Endocrine regulation of sex differences in hepatic histidase activity

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Hepatic histidase activity in adult female rats is twice that in adult male rats. Hypophysectomy and thyroidectomy result in a significant increase in hepatic histidase activities in males, but not in females. This effect on histidase is reversed by the exogenous administration of tri-iodothyronine, but not by ectopic pituitary glands or purified pituitary hormones.

INTRODUCTION

The endocrine regulation of hepatic histidase is mediated via the hypothalamic–hypophyseal–gonadal axis. From birth through the early part of the pre-pubertal period, hepatic histidase activities are similar in male and female rats, but, beginning at puberty and extending into adulthood, histidase activities are higher in adult females than in adult males (Feigelson, 1973). Oestrogen is a positive modulator, whereas testosterone is a negative effector, of histidase (Feigelson, 1973). In intact animals, glucocorticoids, glucagon via cyclic AMP, and amino acids (Feigelson, 1973) and growth hormone (somatotropin) (Skett & Gustafsson, 1979), result in increased activity. The thyroid is suppressive to the expression of histidase (Freedland *et al.*, 1968; Armstrong & Feigelson, 1980). Hypophysectomy results in increased histidase activity in adult male rats, whereas that in females remains unchanged (Feigelson, 1971a). The actions of the gonadal steroids have not been demonstrated in hypophysectomized animals. We have therefore re-investigated the endocrine regulation of hepatic histidase, emphasizing the role of the pituitary and of thyroid hormone.

MATERIALS AND METHODS

Sprague–Dawley CD rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) were maintained on synthetic diet (NIH Feed-31) and allowed free access to water. The animals were housed in a controlled environment (21 °C; 12 h light/12 h dark cycle). Hypophysectomy, adrenalectomy and thyroidectomy were performed by Charles River Breeding Laboratories. Control animals were sham-operated and injected with vehicle.

In Expt. 1, male and female rats were hypophysectomized on day 42. These animals received 5% (w/v) glucose in 0.9% NaCl for 10 days after the surgery only. Pituitaries of age- and sex-matched donors were transplanted under the kidney capsule on day 55 under sodium pentobarbital-induced anaesthesia. Testosterone propionate and oestradiol benzoate (Steraloids Inc., Wilton, NH, U.S.A.) were dissolved in propylene glycol and injected (subcutaneously) daily at 2 mg/kg and 10 µg/kg body wt. respectively (Illsley & Lamartiniere, 1981) on days 56–62. These animals were killed on day 63.

In Expt. 2, male and female rats were hypophysectomized on day 56 and injected subcutaneously on days 62–70 once daily with 30 µg of tri-iodothyronine/kg body wt., or 20 or 100 µg of thyroid-stimulating hormone (thyrotropin; from bovine pituitary; 1 i.u./mg)/kg body wt. (Lax *et al.*, 1976), or 1, 8 or 25 mg of cortisol acetate/kg body wt. (Lamartiniere & Feigelson, 1977). These hormones were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., and the following pituitary hormones were obtained from the National Pituitary Agency, Baltimore, MO, U.S.A. Rat growth hormone (2.1 i.u./kg body wt.; NIAMD-Rat-GH-B-5, 1.7 i.u./mg) was injected (subcutaneously) twice daily into hypophysectomized female rats, and sheep growth hormone (1.0 and 3.0 i.u./kg body wt.; NIH-GH-S-11, 0.56 i.u./mg) was injected into hypophysectomized male rats (Rumbaugh & Colby, 1980). Rat prolactin (5.4 i.u./kg body wt.; NIAMD-RAT Prolactin B-1, 7 i.u./mg) was injected (subcutaneously) twice daily into hypophysectomized female rats, and bovine prolactin (50 and 201 i.u./kg body wt.; NIH-P-B-5, 32.2 i.u./mg) was injected into hypophysectomized male rats (Lamartiniere, 1981). Bovine luteinizing hormone (lutropin; NIH-LH-B-10; 1.06 units/mg) was injected once daily at 0.088 and 0.353 unit/kg body wt. into hypophysectomized female rats and then at 0.55 and 2.2 units/kg body wt. into hypophysectomized male rats. Rat follicle-stimulating hormone (follicitropin; 0.62 and 1.87 units/kg body wt.; NIAMD-Rat FSH-B-1, 3.7 units/mg) was injected once daily into hypophysectomized female rats, and sheep follicle-stimulating hormone (0.2 and 0.64 unit/kg body wt.; NIH FSH-S-12, 1.25 units/mg) was injected into hypophysectomized male rats. The animals were killed 2 h after the last injection. The preparations and concentrations of hormones used depended on the availability. When a hormone dose did not exert a significant effect on the measured biological end-points, larger doses were used in subsequent experiments when possible.

