*Pharmacology Biochemistry & Behavior*, Vol. 24, pp. 9–13, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

# Interstrain Correlation Between Behavioural Effects of Lithium and Effects on Cortical Cyclic AMP

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HAMBURGER-BAR, R., M. ROBERT, M. NEWMAN AND R. H. BELMAKER. *Interstrain correlation between behavioural effects of lithium and effects on cortical cyclic AMP.* PHARMACOL BIOCHEM BEHAV **24**(1) 9–13, 1986.—Six inbred mouse strains were studied to explore possible correlations between lithium effects on behaviour and on cortical cyclic AMP. The strains were fed lithium in ground food for 3 weeks before behavioural tests and ex vivo evaluation of cyclic AMP accumulation. Replicating previous reports, there was a significant inverse correlation ($r=0.73$, $n=6$) between spontaneous activity and noradrenaline-induced cyclic AMP, and an almost significant correlation ($r=0.67$, $n=6$) between spontaneous activity and adenosine-induced cyclic AMP accumulation. The effect of lithium to depress spontaneous activity correlated with its effect to inhibit adenosine-induced rises in cyclic AMP ($r=0.716$, $n=6$). There were significant strain differences in the behavioural responses to amphetamine. In two strains where amphetamine raised activity and lithium inhibited the amphetamine-induced rise, lithium also significantly inhibited the adenosine-induced rise in cyclic AMP. Two other strains showed amphetamine-induced rises in activity that were not inhibitable by lithium, and these strains showed no significant inhibition by lithium of adenosine-induced cyclic AMP accumulation. The remaining two strains showed no behavioural activity increase with amphetamine.

Lithium Amphetamine Behaviour Noradrenaline Adenosine Cyclic AMP Mouse

AMONG the numerous behavioural effects of lithium in animals, effects on stimulant-induced activity have remained of central interest. Lithium interactions with changes in behaviour induced by amphetamine, for instance, appear a more reasonable model of lithium effects on manic behaviour than lithium effects on unstimulated or normal rodent behaviour [20], since lithium has few effects on normal human behaviour [11]. Many groups have studied the effects of chronic lithium on stimulant induced behaviours in rats or mice (for review see Ebstein *et al.* [8]). While usually lithium has been found to depress amphetamine or methylphenidate induced activity, other studies have found different results [8, 10, 13]. Lerer *et al.* [13] reported that amphetamine dosage is a critical variable, and that low-dose amphetamine effects are inhibitable by lithium whereas high dose effects are not. Given the known pharmacogenetic factors involved in lithium response in human manic-depressive patients [14,15], we decided to look for possible strain differences as an additional factor affecting reports of lithium inhibition of amphetamine induced activity in rodents.

Inhibition of neurotransmitter-stimulated adenylate cyclase is a prominent biochemical effect of lithium treatment [9]. Among the adenylate cyclases inhibitable by lithium, only the noradrenaline-stimulated and the adenosine-stimulated cyclase are inhibitable by lithium at clearly therapeutic concentrations [6, 7, 9]. Human brain noradrenaline-stimulated adenylate cyclase is considerably more sensitive to lithium inhibition than rat brain cyclase [1,17]. This species difference prompted us to look for strain differences in sensitivity to lithium inhibition of noradrenaline and adenosine-induced rises in cyclic AMP in mouse cerebral cortical slices. The existence of mouse strain differences in noradrenaline and adenosine-stimulated adenylate cyclase activity has been previously reported [21]. An inverse correlation between spontaneous behavioural activity and adenosine, but not noradrenaline, induced cyclic AMP accumulation in five inbred mouse strains was reported by Stalvey *et al.* [25]. A similar finding had been obtained by Southwick and Clark [24] in six strains. In four strains of rat, however, Skolnick and Daly [23] found a positive correlation between noradrenaline-induced cyclic AMP accumulation in midbrain-striatum and spontaneous behavioural activity, although in cortex there was an inverse correlation between the two parameters.

