Pergamon

0960-0760(95)00037-2

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1-6, pp. 27-31, 1995

0960-0760/95 $9.50 + 0.00

Potential Interactions Between Estrogen Receptor and Thyroid Receptors Relevant for Neuroendocrine Systems

Tammy L. Dellovade, Yuan-Shan Zhu and Donald W. Pfaff*

Laboratory of Neurobiology and Behavior, The Rockefeller University, 1230 York Avenue, New York, NY 10021, U.S.A.

Environmental signals can profoundly affect reproductive behavior, physiology and responses to steroids. One consequence of nutritional or temperature stress is altered plasma concentrations of thyroid hormone. Recent *in vivo* and *in vitro* data indicate that manipulations of estrogen and thyroid hormone levels can alter each other's functions. One possible mechanism for interaction may be that thyroid and estrogen receptors bind to parts of the same hormone response elements of target genes and compete with each other, thus serving to integrate environmental signals with neuroendocrine responses.

J. Steroid Biochem. Molec. Biol., Vol. 53, No. 1-6, pp. 27-31, 1995

INTRODUCTION

Reproduction is energetically expensive for female mammals. Therefore it is beneficial to delay reproduction in the presence of adverse environmental conditions. Environmental stressors such as low food availability and changes in ambient temperature are profound regulators of female reproduction [1]. Food restriction has been demonstrated to delay puberty in juveniles and disrupt estrous cycles in adult rodents [1, 2]. Chronic or acute food deprivation also decreases copulatory behavior, inhibits ovulation and decreases parental behavior in hamsters [1-4]. When metabolic fuels must be diverted to other physiological processes, such as thermoregulation or increased foraging, reproduction is also affected. Prolonged exposure to cold temperatures can inhibit estrous cycles and decrease ovulation rate and litter size [1, 5]. The inhibitory effects of cold temperatures on reproduction occur more slowly when food is readily available. Likewise, when increased foraging is required or the food supply is diminished for prolonged periods, cold temperatures more rapidly alter neuroendocrine function [6].

The neural mechanisms that mediate the suppression of reproduction in response to adverse environmental signals are not well understood. In ovariectomized Syrian hamsters, metabolic fuel deprivation decreased lordosis response to estradiol + progesterone treatment [4]. In this study 48 h of food restriction either before or immediately following estradiol administration decreased sexual receptivity in ovariectomized hamsters. Thus, decreased metabolic fuels may alter neural responsiveness to estradiol and/or progesterone either directly, by acting on steroid concentrating neurons, or indirectly, by altering other neurotransmitter or hormonal systems. In addition, food deprivation and cold temperatures alter gonadotropin releasing-hormone and luteinizing hormone secretion, which ultimately affects follicular development [2, 7]. Recently, hamsters which had been food restricted for the first 2 days of the estrous cycle had fewer gonadotropin-releasing hormone immunoreactive neurons that co-expressed c-*fos* immunoreactivity as compared to controls [8]. Taken together, these data indicate that environmental challenges profoundly affect reproductive behavior, physiology and responses to steroids. Although changes in steroid sensitivity and pituitary responses are indicative of environmental regulation of neural functioning, the molecular mechanisms that integrate environmental signals and physiological responses remain unclear.

MOLECULAR BACKGROUND

In general, steroid and thyroid hormones function through hormone binding to their nuclear receptors [9]. Thyroid hormone receptors (TR) are members of

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24-29 September 1994.
*Correspondence to D. W. Pfaff.

a steroid/thyroid superfamily of nuclear receptors [10,11]. Activation of gene transcription can occur when the ligand-activated receptor binds to specific response elements in the promoter region of target genes. The DNA response elements for steroid hormone actions fall mainly into two categories, i.e. glucocorticoid and estrogen response elements (GRE and ERE). The consensus sequence of the ERE (5′-AGGTCAnnnTGACCT) is palindromic, while that of GRE (5′-GGTACAnnnTGT$^{T}/_{C}$CT) is an "imperfect" palindrome [12, 13]. It was recently demonstrated that thyroid hormone receptors (TRs) can bind to a consensus ERE [14, 15, Zhu and Pfaff, unpublished data]. The consensus sequence of the ERE half-site is the same as that of a TRE (AGGTCA) [13, 16]. It was found that TR homo- or heterodimers can bind to TREs arranged as inverted palindromes, direct repeats and palindromes with different numbers of spaces. In addition, vitamin D receptors (VDR), retinoic acid receptors (RAR) and retinoid X receptors (RXR) can bind to similar sequences as TRs and ER [9, 10]. VDRs preferentially bind to hormone response elements (HREs) with a gap of three nucleotides, TRs, 4 nucleotides and RARs, 5 nucleotides, generating the so-called 3-4-5 rule [17]. However, it is apparent that the specificity and direction of steroid actions are not only dependent on HRE and receptors themselves, but also on the protein–protein interactions and competition of multiple factors at the DNA level. Since elevated levels of thyroid hormone can signal temperature changes, nutritional changes or stress, the liganded receptor interfering with the estrogen receptor dimer could have biologically important signalling value.

