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The Effects of Hypophysectomy and Testosterone on Testicular Prostaglandin F₂ α

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THE EFFECTS OF HYPOPHYSECTOMY AND
TESTOSTERONE ON TESTICULAR PROSTAGLANDIN
F₂ α CONCENTRATION IN RATS

by

Diane Charron Beuving

A Thesis
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Diane Charron Beuving

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INTRODUCTION

The presence of prostaglandins (PG's) in the mammalian male reproductive system has been well documented (1), however their physiological importance remains speculative. PG's have been shown to either increase or decrease testosterone production by decapsulated testes in vitro from rats and mice depending upon the concentration of PG added to the incubation media. Increased androgen synthesis was observed when 10^{-5} to 10^{-7} M prostaglandin was added to the media whereas 10^{-3} to 10^{-4} M PG caused a suppression in steroidogenesis (2).

Prostaglandins may also play a role in the transport of sperm from the testis to the epididymus. Prostaglandins have been shown to stimulate testicular capsule contractions in vitro. This stimulation was inhibited, in a dose dependent manner, by the addition of testosterone to the incubation media at concentrations of 4-20 μ M. It was therefore postulated that there is an interaction between androgens and prostaglandins in vivo that modulates testicular contractility (3).

It is difficult to assess the physiological importance of prostaglandins in vivo. Inhibition of PG synthesis by compounds such as indomethacin or aspirin cause a suppression of PG synthesis in the central nervous system as well as in the reproductive tract. This results in a suppression of gonadotropin release (4). Similarly, administration of exogenous PG causes an increase in gonadotropin and prolactin release (2).

The control of prostaglandin concentrations in the male reproductive tract remains as illusive as their function. Bartke and Koerner (5) measured PGF in the testes, epididymides, vas deferentia, seminal vesicles, coagulating glands, and prostates of intact and castrated rats, as well as castrated rats treated with testosterone propionate (500 μ g/day). They reported that castration caused a significant suppression in PGF concentrations which were restored by testosterone propionate. They have proposed a direct androgenic control of prostaglandin synthesis in the male reproductive tract.

Sutherland et al. (6) have also proposed an androgenic control of prostaglandins in the male reproductive tract. They measured PGF in the prostate and seminal vesicles of mature intact and castrated rats, and in castrated rats treated with testosterone. They observed an increase in PGF concentration in the tissues from castrated rats. Prostaglandin F concentrations in the castrated rats treated with testosterone were reported to be suppressed to the normal concentrations. They have proposed an androgenic control mechanism whereby androgens inhibit PG synthesis in the male reproductive tract.

Ellis et al. (7) measured prostaglandin synthetase activity in the testes of immature intact and hypophysectomized rats. They reported a decrease in prostaglandin synthetase activity in the hypophysectomized rats. From these data they concluded that prostaglandin synthesis in the testes is controlled by gonadotropins.

These authors also measured prostaglandin synthetase activity in the testes from mature intact and adrenalectomized rats. They

observed a decrease in prostaglandin synthetase activity following adrenalectomy. They concluded from these data that prostaglandin synthesis is also under an adrenal dependency (7).

In an attempt to further clarify the control mechanism of testicular $\text{PGF}_2\alpha$ concentration, the effect of hypophysectomy on testicular concentrations of $\text{PGF}_2\alpha$ in mature rats was examined. The effect of testosterone on testicular $\text{PGF}_2\alpha$ in mature hypophysectomized and intact rats was also examined.

MATERIALS AND METHODS

Protocol

Silastic implants containing either testosterone or cholesterol were made by packing 4.5 cm of Silastic tubing (0.062" i.d., 0.125" o.d.) with the crystalline steroid (The Upjohn Company). The ends were sealed with Dow Corning Medical Adhesive. Testosterone propionate (TP) was obtained from The Upjohn Company as a 50 mg/ml solution in cottonseed oil, and was further diluted to 20 mg/ml with cottonseed oil.

Hypophysectomized rats (250-300 g) were obtained from Hormone Assays, Inc. within 24 hours after surgery. They were housed in individual cages in a temperature and humidity controlled room (23°C) with a light cycle of 13 hours of light, 11 hours of dark. The rats were maintained on 5% glucose and Purina rat chow ad libitum. The diet was supplemented with apple and/or orange slices daily.

Intact rats of the same strain and size range were also obtained from Hormone Assays, Inc. They were housed and maintained identically to the hypophysectomized rats with the exception that water was provided in place of 5% glucose.

