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Pineal-Induced Depression of Free Thyroxine in Syrian Hamsters

George M. Vaughan and Basil A. Pruitt, Jr.

U.S. Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas

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Blind hamsters had no alteration of the dialyzable fraction of serum thyroxine (T₄) but had depressed total and free T₄ concentrations compared to controls. Prevention of the effects of blinding by pinealectomy indicates pineal influence on circulating free T₄ concentration. Parallel changes in free T₄ and the free T₄ index indicate adequacy of the index in representing pineal-induced changes in free T₄.

Key words: pineal, free thyroxine, thyroxine, hamsters, blinding

INTRODUCTION

When Syrian hamsters are exposed to a short photoperiod (less than 12 hr light per day) for 2-3 months or are subjected to bilateral orbital enucleation, their circulating total thyroxine (T₄) concentration falls. This effect is brought about by the pineal gland; pinealectomy prevents it [Vriend et al., 1979; Vriend, 1983]. In the above and other studies [G.M. Vaughan et al., 1982; M.K. Vaughan et al., 1982] of light restriction, the pineal, and T₄ in this species the status of circulating free T₄ has been approached by

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simultaneous observations of the free T4 index (FT4I), which depends on the in vitro uptake (T3U) of added radiotracer triiodothyronine (T3) from the serum onto a solid matrix. The FT4I ($T4 \times T3U$) thus contained a correction for possible alterations of protein binding of T4 and provided an indirect assessment of its free concentration. Because the FT4I changed with the same pattern exhibited by T4 in the above studies, it was tentatively concluded that the pineal gland, in the condition of light deprivation, is capable of influencing circulating free T4 concentration (FT4).

However, the general use of the FT4I to represent changes of FT4 has recently been called into question. For example, in a number of conditions and species, including various nonthyroidal illnesses in humans [Chopra et al., 1979, 1984; Woeber and Maddux, 1981; Oppenheimer et al., 1982] and burn injury in rats [Shirani et al., 1985], human [Vaughan et al., 1985a], and hamsters [Vaughan et al., 1985b], the total T4 and FT4I were often depressed but the free or dialyzable fraction of T4 (T4DF) was elevated out of proportion to any change in T3U. This often resulted in free T4 concentrations ($FT4 = T4 \times T4DF$) that were not depressed or were less depressed than predicted by the FT4I in these conditions. The T4DF is that portion of radiotracer T4 that crosses a protein-impermeable membrane from the serum into buffer without involvement of a solid matrix. Thus such discrepancy between the T3U and the T4DF (and hence between the FT4I and FT4) might be due to a circulating factor in some conditions that not only inhibits binding of T4 to serum proteins but also to the solid matrix of the T3U test [Oppenheimer et al., 1982]. It is thus necessary to show that, in the experimental condition under study, the changes in FT4I do reflect those of FT4 directly assessed by observations of the T4DF.

Because of these considerations, in the present study we have assessed whether or not the pineal gland, in the condition of its activation by blindness in Syrian hamsters, can influence free T4 concentration.

MATERIALS AND METHODS

Male golden hamsters, *Mesocricetus auratus*, were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Massachusetts), at about 90 gm body weight (young adults), maintained in our animal quarters in a light/dark (L/D) cycle of 14/10 hr at 21°C with lights on at 0700 hr and given standard laboratory chow and tap water ad libitum. After 2 weeks, they were divided into three groups, according to surgical procedures performed under pentobarbital anesthesia: sham-pinelectomy (SHPX), blinding by bilateral orbital enucleation together with sham-pinelectomy (BL + SHPX), and blinding plus pinelectomy (BL + PX). Pinelectomy was performed according to the method of Hoffman and Reiter [1965], and the sham procedure involved replacing the disc of skull bone without performing pinelectomy. The animals were maintained in the L/D 14/10 cycle until sacrifice by guillotine 11 weeks after surgery, when trunk serum was obtained and both testes and the dorsal prostate were cleaned of fat and connective tissue and weighed. Successful pinelectomy without disruption of the brain

structures in the vicinity was verified grossly at autopsy. T₄ was measured by radioimmunoassay and T₃U by radioassay (charcoal matrix) with kits from Diagnostic Products (Los Angeles, California). The dialyzable fraction of T₄ (T₄DF) was determined in duplicate serum aliquots at the Nichols Institute, San Juan Capistrano, California, by equilibrium dialysis at 37°C [Sterling and Brenner, 1966]. The FT₄I was calculated as the product of T₄ and T₃U, and the FT₄ as the product of T₄ and T₄DF (Sterling and Brenner, 1966). Data were analysed by the Student-Newman-Keuls test [Bruning and Kintz, 1977] and analysis of covariance [Dixon, 1983].