Thyroid receptors are derived from two genes and have at least four known isoforms [11]. TRβ2 mRNA is strongly expressed in the anterior pituitary [18]. The remaining isoforms are expressed in many tissues of the body, but they have preferential expression in specific regions. TRα1 mRNA is most abundant in skeletal muscle and brown fat [19]. The non-thyroid hormone (T_3) binding isoform, c-erbAα2, is highly expressed in the brain [19]. TRβ1 mRNA is abundant in the brain, liver and kidney [20]. In addition, it was recently found that neurons contain TRβ1, while c-erbAα2 is located in astrocytes [21].

Recent evidence suggests that manipulations of estrogen and thyroid hormone levels *in vivo* and *in vitro* may mutually alter each others' action [22–29]. Thyroid hormone has been demonstrated both to potentiate and inhibit estrogen-induced gene expression. In adult *Xenopus* liver cells, thyroid hormone treatment potentiated estradiol activation of vitellogenin genes [22]. However, in chick embryonic liver tissue, T_3 inhibited estrogen-induced protein synthesis, including vitellogenin, and reduced the level of nuclear estrogen receptors [23]. Zhuo-Li *et al.* [24] also demonstrated presumed TR–ER interactions in a rat estrogen sensitive pituitary tumor cell line. *In vitro*, T_3 or estrogen alone stimulated proliferation of these cells. However, simultaneous administration of both hormones resulted in mutual inhibition [24]. Conversely, using a transfection assay, Graupner *et al.* [25] demonstrated that thyroid response element in the absence of TR can act as an imperfect ERE and mediate estrogen-dependent activation of transcription. In the absence of ligand, TR itself inhibited the activation of TRE by estrogen receptor. These data demonstrate the possibility of complex interactions between estrogen and thyroid hormones on gene expression.

The mechanisms responsible for the interactions between estradiol and thyroid hormones are not yet known. Tata's group demonstrated that T_3 potentiation of *Xenopus* liver vitellogenin gene expression also increased estrogen receptors in hepatocytes *in vitro* [22]. Similar increases in estrogen receptors have been found in rat pituitary tumor and rat liver after T_3 treatment [26, 27]. However, in rat uterine and hypothalamic tissues thyroid hormone did not alter ER levels [28, 29]. Therefore, the potentiation of gene expression and/or biological function by estrogen and thyroid hormone can not be explained solely by alterations at the receptor level. Graupner *et al.* [25] used truncated TR mutants and transfection assay to demonstrate that suppression of estrogen receptor activation by TRs was not due to ER–TR heterodimer formation. Rather, these authors suggested that in the absence of T_3, TRs may protect their own response elements from activation by other receptors by preventing their access to TREs.

A possible formulation is that TR and ER bind to parts of the same hormone response elements of a target gene and can compete with each other. In this way, ER and TR interactions would be either potentiating or mutually inhibitory depending on the absence or presence of ligands, and both the affinity for and the consequences of protein–DNA interactions. Glass *et al.* [14] found that TR binds to an ERE with high affinity, but fails to activate transcription *in vivo*. In addition, TR binding inhibits estrogen dependent transactivation, suggesting that TR may bind in a transcriptionally inactive manner and compete for ER binding [14]. Hirst *et al.* [30] synthesized DNA-binding domains of hybrid ERs and TRs and found that each bound to the other's response element as a monomer, rather than a dimer, and less effectively activated transcription. Thus, TR may bind to a half-site of the ERE and thereby compete against ER for binding. Finally, the oxytocin gene promoter is believed to have a common response element because it is stimulated by several members of the steroid/thyroid hormone nuclear receptor family [31, 32]. Such common hormone response elements, which have also been proposed in other genes [33], would enable interactions among transcription factors to integrate hormonal with non-hormonal signals to elicit complex, specific, hormonal responses.

