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Biochemistry

Selective hormonal control of *myo*-inositol biosynthesis in reproductive organs and liver of the male rat*

(*myo*-inositol-1-phosphate synthase/hypophysectomy/thyroidectomy/gonadotropins/thyroxine)

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ABSTRACT *myo*-Inositol biosynthesis has been examined in hypophysectomized and thyroidectomized male rats. After hypophysectomy, inositol-1-phosphate synthase [1L-*myo*-inositol-1-phosphate lyase (isomerizing), EC 5.5.1.4] in the reproductive organs and liver decreased markedly. At the same time, testicular acid phosphatase [orthophosphoric-monoester phosphohydrolase (acid optimum), EC 3.1.3.2] and β-glucuronidase (β-D-glucuronide glucuronosohydrolase, EC 3.2.1.31) increased. Thyroidectomy caused a similar decrease in inositol-1-phosphate synthase in the liver but not in the reproductive organs. Follicle-stimulating hormone (follitropin) and luteinizing hormone (lutropin) restored the activity to at least normal levels in the testis, prostate, and seminal vesicle but not in the liver of hypophysectomized animals. Triiodothyronine and thyroxine stimulated liver synthase 30-fold in hypophysectomized animals. We conclude that inositol-1-phosphate synthase in the reproductive organs is under more or less direct control of the pituitary; in the liver, the control is mediated through the thyroid.

Among the many tissues capable of synthesizing *myo*-inositol, the testis ranks as the richest mammalian source of inositol-1-phosphate synthase [1L-*myo*-inositol-1-phosphate synthase, EC 5.5.1.4], the NAD$^+$-dependent enzyme that isomerizes glucose 6-phosphate to *myo*-inositol 1-phosphate (1) referred to here as simply inositol 1-phosphate. Because the testis is the target organ for various hormones and is itself the site of androgen biosynthesis, we were led to inquire into possible hormonal control of inositol biosynthesis. Although there have been sporadic reports on the effects of various physiological and pathological states on the levels of free inositol and inositol-1-phosphate synthase in certain tissues (2–7), there has been no systematic study of hormonal control of the enzyme. Here we report the effect in male rats of hypophysectomy, adrenalectomy, and thyroidectomy on tissue levels of inositol-1-phosphate synthase and preliminary experiments in the reversal of these effects by gonadotropic and thyroid hormones.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing approximately 200 g were obtained from Taconic Farms (Germantown, NY) and maintained as follows. Normal animals received regular tap water; hypophysectomized, 5% dextrose; thyroidectomized, 1% calcium lactate; and adrenalectomized, isotonic saline. Sham-operated rats were given the same fluids; all animals received regular laboratory chow ad lib. Tissues for enzyme assay were removed and weighed immediately after killing by decapitation. Seminal vesicles were washed with cold isotonic saline after removal of seminal fluid through an incision. Brain, liver, spleen, thymus, pancreas, kidney, testis, and ventral prostate were homogenized in a Potter–Elvehjem homogenizer with 2–3 vol of 0.154 M KCl/0.2 mM dithiothreitol. Epididymis and seminal vesicles were minced and then homogenized in a Polytron homogenizer with 3–5 vol of the same solution. The homogenates were centrifuged 20 min at 27,000 × *g*, and the supernatants were heated at 60°C for 2 min exactly, chilled in ice, and centrifuged at 105,000 × *g* for 1 hr. Solid ammonium sulfate was added to 40% of saturation (0.226 g/ml). The precipitate was collected by centrifugation, dissolved in 0.6–0.7 ml of 50 mM Tris acetate, pH 7.4/0.2 mM dithiothreitol and dialyzed against the same buffer overnight. The suspension was freed of insoluble material by centrifugation and assayed for inositol-1-phosphate synthase by incubation with glucose 6-phosphate (5 mM) and NAD$^+$ (1 mM); the resulting inositol 1-phosphate was determined as inorganic phosphate by the periodate method of Barnett *et al.* (8). Protein was determined by the method of Lowry *et al.* (9), with correction for the effect of Tris.

