Selective induction of hepatic drug metabolizing enzymes by lithium treatment in dogs

Recent studies have provided evidence for the induction of hepatic drug metabolizing enzymes (DME) by lithium treatment in rats (Parmar, Ali & others,1974). These observations prompted us to investigate the effects of prolonged lithium treatment on the activity of dog liver DME. The present study provides evidence for the selective induction of dog liver DME during *O*-dealkylation reaction on prolonged treatment with lithium carbonate for 42 days.

Female mongrel dogs weighing 14–22 kg had free access to laboratory chow and water. The dogs were fed lithium carbonate orally in capsules in a dose of 30 mg kg^{-1} twice a day at an interval of 8 h. Four normal dogs were used for the control group. Animals were killed by intrathoracic electrodes (70 V per 10 m s). The livers were removed within 2 min of death, and washed and homogenized separately in ice-cold 1·15% KCl solution in the ratio of 1:4 (w/v). The homogenates were centrifuged separately at 9000 *g* for 15 min at 4° and the supernatant fractions thus obtained were used as the source of DME. All reagents were of analytical grade and the solutions were made in deionized water and adjusted to pH 7·4. The activity of DME was determined by following the methods reported earlier (Parmar & others, 1974).

The reaction mixture in a total volume of 3 ml consisted of phosphate buffer (0·1M, pH 7·4), 10 μ mol of glucose-6-phosphate, 0·48 μ mol of NADP, 15 μ mol of $MgCl_2$, 20 μ mol of nicotinamide, 10 μ mol of various substrates and 1 ml of 9000 *g* supernatant fraction. The reaction mixture was preincubated at 37° for 15 min in oxygen to allow the sufficient formation of NADPH. The reaction was started by adding the substrate and the mixture was further incubated for 20 min. In experiments for the determination of the activity of DME during the *N*-dealkylation reaction 40 μ mol of semicarbazide hydrochloride was also added to the reaction mixture to bind the formaldehyde formed during oxidation. The reaction was stopped by the addition of 1 ml of 20% trichloroacetic acid solution (w/v). In *N*-dealkylation reactions the proteins were precipitated by heating the mixture after addition of 2 ml of Nash reagent (30% ammonium acetate containing 0·4 ml of acetylacetone) in a boiling water bath for 10 min. The precipitated proteins were separated by centrifu-

Table 1. *Effect of prolonged lithium carbonate treatment on dog liver drug metabolizing enzymes. Each experiment was done in triplicate and the values are the mean values with ± standard error of the mean of separate enzyme preparations obtained from four control and three lithium treated dogs.*

Reaction	Substrate	Product formed ($ng\ mg^{-1}$ protein in 20 min)		Increase
		Control	Experimental	%
Aromatic hydroxylation	Aniline	449±12	440±15	Nil
O-Dealkylation	*p*-Nitroanisole	515±15	767±22 ($P<0{\cdot}001$)	48·9
N-Dealkylation	Amidopyrine	250±5	243±13	Nil
N-Dealkylation	Cocaine	437±8	439±15	Nil
N-Dealkylation	Morphine	33±6	33±3	Nil

gation. The activity of dog liver DME was determined by estimating the amount of product formed in suitable aliquots of the supernatant fractions using colorimetric techniques (Mazel, 1971). Protein determinations were carried out by following the method reported earlier (Lowry, Rosebrough & others, 1951) using bovine serum albumin as the standard.

The *in vivo* effects of prolonged treatment with lithium carbonate for 42 days on the activity of the DME are shown in Table 1. Lithium treatment caused statistically significant induction of DME responsible for *O*-dealkylation of *p*-nitroanisole. Such a selective increase in DME activity was 49% compared to the activity of DME during *O*-dealkylation using liver preparations from untreated dogs. The activity of DME during aromatic hydroxylation of aniline and *N*-dealkylation of amidopyrine, cocaine and morphine remained unaltered. Thus, such a selective induction of DME in dogs compared with the induction of DME during aromatic hydroxylation, *O*-dealkylation and *N*-dealkylation observed in rats treated with lithium carbonate for 21 days (Parmar & others, 1974), may presumably account for the duration of lithium treatment or species differences in drug metabolism (Quinn, Axelrod & Brodie, 1958). The present study provides evidence that the selective induction of DME during *O*-dealkylation may reflect some of the biochemical effects of prolonged lithium carbonate treatment in dogs.

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