and without digitonin activation of the enzyme preparations (Table 1). Significant increases in hepatic BP, zoxazolamine and antipyrine hydroxylase activities (70, 44 and 81 per cent) were also observed after prolonged LiCl treatment (12 days) of rats (Table 1). In addition, a small but statistically significant increase was obtained in 4-MU–UDPglucuronosyltransferase activity (without digitonin—15 per cent, with digitonin—17 per cent) (Table 1).

Table 1. Effect of lithium treatment on the metabolism of benzo[a]pyrene, zoxazolamine, antipyrine and 4-methylumbelliferone in rat liver

	Control	4 days Lithium	Increase (%)	Control	12 days Lithium	Increase (%)
Benzo[a]pyrene hydroxylation (pmoles phenol/mg protein/min)	28 ± 1 (n = 16)	37 ± 2 (n = 16)	32***	27 ± 1 (n = 35)	46 ± 2 (n = 44)	70***
Zoxazolamine hydroxylation (pmoles ^3H_2O/mg protein/min)	123 ± 5 (n = 8)	192 ± 19 (n = 8)	56**	148 ± 7 (n = 8)	213 ± 10 (n = 8)	44***
Antipyrine hydroxylation (pmoles 4-hydroxy antipyrine/mg protein/min)	44 ± 7 (n = 8)	94 ± 19 (n = 8)	114*	80 ± 6 (n = 8)	145 ± 9 (n = 8)	81***
4-Methylumbelliferone conjugation (nmoles 4-MU glucuronide/mg protein/min)						
without digitonin	3.8 ± 0.2 (n = 16)	4.0 ± 0.3 (n = 16)		3.4 ± 0.1 (n = 35)	3.9 ± 0.1 (n = 44)	15***
with digitonin	29.4 ± 2.0 (n = 8)	29.7 + 1.7 (n = 8)		44.2 ± 1.3 (n = 8)	51.9 ± 1.0 (n = 8)	17***

Rats were administered LiCl (2.5 mEq/kg/day, i.p.) for 4 or 12 days. Controls received isoequivalent amounts of NaCl. The enzymatic activities were measured in 10,000 *g* supernatants of rat liver, as described in Materials and Methods. Enzymatic activities are expressed as mean ± S.E. Numbers in parentheses indicate number of animals studied.

* $P < 0.05$.
** $P < 0.005$.
*** $P < 0.001$.

Table 2. Effect of lithium treatment on daily water intake and body weight

Treatment	Water intake (ml/day) Before treatment	4 days	12 days	Body weight (g) Before treatment	4 days	12 days
Control	25.0 ± 1.6 (n = 4)	26.0 ± 1.4	25.3 ± 1.3	140 ± 2 (n = 16)	140 ± 2	169 ± 3
Lithium	27.7 ± 1.2 (n = 6)	61.2 ± 2.9	77.8 ± 5.8	140 ± 1 (n = 24)	140 ± 2	163 ± 3
Increase (%)		135*	207*			

Rats were administered LiCl (2.5 mEq/kg/day) for 4 or 12 days. Controls received isoequivalent amounts of NaCl. Values are expressed as mean ± S.E. Numbers in parentheses indicate number of animals studied.

* $P < 0.001$.

Table 3. Effect of lithium or cesium treatment on the metabolism of benzo[a]pyrene and 4-methylumbelliferone in rat liver

Treatment	Benzo[a]pyrene hydroxylation (pmoles phenol/ mg protein/min)	4-Methylumbelliferone conjugation (nmoles 4-MU glucuronide/ mg protein/min)
Control	26 ± 2 (12)	3.6 ± 0.2 (12)
Lithium	52 ± 4 (17)	4.3 ± 0.2 (17)
% increase	100***	19*
Cesium	35 ± 3 (11)	3.9 ± 0.2 (11)
% increase	35**	

Rats were administered LiCl or CsCl (2.5 mEq/kg/day) for 12 days. Controls received isoequivalent amounts of NaCl. The enzymatic activities were measured in 10,000 *g* supernatants of rat liver, as described in Materials and Methods (4-methylumbelliferone conjugation was studied in supernatants without prior treatment with digitonin). Enzymatic activities are expressed as mean ± S.E. Numbers in parentheses indicate number of animals studied.

* $P < 0.02$.

