Injection of Thyrotrophin-Releasing Hormone in Turkey Embryos Elevates Plasma Thyroxine Concentrations[1]

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ABSTRACT The effectiveness of thyrotrophin-releasing-hormone (TRH) as a secretagogue in turkey embryos was tested. Fertilized turkey eggs were injected with TRH after 24 d of incubation. In an experiment to determine an effective route and dose for TRH administration, it was shown that a single manual injection of 200 μL containing 2.15 μg of TRH, into the air cell or the same injection containing 5.0 μg through the bottom of the egg, was effective in elevating plasma concentrations of thyroxine (T_4) 60 min after injection. In a second experiment, 5 μg of TRH in a volume of 200 μL was injected through the bottom of each egg. Injections were performed mechanically into eggs held in a commercial incubator. The injection increased blood plasma T_4 for 5 h after a 30-min lag. Eggs from two genetic strains of turkeys were injected in Experiment 3. The TRH elicited a persistent response for 120 min from one strain but resulted in a slightly depressed response from the other, suggesting that subtle differences in the maturation of the hypothalamo-hypophyseal-thyroid axis may exist in commercial strains of turkeys.

We concluded that TRH is an effective secretagogue for T_4 in 24-d-old turkey embryos.

(*Key words*: turkey, embryo, thyroid, thyrotropin-releasing hormone)

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INTRODUCTION

The tripeptide, thyrotrophin-releasing-hormone (TRH; pyro Glu-His-Pro NH_2) increased plasma triiodothyronine (T_3) and thyroxine (T_4) when injected into chick embryos during pipping but failed to do so when injected into 17- and 19-d-old prepipping chick embryos (Decuypere and Scanes, 1983). Seventeen-day-old chicken embryos are developmentally similar to 21- and 22-d-old turkey embryos (Abbott, 1967). The injection of TRH into 24-d-old turkey embryos improved their hatchability (Christensen, 1985) as did injection of T_4. Improved hatchability following TRH injection was attributed to its action on the pituitary-thyroid axis.

Because of the involvement of growth hormone with thyroid hormone metabolism, we hypothesized that the response to TRH might differ between embryos from two strains of turkeys with different growth rates. An interaction between thyroid hormones and growth hormone has been observed in young chickens (Harvey, 1983). It was of interest to determine if prepipping poult embryos (24 d of incubation) would respond to TRH injections by increasing plasma T_4 levels and if the response to TRH would be different in embryos from two strains. It is noteworthy that embryos from the fast growth strain survived better when breeder hens were fed additional iodine (Christensen et al., 1991).

MATERIALS AND METHODS

Experiment 1

Approximately 3,000 fertilized turkey eggs with viable embryos were used. One-half of the eggs was divided randomly into six treatment groups of 200 eggs each at 24 d of incubation. Different doses were injected via each route of injection to test the efficacy of the injection procedure in releasing T_3 and T_4. Eggs were injected manually with 1-cc syringes. The doses were 0.1, 0.5, 1.0, 2.5 or 5.0 μg TRH per egg. The TRH solution (200 μL per egg) was deposited onto the air cell membrane covering the embryo. The remaining half in treatment groups of 200 eggs was injected similarly through the small end of the egg. Appropriate vehicle and noninjected groups were also observed.

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Abbreviation Key: TRH = thyrotropin releasing hormone; T_4 = thyroxine; T_3 = triiodothyronine.

Blood samples (between 10 and 24 per treatment) were collected from extra-embryonic blood vessels at 60 min postinjection using procedures described previously (Christensen, 1985). Plasma was recovered by centrifugation under refrigeration (4 C) immediately after collection.