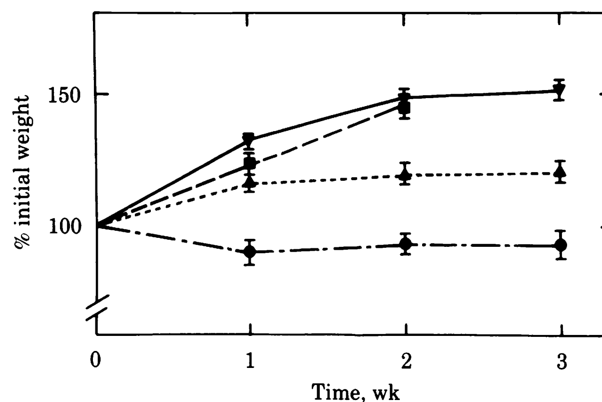

FIG. 1. Growth of rats under various hormonal conditions. ▼——▼, Normal and sham operated; ■– – –■, adrenalectomized; ▲– – –▲, thyroidectomized; ●— · —●, hypophysectomized.

RESULTS

Fig. 1 shows the growth of rats up to 3 wk after surgical removal of various hormonally active organs compared with normal and sham-operated animals. Adrenalectomized rats reached normal weight after 2 wk. Thyroidectomized animals stopped growing after 1 wk, and hypophysectomized animals failed to grow at all, results being consistent with previous observations (10). Sham-operated rats grew normally.

Fig. 2 shows the effect of such organ removal on the specific

Abbreviations: FSH, follicle-stimulating hormone (follitropin); LH, luteinizing hormone (lutropin); T3, triiodothyronine; T4, thyroxine.

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activity of inositol-1-phosphate synthase and on organ weight relative to body weight in testis, epididymis, accessory sex organs, liver, and kidney. Within 2 wk, both enzyme activity and relative weight reached constant values in normal animals. Sham-operated rats in all studies were indistinguishable from normals. Adrenalectomy uniformly produced no effect in any organ. Thyroidectomy had no effect upon the reproductive organs and the kidney but caused a 64% reduction in synthase activity in the liver with only 16% reduction in relative organ weight in 2 wk.

Most of the changes seen were the result of hypophysectomy. The greatest losses were found in the prostate and seminal vesicle, in both of which the decline in enzyme activity paralleled the decrease in relative weight. After 3 wk, synthase activity was undetectable in either organ. By then, less than 10% of the prostate gland remained, while the seminal vesicle decreased 71% in proportion to body weight. The testis and epididymis behaved similarly although differently from the seminal vesicle and prostate gland. In the testis, synthase activity declined 78% in 3 wk, while relative weight decreased 68%; the respective values for the epididymis were 55% and 60%. The larger decreases in synthase activity observed in the prostate gland and seminal vesicle compared with the testis and epididymis suggest different hormonal control between the primary and secondary sex organs, consistent with the known dependence of the secondary sex organs on the testis. Our results in normal animals fail again to confirm the finding of Robinson and Fritz that epididymis is a richer source of synthase than is whole testis (7, 11).

In the liver, hypophysectomy resembled thyroidectomy in causing a 90% decline in synthase activity, accompanied by only a 22% drop in relative weight. The kidney showed no significant change in synthase activity, although relative weight decreased 39%. The low precision of measurement in kidney was the result of phosphatase activity leading to high inorganic phosphate blanks, which interfere in the Barnett method.