We note that heterologous nuclear receptor binding to an ERE is not an exclusive feature of TRs. Retinoid X receptor β (RXRβ) can bind to several different hormone response elements, including EREs [34]. Also, RXRs can form heterodimers with TR, RAR and VDR [35–38]. Recently, Segars *et al.* [39] demonstrated that over-expression of RXRβ in the human breast cancer cell line MCF-7 inhibited ERE-driven reporter activity in a dose-dependent manner. They further demonstrated that TR/RXRβ heterodimers bind to the ERE and TR alone or in combination with RXRβ inhibited ERE reporter activity [39].

Steroid hormones profoundly regulate proenkephalin gene expression. Estrogen has been demonstrated to induce proenkephalin expression in the female rat hypothalamus and has further been correlated with lordosis behavior [40–43]. Recently, we examined possible interactions between estrogen and thyroid hormone on the regulation of proenkephalin gene expression both *in vivo* and *in vitro*, following an investigation of possible ER binding to the proenkephalin promoter [15, Zhu *et al.*, unpublished data]. In hypophysectomized female rats, administration of estrogen increased proenkephalin mRNA levels in the hypothalamus and coadministration of T_3 attenuated this effect. Administration of T_3 alone had only a small effect on proenkephalin expression. Using gel mobility shift assay we found that ER and TR both could bind to a probe with a consensus ERE, as well as a putative ERE from the proenkephalin gene promoter region (Zhu *et al.*, unpublished data). In addition, both ER and TR contributed to the ERE binding activity in the nuclear protein extracts from female rat hypothalamus. These data suggest that both ER and TR are present in the female hypothalamus and can bind to an ERE. Also, the interactions of ER and TR with DNA binding sequences of proenkephalin gene may account for inhibitory actions of thyroid hormone on estrogen induction of gene expression.

PHYSIOLOGICAL RESULTS

Altered concentrations of thyroid hormone in plasma have profound effects on a variety of metabolic functions, including heart rate and oxygen consumption. In addition, it has been realized for many years that altered thyroid function can affect mammalian reproduction [44, 45]. Chronic or acute hypo- and hyperthyroidism can affect female reproductive organs, as well as fertility, gestation and litter size. In rodents, thyroidectomy on the day of birth delays puberty in females, as measured by vaginal opening [44–46]. Once vaginal opening occurs, the ovaries, uterus and vagina are underdeveloped and only a few, small follicles are present. Krohn and White [47] demonstrated that thyroidectomy increased the length and variability of estrous cycle. Thyroidectomized females ovulated and conceived, but the number of pregnancies was reduced and gestation tended to be prolonged. In addition, after thyroid removal litter sizes are smaller, largely due to resorption of fetuses during the 2nd trimester [47].

Hyperthyroidism during prepubertal development in rats prevents maturation of the ovary and in adults will cause ovarian atrophy and a cessation of estrous cycles [45]. In contrast to rats, Hoar *et al.* [48] and others [49] found in adult guinea pigs, excess concentrations of thyroid hormone had little effect on reproductive behavior or gestation. However, removal of the thyroid impaired reproductive behavior, estrous cycles, uterine responsiveness to estrogens and pregnancy [44, 45, 48, 49]. Thus, certain species differences in responses to thyroid hormone may exist.

Several studies have also examined the responsiveness of the uterus to estradiol treatment after induced hypo- and hyperthyroidism. Ruh *et al.* [50] demonstrated that uterine tissue from hypothyroid rats have increased *in vivo* retention and *in vitro* uptake of estrogen as compared to controls. Excess levels of thyroxine reduced retention and uptake of estrogen by the uterus and reduced the metabolic response of the uterus to estrogens [50, 51]. These data suggest that T_3 may inhibit estrogen uptake in the uterus. However, propylthiouracil-induced hypothyroid rats are reported to have diminished late uterine responses to estradiol, including decreased wet weights, dry weight, protein content, RNA content, and incorporation of thymidine into uterine DNA [52]. In this study T_3 or estradiol treatment alone had no effect on uterine responses in hypothyroid rats. However, animals treated with both hormones demonstrated restored uterine responses in a time-dependent and dose-dependent manner [52]. These data suggest that the way in which thyroid function is interrupted, as well as the time course of changes observed may be important for analyzing physiological responses.