RESULTS

Reduction of testicular and prostatic mass was observed in the blind animals, an effect obliterated by additional pinealectomy (Fig. 1). Blinding produced no detectable effect on T₃U or T₄DF, though a small reduction of mean T₃U was seen in the BL+PX group (Table 1). T₄, FT₄I, and FT₄ were all depressed after blinding, unless the animals were additionally pinealectomized (Fig. 1). The correlation of FT₄I with FT₄ was significant (Fig. 1), and there were no significant differences among slopes for individual groups (not shown).

DISCUSSION

Compared to values in controls, lower mean serum T₄ without major alteration of T₃U has been reported in blind humans [Dieckhues, 1974], an effect mediated by the pineal gland in Syrian hamsters [Vriend et al., 1977; Vriend et al., 1979; G.M. Vaughan, et al. 1982; M.K. Vaughan, et al., 1982; Vriend, 1983]. We now report that free T₄ concentration is depressed by blinding and that this effect appears to be mediated by the pineal gland. The small reduction of mean T₃U in BL+PX is not explained, but blinding alone appeared to have no effect on T₃U, and FT₄I appeared to be a relatively reliable index of FT₄ (Fig. 1).

The observed blinding-induced suppression of the reproductive system and the prevention of this by pinealectomy serve to indicate that the animals in this study exhibited the expected pineal-mediated reproductive responses [Reiter, 1980] and thus adequately represent the usual hamster model for demonstrating pineal function. The parallel changes between the reproductive system and circulating T₄ have been noted previously, and the possibility has been considered that the changes in T₄ passively result from pineal-induced hypogonadism and the possible resultant effects on T₄ serum binding proteins [Vriend et al., 1977, 1979; G.M. Vaughan et al., 1982]. It was interpreted that the effects of the pineal on T₄ and free T₄ were likely independent of such a potential effect of hypogonadism, because the FT₄I varied with the same pattern as did total T₄. This interpretation is confirmed in the present study, which shows that the free fraction (T₄DF) of T₄ and hence its serum transport binding in the hamster was not significantly altered in the presence of pineal-induced hypogonadism and that, like T₄ and FT₄I,

