THE EFFECT OF PROSTAGLANDINS $F_{2\alpha}$ AND E_2 ON THYROID HORMONE RELEASE IN THE MOUSE

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INTRODUCTION

Thyroid hormone is primarily required in the regulation of basal metabolic rate. There are four major tissues which are sensitive to it. These are the liver, kidney, heart, and the skeletal muscles. The regulation of the release of thyroid hormone to these tissues has been studied, and several theories have been prepared. These include the role of mast cells, c-AMP, and receptor sites.

Melander and Sundler (1) have studied the relationship of mast cells as a type of "second messenger" in the mechanism of release of thyroid hormone. They used 48/80 (polymer of N-methylhomoanisylamine and formaldehyde) to deplete mast cells of 5-hydroxytryptamine (5-HT), histamine, and metachromatic granules, and found that this depletion caused a decrease in the number of thyroid mast cells. Paoletti (2) found that when mast cells were incubated in vitro with prostaglandin E_1 (PGE_1) or 48/80, there was a significant increase in the depletion of histamine and heparin from these cells.

The effects of Thyroid Stimulating Hormone (TSH) on thyroid hormone release in mice have been studied following pre-treatment with single injections of prostaglandins. Burke (3) observed that TSH and prostaglandins did not have an additive effect on thyroid hormone release. Burke (4) also observed that TSH and PGE_1 both stimulated

thyroid hormone release via increase in thyroid c-AMP. No additive effect was noticed when both hormones were administered simultaneously.

Burke (3) also concluded that the prostaglandins PGE_1, PGE_2, $PGF_{1\alpha}$, and $PGF_{1\beta}$ had a common receptor site with TSH and were competitive for it causing the antagonistic effect on hormone release. Burke (4) later questioned the competition of TSH and the prostaglandins for a common receptor site, and he proposed that the prostaglandins have the receptor site that is essential for TSH action on the thyroid.

The purpose of this research was to elucidate which, if any, of the theories applies to thyroid hormone release.

MATERIALS AND METHODS

Ninety 25 day old female mice of Carworth's CFW strain were used. The animals were separated into 9 groups of 10 animals each (3 groups of controls, 3 groups for PGE_2 treatment, and 3 groups for $PGF_{2\alpha}$ treatment).

An injection timetable similar to that of Melander and Sundler (1) was used. The controls received all injections that the test animals received, except for the prostaglandins. In place of the prostaglandin solution, the control animals only received the carriers for this solution (Appendix A).

Thyroid hormone (Pentahydrate-Sodium Salt of L-Thyroxine) was used as a means of inhibiting endogenous TSH release, and was administered subcutaneously at a concentration of 20 µg in 0.2 ml injection in a saline carrier. TSH (Bovine, NIH-TSH-B6), obtained from NIH, was administered intraperitoneally at a concentration of 0.04 mU in 0.1 ml injection of distilled water carrier. Radioactive iodine (^{125}I) was obtained from New England Nuclear, and was administered intraperitoneally at a concentration of 40 µCi in 0.2 ml injection of physiological saline carrier. The prostaglandins were obtained through the courtesy of Dr. John Pike, Upjohn Laboratories. They were administered subcutaneously in a 0.1 ml injection in increasing dosages of 1.0, 1.2, 1.4, and 1.6 ug in 10 % ETOH and 10 % $NaCO_3$ carrier solution (Appendix A).

The mice were sacrificed by cervical dislocation and 0.2 ml of blood was collected by cardiac puncture. The blood was placed in scintillation vials for determination of ^{125}I activity. Mice from each treatment (control, PGE_2, and $PGF_{2\alpha}$) were sacrificed on the seventh day of treatment before exogenous TSH injection, 2 hours post-injection, and 6 hours post-injection.

The results were analyzed using "F" test for variance and Duncan's New Multiple Range Test using computer program DMV 07V (5). At the time of sacrifice, the thyroids were extracted, placed in Bouin's Solution (6), and prepared for light microscopy as outlined in Appendix B.

RESULTS

There was a noticeable difference in the external appearance of the experimental animals in comparison with the control animals. The control generally maintained a normal coat, while the experimental animals experienced hair loss. Another noticeable difference between the groups of animals was that the prostaglandin groups had a decreased appetite as compared to the control group.

The results of prostaglandin pre-treatment on TSH mediated thyroid hormone release are presented in Figure 1. Analysis of variance (F test) revealed highly significant differences (F=33.0444). Further analysis for mean separation revealed the following results.