In Expt. 3, male and female rats were thyroidectomized on day 42 and injected subcutaneously on days 62–69 with 6 or 30 µg of tri-iodothyronine/kg body wt. once daily and killed on day 70.

In Expt. 4, intact female rats were injected on days 62–69 with 6 or 30 µg of tri-iodothyronine/kg body wt. once daily and killed on day 70.

Animals were killed by decapitation, allowed to bleed,

Table 1. Endocrine regulation of rat liver histidase

Details of methodology, including dosages, are contained in the Materials and methods section. Results are expressed as means $\pm$ S.E.M. ($n = 8$ for Expts. 1–3; $n = 4$ for Expt. 4). ^a $P < 0.0001$ compared with sham-operated males; ^b $P < 0.0001$ compared with hypophysectomized males; ^c $P < 0.0001$ compared with hypophysectomized females; ^d $P < 0.001$ and ^e $P < 0.0001$ compared with thyroidectomized males; ^f $P < 0.05$ and ^g $P < 0.001$ compared with intact females.

Expt. no.	Treatment	Histidase activity ($\mu\text{mol}/\text{min per mg}$)	
		Males	Females
1.	Sham-operated	3.8 ± 0.2	8.7 ± 0.9
	Hypophysectomized	10.0 ± 0.4^a	10.0 ± 0.8
	+ ectopic pituitary	10.2 ± 0.4^a	9.9 ± 0.7
	+ pituitary (+ testosterone/oestrogen)	9.8 ± 0.5^a	10.3 ± 0.6
2.	Sham-operated	4.2 ± 0.2^b	11.5 ± 0.6
	Hypophysectomized	10.0 ± 0.4	11.6 ± 0.6
	+ 30 μg of tri-iodothyronine/kg	3.9 ± 0.3^b	4.4 ± 0.5^c
3.	Sham-operated	5.9 ± 0.3^d	14.1 ± 0.8
	Thyroidectomized	9.0 ± 0.5	14.1 ± 1.0
	+ 6 μg of tri-iodothyronine/kg	4.9 ± 0.3^d	—
	+ 30 μg of tri-iodothyronine/kg	2.2 ± 0.1^e	—
4.	Intact females		15.9 ± 1.4
	+ 6 μg of tri-iodothyronine/kg		12.0 ± 0.7^f
	+ 30 μg of tri-iodothyronine/kg		6.8 ± 0.9^g

and their livers were rapidly removed and placed on ice. Liver homogenates (20%, w/v) in 10 mM-Tris/HCl, pH 7.2, containing 14 mM-MgCl₂ and 0.6 M-KCl were prepared in the cold with a motor-driven glass Potter–Elvehjem homogenizer equipped with a Teflon pestle. Histidase activities were determined spectrophotometrically from cytosolic preparations after centrifugation at 105 000 g for 60 min by the method of Tabor & Mehler (1955) as modified by Lamartiniere & Feigelson (1977). One unit of histidase activity is defined as the amount producing 1 μmol of urocanic acid/min. Protein was determined by the method of Lowry *et al.* (1951).

Statistical comparisons between groups were performed by one-way analysis of variance (Winer, 1962), provided that the overall analysis was significant at $P < 0.05$.

