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Effect of Lithium Ions on RNA Synthesis in Mammary Gland Explants of Mice[1] (38854)

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It has been postulated that the stimulatory action of prolactin on RNA synthesis in mammary gland explants of mice may be mediated by an elevated level of cyclic GMP and a reduced or maintained level of cyclic AMP (1, 2). Evidence supporting this hypothesis is as follows. Incubation of explants with cyclic GMP causes a prolactin-like effect on RNA synthesis. In contrast, incubation with dibutyryl cyclic AMP, theophylline, or quinine causes an abolition of the prolactin response. Theophylline and quinine inhibit the enzyme phosphodiesterase and are thereby believed to elevate cyclic nucleotide levels. Thus, cyclic AMP appears to attenuate the prolactin response whereas cyclic GMP mimics it.

Several investigators have demonstrated that lithium ions reduce cyclic AMP levels via an inhibition of adenyl cyclase in several biological systems (3–8). Therefore, if a reduced level of cyclic AMP is an integral part of the mechanism whereby prolactin stimulates RNA synthesis in the mammary gland, it might be predicted that lithium ions would have a prolactin-like effect on RNA synthesis. The studies in this report were designed to test this possibility.

Materials and Methods. Midpregnant (10–14 days) Swiss–Webster mice were purchased from Spartan Research Animals Inc., Haslett, MI. Ovine prolactin (NIH P-S-9) was a gift from the National Institutes of Arthritis and Metabolic Diseases. Porcine insulin (Lot PJ 5682) was donated by Eli Lilly and Company. Other hormones and chemicals were purchased from the following sources: Hydrocortisone from Chas. Pfizer and Co.; nystatin from E. R. Squibb and Sons, Inc.; Medium 199, penicillin, and streptomycin from Microbiological Associates Inc.; ^{3}H-uridine (G, 5 Ci/mmole) from the New England Nuclear Corp.; calf liver RNA (Type III) from the Sigma Chemical Co.; theophylline and guanosine-3′,5′-monophosphate (cGMP) from the Nutritional Biochemicals Corp.; quinine sulfate from Merck and Co.; and N^6-2′ *o*-dibutyryl adenosine-3′,5′-cyclic monophosphate (DBcAMP) from the Boehringer Mannheim Co.

Details of the methods used to prepare and culture mammary gland explants were described earlier (9). In general, explants (about 5 mg each) were prepared and incubated for various periods of time in Medium 199 which, in certain cases, contained 2.5 μg/ml insulin, hydrocortisone, and/or prolactin. When the effects of lithium ions and/or hormones on RNA synthesis were to be studied, 1 μCi/ml ^{3}H-uridine was added to the medium 30 min prior to termination of the incubations. The specific activity of ^{3}H in the RNA was then assessed by methods previously described (9).

Results. The data in Table I show the effects of various concentrations of LiCl on ^{3}H-uridine incorporation into RNA. Low concentrations (1 m*M* and 5 m*M*) of LiCl had prolactin-like effects on RNA synthesis. Further, the magnitude of the 5 m*M* LiCl effect was not different from that of prolactin. In contrast, 50 m*M* LiCl appeared to inhibit RNA synthesis.

Experiments were next carried out to explore the possible relationship of the LiCl and prolactin effects on RNA synthesis. Table II shows the results of five experiments in which the effects of various substances in combination with LiCl were tested. In the first experiment prolactin in combination with LiCl was found to stimulate RNA synthesis to the same degree as either LiCl or prolactin by themselves. The nonadditivity of these effects suggests that these substances are acting via a similar mechanism. Also, LiCl caused a 36.7% increase in the amount of labeled uridine present in 10% TCA-sol-

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uble tissue extracts (data not presented); this response is similar to that previously observed for prolactin (9). Table II also shows that cyclic GMP and LiCl produced non-additive prolactin-like effects on RNA synthesis. In contrast, theophylline, quinine, and DBcAMP abolished the LiCl stimulation of RNA synthesis; these agents similarly suppress the prolactin (1, 2) and cyclic GMP (unpublished data) stimulation of RNA synthesis in the mammary gland.

The time-course for the LiCl stimulation of RNA synthesis also corresponds with the time-courses for the prolactin (9) and cyclic GMP (1, 2) effects. In tissues preincubated with insulin plus hydrocortisone, an effect of LiCl on RNA synthesis was apparent after a 4-hr incubation but not after 2 hr (Table III). Also similar to prolactin is the observation that LiCl has no effect on RNA synthesis in tissues not pretreated with insulin plus hydrocortisone (Table III).

