There is no evidence that either TRH or TSH is capable of directly altering the rate of peripheral conversion of $T_4$ to $T_3$. The daily injection of $T_4$ into man, increased plasma $T_3$ concentration and the clearance rate of both $T_3$ and $T_4$ resulted in a nett increase in the $T_4/T_3$ ratio[8]. On the other hand a single i.v. injection of $T_4$ did not alter the fractional turnover rate of $T_3$ for at least 1 h after injection, although it had decreased by 24 h. Thus increased secretion of $T_4$ alone, while altering the plasma $T_3$ concentration and clearance rate does not lead to an immediate decrease in the $T_4/T_3$ ratio.

It appears therefore that the decrease in the $T_4/T_3$ molar ratio that follows the acute stimulation of the thyroid gland is due to increased secretion of $T_3$ relative to $T_4$. This relative increase in $T_3$ to $T_4$ secretion is probably underestimated. This is because although binding of $T_3$ to thyroid binding globulin does occur[9], it is probably not tightly bound[10] and therefore will be removed more rapidly from the circulation than $T_4$, most of which is bound to plasma proteins. The short term release of stored thyroglobulin ought not cause rapid changes in the $T_4/T_3$ ratio. However, by whatever mechanism this increased $T_3$ secretion is brought about, the nett result is clearly an increase in the active form of the circulating thyroid hormones.

We conclude that in non-pathological conditions 2 mechanisms operate to meet tissue thyroid hormone requirements. Normally, $T_3$ requirements are met largely by peripheral deiodination of $T_4$ to $T_3$, the active form of the hormone. However, under conditions of increased demand for thyroid hormones the release of both hormones is enhanced, with $T_3$ being discharged more rapidly than $T_4$.

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## Effect of growth factors on hepatic drug metabolism in diabetic-hypophysectomized rats

D. E. Cook and C. K. Phares

*Department of Biochemistry, University of Nebraska Medical Center, Omaha (Nebraska 68105, USA),*
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*Summary.* In vivo administration to diabetic-hypophysectomized rats of either the growth factor produced by the plerocercoid larvae of the tapeworm, Spirometra mansonoides, or mammalian growth hormone caused inhibition of hepatic drug metabolism measured in vitro.

Hormonal control of hepatic metabolism is, at best, only vaguely understood. Recent investigations have shown that the growth factor produced by the plercoceroid larvae of the tapeworm, Spirometra mansonoides, causes inhibition of hepatic drug metabolism in hypophysectomized rats[1]. The inhibition of drug metabolism by the plerocercoid growth factor (PGF) is accompanied by enhanced growth[1] and is similar to the response observed when mammalian growth hormone is administered to hypophysectomized rats[2]. Since alloxan diabetes, a condition of abnormally low insulin levels, is known to affect drug metabolism in rats[3,4] and since GH is reported to have some insulin-like as well as anti-insulin-like activity[5], it is important to know whether the effect of growth factors on hepatic drug metabolism in hypophysectomized rats is dependent on or independent of normal insulin levels. Therefore, the present study was designed to determine the effect of PGF and bovine growth hormone (BGH) on hepatic drug metabolism in diabetic-hypophysectomized rats.

*Methods.* 3 weeks after hypophysectomy (Hormone Assay, Chicago, Ill.) male Sprague-Dawley rats (approximately 100 g) were injected i.p. with alloxan monohydrate (Eastman) (250 mg/kg b.wt) to induce diabetes. Serum sugar concentrations were determined[6] with blood collected by orbital sinus puncture. Only those rats with fed serum sugar concentrations greater than 300 mg/100 ml 4 days after alloxan injection were considered to be diabetic. In one experiment rats were treated with PGF by the s.c. injection of 10 plerocercoids/rat[7] 4 days after alloxan treatment. In another experiment rats were treated with BGH (NIH-GH-B-18, a gift of the Endocrine Study Section, NIH, Bethesda, Maryland) by daily s.c. injection of 500 µg of the hormone dissolved in 0.85% NaCl beginning 9 days after alloxan injection. 7 days subsequent to the initiation of either treatment, the rats were sacrificed after a 12-h fast and the livers used for in vitro drug metabolism studies as previously described[1].

Table 1. Effect of in vivo treatment of diabetic-hypophysectomized rats with plerocercoid growth factor (PGF) on hepatic drug metabolism of aminopyrine and aniline in vitro[a]

| Drug substrate | Control | PGF-treated[b] | Inhibition (%) |
|---|---|---|---|
| | (µmoles/min g liver) | (µmoles/min g liver) | |
| Aminopyrine | 51.72 ± 2.86 (9) | 38.05 ± 3.37 (6)[c] | 26 |
| Aniline | 12.81 ± 0.59 (9) | 7.02 ± 0.58 (6)[d] | 45 |

[a] The results are expressed as formaldehyde-formed or p-aminophenol-formed with aminopyrine or aniline as substrate, respectively. The numbers given are mean ± SEM (number of animals). [b] Treatment conditions are described in methods. [c] $< 0.02$ versus control. [d] $p < 0.01$ versus control.

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The significance of difference between means was established by Student's t-test and unless otherwise indicated, data are expressed as the mean ± SEM.