In the $PGF_{2\alpha}$ treatment, there was no significant difference before TSH injection and the control. After 2 hours, there was a significant increase of the $PGF_{2\alpha}$ group over the PGE_2 and control groups. The same observation was seen at 6 hours.

In the PGE_2 group, there was a significant decrease from the control group in the sample taken before TSH injection. After 2 hours, PGE_2 was found to be significantly different from $PGF_{2\alpha}$, but not from the control group. At 6 hours, a significant decrease was seen by the PGE_2 group while $PGF_{2\alpha}$ and control groups were still increasing.

In the histological portion of the study, no mast cells were observed in any of the sections, therefore a treatment effect in

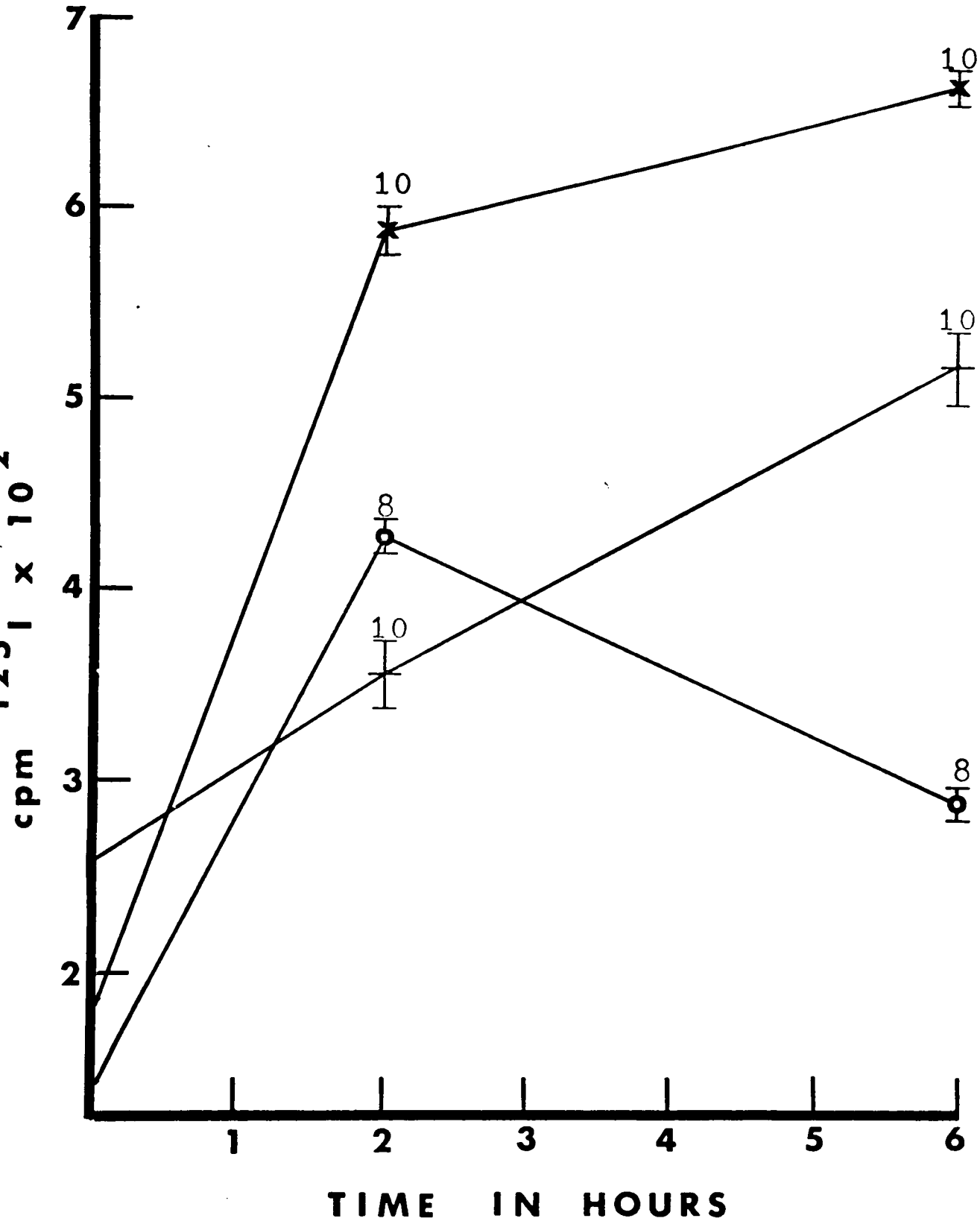
mast cell number was not seen. However, in the control animals, there was an increase in vascularity. This increase became more prominent after TSH injection and increased as time passed. This was also noticed to an even greater extreme in the $PGF_{2\alpha}$ animals. Pre-treatment with PGE_2 produced a marked decrease in vascularity of the thyroid as compared to the other two treatments.