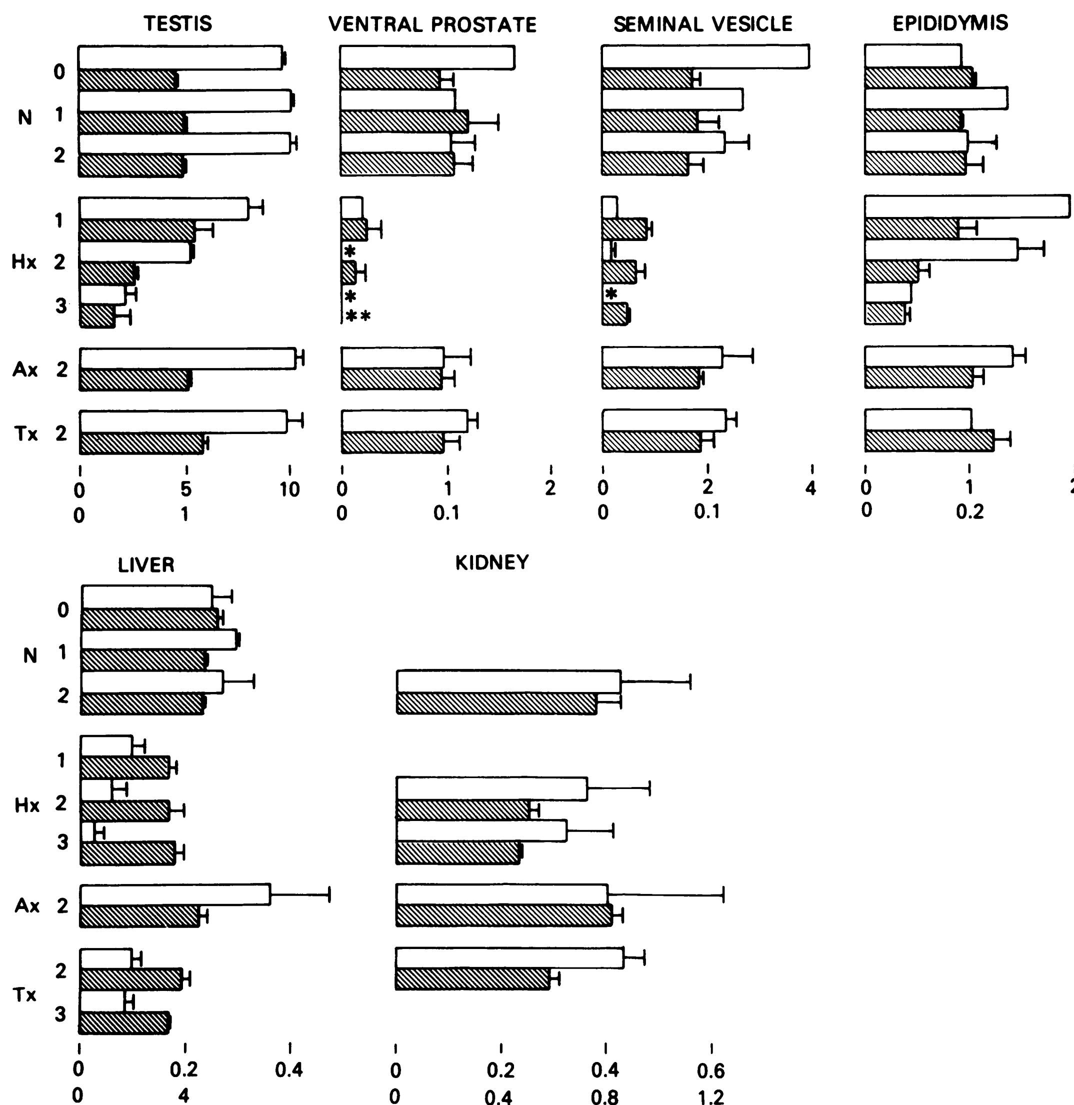

FIG. 2. Effect of hormonal changes on synthase activity and organ weight. N, normal; Hx, hypophysectomized; Ax, adrenalectomized; Tx, thyroidectomized. Ordinate, weeks; abscissa, synthase activity in milliunits/mg of protein (upper abscissa scale, ▭) and percentage of body weight (lower abscissa scale, ▨). Where SD is not indicated, one determination was made; otherwise, for each bar, 3–6 animals were used for sex organ experiments, 3–8 for liver experiments, and 5–8 for kidney experiments. One milliunit of synthase produces 1 nmol of inositol 1-phosphate per min.

* Not detectable.

