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Influence of Prolactin on Thyroxine-Induced Changes in Hepatic and Tail Enzymes and Nitrogen Metabolism in *Rana catesbeiana* Tadpoles[1] (38228)

RANDAL C. JAFFE[2] AND IRVING I. GESCHWIND
(Introduced by H. H. Cole)
Department of Animal Science, University of California, Davis 95616

Following the demonstration that mammalian prolactin could block thyroxine-induced tadpole tail resorption *in vivo* (1, 2) studies were undertaken to determine the biochemical basis of this antagonism between the 2 hormones. It was found that hepatic levels of carbamyl phosphate synthetase (CPS) (3), ornithine transcarbamylase (OTC), and tyrosine aminotransferase (TAT) (4) are increased to similar levels in tadpoles treated with thyroxine or thyroxine plus prolactin. Despite the absence of any antagonism between prolactin and thyroxine on the activities of CPS and OTC, two of the enzymes of the urea cycle, urea excretion in animals treated with prolactin and thyroxine is significantly lower than in tadpoles treated with thyroxine alone (5).

The simultaneous administration of prolactin and thyroxine does prevent the rises in the tail levels of acid phosphatase (3) and β-glucuronidase (4) which are observed when thyroxine is administered alone. It was also found (4) that prolactin-treated tadpoles have increased levels of water-soluble protein and of nitrogen (on a wet wt basis) in the tail. These increases were found to be due to an increased tail water content in prolactin-treated tadpoles.

This study was undertaken to expand the number of enzymes studied in order to determine if prolactin's antagonism of thyroxine's effects was restricted to the tail. It was also of interest to see if prolactin would inhibit a maturative action of thyroxine in a mammal.

Materials and method. *Rana catesbeiana* tadpoles were obtained from Mogul-Ed (Oshkosh, Wisc.) except for the studies on glutamic dehydrogenase when the supplier was Connecticut Valley (Southampton, Mass.). The maintenance of the tadpoles and their experimental treatment were as described previously (4). Sprague–Dawley rats were from our own colony.

Ovine prolactin (NIH-P-S9) was a gift from the Endocrine Study Section of the N.I.H. L-thyroxine (sodium salt), β-diphosphopyridine nucleotide (DPN), and β-diphosphopyridine nucleotide reduced form (DPNH) were purchased from Sigma Chemical Company. Penicillin G and streptomycin sulfate were purchased from Difco Laboratories.

To measure ammonia and urea excretion levels, tadpoles given either daily prolactin (25 μg) or saline injections were placed in individual beakers containing 100 ml of tap water supplemented with 100 units/ml penicillin G and 100 μg/ml streptomycin sulfate. For the thyroxin-treated groups the tap water also contained 25 μg/l thyroxine. The water was changed every day and filtered through Whatman No. 40 filter paper to remove solid waste. Ammonia was determined by the procedure of Chancy and Marbach (6) and urea by

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[2] Present address: Department of Cell Biology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77025.

the procedure of Archibald (7), as modified by Ratner (8).

The supernatants of livers or tails homogenized in 50 m*M* Tris, pH 7.4, 0.15 *M* KCl, 1 m*M* EDTA, and 5 m*M* β-mercaptoethanol, were used for lactic dehydrogenase assays according to the procedure of Neilands (9). Glutamic dehydrogenase was measured using the supernatant of livers homogenized in distilled water. The assay mixture consisted of 100 μ*M* DPNH, 4 m*M* α-ketoglutarate, 50 m*M* NH_4Cl, 100 μ*M* ADP, 100 μ*M* EDTA and 10 m*M* phosphate buffer, pH 7.4. Protein was determined by the method of Lowry *et al.* (10). Levels of significance were determined using the Student–Newman–Keuls Test (11).

Newborn rats were divided into the following experimental groups (30–36 animals per group) to study the effect of simultaneous prolactin-thyroxine administration on day of eye opening:

1. Control: Injected daily with 0.1 ml 0.9% NaCl ip starting on Day 2 and continued until the eyes opened.
2. Prolactin: Injected daily ip with 100 μg prolactin in 0.1 ml 0.9% NaCl from Day 2 until the eyes opened.
3. Thyroxine: Injected daily ip with 1 μg thyroxine per g body wt in 0.9% NaCl from Days 2–5 and then given saline injections until the eyes opened.
4. Prolactin plus thyroxine: Same injections and injection schedule as for the prolactin and thyroxine groups.