Experiment 2

The ability of a single dose of injected TRH to sustain elevated levels of T_4 was tested. Fertilized turkey eggs were obtained from a commercial turkey hatchery.[3] The eggs from Large White[4] turkey breeder hens in their 14th wk of egg production were examined. Eggs were incubated at 37.5 C dry-bulb and 29.3 C wet-bulb temperatures until the 24th d of incubation in incubators[5] at the commercial company. On the 24th d of incubation, the eggs were removed and divided randomly into three groups of 2,200 eggs each. Each egg in a group was injected with 5 μg of TRH in 200 μL of physiological 0.9% saline (TRH), and a second group was injected with only the saline (vehicle). A third group was designated as an uninjected control (noninjected). All injections were performed using a commercial injecting machine[6] that injected eggs in the small end (Christensen, 1985). Following injection, eggs were returned to the incubator. The entire injection process required approximately 30 min.

Blood was collected by decapitation at 30, 60, 120, 180, or 300 min postinjection (n = 10 embryos per time point per treatment group). Approximately 1 mL of blood from each embryo was collected in a tube containing 10 mg EDTA. Capped tubes were immediately placed into an ice bath where they remained until return to the laboratory. After arrival at the laboratory the blood samples were centrifuged for 30 min at 700 $\times$ *g* and 4 C. Plasma was decanted and frozen at – 20 C until analysis. Approximately 3 h elapsed between initial blood collection and freezing.

Experiment 3

The protocol for the third experiment was nearly identical to the first except two strains of turkeys were used, and the embryos were injected manually in the air cell only. The eggs came from two flocks (N and B) that were 14 wk of age. Samples were collected from 30 to 120 h postinjection at 30-min intervals. The injection procedure was changed so the samples could be collected in the laboratory rather than in the hatchery. The time between sampling and centrifugation was less than 10 min in the third experiment.

Hormone Assays

Plasma thyroid hormones were assayed using commercial reagents[7] and the procedures described by Lien and Siopes (1989). Data in Experiment 1 were compared among doses within routes of injection by analysis of variance (SAS Institute, 1989). Orthogonal contrasts were used to compare all doses to the vehicle control.

Data in Experiment 2 were arranged in a 3 $\times$ 5 factorial with three injection treatments and five sampling time as factors. Data from Experiment 3 were arranged in a 2 $\times$ 3 factorial design with two strains of turkey and three injection treatments. Data in Experiment 3 were analyzed within a time. Means that differed significantly were separated using the least squares means procedure. Significant differences in all analyses were based on $P \leq 0.05$.

RESULTS

All samples from Experiments 1 and 2 were analyzed for T_3 and T_4 in a single assay. Samples from Experiment 3 were within a separate assay. The intraassay coefficients of variation (T_4) were 3.8 and 2.4%, respectively. The sensitivity, based on two SD from maximal binding was 0.78 ng/mL and 0.32 ng/mL, respectively. The intraassay coefficients of variation for T_3 were 2.1 and 3.0%, respectively. The sensitivities, based on two SD from maximal binding, were 0.16 ng/mL and 0.10 ng/mL, respectively.

In Experiment 1 (Table 1) an injection dose of 2.5 μg/egg into the air cell (13.6 $\pm$ 5.8 vs. 6.9 $\pm$ 3.9 ng/mL) or a 5.0 μg/egg into the small end of the egg (20.3 $\pm$ 2.5 vs. 10.9 $\pm$ 3.6 ng/mL) increased circulating blood levels of T_4. The dose of 5.0 μg/egg increased circulating T_3 levels (4.94 $\pm$ 0.82 vs. 1.34 $\pm$ 0.75) as well, but the dose of 2.5 μg/egg did not (0.66 $\pm$ 0.33 vs. 0.33 $\pm$ 0.08). More uniform results were obtained by injecting into the air cell.

Injection of TRH at Day 24 of incubation elevated plasma T_4 levels within 30 min postinjection in Experiment 2 (Table 2). No significant injection by sampling time interaction was observed. The noninjected control data did not differ from the vehicle-injected control treatment. Hormone concentrations were still elevated at the final sampling time in each experiment, suggesting that a single injection of TRH was capable of sustaining elevated T_4 concentrations for at least 300 min.

In Experiment 3, a significant strain by injection interaction occurred at 120 min postinjection (Table 3). The TRH injection resulted in significantly ($P \leq 0.05$) greater blood plasma concentration in the N than the B embryos with no corresponding differences between strains in the vehicle-injected controls.

