Aromatase Activity of Human Adipose Tissue Stromal Cells: Effects of Thyroid Hormones and Progestogens (43101)

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Abstract. **In order to determine the direct effects of thyroid hormones and progestogens on extraglandular aromatization, human adipose stromal cells in monolayer culture were used as a model system for this study on the regulation of aromatase enzyme activity. It was found that 1-thyroxine at 2- and 4-fold normal concentrations (220 and 440 n*M*, respectively) and triiodothyronine at 4-fold normal concentration (7.4 n*M*) had no effect on basal, dibutyryl cyclic AMP ($(Bu)_2$ cAMP)-induced, or dexamethasone-induced aromatization. Medroxyprogesterone acetate at a concentration of 25.9 n*M*, but not progesterone, 47.7 n*M*, stimulated basal aromatization slightly but not significantly. In contrast, both medroxyprogesterone acetate and progesterone potentiated the effect of $(Bu)_2$ cAMP on aromatase activity ($P < 0.05$ and $P < 0.01$, respectively) but had no effect on dexamethasone-stimulated aromatase activity. We concluded that (i) the increased peripheral aromatization associated with hyperthyroidism is not due to the direct effect of thyroid hormones on aromatase activity, and (ii) neither progesterone nor medroxyprogesterone acetate inhibit aromatase activity of adipose tissue stromal cells, but may stimulate this activity under certain conditions.** [P.S.E.B.M. 1990, Vol 194]

In men and women aromatization of androgens to estrogens occurs in glandular and extraglandular tissue (peripheral aromatization) (1, 2), and the latter is a major source of circulating estrogens in men and in postmenopausal women (1, 2). Peripheral aromatization is increased in hyperthyroidism (3, 4) and in postmenopausal women (1, 2), although the mechanism causing these increases remains uncertain (2). It has been suggested that in hyperthyroidism the increase in aromatization may be the result of increased tissue blood flow (2), but a direct effect of thyroid hormone on peripheral aromatase activity has not been ruled out. Progestogens have been reported to inhibit the aromatase activity of granulosa cells either directly (5) or after stimulation by dibutyryl cyclic AMP ($(Bu)_2$ cAMP) (6). Since progesterone levels are low in postmenopausal women (7), it is possible that the increased aromatase activity in such women is the result of the loss of progesterone inhibition.

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An important site of peripheral aromatization is adipose tissue, where aromatase activity has been localized primarily to adipose stromal cells (8, 9). These cells have proven to be a useful model to study the effects of hormones on the peripheral aromatase complex. Therefore, we used this system to study the effects of thyroid hormones and progestogens on peripheral aromatization.

Materials and Methods

Source, Preparation, and Culture of Stromal Cells. Subcutaneous adipose tissue was obtained from men and women during indicated elective surgery, usually from the abdominal wall except for one sample which came from breast tissue (female subject, 33 years old). The adipose tissue was minced finely and incubated with collagenase, Type II (Gibco Laboratories, Life Technologies, Inc., Grand Island, NY) 1 mg/ml, for 60 min at 37°C. After incubation, the stromal cells were isolated by using differential centrifugation as described in detail elsewhere (8). The washed stromal cells were suspended in Eagle's minimum essential medium (modified) (Flow Laboratories Inc., McLean, VA) containing Penicillin-G (100 units/ml), streptomycin sulfate (100 μg/ml), and fetal bovine serum (15%

v/v), and placed in 75- to 150-cm^2 tissue culture flasks and incubated at 37°C in a humidified atmosphere of air (95%) and CO_2 (5%). The medium was changed after 3 days and then weekly. After 2–3 weeks, the monolayer cultures were dissociated from the stock flask with 5 ml of trypsin-EDTA (0.25%; Gibco Laboratories) washed with Eagle's minimum essential medium, and plated in flasks (first passage). Cells used for aromatase assay had undergone at least two passages before plating into 60-mm culture dishes. In some cases, after the second passage, cells were frozen by the Cryomed computerized freezing system and stored for later use. In this system dexamethasone stimulation of aromatase activity occurs only in the presence of fetal bovine serum and $(Bu)_2$ cAMP stimulation occurs in the absence of fetal bovine serum (10, 11). Therefore, for all experiments with dexamethasone the final incubations were done in 15% fetal bovine serum and for all experiments with $(Bu)_2$ cAMP the final incubations were done in Eagle's minimum essential medium only.