TABLE I. EFFECT OF VARIOUS CONCENTRATIONS OF LiCl AND PROLACTIN ON RNA SYNTHESIS IN MAMMARY GLAND EXPLANTS.[a]

Addition	^{3}H-Uridine incorporation into RNA (dpm/μg RNA)
Control	212 ± 9[b]
1 m*M* LiCl	344 ± 22
5 m*M* LiCl	382 ± 26
50 m*M* LiCl	108 ± 6
Prolactin	410 ± 29

[a] Explants were preincubated for 2 days in Medium 199 containing insulin plus hydrocortisone. Prolactin or LiCl was then added to certain flasks and incubation was continued for 4 hr. 1 μCi/ml ^{3}H-uridine was added to the medium 30 min prior to termination of the incubations.

[b] Numbers represent mean ± standard errors of explants from seven flasks.

Discussion. Since LiCl is known to inhibit the enzyme adenyl cyclase and is thus presumed to reduce levels of cyclic AMP, it would appear that the action of prolactin on RNA synthesis in the mammary gland may be mediated by a reduced level of cyclic AMP. This conclusion is based on the similarity of the LiCl and prolactin responses which are as follows. First, the time-courses for the LiCl and prolactin effects were the same; second, the effects of LiCl and prolactin were not additive; third, agents which elevate levels of cyclic AMP abolish both responses; and, fourth, neither LiCl nor prolactin affects the rate of RNA synthesis in tissues not properly pretreated with insulin plus hydrocortisone. Although LiCl was found to stimulate labeled-uridine uptake into the mammary explants, it is unlikely that a consequential altered specific activity of the uridine pool is responsible for the enhanced labeling of the RNA pool. If this were the case, then one would expect to observe additive effects of LiCl and prolactin on uridine incorporation into RNA; previous studies have shown that prolactin does not alter the specific activity of ^{3}H in the uridine triphosphate pool when tissues are incubated with ^{3}H-uridine (10, 11).

Evidence was previously published which demonstrates that cyclic GMP also probably mediates the action of prolactin on RNA synthesis in the mammary gland (1, 2). Since

TABLE II. EFFECT OF PROLACTIN, CYCLIC GMP, THEOPHYLLINE, QUININE, DBcAMP AND/OR 5 m*M* LiCl ON RNA SYNTHESIS IN MAMMARY GLAND EXPLANTS.[a]

Agent added	^{3}H-Uridine incorporation into RNA (dpm/μg RNA)			
	Control	LiCl	Agent	Agent + LiCl
Prolactin	189 ± 15[b]	261 ± 24	243 ± 18	243 ± 12
5 m*M* Cyclic GMP	161 ± 7	222 ± 8	213 ± 7	218 ± 6
5 m*M* Theophylline	170 ± 10	224 ± 5	156 ± 7	158 ± 9
0.2 m*M* Quinine	178 ± 10	243 ± 12	164 ± 10	168 ± 7
5 m*M* DBcAMP	159 ± 14	219 ± 6	166 ± 5	174 ± 4

[a] Experimental protocol is the same as is described in Table I except that the combination of agents indicated above was added to the medium after the preincubation period.

[b] Numbers represent means ± standard errors of explants from seven flasks.

TABLE III. TIME-COURSE OF 5 m*M* LiCl STIMULATION OF RNA SYNTHESIS IN MAMMARY GLAND EXPLANTS.[a]

Insulin plus hydrocortisone preincubation	Incubation Time	^{3}H-Uridine incorporation into RNA (dpm/μg RNA)	
		Control	LiCl
Yes	2 hr	184 ± 10[b]	181 ± 6
Yes	4 hr	203 ± 16	352 ± 16
No	1 hr	166 ± 18	160 ± 21
No	4 hr	164 ± 10	173 ± 12

[a] Explants were either preincubated for 1 day with medium containing no hormones or for 2 days with medium containing insulin plus hydrocortisone. LiCl was then added to certain flasks and incubation was continued for 2 or 4 hr. 1 μCi/ml ^{3}H-uridine was added to the medium 30 min prior to termination of the incubations.

[b] Numbers represent means ± standard errors of explants from seven flasks.

cyclic GMP and LiCl were shown to produce nonadditive responses, the actions of these two agents on RNA synthesis probably occur via the same mechanism. Thus, since Beavo *et al.* (12) have previously demonstrated that cyclic GMP at physiological concentrations stimulates cyclic AMP phosphodiesterase, it seems tenable that the action of prolactin on RNA synthesis in the mammary gland may be mediated by an elevated level of cyclic GMP and a consequential reduction in the level of cyclic AMP. This sort of mechanism is similar to that previously proposed by Goldberg *et al.* (13) for metabolic actions of certain other hormones.

Summary. LiCl was found to stimulate RNA synthesis in the mammary gland in a manner similar to that of prolactin. Since LiCl is known to inhibit adenyl cyclase and thus to reduce levels of cyclic AMP, it is concluded that a reduced level of cyclic AMP may be one step in the mechanism whereby prolactin regulates the metabolism of the mammary gland.

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