In the present work the effect of lithium on noradrenaline and adenosine-induced cyclic AMP accumulation in 6 mouse strains was studied in correlation with effects of lithium on behaviour in the same strains. In addition, the agent forskolin, which stimulates adenylate cyclase independently of interaction with a receptor [22] was also used to raise cyclic

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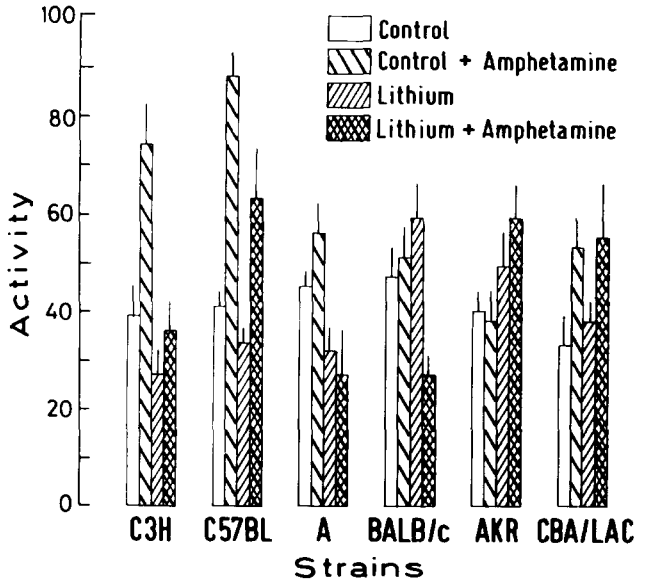


FIG. 1. Strain differences in basal and amphetamine stimulated activity in control and Li-fed mice. Activity is expressed as sum of crossings per 30 minute period.

Statistical Evaluation of Behavioral Test

Analysis of variance Li/amphetamine/strains for 6 strains

| Source of variation | *df* | F | Significance of F |
|---|---|---|---|
| Main effects | 7 | 6.936 | 0.000 |
| food | 1 | 11.560 | 0.001 |
| amphetamine | 1 | 24.890 | 0.000 |
| strain | 5 | 2.785 | 0.018 |
| 2-way interactions | 11 | 6.452 | 0.000 |
| food amph. | 1 | 7.096 | 0.008 |
| food strain | 5 | 6.122 | 0.000 |
| amph. strain | 5 | 7.265 | 0.000 |
| 3-way interactions | 5 | 1.661 | 0.145 |
| food amph. strain | 5 | 1.661 | 0.145 |
| Explained | 23 | 5.558 | 0.000 |
| Residual | 246 | | |
| Total | 269 | | |

AMP levels and the effects of lithium on this rise in the various mouse strains determined. Lithium in vitro has been shown to inhibit the forskolin-induced rise in both cerebral cortical slices and membranes (Newman and Belmaker, submitted for publication).

## METHOD

Male mice of A, $C_{57}$BL, CBA/LAC, $C_3$H, AKR/J and Balb/c strains (40–60 mice for each strain) were obtained from the Hadassah-Hebrew University breeding facilities, at age 2 months of life. The mice were housed 10 per cage with a reversed day-night cycle (dark from 5.00 till 17.00). After 1 week of adaptation half of the mice were fed ad lib with ground chow thoroughly mixed with LiCl to give a final concentration of 0.4% by weight, the other half were fed only with the ground chow. Behavioural testing started after the mice were fed with lithium for three weeks. Body weight after 3 weeks did not differ significantly between the lithium fed mice and controls [6] in any of the strains studied ($C_{57}$BL; control, 22.4±0.3; Li, 22.5±0.3: A; control, 21.0±0.3; Li, 19.6±0.5: $C_3$H; control, 21.7±0.5; Li, 22.3±0.4: CBA/LAC; control, 27.5±0.6; Li, 27.0±0.5: AKR; control, 25.5±0.6; Li, 23.1±0.5: Balb/c; control, 24.2±0.4; Li, 24.0±0.5).

Midline crossings were counted by a photoelectric beam separating the two chambers of the Columbus "Reflex 6" instrument (size of each chamber 29×24×29 cm), and recorded once every 15 seconds for a total of 30 minutes (maximum of 120 possible crossings). Saline or d-amphetamine sulfate 2 mg/kg was administered IP in a volume of 0.02 ml/10 g immediately before testing activity. In each strain there were 4 treatment groups: Control: fed with ground chow and injected with saline before activity test; Control + amphetamine: fed as for the control and injected with amphetamine solution; Lithium: fed with 0.4% lithium and injected with saline; Lithium + amphetamine: fed with 0.4% lithium for 3 weeks and injected with amphetamine before the activity test.

