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Modulation of Nitrogen Dioxide Toxicity by Lithium[1]

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MESSIHA, F S AND J McGRATH *Modulation of nitrogen dioxide toxicity by lithium* PHARMACOL BIOCHEM BEHAV **21**: Suppl 1, 93–97, 1984 —The effect of short-term intake of LiCl in drinking fluid on NO_2 toxicity was studied in mice as a function of mortality and of specific activities of mouse liver alcohol dehydrogenase (L-ADH) and aldehyde dehydrogenases (L-ALDH) Pretreatment with LiCl for 10 days decreased mortality in mice exposed to 60 to 70 PPM NO_2 for 6 hr compared to controls Pretreatment with LiCl for 10 days under continued exposure to 5 PPM NO_2 resulted in a decrease in liver weight compared to control Lithium treated mice exposed to NO_2 showed less gain in body weight than the controls treated with LiCl and exposed to air The latter group showed an induction of mitochondrial but not cytoplasmic L-ALDH and the NO_2 exposure did not alter endogenous L-ALDH from corresponding controls This induction of mitochondrial ALDH was associated with an increase in both V_{max} and the apparent K_m Exposure to NO_2 for 10 consecutive days resulted in inhibition of cytoplasmic L-ALDH The data suggest that Li^+ antagonized NO_2 toxicity A possible mechanism for reduction of NO_2 toxicity by LiCl may be due to Li^+ action on stabilizing cell membranes and/or modifying intercellular pulmonary response to NO_2 injury

Aldehyde dehydrogenase Lithium Mortality Nitrogen dioxide

INCREASED human exposure to nitrogen oxides (NOx) from fossil fuel combustion, as well as tobacco smoking, has caused concern that NO_2 may interact with other materials inhaled or ingested by man. Acute exposure to NO_2 is associated with pulmonary edema which is ascribed to increased capillary permeability [15, 17, 35, 39]. Lithium (Li) has been shown to stabilize cellular membranes in various organ systems [6, 12, 37]. This suggests the possibility that Li^+ may protect against NO_2-induced cellular permeability and reduce morbidity and mortality. This study was conducted to evaluate the effect of short-term pretreatment with LiCl on acute NO_2 toxicity in mice, a species known to be sensitive to NO_2 The effect of LiCl on endogenous hepatic ethanol and acetaldehyde metabolizing enzymes was also studied as a function of NO_2 exposure This was prompted by the wide misuse of alcohol in general and by changes in activities of these enzymes evoked by Li under normal environmental conditions [26]

METHOD

The subjects were adult albino mice of both sexes obtained from Sprague-Dawley Co , Madison, WI. In the first set of experiments, the effect of pretreatment with LiCl on NO_2 induced mortality was studied. Adult male mice received distilled water or 0.2% LiCl solution as the sole drinking fluid ad lib for 10 consecutive days They were then challenged for 6 hr with either 60 or 70 PPM NO_2. The LD_{50} determined in an earlier study was in this range. The number of animals dying in each group immediately and 24 hr after NO_2 exposure was recorded and expressed as percent mortality The data were analyzed by Chi square analysis

In the second set of experiments the effect of LiCl on hepatic alcohol dehydrogenase (L-ADH) and aldehyde dehydrogenase (L-ALDH) was studied as a function of exposure to NO_2. Forty adult female mice were divided into two groups of 20 and given either water or 0.2% LiCl as the only drinking fluid. Each group was divided into two subgroups of ten mice each and housed in environmental chambers and exposed to either filtered air or 5 PPM NO_2. The LiCl treatment was initiated concomitant with the NO_2 exposure and lasted for ten consecutive days. Animals were sacrificed by decapitation 20 min post termination of the ten day NO_2 exposure period. The liver was removed quickly and weighed prior to homogenization in 0 1 M KCl buffer pH 6.8 The homogenate was fractionated into subcellular mitochondrial (MT) and cytoplasmic (CT) fractions [27] for the enzymatic assays of L-ALDH [3] and L-ADH [4], respectively. Body weight was determined before and after termination of the experiment The enzymatic activity was expressed as specific activity, nMol/min/mg protein, measured at 30°C The kinetics was performed by the Lineweaver and Burk method [23]. Statistical significance of the results was assessed by Student's *t*-test.