Effects of Prostaglandins E_2 and $F_{2\alpha}$ on Thyroid Hormone Release with Standard Error and Group Size

x= $PGF_{2\alpha}$

0= PGE_2

+= CONTROL



EFFECTS OF PROSTAGLANDINS E_2 AND $F_{2\alpha}$ ON

THYROID HORMONE RELEASE

DISCUSSION

It was shown by this research that PGE_2, when used in conjunction with TSH, caused an overall effect on thyroid hormone release from the thyroid. It was also shown that $PGF_{2\alpha}$, when used with TSH, caused an increased additive effect in thyroid hormone release from the thyroid.

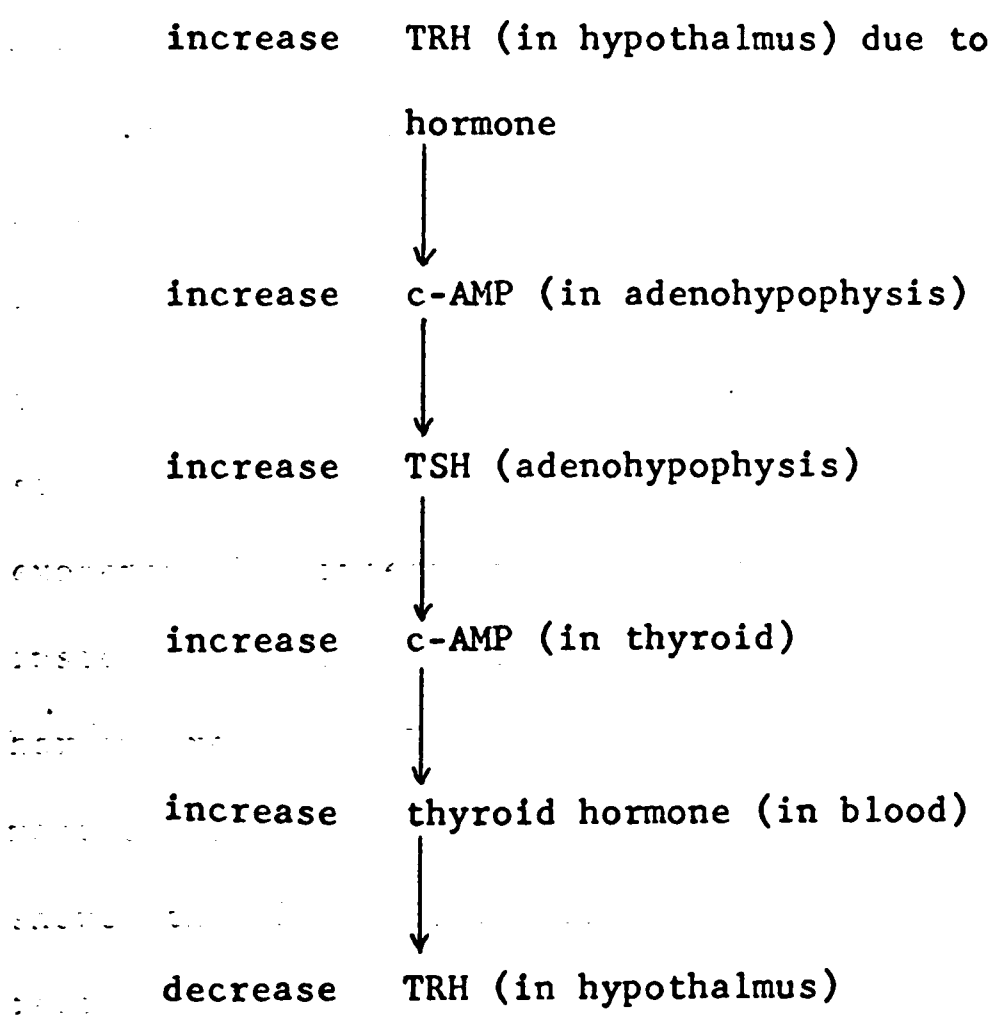
Burke (3) showed that PGE_1, PGE_2, $PGF_{1\alpha}$, and $PGF_{1\beta}$, along with TSH, caused antagonistic effects on thyroid hormone release from the thyroid. Ahn (7) showed that PGE_1 caused an antagonistic effect on the release of thyroid hormone from the thyroids of dogs when used with TSH.