Results. Urea and ammonia excretion. Thyroxine induced an increase in urea excretion which was largely prevented if prolactin was simultaneously administered (Fig. 1), as previous studies by Medda and Frieden (5) have shown. In the same animals the thyroxine group has a higher level of ammonia excretion from Days 4–9 than the control group (Fig. 2). The level of ammonia excretion in the prolactin plus thyroxine group, however, is elevated and maintained to the same extent as that of the thyroxine group.

LDH and GDH activities. Liver protein/g wet wt was examined on the supernatants

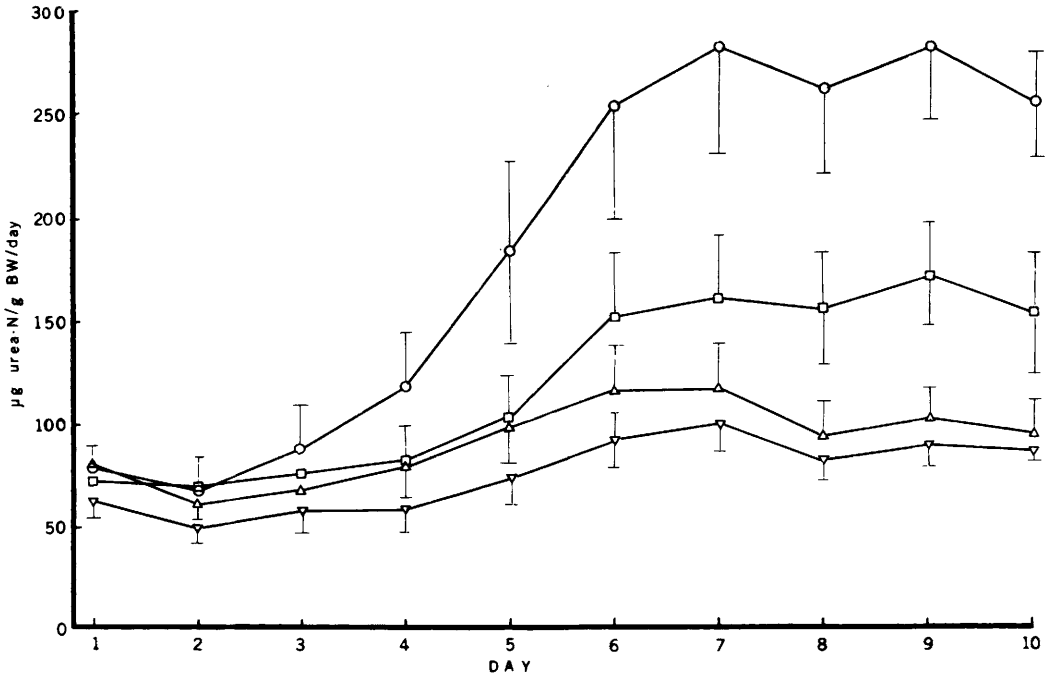

FIG. 1. Effects of prolactin and thyroxine treatment on urea excretion in tadpoles. All points represent the mean of eight tadpoles ± the standard error of the mean except from day 6 on in the thyroxine group, where the number of animals is 7. △—△, control; ▽—▽, prolactin; ○—○, thyroxine; □—□, prolactin + thyroxine.