*Results.* The PGF-treated rats gained 12.5 g (n=6) compared to a loss of 1.3 g (n=9), $p < 0.05$, for the control rats. Blood sugar determined 24 h prior to sacrifice was not significantly altered by PGF treatment; 492 ± 47 mg/100 ml blood for control and 425 ± 52 mg/100 ml blood for treated rats, $p > 0.3$. The data shown in table 1, however, clearly indicate that PGF treatment depressed hepatic drug metabolism in these diabetic-hypophysectomized animals.

In a similar manner, BGH injections resulted in a b.wt increase during the treatment period of 19.4 g (n=7) compared to a loss of 3.8 g (n=4), $p < 0.05$, for controls and was without significant effect on blood sugar determined 24 h prior to sacrifice; 543 ± 49 mg/100 ml blood for controls compared to 441 ± 36 mg/100 ml blood for treated rats, $p > 0.1$. Treatment with BGH also depressed the hepatic metabolism of aniline but did not significantly affect aminopyrine metabolism (table 2).

Table 2. Effect of in vivo treatment of diabetic-hypophysectomized rats with bovine growth hormone (BGH) on hepatic drug metabolism in vitro[a]

| Drug substrate | Control | BGH-treated[b] | Inhibition (%) |
|---|---|---|---|
| | (μmoles/min g liver) | (μmoles/min g liver) | |
| Aminopyrine | 62.45 ± 3.10 (4) | 56.99 ± 1.66 (7) | 4 |
| Aniline | 16.00 ± 1.39 (4) | 10.32 ± 0.43 (7)[c] | 36 |

[a] Results are expressed the same as for table 1. [b] Treatment conditions are described in 'methods'. [c] $p < 0.01$ versus control.

*Discussion.* After injection of alloxan into rats to produce diabetes, a small amount of immunoassayable insulin remains in the plasma even though the ability of beta cells to secrete insulin in response to normal stimuli is greatly diminished[8]. Although insulin is necessary for growth in some species only very small amounts of insulin are necessary for the expression of the growth response to GH in hypophysectomized-pancreatectomized rats[9]. In addition it has previously been shown that PGF stimulates the growth of alloxan diabetic rats[10] and that PGF has some 'insulin-like' activity[11]. In light of these observations it is not surprising that both BGH and PGF were observed to stimulate growth in the diabetic-hypophysectomized rats used in the present experiments.

Alloxan diabetes in rats has also been shown to decrease the in vitro metabolism of aminopyrine but increase the in vitro metabolism of aniline[4]. Insulin treatment of diabetic rats, on the other hand, decreases aniline metabolism but has no effect on aminopyrine metabolism[4]. Likewise, hypophysectomy alone has been shown to decrease hexobarbital[2] and aminopyrine[1] metabolism in rats. The present observations that PGF acted to decrease the rate of hepatic aminopyrine and aniline metabolism without altering blood sugar concentrations during a period of enhanced growth in diabetic-hypophysectomized rats indicate that PGF affects growth and drug metabolism by a mechanism that is not dependent on normal insulin levels. The observation that BGH affected only aniline metabolism under these same conditions, however, suggests that mammalian growth hormone and PGF may affect hepatic drug metabolism by independent mechanisms.

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## A specific binding protein for the moulting hormone ecdysterone in locust haemolymph

R. Feyereisen

*Laboratoire de Biologie Générale, Université Louis Pasteur, 12, rue de l'Université, F–67000 Strasbourg (France), 20 January 1977*

*Summary.* Specific binding of $^3$H-ecdysterone to a high mol. wt protein from Locusta migratoria haemolymph was shown by gel filtration. The hormone-protein complex shows a dissociation constant $K_d \simeq 3.10^{-7}$ M, and the concentration of binding sites varies during the last larval instar.

The moulting hormones of insects, ecdysone (α-ecdysone) and ecdysterone (β-ecdysone, 20-OH-ecdysone), are polyhydroxysteroidal molecules. These hormones circulate in the haemolymph at levels which are at least a 100fold below their solubility in water. It has thus been generally agreed[1] that these hormones circulate in a 'free' form, rather than partially bound to protein carriers as steroid hormones of vertebrates. I report here the occurrence of a specific and saturable binding protein for ecdysterone in Locusta migratoria haemolymph.

Ecdysone disappears quickly from the haemolymph when injected into locust larvae[2], and chemical assay (gas chromatography-mass fragmentography) shows that ecdysterone is the principal hormone molecule circulating in larval haemolymph[3]. Moreover, after injection of $^3$H-ecdysone, in vivo binding of $^3$H-ecdysterone to a macromolecular fraction of the haemolymph of locust larvae has been shown by gel filtration on Sephadex G100[4].

*Material and methods.* In order to allow an in vitro study of this binding, $^3$H-ecdysterone of high specific activity was first synthesized. Fat body and Malpighian tubules are the primary sites of the conversion of ecdysone into ecdysterone in locusts[2]. Malpighian tubules of 50 late fifth instar locusts were dissected and rinsed for at least 2 h at 4 °C in locust saline[5]. 15 nmoles $^3$H-ecdysone

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