The inhalation chambers were constructed of glass and stainless steel and had a volume of 3.24 M^3. Filtered air was drawn into each chamber by an electric blower. The chamber system has been detailed elsewhere [24]. The chamber's NO_2 concentration, temperature, pressure and relative humidity were monitored continuously The airflow was 800 L or 16 0 air changes/hr, at 21±2°C and relative

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TABLE 1

EFFECT OF PRETREATMENT WITH LiCl ON NO_2-PRODUCED MORTALITY IN THE ADULT MALE MOUSE

NO_2 Concentration (PPM)	Percent Mortality			
	Immediately post NO_2 inhalation		24 hr post NO_2 inhalation	
	Controls	Li-treated	Controls	Li-treated
60	67	33	67	50
70	67	17	100	33*

The LiCl was dissolved in distilled water (0 2%) and provided ad lib for 10 consecutive days, (controls received distilled water) Animals were exposed to 60–70 PPM NO_2 for 6 hr Percent mortality was determined immediately, or 24 hr after termination of the NO_2 exposure Each treatment group consisted of 6 mice
*$p<0$ 05

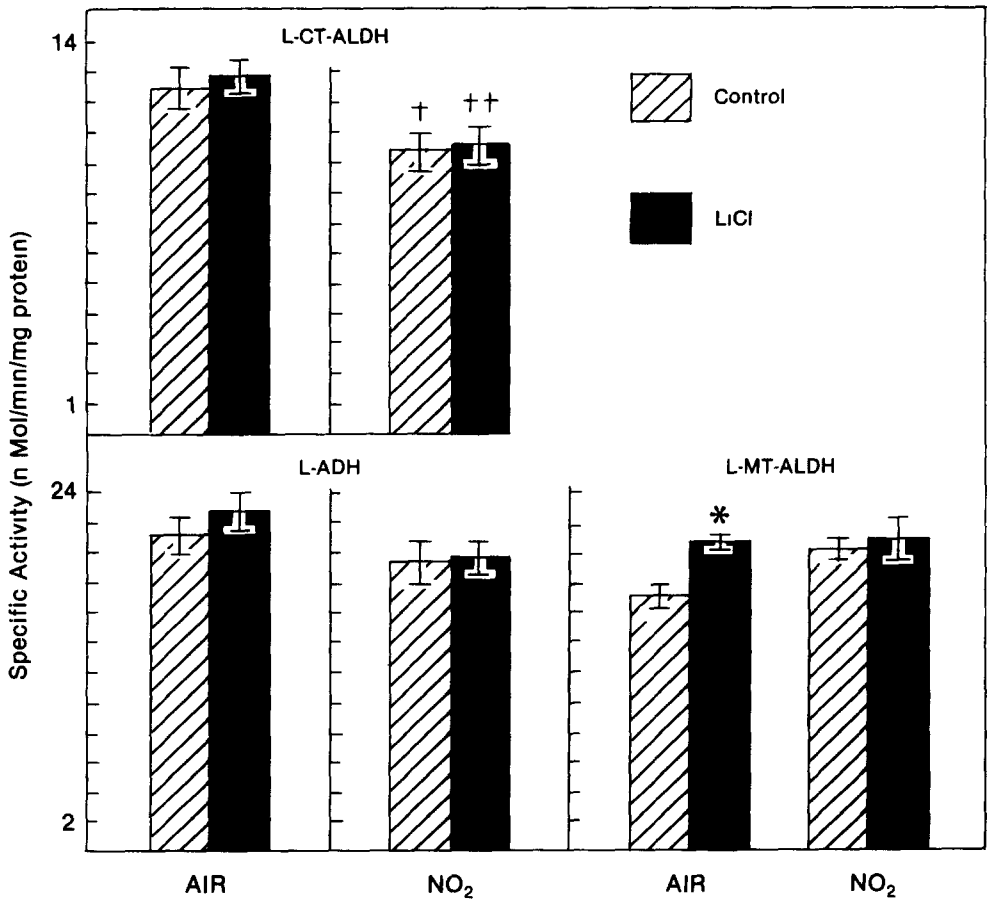

FIG 1 The effect of short-term intake of LiCl on endogenous mouse liver alcohol dehydrogenase (L-ADH) and aldehyde dehydrogenase (L-ALDH) as a function of NO_2 inhalation Animals breathed 5 PPM NO_2 or filtered air for 10 consecutive days They received 0 2% LiCl or distilled water ad lib The L-ALDH was assayed both in cytoplasmic (CT) and in mitochondrial (MT) preparations Values are means of specific activity ±SEM of the mean for 10 independent determinations ††$p<0$ 01, Different from Li-treated mice inhaling air †$p<0$ 05, Different from drug-free mice inhaling air *$p<0$ 05, Different from drug-free controls inhaling air

TABLE 2

EFFECT OF SHORT-TERM EXPOSURE TO NO_2 ON MOUSE BODY AND LIVER WEIGHTS AS A FUNCTION OF TREATMENT WITH LiCl

NO_2 Concentration	Drinking Fluid	Body Weight (g) Initial	Body Weight (g) Terminal	Liver Weight (g) Terminal
0	H_2O	28 2 ± 0 9	29.6 ± 0 9	1.348 ± 0 018
0	$LiCl/H_2O$	29 2 ± 1 2	28 6 ± 0 7	1.298 ± 0 064
5 PPM	H_2O	26 6 ± 1 5	27 2 ± 1 5	1 345 ± 0 067
5 PPM	$LiCl/H_2O$	26 8 ± 0.7	25 9 ± 0 2†	1.141 ± 0 046*

Adult female mice were exposed to 5 PPM NO_2 for 24 hr, for 10 consecutive days Controls were exposed to filtered air. Animals received distilled water or 0 2% (w/v) LiCl solution ad lib Values are means ± SE of the mean of 5 independent determinations