Hormone treatment was initiated as soon as the animals arrived. Hypophysectomized rats received either a testosterone or cholesterol implant subcutaneously, or daily subcutaneous injections of 2 mg TP in 0.1 ml cottonseed oil. Intact rats were treated with TP (2 mg/day) or 0.1 ml cottonseed oil. After 2 weeks of treatment the rats

were decapitated, the trunk blood collected, and the serum harvested. The cranial vaults of hypophysectomized rats were examined grossly for remnants of pituitary tissue. One testis from each animal, chosen at random, was removed, weighed, and immediately frozen on dry ice. The remaining testis was fixed in Bouin's solution for subsequent histological examination.

The mean testicular weight for each group of animals was compared to that of the intact rats treated with cottonseed oil by performing a oneway analysis of variance. Statistical significance was assumed at $p = 0.01$.

Prostaglandin $F_{2\alpha}$ Assay

The testes were homogenized in saline containing indomethacin (0.1 mg/ml) with a PCU-110 Polytron Tissue Homogenizer while submerged in an ice bath. Duplicate aliquots (0.3-0.5 ml) were acidified to pH 4 with 1N HCl and extracted twice with 3 mls ethyl acetate. The extracts were combined and the solvent evaporated at 37°C under nitrogen gas.

The tissue extracts were chromatographed on prepacked Sephadex LH-20 columns (1 g Sephadex LH-20 in methanol, bed dimensions were 8 x 253 mm, Isolab, Inc.). The methanol was drained from the columns, and the columns rinsed with 20 mls methylene chloride:ethyl acetate, 85:15 (MC:EA). The samples were applied to the columns with 2 x 0.1 ml MC:EA with 2% methanol and 0.5% acetic acid. Ten mls of MC:EA with 2% methanol were then applied to each column, and the columns allowed to drain. Fifteen mls of MC:EA with 10% methanol

were then passed through the columns and collected. The elution pattern is illustrated in Figure 1. The solvent was evaporated at 37°C under nitrogen gas. One ml glass distilled absolute ethanol was added to each tube. The tubes were corked and stored at 4°C until the time of assay.

Two 0.1 ml aliquots from each sample were assayed for $\text{PGF}_2\alpha$ with a specific radioimmunoassay (8). The ethanol was dried at 37°C under nitrogen gas and 0.2 ml tris buffer was added to each sample tube. The first antibody dilution was 1:5000, and the tritiated $\text{PGF}_2\alpha$ ($^3\text{H-PGF}_2\alpha$, obtained from Amersham with a specific activity of 14.4 Curies/mole) and second antibody concentrations were adjusted to produce 80% binding in the zero-dose tubes.

Procedural losses, as monitored by the recovery of $^3\text{H-PGF}_2\alpha$, were found to be less than 5%. Therefore the reported values were not corrected for procedural losses.

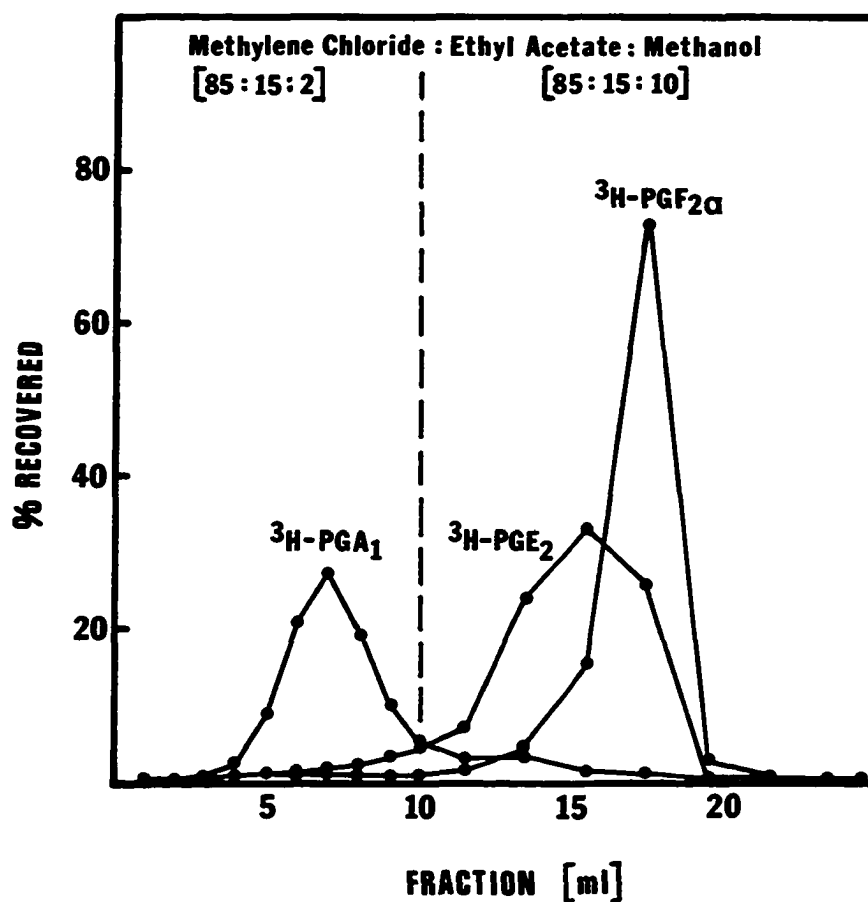
The concentration of $\text{PGF}_2\alpha$ in the testes (ng $\text{PGF}_2\alpha$ /g tissue) was calculated. A oneway analysis of variance with a rank transformation was performed. The Bonferroni method of multiple comparisons (9) was used to determine statistical significance. To assure an overall error rate of $p = 0.05$, the individual comparisons were made at $p = 0.005$.