For determination of the cyclic AMP responses, 2 mice of each strain which had received the lithium diet were killed simultaneously with 2 mice fed a control ground chow, and the cortices from two of the same strain pooled. Three strains were examined in each experiment. The cortices were sliced using a McIlwain tissue chopper set at 0.35 mm, and the slices preincubated at 37°C in Krebs-Ringer bicarbonate medium containing 10 mM glucose and 1.29 mM $CaCl_2$ for 30 min, with continual gassing with 95% 0:5% $CO_2$. At the end of the period the slices were collected on a Buchner funnel and distributed among vials containing 5 ml Krebs-Ringer's alone or with addition of 50 $\mu$M noradrenaline, 40 $\mu$M forskolin or 50 $\mu$M 2-chloro-adenosine for a 20 min incubation period. The slices from each incubation were then collected by centrifugation and homogenized in 2 ml ethanol using a Polytron homogenizer at setting 5 for 5 sec. Fifty $\mu$l aliquots of the supernatants were evaporated to dryness under $N_2$ for determination of cyclic AMP by the method of Brown *et al.* [2].

Brain lithium content was estimated by homogenization in 3 volumes of 0.5 N trichloroacetic acid and assayed by flame photometry (EEL with no internal $K^+$ standard). Carotid blood was also taken for lithium determination at the time of sacrifice but the blood levels obtained were not as stable as those in brain, perhaps because the time between ingestion of Li in food and sacrifice is variable. Mean brain levels in the six strains were: $C_3$H, 1.23 mM; $C_{57}$BL, 1.0 mM; A, 0.76 mM; Balb/c, 0.80 mM; CBA, 0.82 mM; and AKR, 0.80 mM. The corresponding mean blood levels were 0.52, 0.86, 0.40, 0.52, 0.44 and 0.60 mM respectively.

There was an excellent interstrain correlation ($r=0.84$, $p<0.05$) between the levels obtained in 2 separate experiments in which brain lithium levels were determined.

## RESULTS

Figure 1 illustrates the behavioural results. There is a significant main effect of lithium to depress mouse activity, $F(1,269)=11.56$, $p<0.001$, and a significant effect of amphetamine to raise mouse activity, $F(1,269)=24.89$, $p<0.001$. The interaction of these two main effects is significant, $F(1,269)=7.09$, $p<0.008$, suggesting that lithium in mice, as in rats [13], significantly reduces amphetamine-induced activity. There is also a significant main effect of strain, $F(5,269)=2.78$, $p<0.018$, in accord with known strain differences in activity level [19]. There is a trend for three-way

TABLE 1

EFFECT OF Li IN VIVO ON THE CYCLIC AMP RESPONSE TO NORADRENALINE, FORSKOLIN AND 2-Cl-ADENOSINE IN MOUSE CEREBRAL CORTEX SLICES

