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Effect of Zinc Ions on the Actions of Prolactin on RNA and Casein Syntheses in Mouse Mammary Gland Explants (40705)[1]

JAMES A. RILLEMA

Department of Physiology, Wayne State University, School of Medicine, Detroit, Michigan 48201

Introduction. We have demonstrated earlier that cyclic GMP imitates the stimulatory action of prolactin on RNA synthesis in cultured mouse mammary gland explants (1). Deviller *et al.* (2) have shown that zinc is a potent inhibitor of guanylate cyclase activity in homogenates of lymphocytes. The present studies were therefore designed to test whether zinc ions would affect guanylate cyclase activity in homogenates of mouse mammary tissues. In addition, the actions of prolactin and insulin on RNA and casein synthesis in cultured mouse mammary tissues were tested with zinc ions added to the culture medium.

Materials and methods. Pregnant Swiss-webster mice were used for experimentation 10–14 days after Day 1 of pregnancy; they were purchased from Spartan Research Animals Inc., Haslett, Michigan. Ovine prolactin (NIH-P-S-13) was obtained from the National Institute of Arthritis and Metabolic Diseases. Other substances were purchased from the following sources: hydrocortisone from Charles Pfizer and Company; Nystatin from E. R. Squibb and Sons, Inc.; Medium 199, penicillin, and streptomycin from KC Biological Inc.; [5,6-^{3}H]uridine (41.3 Ci/mmol), [4,5-^{3}H]leucine (51–56 Ci/mmol), guanosine 5′-[α-^{32}P]triphosphate (GTP), and [^{3}H]guanosine 3′,5′-cyclic monophosphate (cyclic GMP) from the New England Nuclear Corporation; porcine insulin from the Eli Lilly Company; GTP and cyclic GMP from the Sigma Chemical Company.

Incubations and preparation of mammary gland explants were accomplished by methods employed earlier (3). Briefly, explants (3–5 mg each) were prepared for incubation and three explants were placed on siliconized lens paper floating on 2 ml Medium 199. When the effect of zinc on the insulin stimulation of RNA synthesis was to be studied, the tissues were cultured for 24 hr in the absence of hormones. Insulin (2.5 μg/ml) and/or zinc were then added to the culture medium and incubation was continued for 4 hr. [^{3}H]Uridine was added to the culture medium for the final hour of incubation. The amount of [^{3}H]uridine incorporated into RNA was then assessed as described earlier (3). If the effects of zinc on the prolactin stimulation of RNA and casein syntheses were to be studied, the explants were initially cultured for 2 days with Medium 199 containing insulin (2.5 μg/ml) and hydrocortisone (1 μg/ml). Prolactin (2.5 μg/ml) and/or zinc were then added to the culture medium and incubations were continued for 4 hr (RNA experiments) or 14 hr (casein experiments). The rates of [^{3}H]-uridine incorporation into RNA and [^{3}H]leucine incorporation into a casein-rich protein fraction were used as indices of the rates of RNA and casein syntheses, respectively; these labeled compounds were added to the culture media (1 μCi/ml) 1 hr prior to termination of the incubations. Methods used to isolate and quantitate the labeled macromolecules were described earlier (3). Zinc was added to the culture medium as the chloride salt.

Guanylate cyclase activity in mammary gland homogenates was assessed by the rate of [α-^{32}P]GTP conversion to cyclic [^{32}P]GMP as was described earlier (4). Briefly, tissues were homogenized 1:10 (w/v) and incubated for 15 min at 30°C with 0.1 m*M* [^{32}P]GTP (0.5 μCi/tube). The cyclic GMP formed during the reaction was isolated by ion exchange chromatography and salt precipitation with $Ba(OH)_2$ and $ZnSO_4$. The cyclic GMP formed per 10 mg tissue was then calculated.

Statistical comparisons were made with Student's *t* test or the multiple *t* test where appropriate.

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Results. Table I shows that zinc ion concentrations of 0.025 m*M* and above abolished the prolactin stimulation of [^{3}H]uridine incorporation into RNA. In addition, 1 m*M* zinc reduced by more than 75% the basal rate of [^{3}H]uridine incorporation. Zinc ion concentrations of 0.5 m*M* and above similarly abolished the action of prolactin on the rate of [^{3}H]leucine incorporation into casein (Table II). Zinc, by itself, caused a concentration-dependent inhibition of the rate of [^{3}H]leucine incorporation. Table III shows the effects of various zinc ion concentrations on guanylate cyclase activity in homogenates of mammary tissues. Zinc ion concentrations of 0.05 m*M* and above inhibited guanylate cyclase activity in a concentration-dependent manner. Finally, the effect of zinc ions on the insulin stimulation of RNA synthesis in cultured mammary tissues was tested (Table IV). A concentration-dependent inhibition of insulin's action on RNA synthesis was observed as the zinc ion concentration was increased. With a 0.1 m*M* zinc ion concentration, the insulin response was no longer significant.