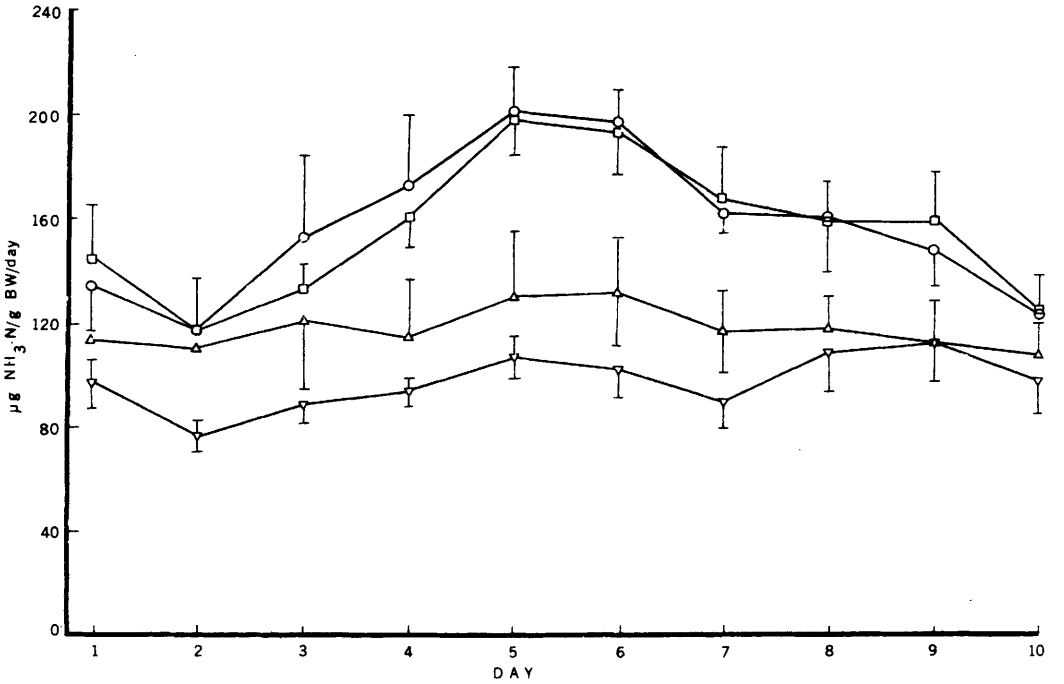

FIG. 2. Effects of prolactin and thyroxine treatment on ammonia excretion in tadpoles. For numbers of tadpoles in each group and for identification of symbols, see legend to Fig. 1.

of extracts used for lactic dehydrogenase analysis. Thyroxine treatment, either alone or when administered with prolactin, caused a significant decrease in protein content after both 7 and 10 days of treatment (Table I). Lactic dehydrogenase (LDH) was chosen for study because its level in the liver was reported to decrease after thyroxine treatment (12). At 7 days there are no significant differences among the 4 groups in LDH activity or specific activity (Table II). After 10 days of treatment, LDH activity and specific activity levels in the prolactin group are elevated above those of the control and thyroxine groups. Treatment with thyroxine for the same period of time led to a decrease in LDH activity. The simultaneous administration of thyroxine with prolactin prevents the prolactin-induced increase in LDH activity. Glutamic dehydrogenase (GDH) was studied because of its role in supplying ammonia for the urea cycle. DeGroot and Cohen (12) have shown that the activity of this enzyme increases with metamorphosis. The data presented in Table III reveal that after 7 days of treatment the thyroxine and prolactin plus thyroxine groups have greater specific activities of this enzyme than those found in both the control and prolactin groups.

TABLE I. The Effects of 7 and 10 Days of Prolactin and Thyroxine Treatment on Protein Content of the Liver.

Group	mg protein/g liver (Mean ± SEM) 7 days	10 days
Control	68.23 ± 2.50[a] (8)	69.50 ± 1.92[a] (8)
Prolactin	63.67 ± 2.32[a,b] (8)	65.62 ± 1.70[a] (8)
Thyroxine	57.87 ± 1.78[b,c] (7)	57.31 ± 1.49[b] (7)
Prolactin + Thyroxine	53.40 ± 1.28[c] (8)	56.72 ± 1.82[b] (8)

[a,b,c] Means with the same superscript in the same column do not differ significantly from each other at $P < 0.05$.

The number of animals in each group is given in parentheses (in all tables).

The response of tail LDH to prolactin and thyroxine treatment is different from the response of hepatic LDH. As can be seen in Table IV, the activity of tail LDH

TABLE II. The Effects of 7 and 10 Days of Prolactin and Thyroxine Treatment on Lactic Dehydrogenase Levels in the Liver.

Group	Enzyme Activity (μmoles NADH formed/min/g liver) 7 days	10 days
Control	33.1 ± 1.7[a] (8)	28.2 ± 1.4[a] (8)
Prolactin	33.9 ± 2.1[a] (8)	33.0 ± 2.0[b] (8)
Thyroxine	27.7 ± 2.4[a] (7)	22.6 ± 1.3[c] (7)
Prolactin + Thyroxine	28.0 ± 1.7[a] (8)	26.4 ± 1.8[a,c] (8)
	Specific Activity (μmoles NADH formed/min/mg protein)	
Control	0.492 ± 0.038[a] (8)	0.409 ± 0.025[a] (8)
Prolactin	0.535 ± 0.030[a] (8)	0.506 ± 0.038[b] (8)
Thyroxine	0.487 ± 0.052[a] (7)	0.394 ± 0.021[a] (7)
Prolactin + Thyroxine	0.526 ± 0.031[a] (8)	0.469 ± 0.024[a,b] (8)

[a,b,c] Means with the same superscript in the same column do not differ significantly from each other at $P < 0.05$.