** Not determined.

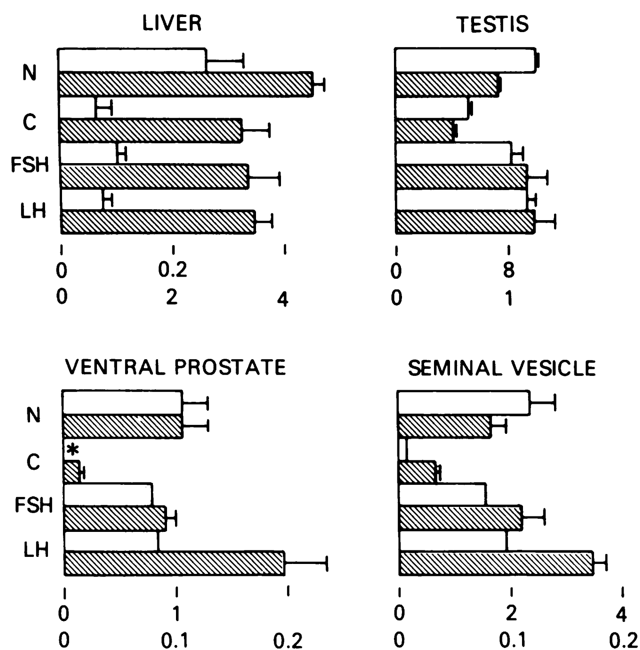

FIG. 3. Effect of gonadotropins on synthase activity and organ weight in liver and sex organs of hypophysectomized rats. N, normal and not injected; C, hypophysectomized and not injected; FSH, hypophysectomized and injected subcutaneously daily for 2 wk with four units of porcine FSH (Sigma) dissolved in sterile saline; LH, same except injected with 600 units of equine LH (Sigma). From 3–8 animals were used in all studies except where SD is not indicated. ▭, Synthase activity in milliunits/mg of protein; ▨, percentage of body weight. * Not detectable.

In other experiments (not shown), thyroidectomy caused about a 50% decrease in synthase activity of the spleen and pancreas but no change in the thymus. In brain from any animal preparation, there was no change in synthase activity, which measured about 0.175 milliunit/mg of protein.

Because a major function of the pituitary is the elaboration of gonadotropic hormones, it was of interest to determine their effect on the synthase activity in reproductive organs after hypophysectomy. The effect on the gonads was of especial interest because of the high synthase activity of the testis, reflected in the high concentration of free inositol in the rete testis amounting to 30–100 times the plasma level (12). Fig. 3 shows the results of administration of follicle-stimulating hormone (follitropin; FSH) and luteinizing hormone (lutropin; LH) to hypophysectomized rats compared with controls, both intact and hypophysectomized, which had received neither hormone. Of the four organs studied, all of which are characterized by substantial decreases in synthase activity and relative weight in the hypophysectomized controls (see also Fig. 2), the liver alone failed to respond to the hormones. In the testis, prostate, and seminal vesicle, both FSH and LH restored the enzymatic activity and the relative organ weight to normal levels, with an even greater-than-normal effect of LH on the relative weight of the secondary sex organs. Based on these findings, we conclude that inositol-1-phosphate synthase of the reproductive organs and liver is subject to control by the pituitary. From the known interrelationships among the reproductive organs and the pituitary, the effect of the gonadotropins on the testicular enzyme is probably direct, whereas that on the synthase of the accessory sex organs is probably indirect and mediated through the testis. In the liver, the effect is also indirect and probably mediated through the thyroid gland because the effect of hypophysectomy is mimicked by thyroidectomy.

The close parallels between loss of synthase activity and reduction in relative weight after hypophysectomy and their restoration by gonadotropins raised a question concerning the specificity of the hormonal effects on synthase activity in the sex organs. Do all enzyme activities decrease with losses in tissue weight after hypophysectomy? To answer this question, two other testicular enzymes were studied: acid phosphatase [orthophosphoric-monoester phosphohydrolase (acid optimum), EC 3.1.3.2] (13), a germ cell marker (14), and β-glucuronidase (β-D-glucuronide glucuronosohydrolase, EC 3.2.1.31) (13), a Sertoli cell marker (14). Table 1 shows the levels of synthase, acid phosphatase, and β-glucuronidase 2 wk after hypophysectomy. While the synthase activity declined, both acid phosphatase and β-glucuronidase increased, showing that the hormonal effect can vary among different enzymes.

If our conclusion above is valid that the liver synthase is under the control of the thyroid, then administration of thyroid hormones should restore synthase activity in hypophysectomized animals. Far from mere restoration to normal levels, we observed greater than 30-fold stimulation when either triiodothyronine (T3) or thyroxine (T4) were given to hypophysectomized rats (Table 2), a result that supports our conclusion but that probably has other implications as well. To insure that the product of this large stimulation was authentic inositol 1-phosphate and not another compound that might interfere in the Barnett assay, a sample of the incubation mixture was analyzed by gas chromotography (15) and showed a peak of expected size with the retention time of inositol-1-phosphate. Treatment of this material with Mg^{2+} and specific inositol-1-phosphatase from testis (16) resulted in the disappearance of this peak and the appearance of a peak of equivalent size with the retention time of free *myo*-inositol.