DISCUSSION

It has been previously reported (Christensen, 1985) that in ovo injections of TRH improved hatchability. It was speculated that this improvement occurred by a compensation for a hypothyroid condition in turkey embryos. The data presented in the current study show that the doses of injected TRH that improved hatchability can also elevate embryonic blood plasma T_4. Plasma T_4 levels were markedly increased within 30 min after TRH injection

[3]Swift-Eckrich Company, Wallace, NC 28466.
[4]Nicholas Turkey Breeding Farms, Sonoma, CA 95476-1209.
[5]Sigma Chemical Company (No. P-9012), St. Louis, MO 63179-9916.
[6]Agrimatic Corporation, Paramount, CA 90723.
[7]Diagnostic Products Corp., 5700 West 96th Street, Los Angeles, CA 90045.

TABLE 1. Effect of TRH[1] injected in ovo into the air cell membrane of 24-d-old embryonated turkey eggs on plasma T_3 and T_4 levels 1 h after administration; Experiment 1

	T_3 (ng/mL)	n	T_4 (ng/mL)	n
Into air cell				
Noninjected vehicle	0.33	10	6.9	13
	0.38	15	8.2	15
0.1 μg TRH	0.54	13	10.3	15
0.5 μg TRH	0.43	16	11.1	15
1.0 μg TRH	0.56	11	11.9	17
2.5 μg TRH	0.66	14	13.6	14[2]
5.0 μg TRH	0.48	15	15.2	16[2]
$\bar{x}$ ± SEM	0.56 ± 0.13		11.0 ± 3.8	
Into bottom				
Vehicle	1.34	24	10.9	24
0.1 μg TRH	1.86	20	14.9	20
0.5 μg TRH	0.64	22	6.9	22
1.0 μg TRH	1.34	22	7.2	22
2.5 μg TRH	0.96	21	7.2	22
5.0 μg TRH	4.94	22[2]	20.3	22[2]
$\bar{x}$ ± SEM	1.85 ± 0.58		11.2 ± 3.9	

[1]TRH = Thyrotrophin-releasing hormone (pyro Glu-His-Pro NH_2).

[2]Significantly different from vehicle-injected controls ($P \leq 0.05$).

into 24-d-old turkey embryos and remained elevated for at least 300 min postinjection.

To the best of the authors' knowledge, these are the first data to show that 24-d-old turkey embryos are capable of responding to TRH. The results of the present study suggest a possible species difference in the thyrotrophic response of the embryonic chick and poult. Previous data (Decuypere and Scanes, 1983; Kuehn et al., 1988) suggested that chicken embryos responded to TRH administration with only small increases in T_4, whereas T_3 increased profoundly. In the current study, T_4 increased fourfold in response to TRH injected into 24-d-old turkey embryos. Blood plasma concentrations of T_3 were not measured in Experiment 2 because of insufficient sample volume, but the data from Experiment 1 comparing TRH injection routes suggest T_3 may be elevated nearly threefold in poult embryos in response to thyrotrophic hormones as well. The route of TRH administration also affected T_3 elevation. Only injection into the bottom of the egg with the largest dose of TRH (5.0 μg) was effective.

A single injection of 5 μg of TRH was sufficient to elevate blood plasma T_4 concentrations for 120 to 300 min. Plasma T_4 levels increased by 30 min after TRH administration (60 min in Experiment 2) but continued to increase until 120 min in Experiment 2 and 300 min after injection in Experiment 1. Thus, the absorption of the 200 μL of vehicle might have occurred over an extended period. These prolonged effects of the treatments were unexpected.