Testosterone Assay

Two 10-100 μl aliquots of serum were extracted with 2 x 2.5 mls benzene:hexane, 1:2. The extracts were combined and dried at 37°C under nitrogen gas. Testosterone concentrations were estimated

Figure 1

Chromatography of Prostaglandins



Tritiated PG's were applied to separate columns and chromatographed as described. Ten 1 ml fractions followed by seven 2 ml fractions of the effluent were collected. The solvent was evaporated, scintillation fluid added, and the amount of radioactive PG recovered was measured.

using a specific radioimmunoassay for testosterone (10). Group means were calculated and compared to that of intact rats treated with cottonseed oil by performing a oneway analysis of variance. Statistical significance was assumed at $p = 0.01$.

RESULTS

The mean concentration of serum testosterone in hypophysectomized rats treated with testosterone implants (6.72 ng/ml) was not significantly different from that of the intact rats treated with cottonseed oil (3.37 ng/ml). Serum testosterone concentrations for the remaining groups of animals are reported in Table 1.

Hypophysectomy caused a significant decrease in testicular weight. Testosterone, when given to hypophysectomized rats, resulted in a dose-dependent increase in testicular weight. Testosterone propionate given to intact rats (2 mg/day) did not affect testicular weight (Table 1).

Microscopic examination of hematoxylin and eosin stained sections of the testes demonstrated the presence of spermatogenesis in all intact rats, as evidenced by maturing sperm in the lumen of the spermatogenic tubules (Figures 2, 3). Spermatogenesis was maintained in hypophysectomized rats by either a testosterone implant or TP injections (Figures 4, 5). Spermatogenesis was severely depressed in hypophysectomized rats treated with cholesterol implants (Figure 6).

Testicular $\text{PGF}_2\alpha$ concentrations are illustrated in Figure 7. Hypophysectomy resulted in a three fold increase in the testicular concentration of $\text{PGF}_2\alpha$. Testosterone given to intact rats at doses sufficient to cause a suppression in gonatropin release (11) did not result in the increase in testicular $\text{PGF}_2\alpha$ concentrations observed in hypophysectomized rats. When testosterone was given to hypophysectomized rats at either physiological doses (testosterone

TABLE 1

Serum Testosterone Concentrations and Testicular Weights

Treatment	n	Serum Testosterone ng/ml	Testicular Weight g
Intact + oil	12	3.67 \pm 0.69	1.73 \pm 0.04
Intact + TP	12	41.14 \pm 2.56*	1.69 \pm 0.04
H + TP	10	69.67 \pm 3.05*	1.42 \pm 0.03*
H + CI	10	1.84 \pm 0.62	0.91 \pm 0.03*
H + TI	10	6.72 \pm 1.00	1.29 \pm 0.04*