DISCUSSION

Although the male gonad has been recognized for nearly 2 decades as the principal site of mammalian inositol biosynthesis (1), there have been no studies on the effects of hormones on this pathway in the testis. Melampy and Mason (17) and later Nixon (18) showed that in the male accessory sex organs the content of free *myo*-inositol is dependent on the level of testosterone, but whether this effect was on the biosynthetic pathway was not investigated. Molitoris *et al.* found no effect of para-

Table 1. Effect of hypophysectomy on rat testis enzymes

Enzyme	Assays, units/mg of protein: Normal	Assays, units/mg of protein: Hypophysectomized	Change, %	P†
Inositol-1-phosphate synthase	0.0101 ± 0.00058 (6)	0.00554 ± 0.00051 (6)	−45.1	0.001
Acid phosphatase*	34.6 ± 1.8 (4)	42.3 ± 1.5 (4)	22.3	0.01
β-Glucuronidase*	0.964 ± 0.073 (4)	1.57 ± 0.28 (4)	62.9	0.05

* Assayed on the supernatant fraction from centrifugation at 100,000 × *g*; 0.1% Triton X-100 was added 30 min before centrifugation.

† Student's *t* test; the number of animals is in parentheses.

Table 2. Effect of thyroid hormones on synthase activity in liver of hypophysectomized rats

Thyroid hormones	Specific activity, milliunits/mg of protein
Control	0.107 ± 0.039
T3	3.49 ± 0.92
T4	3.43 ± 0.29

One week after hypophysectomy, T3 and T4 (Sigma; 100 μg/rat) were administered subcutaneously in separate experiments daily for 6 days; the control rats received saline. Four rats were used in each study.

thyroid hormone on biosynthesis of inositol in the kidney (19). Additionally there have been several inquiries into changes in the biosynthetic pathway in various physiological and experimentally induced pathological states that may reflect hormonal control of the enzyme. These include decrease in testicular synthase activity in cryptorchidism (2), long-term diabetes (3), and, in a developmental study, in the fetal liver, placenta, and neonatal liver with increase in activity in the mammary gland, beginning at parturition (5).

The great capacity of the testis to produce inositol may be a consequence of the blood/testis barrier, which prevents the entry of certain blood substances including inositol (20). To maintain the high concentration of inositol found in the rete testis (100 times the plasma concentration), the organism requires a nearby synthetic system (21). Although the role of free inositol as the precursor of phosphatidylinositol is well established (22), what additional function this concentration of free inositol serves in the male reproductive tract (23), as in the brain (24), remains unexplained.

With the demonstration that the seminiferous tubules are the chief site of testicular inositol synthesis (1), other investigators have attempted to define the specific cellular locus of the enzyme. Morris and Collins (2) concluded from studies of experimental cryptorchidism that mature germ cells were the site, whereas Robinson and Fritz (11) in more recent studies with Sertoli cell-enriched preparations and cultures attribute the synthesis to the Sertoli (somatic) cells, although these results should be viewed with caution until the unprecedentedly high specific activities reported can be verified (7). If, as Robinson and Fritz contend, control of spermatogenesis is mediated through hormone-responsive somatic cells, then inositol-1-phosphate synthase might be expected to be under hormonal control. Thus, our results are consistent with the conclusion of Robinson and Fritz that synthase originates in the somatic cells of the testis and probably not in the germ cells.

The hormonal response of inositol-1-phosphate synthase in the liver is different from that observed in the sex organs. Unresponsive to the gonadotropic hormones, the liver showed an unexpectedly large response to thyroid hormones. The significance of this observation is unknown because ordinarily the contribution of the liver to inositol biosynthesis is small (1). Apparently under certain conditions the liver can be called upon to produce inositol, an essential membrane precursor, at a rate beyond the capacity of the diet to supply. The extent of this change in synthase activity is large compared with other liver enzymes studied under similar conditions: histidase doubled after hypophysectomy and returned to normal with T3 (25); acetyl CoA carboxylase decreased 90% after hypophysectomy and increased 9-fold with T3 (26); NAD^+ pyrophosphorylase was unchanged by thyroidectomy followed by administration of T3 (27).

Because inositol-1-phosphate synthase is the same protein in all tissues as inferred from immunological evidence (7), other tissue factors as yet unidentified must account for the selectivity of the hormonal effects we have observed in the liver and sex organs and the absence of these controls in the brain and kidney.

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