** $P < 0.025$.

*** $P < 0.001$.

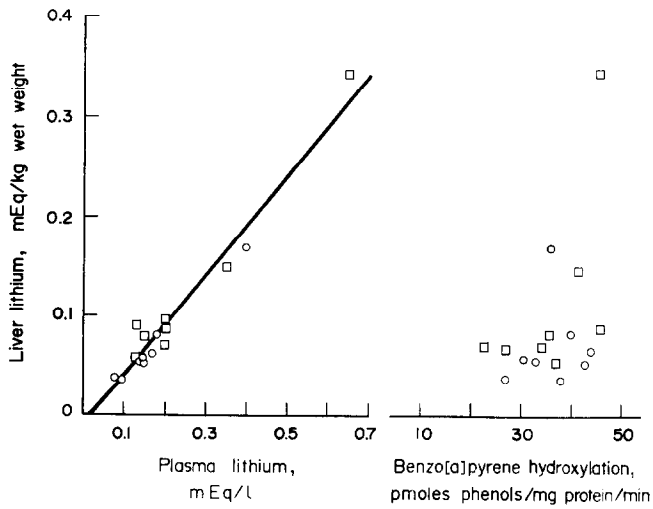

Fig. 1. Relationship between liver and plasma lithium levels and between liver lithium levels and hepatic benzo[a]pyrene hydroxylase activities in rats treated with LiCl (2.5 mEq/kg/day) for 4 (□) or 12 days (○).

Administration of LiCl also increased the daily water intake of rats by 135 per cent after 4 days, and by 207 per cent after 12 days of treatment (Table 2). No significant changes were observed in body weight during treatment with lithium (Table 2).

Lithium concentrations in the liver highly correlated with those in plasma ($r = 0.98$; $P < 0.001$) (Fig. 1). However, no correlation was observed in the individual livers between BP hydroxylase activity and liver lithium levels ($r = 0.36$; N.S.) (Fig. 1). When 10,000 *g* supernatant from a control rat (equivalent to 10 mg of wet weight liver) was incubated for 10 min with BP (as described in Materials and Methods), no change was observed in the rate of hydroxylation of BP after adding to the incubation mixture amounts of lithium equivalent to 0.1–10 mEq/kg wet weight of liver.

In another series of experiments rats were administered cesium which, together with lithium, belongs to group IA of the Periodic Chart of Elements. After 12 days of treatment cesium increased hepatic BP hydroxylase activity by 35 per cent compared to a 100 per cent increase produced by a 12 day-long lithium treatment (Table 3). 4-MU-UDPglucuronosyltransferase activity was only slightly raised by lithium treatment (19 per cent) and did not change significantly after cesium administration.

DISCUSSION

Chronic treatment of rats with lithium chloride increased the hepatic cytochrome P-450-dependent monooxygenation of the chemical carcinogen benzo[a]pyrene (by up to 100 per cent) and that of the drugs antipyrine (114 per cent) and zoxazolamine (56 per cent) (Tables 1 and 3). Lithium treatment caused only a slight increase (19 per cent) in the microsomal conjugation of 4-methylumbelliferone with glucuronic acid (Tables 1 and 3). Increases in drug metabolizing enzyme activity such as those found in the present study may be of clinical importance in the routine chronic treatment of patients with the manic-depressive syndrome who receive other drugs in addition to lithium salts. Thus, it was recently shown that lithium administration caused delayed gastric emptying of chlorpromazine in rats [11] but not in man [12]. Rivera-Calimlim *et al.* suggested that other factors (e.g. increased metabolism of chlorpromazine) may be responsible for the lower plasma chlorpromazine levels found in psychiatric patients [2] and in normal volunteers [13] treated simultaneously with lithium and chlorpromazine as compared with those treated with chlorpromazine alone. Similar interactions are possible between lithium and other drugs used in psychiatric practice. This will require careful monitoring of plasma levels of these drugs and readjustment of their dosage in order to prevent either a lack of effect or a toxic response to the above drugs during or after concomitant treatment with lithium.

Of special interest is the lithium-induced duplication of BP hydroxylase activity since this enzyme is responsible for the formation of mutagenic and carcinogenic metabolites from polycyclic aromatic hydrocarbons, which are ubiquitous in man's environment [14].

Similar, although less marked, effects on drug metabolizing enzyme activity were reported by Parmar *et al.* [3]. Lithium therapy was also shown to alter stress-mediated induction of hepatic tyrosine aminotransferase and tryptophan oxygenase activities [4].

Cesium, which together with lithium, belongs to group IA of the Periodic Chart of Elements, also increased hepatic BP hydroxylase activity but its effect was less marked than that of lithium (Table 3). Hietanen has recently shown that administration of cadmium chloride to rats for 14 days increased hepatic BP hydroxylase activity to an extent similar to that observed with lithium chloride in the present study, without affecting the conjugation of *p*-nitrophenol with glucuronic acid [15].

The results of the current study suggest that the effect of lithium on drug metabolizing enzyme activity is probably not due to enzyme activation. *In vitro* additions of high amounts of lithium to liver 10,000 *g* supernatant, which were in great excess of those found in rat liver after *in vivo* administration, did not affect BP hydroxylase activity. In addition, there was no correlation in lithium-treated rats between hepatic BP hydroxylase activity and lithium levels (Fig. 1).

Since chronic lithium administration to rats increased plasma corticosterone levels [4], it is possible that the lithium-induced increase in hepatic drug metabolism is mediated by this augmented release of corticosteroids, which are known inducers of microsomal drug metabolizing enzymes [16]. Experiments are now in progress to clarify this possibility and to determine the nature of the cytochrome involved (P-450 or P-448).

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