BEHAVIORAL POSSIBILITIES

A review of the literature revealed that virtually no studies over the past 50 years have directly examined the implications of thyroid function for sexual behavior in mammals. However, it is apparent from reports on gestation length and litter size that removal of the thyroid or excess thyroid hormone levels in adult rodents does not eliminate sexual behavior [44, 45]. Hypophysectomy does not reduce sexual behavior in ovariectomized female rats treated with estradiol and progesterone [53]. Peterson *et al.* [49] found that thyroidectomy slightly decreased the frequency of cyclic vaginal openings and the percent of guinea pigs found in heat. In addition, the percent of fertile matings and young born alive was decreased after thyroid removal. However, reproductive performance was equal or increased in hyperthyroid guinea pigs [49]. Interestingly, in a paper by Langham and Gustav-

son [54] there is a suggestion of possible mutual inhibition by estrogen and thyroid hormone on rat vaginal estrous. They found that thyroxine treatment decreased the estrus response, as measured by vaginal estrous, of ovariectomized rats treated with estrone. Thyroidectomy increased the estrus response to estrone administration in ovariectomized animals and the increased sensitivity was corrected following thyroxine replacement.

We are presently investigating interactions between thyroid hormones and estrogen by examining changes in estrogen-dependent sexual behavior in hypo- and hyperthyroid female rats. There are several possible levels of interaction. Since altered thyroid hormone concentrations in plasma affect metabolism, it is possible that the distribution, absorption and/or clearance of estradiol may be affected and thereby alter sexual behavior. In addition, manipulations of thyroid hormones and estrogen may affect neural receptor levels directing reproductive behavior or may result in competing behavioral responses. However, based on the discussion above, we hypothesize that thyroid hormone and estrogen interactions, as are potentially important for the integration of environmental signals with neuroendocrine substances, can occur by competing for hormone response elements of target genes in forebrain neurons.