H - Hypophysectomized

TP - Testosterone Propionate

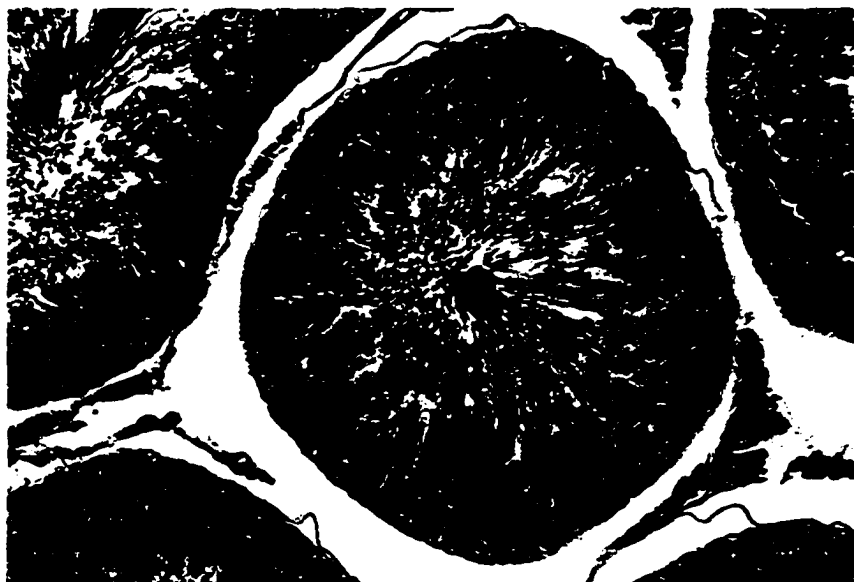
CI - Cholesterol Implant

TI - Testosterone Implant

* - $p < 0.01$ as compared to intact rats treated with cottonseed oil

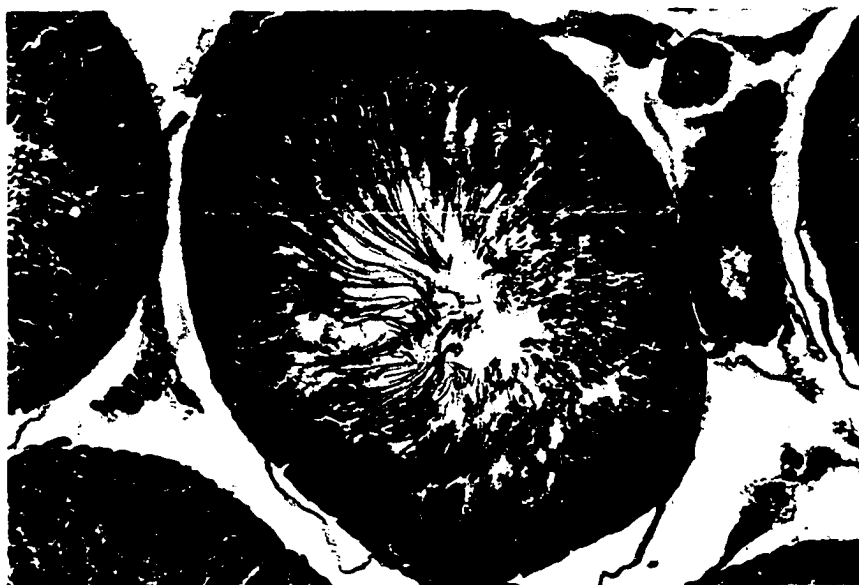
Reported values represent group means \pm SEM.

Figure 2



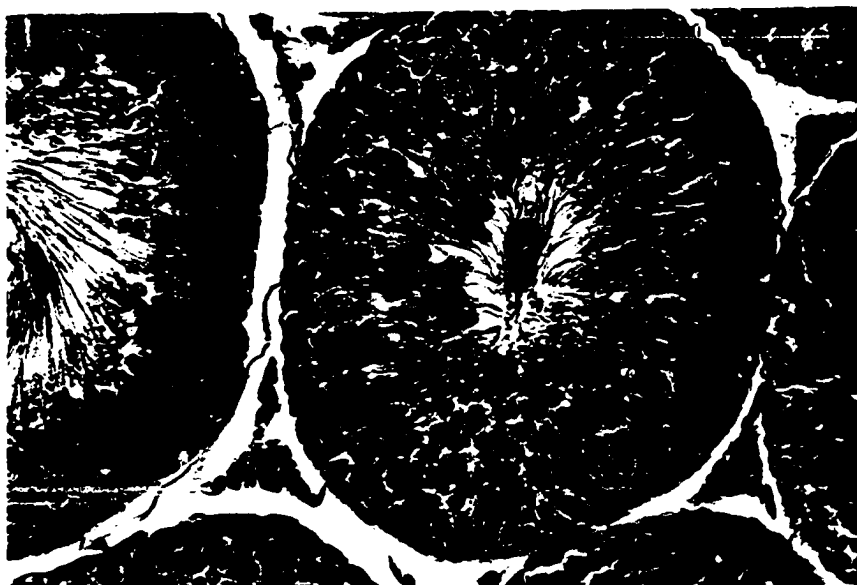
Hematoxylin and eosin stain section of the testis from an intact rat treated with cottonseed oil (250x).