RESULTS

Hepatic histidase activities in adult female rats are more than twice those in adult male rats (Table 1). Hypophysectomy abolishes these sex differences as a result of a significant increase in male histidase activity and no significant change in female activity (Expt. 1). We subsequently made attempts to elucidate the nature of the pituitary factor(s) responsible for the metabolic regulation and sexual differentiation of hepatic histidase. Age- and sex-matched pituitaries were transplanted under the kidney capsule of hypophysectomized rats in order to see whether the negative modulation of hepatic histidase by the pituitary could be re-established. As evident from the results of Expt. 1, ectopic pituitary secretions were not capable of altering histidase activities in hypophysectomized animals. Since testosterone has been shown to be a negative modulator of histidase in intact and castrated animals, but not in hypophysectomized rats, we treated hypophysectomized male rats receiving an ectopic pituitary with testosterone as an attempt to reconstitute the system and show that androgen action is exerted via

secretions of the ectopic pituitary. This treatment did not reverse the effect of hypophysectomy, either. Likewise, hypophysectomized female rats with an ectopic pituitary, with or without oestrogen treatment, had activities similar to those of hypophysectomized female rats.

We subsequently investigated the effect of exogenously administered purified hormones on hepatic histidase in hypophysectomized animals. Tri-iodothyronine administered to hypophysectomized male and female rats resulted in significantly decreased histidase activities in both sexes (Expt. 2), to values similar to those of control males. Further investigations revealed that thyroidectomy results in an increase in histidase activities in males only and that exogenously administered tri-iodothyronine reverses the effect of thyroidectomy in these male rats (Expt. 3). In female rats thyroidectomy had no effect on histidase activity, but exogenous administration of tri-iodothyronine to intact females resulted in lower histidase activities (Expt. 4).

Exogenously administered growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone and cortisol acetate to hypophysectomized male and female rats did not alter histidase activities. The exogenously administered growth hormone was, however, of sufficient concentration to result in increased body and testicular weights. Luteinizing hormone and follicle-stimulating hormone resulted in increased testicular weights, and follicle-stimulating hormone resulted in increased uterine wet weights. Adrenalectomy of male and female rats also had no effect on histidase (results not shown).

DISCUSSION

Hepatic histidase activities are higher in adult female rats than in adult male rats. Hypophysectomy results in increased activity in male rats, but no changes in female rats (Feigelson, 1971a). In an attempt to elucidate the nature of the pituitary factor(s) responsible for the

metabolic regulation and sexual differentiation of this enzyme, we implanted age- and sex-matched pituitaries under the kidney capsules of hypophysectomized rats. We have previously demonstrated the physiological function of the ectopic pituitary by the measurements of serum prolactin (Lamartiniere *et al.*, 1979), body-weight increases (Lamartiniere, 1981) and the modulation of hepatic glutathione transferase (Lamartiniere, 1981) and monoamine oxidase (Illsley *et al.*, 1980). Since androgen and oestrogen have been demonstrated to be negative and positive modulators, respectively, of histidase in intact and gonadectomized rats, we also treated these hypophysectomized male and female rats receiving the ectopic pituitaries with the appropriate sex steroid. These treatments were not able to alter histidase activities from those of hypophysectomized animals. These results demonstrate that an intact pituitary (regulated by the hypothalamus) is necessary for sex steroids to exert endocrine action in the regulation of hepatic histidase. Our results also lead us to suggest that the pituitary factor responsible for the negative modulation of hepatic histidase is probably dependent on a hypothalamic releasing factor, since autonomous secretions of the ectopic pituitary were not sufficient to modulate hepatic histidase.

Exogenously administered growth hormone at concentrations that modulate hepatic enzymes (Wong & Dunn, 1977; Rumbaugh & Colby, 1980; Lamartiniere, 1981) and prolactin to hypophysectomized rats did not modulate hepatic histidase. This is consistent with the ectopic pituitary secretions (growth hormone and prolactin) not modulating histidase. These two hormones are in part regulated by hypothalamic inhibiting factors.

Luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone, pituitary factors that are regulated via hypothalamic releasing hormones, when administered to hypophysectomized rats were also not able to modulate hepatic histidase activities. Feigelson (1971*b*) has previously shown that these peptides do not affect histidase, and we are not aware of any evidence of these pituitary hormones directly affecting any other hepatic enzymes.