TABLE IV. The Effects of 7 and 10 Days of Prolactin and Thyroxine Treatment on Lactic Dehydrogenase Levels in the Tail.

Group	Enzyme Activity (μmoles NADH formed/min/g tail) 7 days	10 days
Control	35.7 ± 2.1[a] (8)	40.2 ± 3.1[a] (7)
Prolactin	31.7 ± 1.1[a] (8)	33.4 ± 2.5[a] (8)
Thyroxine	42.7 ± 1.8[b] (7)	50.5 ± 3.1[b] (7)
Prolactin + Thyroxine	36.0 ± 1.4[a] (8)	37.7 ± 2.1[a] (8)
	Specific Activity (μmoles NADH formed/min/mg protein)	
Group	7 days	10 days
Control	1.37 ± 0.07[a] (8)	1.58 ± 0.08[a] (8)
Prolactin	1.46 ± 0.04[a,b] (8)	1.55 ± 0.08[a] (8)
Thyroxine	1.59 ± 0.06[b] (7)	1.57 ± 0.11[a] (7)
Prolactin + Thyroxine	1.52 ± 0.05[a,b] (8)	1.59 ± 0.08[a] (8)

[a,b] Means with the same superscript do not differ significantly from each other at $P < 0.05$.

is increased following thyroxine treatment. Only at 7 days is the specific activity of tail LDH higher in the thyroxine group than in the control group.

Eye-opening in the rat. Thyroxine has a role in the development of higher vertebrates, such as the rat (13), and the possibility that prolactin might antagonize thyroxine's effect on the day of eye opening in the rat was investigated. Thyroxine caused precocious eye opening in the rat two days, on the average, before untreated or prolactin-treated control animals opened their eyes (12.3 ± 0.2 days vs 14.2 ± 0.1 days for the combined control groups), as was observed previously by Schapiro (14). Animals administered both prolactin and thyroxine opened their eyes as early as those treated with thyroxine alone (12.1 ± 0.2 days). (By day 13 only 8 of 66 rats in the 2 control groups had opened their eyes, whereas 67 of 73 rats in the 2 groups receiving thyroxine had opened theirs.)

TABLE III. The Effects of 7 Days of Prolactin and Thyroxine Treatment on Glutamic Dehydrogenase Levels in the Liver.

Group		Enzyme Activity (μmoles NADH formed/min/g liver)	Specific Activity (mμmoles NADH formed/min/mg protein)
Control	(8)	13.3 ± 1.6[a]	172 ± 22[a]
Prolactin	(7)	13.3 ± 2.1[a]	188 ± 26[a]
Thyroxine	(7)	17.1 ± 1.2[a]	284 ± 16[b]
Prolactin + Thyroxine	(7)	18.6 ± 1.6[a]	290 ± 21[b]

[a,b] Means with the same superscript in the same column do not differ significantly from each other at $P < 0.05$.

Discussion. Previous studies on prolactin inhibition of thyroid hormone-induced changes in urea and ammonia excretion have employed either a total of only 3 injections of prolactin at a daily dosage of 1–20 μg/g body wt in *R. catesbeiana* (5) or 1 or 2 injections of 100 μg/g body wt

in *R. grylio* (5, 15). Tadpoles of both species received a single ip injection of triiodothyronine (65 ng/g). The possibility existed that because of a limited duration of action of prolactin (as well as of thyroid hormone), results different from those previously found might be obtained if prolactin and thyroxine were administered throughout the whole course of the experiment. The results presented in Figs. 1 and 2 confirm the earlier findings of Medda and Frieden (5, 15) that prolactin prevents the increased excretion of urea found for thyroxine-treated tadpoles. We also observed a transient rise in ammonia excretion similar to that found by Medda and Frieden (15), in both the thyroxine and prolactin plus thyroxine groups. However, unlike Medda and Frieden (15) who reported that the ammonia excretion of the doubly-treated group declined more rapidly after the fifth day, we found no difference in the ammonia excretion of the 2 groups after the fifth day. This difference in results, and our failure to observe the decrease in urea excretion in the thyroxine-treated group after the seventh day which had been reported by Medda and Frieden (5), may very well have arisen from the more constant hormonal environment provided by our experiments.