Figure 3



Hematoxylin and eosin stain section of the testis from an intact rat treated with testosterone propionate (250x).

Figure 4



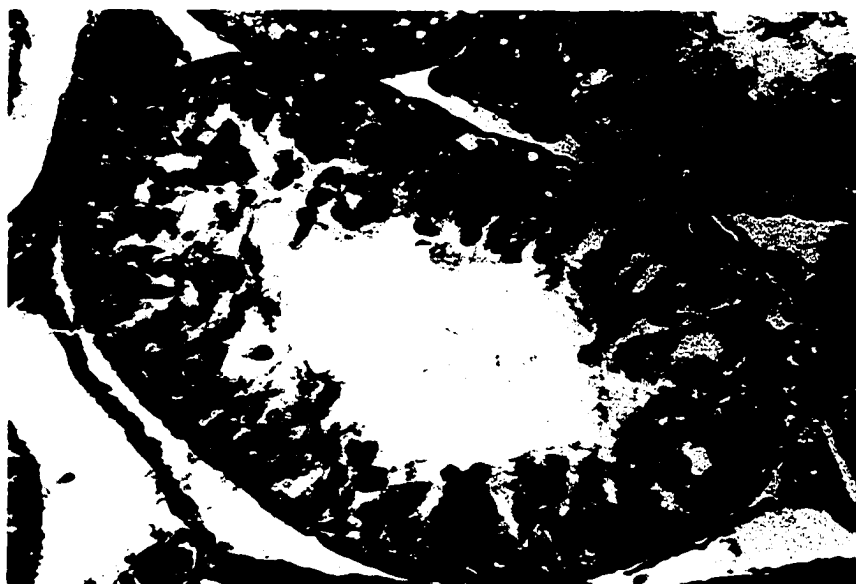
Hematoxylin and eosin stain section of the testis from a hypophysectomized rat treated with a testosterone implant (250x).

Figure 5



Hematoxylin and eosin stained section of the testis from a hypophysectomized rat treated with testosterone propionate (250x).