Assuming that hypophysectomy causes adrenal and thyroid hypotrophy, we treated hypophysectomized animals with cortisol acetate and tri-iodothyronine. Exogenously administered cortisol did not affect hepatic histidase in hypophysectomized adult male or female rats. This same treatment did, however, reverse the effect of hypophysectomy on glutathione transferase and alcohol dehydrogenase (C. A. Lamartiniere, unpublished work). Adrenalectomy of male and female rats also had no effect on histidase. The only hormone shown definitively to reverse the effect of hypophysectomy on hepatic histidase is tri-iodothyronine (Armstrong & Feigelson, 1980). Using a tri-iodothyronine concentration that is not thyrotoxic (as evidenced by no changes in whole-body and testicular weights), we observed significantly decreased hepatic histidase activities in hypophysectomized males and females, activities that are similar to those of control males. Since tri-iodothyronine exerts a negative effect on histidase activity in hypophysectomized animals, we investigated the effect of thyroid removal. Interestingly, thyroidectomy resulted in an increase in histidase activities in males, but not in females, and exogenously

administered tri-iodothyronine reversed the effect of thyroidectomy in the male rats. However, exogenously administered tri-iodothyronine to intact females results in lower histidase activities, indicating that, beyond some threshold, higher thyroid-hormone concentrations will suppress histidase activity in the female, even in the presence of the pituitary.

Even though thyroid hormone exerts a major suppressive effect on hepatic histidase, this hormone is not adequate to restore oestrogen action in the hypophysectomized rat (Armstrong & Feigelson, 1980). Prolactin, a pituitary peptide that can restore depleted oestrogen receptors in hypophysectomized animals (Chamness *et al.*, 1975), is also not the pituitary hormone that plays a central role in the expression of hepatic histidase. Growth hormone has been shown to cause a small but significant suppression of hepatic histidase in hypophysectomized rats (Feigelson, 1971*a*), but we were not able to confirm this. In intact animals, Skett & Gustafsson (1979) noted an increase in histidase activity after exogenous administration of growth hormone, and I have reported an increase in activity in animals bearing a growth-hormone-secreting tumour (Lamartiniere, 1985). The identity of the pituitary factor that plays a central role in the ontogeny and expression of sex-differentiated hepatic histidase remains to be elucidated.

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REFERENCES

- Armstrong, E. G. & Feigelson, M. (1980) *J. Biol. Chem.* **255**, 7199–7203
- Chamness, G. C., Costlow, M. E. & McGuire, W. L. (1975) *Steroids* **26**, 363–371
- Feigelson, M. (1971*a*) *Biochim. Biophys. Acta* **230**, 296–308
- Feigelson, M. (1971*b*) *Biochim. Biophys. Acta* **230**, 309–318
- Feigelson, M. (1973) *Enzyme* **15**, 169–197
- Freedland, R. A., Avery, E. H. & Taylor, A. R. (1968) *Can. J. Biochem.* **46**, 141–150
- Illsley, N. P. & Lamartiniere, C. A. (1981) *Endocrinology* (Baltimore) **108**, 1737–1743
- Illsley, N. P., Kita, E. & Lamartiniere, C. A. (1980) *Endocrinology* (Baltimore) **106**, 798–804
- Lamartiniere, C. A. (1981) *Biochem. J.* **198**, 211–217
- Lamartiniere, C. A. (1985) *Endocrinology* (Baltimore) **117**, 523–526
- Lamartiniere, C. A. & Feigelson, M. (1977) *J. Biol. Chem.* **252**, 3234–3239
- Lamartiniere, C. A., Dieringer, C. S., Kita, E. & Lucier, G. W. (1979) *Biochem. J.* **180**, 313–318
- Lax, E. R., Ghraf, R., Schriefers, H., Herrmann, M. & Petutschnig, D. (1976) *Acta Endocrinol. (Copenhagen)* **82**, 774–784
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Rumbaugh, R. C. & Colby, H. D. (1980) *Endocrinology* (Baltimore) **107**, 719–724
- Skett, P. & Gustafsson, J.-Å. (1979) *Med. Biol.* **57**, 374–389
- Tabor, H. & Mehler, A. H. (1955) *Methods Enzymol.* **2**, 228–233
- Winer, B. J. (1962) *Statistical Principles in Experimental Research*, McGraw-Hill, New York
- Wong, B. S. & Dunn, A. (1977) *Biochem. Biophys. Res. Commun.* **79**, 876–884