REFERENCES

1. Bronson F. H.: Mammalian reproduction: an ecological perspective. *Biol. Reprod.* **32** (1985) 1–26.
2. Wade G. N. and Schneider J. E.: Metabolic fuels and reproduction in female mammals. *Neurosci. Biobehav. Rev.* **16** (1992) 235–272.
3. Schneider J. E. and Wade G. N.: Decreased availability of metabolic fuels induces anestrus in golden hamsters. *Am. J. Physiol.* **258** (1990) R750–R755.
4. Dickerman R. W., Li H-Y. and Wade G. N.: Decreased availability of metabolic fuels suppresses estrous behavior in Syrian hamsters. *Am. J. Physiol.* **264** (1993) R568–R572.
5. Piacsek B. E. and Nazian S. J.: Thermal influences on sexual maturation in the rat. In *Environmental Factors in Mammalian Reproduction* (Edited by D. Gilmore and B. Cook). Macmillan, London (1981) pp. 215–231.
6. Schneider J. E. and Wade G. N.: Effects of diet and body fat content on cold-induced anestrus in Syrian hamsters. *Am. J. Physiol.* **259** (1990) R1198–R1204.
7. Bronson F. H.: Effect of food manipulation on gonadotropin releasing hormone-luteinizing hormone-estradiol axis of the young female rat. *Am. J. Physiol.* **23** (1988) R616–R621.
8. Berriman S. J., Wade G. N. and Blaustein J. D.: Expression of fos-like proteins in gonadotropin-releasing hormone neurons of syrisn hamsters: effects of estrous cycles and metabolic fuels. *Endocrinology* **131** (1992) 2222–2228.
9. Beato M.: Gene regulation by steroid hormones. *Cell* **56** (1989) 335–344.
10. Evans R. M.: The steroid and thyroid hormone receptor superfamily. *Science* **240** (1988) 889–895.
11. Lazar M. A.: Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocr. Rev.* **4** (1993) 184–193.
12. Truss M. and Beato M.: Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. *Endocr. Rev.* **14** (1993) 459–479.
13. Gronemeyer H.: Transcription activation by estrogen and progesterone receptors. *Ann. Rev. Genet.* **25** (1991) 89–123.
14. Glass C. K., Holloway J. M., Devary O. V. and Rosenfeld M. G.: The thyroid hormone receptor binds with opposite transcriptional effects to a common sequence motif in thyroid hormone and estrogen response elements. *Cell* **54** (1988) 313–323.
15. Zhu Y. S. and Pfaff D. W.: DNA binding of hypothalamic proteins on estrogen response element and preproenkephalin promoter: modification by estrogen. *Neuroendocrinology* (1994) submitted.
16. Yen P. M. and Chin W. W.: New advances in understanding the molecular mechanisms of thyroid hormone action. *Trends Endocr. Metab.* **5** (1994) 65–72.
17. Umesono K., Murakami K. K., Thompson C. C. and Evans R. M.: Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. *Cell* **65** (1991) 1255–1266.
18. Hodin R. A., Lazar M. A., Wintman B. I., Darling D. S., Koenig R. J., Larsen P. R., Moore D. D. and Chin W. W.: Identification of a thyroid hormone receptor that is pituitary-specific. *Science* **244** (1989) 76–79.
19. Mitsuhashi T. G., Tennyson G. E. and Nikodem V. M.: Alternative splicing generates messages encoding rat c-erbA proteins that do not bind thyroid hormone. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 5804–5808.
20. Koenig R., Warne R. L., Brent G. A., Harney J. W., Larsen P. R. and Moore D. D.: Isolation of a cDNA clone encoding a biologically active thyroid hormone receptor. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 5031–5035.
21. Leonard J. L., Farwell A. P., Yen P. M., Chin W. W. and Stula M.: Differential expression of thyroid hormone receptor isoforms in neurons and astroglial cells. *Endocrinology* **135** (1994) 548–555.
22. Rabelo E. M. and Tata J. R.: Thyroid hormone potentiates activation of vitellogenin genes and autoinduction of estrogen receptor in adult *Xenopus* hepatocytes. *Molec. Cell. Endocr.* **96** (1993) 37–44.
23. Elbrecht A. and Lazier C. B.: Selective inhibitory effects of thyroid hormones on estrogen-induced protein synthesis in chick embryo liver. *Can. J. Biochem. Cell Biol.* **63** (1985) 1206–1211.
24. Zhuo-Li F., Skalli M., Albaladejo V., Joly-Pharaboz M-O., Nicolas B. and Andre J.: Interference between estradiol and L-triiodothyronine in the control of the proliferation of a pituitary tumor cell line. *J. Steroid Biochem. Molec. Biol.* **45** (1993) 275–279.
25. Graupner G., Zheng X-K., Tzukerman M., Wills K., Hermann T. and Pfahl M.: Thyroid hormone receptors repress estrogen receptor activation of a TRE. *Molec. Endocr.* **5** (1991) 365–372.
26. Fujimoto N., Roy B., Watanaba H. and Ito A.: Increase of estrogen receptor level by thyroxine in estrogen dependent pituitary tumor (MTT/F84) in rat. *Biochem. Biophys. Res. Commun.* **152** (1988) 44–48.
27. Freyschuss B., Sahlin L. and Eriksson H. A.: Regulatory effects of growth hormones, glucocorticoids, and thyroid hormone on the estrogen receptor level in the rat liver. *Steroids* **56** (1991) 367–374.
28. Kirland J. L., Gardner R. M., Ireland J. S. and Stancel G. M.: The effect of hypophysectomy on the uterine response to estradiol. *Endocrinology* **101** (1977) 403–410.
29. Cidlowski J. A. and Muldoon T. G.: Modulation by thyroid hormones of cytoplasmic estrogen receptor concentrations in reproductive tissues of the rat. *Endocrinology* **97** (1975) 59–67.
30. Hirst M. A., Hinck L., Danielsen M. and Ringold G. M.: Discrimination of DNA response elements for thyroid hormone and estrogen is dependent on dimerization of receptor DNA binding domains. *Proc. Natn. Acad. Sci.* **89** (1992) 5527–5531.
31. Adan R. A. H., Cox J. J., van Kats J. P. and Burbach J. P. H.: Thyroid hormone regulates the oxytocin gene. *J. Biol. Chem.* **267** (1992) 3771–3777.
32. Adan R. A. H., Cox J. J., Beischlag T. V. and Burbach J. P. H.: A composite hormone response element mediates the transactivation of the rat oxytocin gene by different classes of nuclear hormone receptors. *Molec. Endocr.* **7** (1993) 47–57.
33. Lucas P. C. and Granner D. K.: Hormone response domains in gene transcription. *Ann. Rev. Biochem.* **61** (1992) 1131–1173.
34. Marks M. S., Levi B-Z., Segars J. H., Driggers P. H., Hirschfeld S., Nagata T. and Ozato K.: H-2RIIBP expressed from baculovirus vector binds to multiple hormone response elements. *Molec. Endocr.* **6** (1992) 219–230.