Holoprotein and glycosylated chicken growth hormone were shown to be effective in increasing the hepatic 5′-deiodinase activity in chick embryos (Berghman et al., 1987, 1989). TRH and growth hormone-releasing factor, which are known to release growth hormone in avian species, mimic the effect of growth hormone on hepatic 5′-monodeiodinase (Kühn et al., 1988). Because of the involvement of growth hormone with thyroid hormone metabolism, we proposed the hypothesis that embryos from N and B strain embryos might differ in their responses to injected TRH. The N strain grows faster than does the B (Warnick, 1989; Christensen et al., 1991). The interaction between injection and strain that occurred at 120 min postinjection suggests that by 120 min after TRH administration, the N strain embryos may be more effec-

TABLE 2. Effect of in ovo TRH[1] injection on thyroxine (T_4) concentration (ng/mL) in 24-d-old turkey embryos

	Time postinjection, min (n)					
Treatment[2]	30	60	120	180	300	$\bar{x}$
Control	3.44 (9)	9.22 (10)	6.07 (10)	7.21 (10)	9.63 (10)	7.15[b]
SHAM	4.63 (7)	5.68 (10)	4.89 (10)	7.39 (10)	5.65 (10)	5.68[b]
TRH	63.98 (10)	12.09 (10)	48.25 (10)	9.76 (10)	31.99 (10)	32.92[a]

[a,b]Pooled means with no common superscripts differ significantly ($P \leq 0.001$). The T_4 overall $\bar{x}$ ± SEM = 15.24 ± 1.41.

[1]TRH = Thyrotrophin-releasing hormone (pyro Glu-His-Pro NH_2).

[2]TRH = Injected in ovo with 5 μg in 200 μL saline; SHAM = injected with 200 μL of saline; and Control = not injected.

TABLE 3. Effect of in ovo injection of TRH[1] and strain of turkey on 24-d-embryonic plasma concentration of thyroxine (n = 15)

	Min postinjection			
Injection[2]	30	60	90	120
TRH				
B strain	5.8	10.8	19.9	23.7[b]
N strain	6.2	11.7	18.8	28.4[a]
$\bar{x}$	5.9	11.2[a]	19.3[a]	26.1
Vehicle				
B strain	6.0	5.3	9.9	13.7[c]
N strain	6.0	6.3	7.7	11.1[c]
$\bar{x}$	6.0	6.0[b]	8.7[b]	12.4
$\bar{x}$ ± SEM	6.0 ± 2.1	9.0 ± 2.8	13.8 ± 4.9	19.3 ± 2.7
Probabilities				
Strain (S)	NS	NS	NS	NS
Injection (I)	NS	0.0001	0.0001	0.0001
S × I	NS	NS	NS	0.05

[a–c]Pooled means with no common superscripts differ significantly ($P \leq 0.05$). NS = not significantly different ($P > 0.05$).

[1]TRH = Thyrotrophin-releasing hormone (pyro Glu-His-Pro NH_2).

[2]TRH = Injected in ovo with 2.5 μg in 200 μL saline; Vehicle = injected with 200 μL of saline. B strain = grows slowly; N strain = grows rapidly.

tive in elevating T_4 than are B strain embryos. The differences observed between N and B embryos were very slight or subtle and should be investigated further to verify this observation.

Because of the observations that strains of turkeys selected for growth have depressed plasma concentrations of growth hormone (Proudman and Wentworth, 1980; Bacon et al., 1989), it is suggested that a critical time exists perinatally in strains of turkeys selected for rapid growth when thyroid hormones and possibly glucocorticoids become limiting. Both of these hormone types are necessary for the final maturation of many tissues prior to hatching (Decuypere and Scanes 1983; Decuypere et al., 1991) and are elevated in turkey embryo blood plasma at pipping (Christensen and Biellier, 1982; Wentworth and Hussein, 1985). The maturation effects of thyroid and adrenal hormones may also influence changes in growth hormone (Harvey, 1983). This hypothesis remains to be tested in turkeys, although similar changes have been noted in dwarf strains of chickens (Decuypere and Scanes, 1983).

From the data in the present study, it can be concluded that TRH is an effective T_4 secretagogue in 24-d-old poult embryos. Additionally, T_4 levels were elevated as long as 300 min following a single in ovo injection of TRH. Physiological doses of TRH were shown to be effective secretagogues for T_3 as well but only when injections of large doses occurred through the bottom of the egg. It can additionally be concluded that the embryonic response to TRH may differ between different commercial strains of turkeys.

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