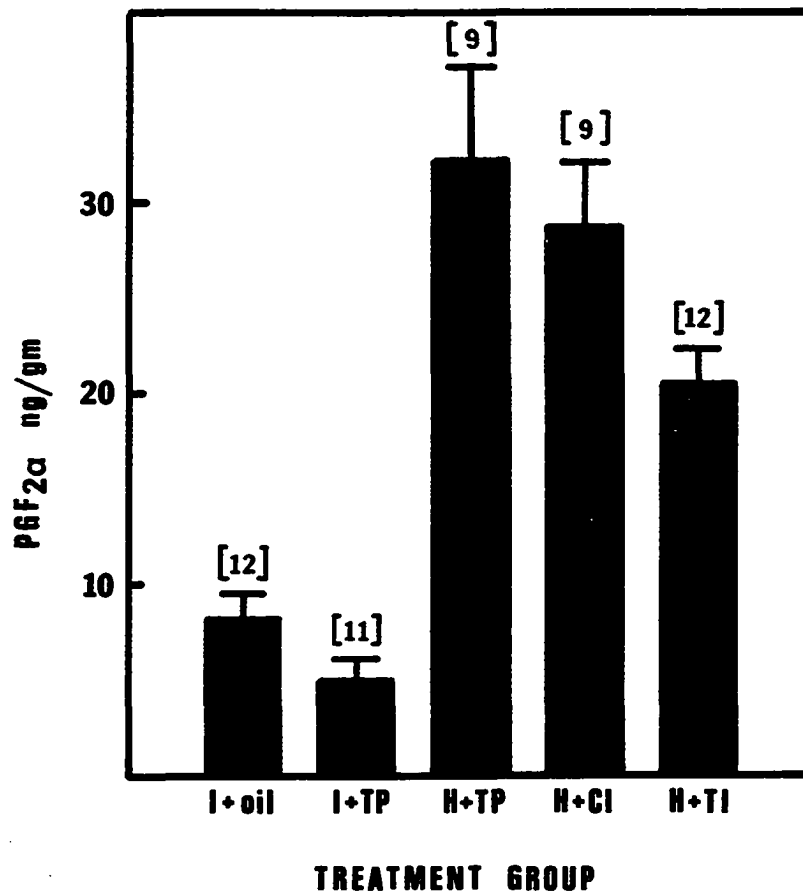
Figure 6



Hematoxylin and eosin stained section of the testis from a hypophysectomized rat treated with a cholesterol implant (250x).

Figure 7

Testicular $\text{PGF}_{2\alpha}$ Concentrations in Hypophysectomized
and Androgen Treated Rats



I - Intact

H - Hypophysectomized

TP - Testosterone Propionate

CI - Cholesterol Implant

TI - Testosterone Implant

The reported values represent group means \pm SEM. The number of animals in each group is given in parentheses.

implants) or pharmacological doses (TP injections) the increase in testicular $\text{PGF}_2\alpha$ concentrations caused by hypophysectomy was not suppressed (Table 2).

Table 2

Testicular PGF₂α Concentrations in
Hypophysectomized and Androgen Treated Rats

Treatment	n	Testicular PGF ₂ α ng/g	Testicular PGF ₂ α Mean Rank
Intact + Oil	12	8.14 ± 1.32	15.83 ± 1.93
Intact + TP	11	5.70 ± 1.33	9.32 ± 2.42
H + TP	9	32.32 ± 5.00	41.67 ± 3.23
H + CI	9	28.79 ± 3.40	40.61 ± 2.91
H + TI	12	20.48 ± 2.32	33.17 ± 2.62

H - Hypophysectomized
 TP - Testosterone Propionate
 CI - Cholesterol Implant
 TI - Testosterone Implant

The reported values represent group means ± SEM. The analysis of variance was performed on the ranked data. The mean rank for each group of hypophysectomized rats was different from that of both groups of intact rats. The mean rank for each group of hypophysectomized rats was not different from that of the other groups of hypophysectomized rats. The mean for the intact rats also were not significantly different from each other.

DISCUSSION

The results reported here demonstrate a highly significant increase in testicular $\text{PGF}_2\alpha$ concentrations in mature hypophysectomized rats as compared with intact rats ($p < 0.005$), suggesting that the testicular $\text{PGF}_2\alpha$ is under inhibitory hypophyseal control. This conclusion is further supported by the observation that testosterone given to hypophysectomized rats did not suppress this increase.

The release of testosterone by the testis of normal rats has been shown to be pulsatile causing large irregular fluctuations in plasma testosterone concentrations (16). No attempt was made to mimic this release pattern in hypophysectomized rats treated with testosterone. The effects of these fluctuations on testicular $\text{PGF}_2\alpha$ concentrations has not yet been investigated, but their role cannot be discounted.

It is assumed that gonadotropin secretion was suppressed in intact rats by testosterone propionate. This suppression did not produce the increase in $\text{PGF}_2\alpha$ observed in hypophysectomized rats. These data suggest that testicular $\text{PGF}_2\alpha$ is not controlled by gonadotropins.

The actions of several hypophyseal hormones have been associated with altered tissue levels of PG's. For example, some effects of prolactin have been reported to be associated with increased $\text{PGF}_2\alpha$ synthesis in the mouse mammary gland (12). Prostaglandins have also been shown to mediate the effects of prolactin in rat blood vessels

(13, 14). Horrobin et al. (15) have proposed a control mechanism for prostaglandin synthesis that involves glucocorticoids, prolactin, growth hormone, and thyroid hormones. They suggest that prolactin, growth hormone, and thyroid hormones have a "permissive action" on PG synthesis whereas glucocorticoids inhibit PG synthesis. Their hypothesis is based on limited data from in vitro measurements on blood vessels, mammary glands, and renal tissue. As a consequence, they should not be generalized to all mammalian tissues. Hypophysectomy either directly or indirectly affects the secretion of all of these hormones. Further study is needed to determine which hypophyseal hormones have the principle role in regulating testicular $\text{PGF}_2\alpha$ concentrations, in either a direct or indirect manner, in the mature rat.

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