Thyroxine-induced metamorphosis increases the liver content of many enzymes (16). In spite of the increased enzyme levels the protein content/g wet liver decreases. The decrease in protein/g wet liver resulting from thyroxine and prolactin plus thyroxine treatments could be due to a decrease in the soluble protein or an increase in nonproteinaceous material. This contrasts with our previous finding (4) that both the prolactin and prolactin plus thyroxine-treated tadpoles have decreased protein/g wet *tail* while the thyroxine group has no such decrease.

DeGroot and Cohen (12) have shown that liver LDH specific activity decreases slightly after 10 days of treatment with $2.6 \times 10^{-8} M$ thyroxine while in our experiments, using a slightly higher concentration of thyroxine ($3.1 \times 10^{-8} M$), LDH activity, but not LDH specific activity, decreased after 10 days of treatment (Table II). The difference in results is probably due to differences in the animals' responsiveness to thyroxine. Of special interest is the significant increase in LDH activity and specific activity after 10 days of treatment with prolactin. Prolactin plus thyroxine-treated animals also showed an increase in LDH specific activity but this increase was not significantly different from the control value. These findings indicate that prolactin has as effect on a liver enzyme.

As mentioned earlier, despite the failure of prolactin to inhibit the thyroxine-induced increases in CPS (3) and OTC (4), 2 of the enzymes of the urea cycle, prolactin does prevent the thyroxine-induced increase in urea excretion. It was therefore deemed important to determine if prolactin's effect on urea excretion resulted from an inhibition of hepatic ammonia production which is required in the urea cycle. As shown in Table III, GDH which is the enzyme which converts glutamic acid to α-ketoglutaric acid and ammonia, which enters the urea cycle, is raised to the same levels after thyroxine and thyroxine plus prolactin treatment. Thus, none of the results points to an effect of prolactin on the formation of urea, and the decreased urea excretion apparently is a consequence of an effect of prolactin at another level, possibly in the kidney.

In the tail, LDH activity levels increased in response to thyroxine (Table IV) while in the liver they decreased (Table II). The increase in tail LDH levels was prevented if prolactin was administered simultaneously, as was the case for the tail lysosomal enzymes acid phosphatase (3) and β-glucuronidase (4).

We have confirmed Schapiro's finding (14) that thyroxine causes precocious eye epening in the rat. We have also found that prolactin, in what must be considered a high dose for a newborn rat (100 μg), when administered simultaneously with thyroxine does not prevent the precocious eye opening seen with thyroxine alone. Prolactin, thus, may have no antagonistic control over thyroxine in the immature rat, although this one study, at 1 dose level of prolactin and measuring a single parameter, can hardly be considered definitive.

This study extends the number of biochemical systems which have been investigated during prolactin inhibition of thyroxine-induced metamorphosis. In the liver prolactin does not antagonize any of those effects of thyroxine which have been examined, while in the tail the opposite is true; prolactin apparently antagonizes all of thyroxine's actions. How prolactin inhibits the thyroxine-induced increase in urea excretion remains a mystery. It may be due to an anabolic effect, as proposed by Medda and Frieden (5), or to an action of prolactin on the kidney inhibiting excretion.

Summary. Thyroxine administered either alone or in conjunction with prolactin caused a decrease in hepatic protein concentration and an increase in hepatic glutamic dehydrogenase activity and specific activity in *Rana catesbeiana* tadpoles. *Hepatic* lactic dehydrogenase activity is decreased after thyroxine treatment, while prolactin alone caused an increase in the activity and specific activity of this enzyme. *Tail* lactic dehydrogenase activity, on the other hand, increased following thyroxine treatment. Thyroxine-treated animals were shown to have increased urea excretion which is prevented when prolactin is simultaneously administered, confirming the findings of Medda and Frieden (5). On the other hand, the pattern of ammonia excretion is identical in tadpoles treated with thyroxine or prolactin plus thyroxine.

The possibility of a prolactin-thyroxine antagonism in mammals was examined in rats. It was found that prolactin, in the dose administered, had no antagonistic action on thyroxine-induced precocious eye opening.

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