One theory for thyroid hormone release from the thyroid has been that proposed by Melander and Sundler (1) using mast cells as the intermediates. They found that the rats have mast cells in their thyroids before and after treatment with 48/80 and TSH. They also stated that mice only have mast cells present in their thyroids after exogenous TSH injection. These workers used the compound 48/80, instead of prostaglandins, and found this substance to block thyroid hormone release. They speculated that the 48/80 was depleting the mast cells of histamine, 5-HT, and its metachromatic granules. They showed that 5-HT was the compound instrumental in thyroid hormone release. This theory could be applied in the present study if there had been mast cells found in the $PGF_{2\alpha}$ group. Since there were no

mast cells in the thyroid such as found by Melander and Sundler (1) with 48/80, this theory appears inappropriate in light of the above results.

While this theory might apply to rats, where mast cells are present, it could not be used for mice, where mast cells were only found after exogenous TSH injection as reported by Melander and Sundler (1).

Another mechanism proposed for thyroid hormone release from the thyroid involves c-AMP. TSH is known to cause an increase in adenyl cyclase, which will effect an increase in the amount of thyroid hormone released from the thyroid. The mechanism (8) postulated is:



Burke (3) showed that TSH and the prostaglandins both caused an increase in adenyl cyclase when administered separately. There was

also an additive effect when the prostaglandins were given together with TSH. However, he found that they did not have an additive effect on thyroid hormone release. In this study, PGE_2, when given with TSH, agreed with Burke's findings, but $PGF_{2\alpha}$ plus TSH showed an additive effect on thyroid hormone release. This theory cannot be discounted since the data of this research neither supports nor negates the theory. Thyroid hormone release and not c-AMP was the parameter measured.

Burke's hypothesis of receptor sites (4) remains the main theory of thyroid hormone release. The PGE_2 group would be surmised as being competitive with the TSH group, while the $PGF_{2\alpha}$ group could possibly have a completely different receptor site. This would explain its increased additive effect with TSH on thyroid hormone release.

In summary, the thyroid mast cell theory probably does not apply to the data of this research, while the c-AMP theory cannot be discounted or supported. This leaves receptor sites as perhaps the most plausible avenue for future research.

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APPENDIX

A. INJECTION TIMETABLE

B. HISTOLOGICAL PREPARATION OF THYROID MAST CELLS

APPENDIX A

INJECTION TIMETABLE

Experimental

Day	T_4 Dosage	TSH Dosage	Prostaglandin Dosage	^{125}I Dosage
0	20/µg/mouse	----------	----------	40 µCi/mouse
1	----------	----------	----------	----------
2	20 µg/mouse	----------	1.0 µg/mouse	----------
3	----------	----------	1.2 µg/mouse	----------
4	20 µg/mouse	----------	1.4 µg/mouse	----------
5	----------	----------	1.6 µg/mouse	----------
6	20 µg/mouse	----------	----------	----------
7	----------	0.04 mU/mouse	----------	----------

Control

0	20 µg/mouse	----------	----------	40 µCi/mouse
1	----------	----------	----------	----------
2	20 µg/mouse	----------	----------	----------
3	----------	----------	----------	----------
4	20 µg/mouse	----------	----------	----------
5	----------	----------	----------	----------
6	20 µg/mouse	----------	----------	----------
7	----------	0.04 mU/mouse	----------	----------

APPENDIX B

HISTOLOGICAL PREPARATION OF THYROID MAST CELLS

	SOLUTION	TIME IN SOLUTION
1.	Bouins Solution	24 hours
2.	50% ETOH	10 minutes
3.	70% ETOH	15 minutes
4.	85% ETOH	15 minutes
5.	95% ETOH	15 minutes
6.	100% ETOH	10 minutes
7.	100% ETOH	15 minutes
8.	Xylene-ETOH (1:1 Mixture)	5 minutes
9.	Xylene	5 minutes
10.	Xylene	5 minutes
11.	Xylene-Paraffin	10 minutes
12.	Paraffin	30 minutes
13.	Paraffin	30 minutes
14.	Embed in Paraffin	----------
15.	Trim Block	----------
16.	Section	----------
17.	Xylene	5 minutes
18.	100% ETOH	5 minutes
19.	95% ETOH	5 minutes
20.	70% ETOH + $LiCO_3$ (Few Drops)	5 minutes
21.	60% ETOH	5 minutes

APPENDIX B--(continued)

	SOLUTION	TIME IN SOLUTION
22.	Toluidine Blue	1½ minutes
23.	Rinse in Tap Water	----------
24.	Acetone	3 minutes
25.	Acetone	2 minutes
26.	Xylene	----------
27.	Mount in Permount	----------
28.	Observe under oil immersion	----------