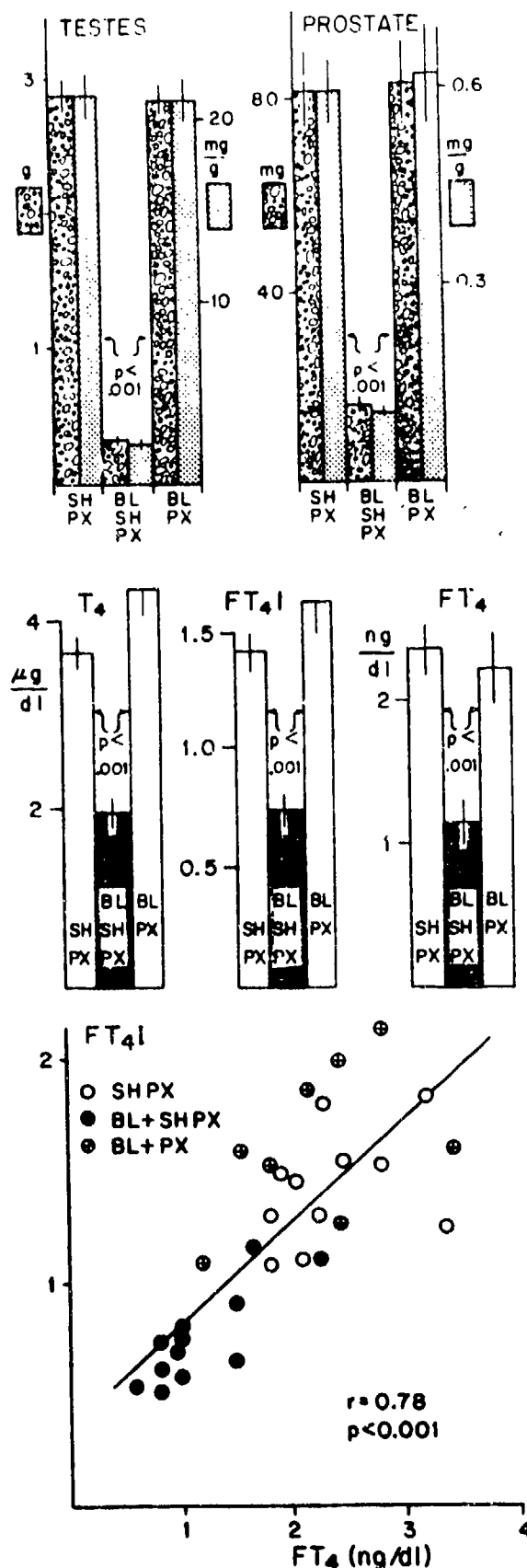


Fig. 1. Testicular and prostate weights as absolute (g) or relative to body weight (mg/g), T₄, FT₄I, and FT₄ after sham pinealectomy (SHPX), blinding (BL + SHPX), or combined blinding and pinealectomy (BL + PX). Means $\pm$ SE (top and middle panels) and rectilinear regression (bottom panel).

TABLE 1. T₃U and T₄DF After Sham Pinealectomy (SHPX), Blinding (BL), and/or Pinealectomy (PX)

	SHPX	BL + SHPX	BL + PX
n in group	11	12	8
T ₃ U (%)	38.04 ±.319	38.66 ±.293	36.63** ±.404
T ₄ DF (%)	.0638 ±.004	.0581 ±.004	.0504 ±.006

**P < 0.01 vs. SHPX and BL + SHPX.

Means ± SE.

FT₄ was actually depressed in this condition. Furthermore, it was previously shown that the depressed T₄ in blind hamsters was not a result of their gonadal regression, because castration alone did not alter serum T₄ [Vriend et al., 1979]. Of course, the possibility remains that the pineal-dependency of the blinding-induced suppressions of both the reproductive and thyroidal systems results from some pineal-dependent common central neural control mechanism [Vriend, 1983].

The magnitude of the dialyzable fraction (T₄DF) in hamsters appears to be similar to that in humans [Sterling and Brenner, 1966]. Because humans with altered levels of serum thyroxine-binding protein (and consequently abnormal total serum T₄ concentration) often have normal free (dialyzable) concentrations but are euthyroid, it is thought that the free concentration is the bioavailable portion important for hormone action [Ingbar and Woeber, 1981]. Although somewhat more than the dialyzable fraction can be transported into some rat tissues [Pardridge, 1981], the precise fraction of T₄ that is active in hamsters particularly at the level of the pituitary has not been elucidated. In the rat and human with primary reduction of thyroid function, it appears that T₄ rather than T₃ in the circulation determines feedback in the pituitary for thyrotrophin (TSH) secretion, via local T₄ to T₃ conversion [Connors and Hedge, 1981; Larsen, 1982]. The observation that both total and free T₄ in serum are lower in blind than in control hamsters is important, because it indicates that the pineal gland can induce a condition in which bioavailable T₄ in the circulation is reduced, a finding previously only suspected on the basis of indirect FT₄I results [Vriend et al., 1977]. Whether or not the reduced FT₄ results in other physiologic changes in these animals remains to be determined.

The site of the suppressive action of the pineal on serum T₄ might be some element of the hypothalamopituitary unit (central), the thyroid, the organs responsible for T₄ conversion or degradation (peripheral), or a combination of the above. TSH was not determined in the present animals because of an insufficient quantity of serum. As reviewed previously for the Syrian hamster [G.M. Vaughan, et al., 1982], in animals blind for 2–3 months, mean TSH is not consistently altered at a time when serum T₄ is always low. Thus it is not unreasonable to suggest that the effect of the pineal is at least partly at the level of the pituitary or hypothalamus [Vriend, 1983], causing an inadequacy in the compensatory elevation of TSH, which normally should be a response to a fall in free T₄ concentration [Larsen, 1982].