Discussion. These studies show that zinc abolishes the actions of prolactin on RNA and casein syntheses in mouse mammary gland explants. These inhibitory actions of zinc ions occurred at zinc concentrations which inhibited guanylate cyclase activity in broken cell preparations of mammary glands. It was postulated earlier that because cyclic GMP mimics the action of prolactin on RNA synthesis in the mammary gland (1), cyclic GMP may be involved in certain of the actions of prolactin in mammary tissues. Compatible with this hypothesis were the observations that zinc ions both inhibit guanylate cyclase activity and abolish certain of prolac-

TABLE I. EFFECT OF VARIOUS ZINC ION CONCENTRATIONS ON THE PROLACTIN STIMULATION OF RNA SYNTHESIS.[a]

$ZnCl_2$ concentration (*M*)	[^{3}H]Uridine incorporation into RNA (dpm/μg RNA)		
	Control	Prolactin	*P*
Experiment I			
0	171 ± 5	249 ± 11	<0.01
1×10^{-3}	39 ± 4	33 ± 3	N.S.
5×10^{-5}	140 ± 8	132 ± 7	N.S.
2.5×10^{-5}	181 ± 10	180 ± 13	N.S.
1×10^{-5}	190 ± 13	271 ± 10	<0.01
Experiment II			
0	180 ± 11	320 ± 20	<0.01
1×10^{-5}	170 ± 10	324 ± 8	<0.01
1×10^{-6}	158 ± 11	332 ± 16	<0.01
4×10^{-7}	168 ± 10	322 ± 14	<0.01
1×10^{-7}	165 ± 10	320 ± 22	<0.01

[a] Explants were cultured for 2 days with insulin plus hydrocortisone. Prolactin and/or zinc were then added to certain flasks and incubation was continued for 4 hr. [^{3}H]Uridine, 1 μCi/ml, was added to the medium 1 hr prior to termination of the incubations. Numbers represent the means ± standard errors of explants from seven flasks.

TABLE II. EFFECT OF VARIOUS ZINC ION CONCENTRATIONS ON THE PROLACTIN STIMULATION OF CASEIN SYNTHESIS.[a]

$ZnCl_2$ concentration (*M*)	[^{3}H]Leucine incorporation into casein (dpm/mg tissue)		
	Control	Prolactin	*P*
Experiment I			
0	380 ± 14	630 ± 37	<0.01
1×10^{-4}	145 ± 10	154 ± 9	N.S.
5×10^{-5}	257 ± 9	266 ± 18	N.S.
2.5×10^{-5}	350 ± 16	442 ± 13	<0.01
1×10^{-5}	407 ± 22	760 ± 47	<0.01
Experiment II			
0	532 ± 29	877 ± 21	<0.01
1×10^{-5}	490 ± 32	787 ± 32	<0.01
1×10^{-6}	471 ± 17	795 ± 44	<0.01
4×10^{-7}	477 ± 21	796 ± 19	<0.01
1×10^{-7}	522 ± 24	830 ± 25	<0.01

[a] Explants were cultured for 2 days with insulin plus hydrocortisone. Prolactin and/or zinc were then added to certain flasks and incubation was continued for 14 hr. [^{3}H]Leucine, 1 μCi/ml, was added to the medium 1 hr prior to termination of the incubations. Numbers represent the mean ± standard error of explants from seven flasks.

TABLE III. EFFECT OF VARIOUS CONCENTRATIONS OF ZINC ON GUANYLATE CYCLASE ACTIVITY IN HOMOGENATES OF MOUSE MAMMARY TISSUES.[a]

$ZnCl_2$ concentration (m*M*)	Guanylate cyclase activity (pmol/15 min/10 mg)
1	30.6 ± 6.2
0.5	38.0 ± 2.8
0.2	60.9 ± 3.6
0.1	72.1 ± 2.1
0.05	83.5 ± 3.5
0.01	89.8 ± 1.9
0.005	87.9 ± 2.1
0	93.5 ± 5.1

[a] Guanylate cylase activity in 1:10 (w/v) tissue homogenates was measured as described in the Materials and Methods section. Numbers represent the mean ± standard error of quadruplicate determinations.

TABLE IV. EFFECT OF VARIOUS ZINC ION CONCENTRATIONS ON THE INSULIN STIMULATION OF RNA SYNTHESIS.[a]

$ZnCl_2$ concentration (M)	[^{3}H]Uridine incorporation into RNA (dpm/μg RNA)		
	Control	Insulin	P
0	152 ± 11	345 ± 19	<0.01
1×10^{-4}	178 ± 13	210 ± 15	N.S.
5×10^{-5}	158 ± 12	262 ± 29	<0.01
2.5×10^{-5}	157 ± 8	306 ± 15	<0.01
1×10^{-5}	164 ± 13	298 ± 19	<0.01

[a] Explants were cultured for 1 day in the absence of hormones. Insulin and/or zinc was then added to the culture medium and incubation was continued for 4 hr. [^{3}H]Uridine, 1 μCi/ml, was added to the medium 1 hr prior to termination of the incubations. Numbers represent the mean ± standard error of explants from seven flasks.

tin's actions in mammary tissues. It is also possible, however, that other actions of zinc ions may be responsible for its inhibition of prolactin's effects. It was also of interest that the action of insulin on RNA synthesis was abolished by incubation with zinc ions. It is thus possible that the actions of insulin in mammary tissues may be influenced by cyclic GMP.

Recently, Manku *et al.* (5) in Horrobin's laboratory demonstrated that zinc ions at 0.012 mM imitate the actions of prolactin on vascular reactivity in the rat mesenteric vascular bed. It is indeed interesting the zinc ions at 10^{-5} M have no actions in mammary tissues, but imitate the actions of prolactin in the mesenteric vascular bed.

Summary. Zinc ions, at concentrations which were shown to inhibit guanylate cyclase activity, abolished the actions of prolactin on RNA and casein synthesis in cultured mouse mammary gland explants. These observations are compatible with the hypothesis that certain of the actions of prolactin in the mammary gland may be mediated by cyclic GMP.

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