*Different from drug-free mice inhaling NO_2, $p<0$ 05

†Different from air-inhaling mice treated with LiCl, $p<0$ 05

humidity of 48±20% The chamber's NO_2 concentration was produced by mixing NO_2 from heated cylinders (Scientific Gas Products) with air by means of a mass flow controller. The NO_2 concentration was determined daily with a chemiluminescence analyzer (Thermoelectron) verified by the spectrophotometric method of Saltzman [33] The analyzer was calibrated weekly by gas-phase titration.

RESULTS

Table 1 summarizes the effects of 6 hr exposure to NO_2 (60–70 PPM) as a function of pretreatment with LiCl. A 67% mortality was recorded in controls immediately after exposure to 60 or 70 PPM NO_2 compared to 33% and 17% ($p<0.1$) in the LiCl pretreated mice, respectively. Additional death occurred in the group inhaling 70 PPM during the subsequent 24 hr period. Total mortality at this time was 100% in controls and 33% in the Li-treated mice ($p<0.025$). The Li-pretreatment exerted a protective action against the 60 PPM NO_2 exposure which was not statistically significant.

The effect of LiCl treatment on mouse body and liver weights during NO_2 inhalation for 10 days is given in Table 2. The Li treatment had little effect on body or liver weight in animals breathing filtered air However, there was a small but a statistically significant ($p<0$ 05) decrease in both body and liver weights in Li-treated mice breathing NO_2 compared to their controls inhaling air. Treatment with LiCl during NO_2 exposure resulted in a decrease in liver weight of approximately 15% ($p<0.05$) compared to drug-free mice breathing the same NO_2 concentration.

Figure 1 shows the effect of administration of LiCl for 10 consecutive days on mouse L-ADH and L-ALDH as a function of exposure to 5 PPM NO_2 during drug treatment. The Li-treatment induced mitochondrial but not cytoplasmic L-ALDH by approximately 21% ($p<0.05$) in mice housed under air. This effect was not apparent in Li-treated mice breathing NO_2. Conversely, L-CT-ALDH was inhibited by 17 7% ($p<0$ 05) in drug-free mice breathing NO_2. This inhibition was present also after the Li-treatment ($p<0.01$). No changes in specific activity of L-ADH were noted under any of experimental conditions used