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LITERATURE CITED

1. Bruning, J.L., B.L. Kintz (1977) Computational Handbook of Statistics. Scott, Foresman and Company, Glenview, Illinois.
2. Chopra, I.J., T-S. Huang, R.E. Hurd, A. Beredo, D.H. Solomon (1984) A competitive ligand binding assay for measurement of thyroid hormone-binding in serum and tissues. *J. Clin. Endocrinol. Metab.* 58:619-628.
3. Chopra, I.J., D.H. Solomon, G.W. Hepner, A.A. Morgenstein (1979) Misleadingly low free thyroxine index and usefulness of reverse triiodothyronine measurement in nonthyroidal illness. *Ann. Intern. Med.* 90:905-912.
4. Connors, J.M., and G.A. Hedge (1981) Feedback regulation of thyrotropin by thyroxine under physiological conditions. *Am. J. Physiol.* 240:E308-E313.
5. Dieckhues, B. (1974) Die Bedeutung der Lichtperzeption durch das Auge auf den Hormonhaushalt des Menschen. *Klin. Monatsbl. Augenheilkd.* 165:291-296.
6. Dixon, W.J. (ed) (1983) BMDP Statistical Software. University of California Press, Berkeley.
7. Hoffman, R.A., and R.J. Reiter (1965) Rapid pinealectomy in hamsters and other small rodents. *Anat. Rec.* 153:19-21.
8. Ingbar, S.H., and K.A. Woeber (1981) The thyroid gland. In: *Textbook of Endocrinology*. R.H. Williams, ed. W.B. Saunders, Philadelphia, p. 117.
9. Larsen, P.R. (1982) Thyroid-pituitary interaction: Feedback regulation of thyrotropin secretion by thyroid hormones. *N. Engl. J. Med.* 306:23-32.
10. Oppenheimer, J.H., H.L. Schwartz, C.N. Mariash, F.E. Kaiser (1982) Evidence for a factor in the sera of patients with nonthyroidal disease which inhibits iodothyronine binding by solid matrices, serum proteins, and rat hepatocytes. *J. Clin. Endocrinol. Metab.* 54:757-766.
11. Pardridge, W.M. (1981) Transport of protein-bound hormones into tissues in vivo. *Endocrinol. Rev.* 2:103-123.
12. Reiter, R.J. (1980) The pineal and its hormones in the control of reproduction in mammals. *Endocrinol. Rev.* 1:109-131.
13. Shirani, K.Z., G.M. Vaughan, B.A. Pruitt, Jr., A.D. Mason, Jr. (1985) Reduced serum T₄ and T₃ and their altered transport binding after burn injury in rats. *J. Trauma* (in press).
14. Sterling, K., and M.A. Brenner (1966) Free thyroxine in human serum: Simplified measurement with the aid of magnesium precipitation. *J. Clin. Invest.* 45:155-163.
15. Vaughan, G.M., A.D. Mason, Jr., W.F. McManus, B.A. Pruitt, Jr. (1985a) Alterations of mental status and thyroid hormones after thermal injury. *J. Clin. Endocrinol. Metab.* 60:1221-1225.
16. Vaughan, G.M., K.Z. Shirani, M.K. Vaughan, B.A. Pruitt, Jr., A.D. Mason, Jr. (1985b) Hormonal changes in a hamster burn model. *Endocrinol.* (in press).
17. Vaughan, G.M., M.K. Vaughan, L.G. Serraille, R.J. Reiter (1982) Thyroid hormones in male hamsters with activated pineals or melatonin treatment. *Prog. Clin. Biol. Res.* 92:187-196.
18. Vaughan, M.K., M.C. Powanda, G.C. Brainard, L.Y. Johnson, R.J. Reiter (1982) Effects of blinding or afternoon melatonin injections on plasma cholesterol, triglycerides, glucose, TSH and thyroid hormone levels in male and female Syrian hamsters. *Prog. Clin. Biol. Res.* 92:177-186.
19. Vriend, J. (1983) Evidence for pineal gland modulation of the neuroendocrine-thyroid axis. *Neuroendocrinology* 36:68-78.
20. Vriend, J., R.J. Reiter, G.R. Anderson (1979) Effects of the pineal and melatonin on thyroid activity of male golden hamsters. *Gen. Comp. Endocrinol.* 38:189-195.
21. Vriend, J., J.W. Sackman, R.J. Reiter (1977) Effects of blinding, pinealectomy and superior cervical ganglionectomy on free thyroxine index of male golden hamsters. *Acta Endocrinol* 86:758-762.
22. Woeber, K.A., and B.A. Maddux (1981) Thyroid hormone binding in non-thyroidal illness. *Metabolism* 30:412-416.