35. Marks M. S., Hallenbeck P. L., Nagata T., Segars J. H., Appella E., Nickodem V. M. and Ozato K.: H-2RIIBP (RXRβ) heterodimerization provides a mechanism for combinatorial diversity in the regulation of retinoic acid and thyroid hormone responsive genes. *EMBO J.* **11** (1992) 1419–1435.
36. Bugge T. H., Pohl J., Lonnoy O. and Stunnenberg H. G.: RXRα, a promiscuous partner of retinoic acid and thyroid hormone receptors. *EMBO J.* **11** (1992) 1409–1418.
37. Kliewer S. A., Umesono K., Mangelsdorf D. J. and Evans R. M.: Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D_3 signalling. *Nature* **355** (1992) 446–449.
38. Yu V. C., Delsert C., Andersen B., Holloway J. M., Devary O. V., Naar A. M., Kim S. Y., Boutin J. M., Glass C. K. and Rosenfeld M. G.: RXRβ: a coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. *Cell* **67** (1991) 1251–1266.
39. Segars J. H., Marks M. S., Hirschfeld S., Driggers P. H., Martinez E., Grippo J. F., Wahli W. and Ozato K.: Inhibition of estrogen-responsive gene activation by the retinoid X receptor β: evidence for multiple inhibitory pathways. *Molec. Cell. Biol.* **13** (1993) 2258–2268.
40. Romano G. J., Harlan R. E., Shivers B. D., Howells R. D. and Pfaff D. W.: Estrogen increases proenkephalin messenger ribonucleic acid levels in the ventromedial hypothalamus of the rat. *Molec. Endocr.* **2** (1988) 1320–1328.
41. Romano G. J., Mobbs C. V., Howells R. D. and Pfaff D. W.: Estrogen regulation of proenkephalin gene expression in the ventromedial hypothalamus of the rat: temporal qualities and synergism with progesterone. *Molec. Brain Res.* **5** (1989) 51–58.
42. Romano G. J., Mobbs C. V., Lauber A., Howells R. D. and Pfaff D. W.: Differential regulation of proenkephalin gene expression by estrogen in the ventromedial hypothalamus of male and female rats: implications for the molecular basis of a sexually differentiated behavior. *Brain Res.* **536** (1990) 63–68.
43. Lauber A. H., Romano G. J., Mobbs C. V., Howells R. D. and Pfaff D. W.: Estradiol induction of proenkephalin messenger RNA in hypothalamus: dose–response and relation to reproductive behavior in the female rat. *Molec. Brain Res.* **8** (1990) 47–54.
44. Leathem J. H.: The role of the thyroid. In *Reproductive Biology* (Edited by H. Balin and S. Glasser). Excerpta Medica, Amsterdam (1972) pp. 857–876.
45. Young W. C.: The hormones and mating behavior. In *Sex and Internal Secretions* (Edited by W. C. Young). Williams and Wilkins Co., Baltimore (1961) pp. 1210–1212.
46. Hagino N.: Influence of hypothyroid state on ovulation in rats. *Endocrinology* **88** (1971) 1332–1336.
47. Krohn P. L. and White H. C.: The effect of hypothyroidism on reproduction in the female albino rat. *J. Endocr.* **6** (1949) 375–385.
48. Hoar R. M., Goy R. W. and Young W. C.: Loci of action of thyroid hormone on reproduction in the female guinea pig. *Endocrinology* **60** (1957) 337–346.
49. Peterson R. R., Webster R. C., Rayner B. and Young W. C.: The thyroid and reproductive performance in the adult female guinea pig. *Endocrinology* **51** (1952) 504–518.
50. Ruh M. F., Ruh T. S. and Klitgaard H. M.: Uptake and retention of estrogens by uteri from rats in various thyroid states. *Proc. Soc. Exp. Biol. Med.* **134** (1970) 558–561.
51. Schultze A. B. and Noonan J.: Thyroxin administration and reproduction in rats. *J. Animal Sci.* **30** (1970) 774–777.
52. Gardner R. M., Kirkland J. L., Ireland J. S. and Stancel G. M.: Regulation of the uterine response to estrogen by thyroid hormone. *Endocrinology* **103** (1978) 1164–1172.
53. Pfaff D. W.: Mating behavior of hypophysectomized rats. *J. Comp. Physiol. Psych.* **72** (1970) 45–50.
54. Langham W. and Gustavson R. G.: Effect of level of thyroid activity on response of ovariectomized rats to estrone. *Am. J. Physiol.* **150** (1947) 761–767.