Figure 2 illustrates the reciprocals of the velocity of the reaction of L-MT-ALDH against substrate concentration as a function of LiCl treatment in mice breathing filtered air

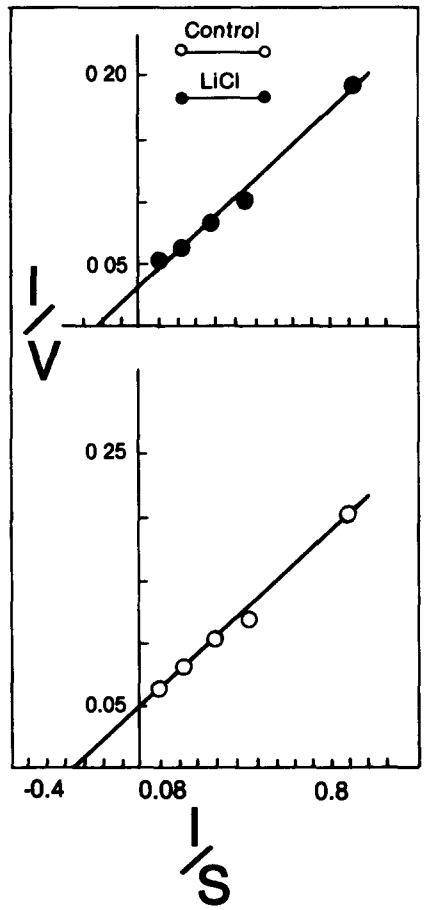

FIG 2 The effect of short-term treatment with LiCl on kinetics of mouse liver mitochondrial aldehyde dehydrogenase. Mice breathed filtered air and received 0 2% LiCl ad lib for 10 days Reciprocal value of the velocity of the reaction (1/v) is plotted against substrate concentration (1/s)

The V_{max} increased from 21.6 to 31.9 units after the Li-treatment Concomitantly, there was an increase in the apparent K_m from 3 9 in controls to 5 9 units in LiCl treated animals

DISCUSSION

This study demonstrates that treatment with LiCl for 10 days prior to exposure to high concentrations of NO_2 (60 and 70 PPM, for 6 hr) protected against NO_2-induced mortality This Li effect may be of clinical relevance if it occurred in man For example, NO_2 toxicity is associated with pulmonary dysfunction, respiratory infection and increased susceptibility to lung tumor development [5, 7–9, 11, 13, 14, 30, 36] Noteworthy, Li-salts possess certain clinical potential in counteracting some of these symptoms Lithium decreases the incidence of infection by various agents [21, 22, 38] causes mild leukocytosis [32], interferes with certain tumor growth [18] and ameliorates asthma attacks [29,31]. Other immunological actions have been reviewed recently [10] It is possible that the pulmonary effects evoked by Li^+ may have minimized the lethal effects of high concentration of NO_2 Moreover, it is likely that the Li-stabilizing effects on cellular membrane [6, 12, 37] may have counteracted the NO_2-mediated increase in vascular capillary permeability and edema formation [15, 17, 35, 39]

The mechanism(s) underlying NO_2 toxicity and its modulation by short-term pretreatment with LiCl, remains to be elucidated Experimental studies on the lethal effect of acute exposure to high concentrations of NO_2 have been focused primarily on pathological changes in the pulmonary system [16, 28, 42] One biochemical mechanism advanced has been related to NO_2-induced decrease in pulmonary lipid content [1,20] due to intercellular formation of free radicals [34, 39, 41] This can be partially prevented by pretreatment with antioxidants [40]. The NO_2-produced decrease in catalase [41] would tend to support this assumption Reduced catalase activity decreases detoxification of peroxides and other free radicals These compounds may accumulate and augment cellular toxicity Therefore, it appears likely that the NO_2 inhibition of L-C-ALDH noted in this study may reflect derangement in some NAD-dependent dehydrogenases as noted for lactate dehydrogenase [2, 9, 25] Lithium produced induction of L-MT-ALDH is consistent with previous reports obtained after prolonged ingestion of LiCl by the mouse [26] In conclusion, Li salts may conceivably have a prophylactic action against NO_2 toxicity

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