| | Cyclic AMP, pmol/mg protein (mean ± S.E.M.) | | | |
|---|---|---|---|---|
| | | Incubation with | | |
| Strain | Basal (no addition) | 50 μM noradrenaline | 40 μM forskolin | 50 μM 2-Cl-adenosine |
| | | Control Animals | | |
| A | 18.0 ± 3.1 | 46.0 ± 6.8 | 66.6 ± 13.1 | 68.2 ± 4.1 |
| Balb/c | 24.2 ± 5.8 | 61.8 ± 16.0 | 59.3 ± 8.6 | 58.7 ± 12.5 |
| CBA/Lac | 16.6 ± 3.3 | 74.6 ± 10.3 | 86.3 ± 35.6 | 91.9 ± 20.1 |
| C3H | 11.3 ± 2.0 | 62.5 ± 8.7 | 125.4 ± 20.4 | 132.2 ± 23.9 |
| C57Bl | 33.5 ± 1.3 | 66.4 ± 10.4 | 95.4 ± 26.2 | 69.2 ± 8.3 |
| AKR | 24.3 ± 9.6 | 140.4 ± 15.0 | 130.2 ± 22.3 | 108.5 ± 16.8 |
| | | Li-Fed Animals | | |
| A | 13.4 ± 4.5 | 52.0 ± 9.9 | 78.8 ± 7.0 | 44.5 ± 7.2* |
| Balb/c | 22.0 ± 6.5 | 51.6 ± 2.9 | 77.7 ± 16.3 | 69.0 ± 9.6 |
| CBA/Lac | 27.5 ± 5.4 | 78.2 ± 13.0 | 91.8 ± 26.7 | 108.7 ± 9.1 |
| C3H | 14.4 ± 2.0 | 43.7 ± 11.7 | 136.7 ± 32.2 | 60.6 ± 11.7* |
| C57Bl | 33.4 ± 4.9 | 91.9 ± 9.7 | 96.7 ± 9.7 | 91.9 ± 13.4 |
| AKR | 17.7 ± 1.0 | 176.3 ± 23.6 | 145.1 ± 7.6 | 132.5 ± 7.5 |

Results are given as means ± S.E.M. of at least 4 observations in each case.

*Significantly different from corresponding data for control animals by Student's $t$ test, $p<0.05$.

2-Way analysis of variance of cyclic AMP accumulation.

Noradrenaline stimulation: strain effect, $F(5,51)=13.54$, $p<0.05$.

Forskolin stimulation: strain effect, $F(5,50)=2.64$, $p<0.05$.

Cl-adenosine stimulation: Li effect, $F(1,40)=2.85$; Strain effect, $F(5,48)=3.80$, $p<0.01$; Interaction, $F(5,48)=3.31$, $p<0.05$.

interaction of lithium, amphetamine, and strain, which is almost significant, $F(5,269)=1.661$, $p=0.145$. There are two strains ($C_3H$ and A) where amphetamine clearly stimulates activity and lithium blocks this hyperactivity, two strains (Balb/c and AKR) where amphetamine has no clear effect to enhance activity and a lithium effect is not possible to evaluate, and two strains ($C_{57}BL$ and Balb/c) where amphetamine enhances activity but lithium does not block the effect. In one strain (Balb/c) the strong reduction in activity only in the Li + amphetamine treated group might be due to synergism of the toxicity of Li by amphetamine ([4,20], pp. 161–162).

Table 1 summarizes the effects of Li on stimulation of adenylate cyclase activity in the six mouse strains and the appropriate two-way ANOVA's. There are significant strain differences for the noradrenaline-induced rise, $F(5,51)=13.54$, $p<0.01$, the forskolin-induced rise, $F(5,50)=2.64$, $p<0.05$, and the 2-Cl-adenosine-induced rise, $F(5,48)=3.80$, $p<0.01$. There is a borderline significant lithium inhibition of the cyclase only for the Cl-adenosine-stimulated cAMP accumulation, $F(1,48)=2.85$. There is a significant interaction with strains (differential effect by strains of lithium on the cyclase) only for the Cl-adenosine-induced rise, $F(5,48)=3.31$, $p<0.05$.

Comparison of the behavioural and biochemical data show an inverse correlation between spontaneous activity and the noradrenaline-induced cyclic AMP rise ($r=0.73$, $n=6$, $p<0.05$) and an almost significant inverse correlation between spontaneous activity and Cl-adenosine induced cyclic AMP ($r=0.67$, $n=6$, $p=0.06$). There was no correlation of amphetamine-induced activity with either noradrenaline or Cl-adenosine induced cyclic AMP, or of either basal or amphetamine-induced activity with forskolin-induced cyclic AMP. The effect of lithium in reducing basal activity behaviour correlated with its effect in reducing the Cl-adenosine induced cyclic AMP rise ($r=0.716$, $n=6$, $p=0.055$) while there was no correlation with either the noradrenaline or forskolin effects on cyclic AMP. In the case of amphetamine-stimulated activity, the Li inhibition correlated with inhibition both of the noradrenaline-induced rise in cyclic AMP ($r=0.66$, $n=6$, $p=0.07$) and the Cl-adenosine induced rise ($r=0.68$, $n=6$, $p=0.06$).

## DISCUSSION

The present results support the possibility that strain differences may contribute to inter-study variance in the effect of lithium on amphetamine-induced behaviour. Rats in various laboratories, notwithstanding the fact that they do not represent pure strains, may vary as much as the inbred mice strains studied here. The dose of amphetamine in the behavioural studies was chosen in accordance with the report of Lerer *et al.* [13] to be a low dose, in order to maximize the chances that lithium might successfully inhibit the amphetamine effect. However, as reported in several previous studies [3, 5, 16, 19], mice strains vary greatly in their re-

sponse to amphetamine and some strains show no response or depression rather than enhancement of activity. This fact, also noted in the present results where strains A, AKR and Balb/c showed no amphetamine response, tends to obscure the statistical analysis. Two mg/kg of d-amphetamine induced hyperactivity only in the pigmented strains ($C_{57}$BL, $C_3$H and CBA/LAC, see Fig. 1). This confirms data previously reported in the open field situation [12] where 1 mg/kg induced hyperactivity in pigmented strains and hypoactivity or no change in albino strains (Balb/c, A and AKR).

Lithium inhibition of noradrenaline-induced adenylate cyclase in vitro in rats occurs at 2 mM lithium, and in vivo inhibition after chronic lithium treatment was reported only at lithium plasma levels of 1.7 mM [9]. Thus it is not surprising that no significant lithium inhibition of noradrenaline-sensitive adenylate cyclase was found in the present in vivo study, with plasma lithium levels of 0.4–0.8 mM.

Inhibition of adenosine-induced rises in cyclic AMP was significant in vitro at 1 mM lithium in rats [7], but chronic treatment of rats with plasma lithium levels of 0.78 mM did not lead to significant ex vivo inhibition of the adenosine-induced rise [18]. The present results, however, reveal a clear strain effect in this property and two mouse strains have adenosine-linked cyclases that are markedly inhibited by chronic lithium at brain levels of 0.76 and 1.23 mM respectively. However, in vitro addition of 1, 2, or 5 mM lithium led to no inhibition of mouse brain noradrenaline-sensitive adenylate cyclase (results not shown). This is unlike results in rat, rodent or human and is at present unexplained. Forskolin-induced increases in cyclic AMP accumulation show significant strain differences, suggesting the existence of genetic effects on components of the adenylate cyclase complex distal to the receptor. Unlike in vitro results in rat cortex (Newman and Belmaker, submitted), the present ex vivo biochemical evaluation did not reveal lithium inhibition of the forskolin-induced cyclic AMP accumulation.

The inverse correlation between spontaneous behavioural activity and noradrenaline or CL-adenosine induced cyclic AMP accumulation found in the present results agrees with previously published results both in mice [24,25] and rats [23]. Interestingly, the two strains with clear in vivo lithium inhibition of adenosine-stimulated rises in cyclic AMP were the two strains with clear inhibition of spontaneous basal activity by lithium. This suggests that lithium inhibition of adenosine-stimulated cyclase may be the mechanism of the lithium inhibition of spontaneous behavioural activity. However, the range of strains studied showed a restricted variance for the lithium effect on noradrenaline-induced cAMP (not a single strain with clear sensitivity to lithium) and this may have prevented the appearance of a similar correlation with noradrenaline. For lithium inhibition of amphetamine-induced hyperactivity, there was a correlation with the inhibition of both noradrenaline and Cl-adenosine induced cyclic AMP accumulation. Again the two strains in which Li significantly inhibited adenosine-stimulated cyclic AMP accumulation, A and $C_3$H, were those in which Li inhibition of amphetamine-induced activity was greatest. The correlation between lithium inhibition of adenosine-induced cyclic AMP rises and lithium inhibition of amphetamine-induced hyperactivity suggests that these effects may be mechanistically related. Although the correlation between lithium effects on noradrenaline-induced cyclic AMP accumulation and on amphetamine-induced hyperactivity was of similar magnitude, the observation that no single strain showed clear inhibition of the noradrenaline effect at the lithium levels reached in the study, makes this correlation more difficult to interpret.

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