Reagents. [1β-^{3}H]androstenedione (27.4 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, MA) and used without further purification. Dexamethasone, $(Bu)_2$ cAMP, 1-thyroxine, triiodothyronine, progesterone, and medroxyprogesterone acetate were obtained from Sigma Chemical Co. (St. Louis, MO).

Incubation of Stromal Cells with Thyroid Hormones or Progestogens and Radiolabeled Steroid. When the stromal cells in 60-mm culture dishes became confluent, the cells were treated with 1-thyroxine, triiodothyronine, progesterone, or medroxyprogesterone acetate in the presence or absence of $(Bu)_2$ cAMP or dexamethasone for 24 hr before adding [1β-^{3}H]androstenedione (150 nM) as a substrate. After radiolabeled steroid was added, the incubation was continued for 4 hr at 37°C in an atmosphere of air (95%) and CO_2 (5%).

Assay of Aromatase Activity. Aromatase activity was assayed by measuring ^3H_2O as described (8, 12), after incubation of stromal cells for 4 hr at 37°C with [1β-^{3}H]androstenedione (150 nM). Each series of incubations included culture dishes without cells, and the ^3H_2O generated in these dishes was subtracted from the sample dishes to determine net ^3H_2O formed. After 4-hr incubation, the culture dishes were placed on ice for 15 min to condense water vapor. The medium (2.0 ml) was transferred to Centricon tubes (Amicon; W. R. Grace & Co., Danvers, MA) and centrifuged at 5000g for 30 min. The protein-free filtrate was transferred to tubes containing 5.0 ml of chloroform. The monolayers of stromal cells were then rinsed with 1.0 ml of normal saline and these rinsings were added to the medium. The cells were then scraped, homogenized, and assayed for protein according to the method of Lowry *et al.* (13).

The combined medium and rinsings were extracted twice with chloroform which was discarded. A 2.0-ml aliquot of the medium was removed and added to 2.0 ml of an aqueous suspension of Norit-A charcoal (5%) and dextran (0.5%). The tubes were vortexed and incubated at 4°C for 2 hr. They were then centrifuged at 1700g for 15 min. An aliquot (2.0 ml) of the supernatant was added to 15.0 ml of Optifluor (Packard Instrument Co., Downers Grove, IL) in scintillation vials, vortexed, chilled and assayed for radioactivity. The amount of radioactivity in ^3H_2O thus measured was corrected by subtracting the blank values from each sample. The values also were corrected by multiplying by 3.0 to correct for dilution of initial medium and dividing by the fractional retention of tritium in medium water throughout the procedure in incubation and processing. The aromatization rate was expressed as picomoles [1β-^{3}H]androstenedione metabolized per milligram of cell protein per 4 hr.

Statistics. Comparisons between mean values were made using a one-way analysis of variance and the Student-Newman-Keuls test for multiple comparisons between means (Statistical Package for the Social Sciences, SPSSX) (14).

Results

Thyroid Hormones. As shown in Tables I and II, 1-thyroxine at 220 and 440 nM resulted in no stimulation of aromatase enzyme activity over that of the control dishes, and had no effect on the stimulation of enzyme activity caused by $(Bu)_2$cAMP (1 mM) or dexamethasone (2.5×10^{-7} M). Similarly, 1-triiodothyronine at 7.4 nM had no effect on basal, $(Bu)_2$ cAMP-induced, or dexamethasone-induced aromatization of human adipose stromal cells.

Progestogens. As shown in Table III and IV, progesterone at the concentration of 47.7 nM had neither an inhibitory nor a stimulatory effect on aromatase activity of human adipose stromal cells when compared with control dishes, nor did it affect dexamethasone-stimulated aromatization. However, progesterone did increase the effect of $(Bu)_2$ cAMP on the aromatase enzyme system ($P < 0.01$).

Medroxyprogesterone acetate (25.9 nM) resulted in a slight but not significant increase in basal aromatization and had no effect on the aromatase activity stimulated by dexamethasone (2.5×10^{-7} M). However, medroxyprogesterone acetate did accentuate the effect of $(Bu)_2$ cAMP (1 mM) on the aromatase activity of the cultured stromal cells ($P < 0.05$).

Discussion

Adipose tissue has been shown to be an active site of peripheral aromatization (8, 15, 16) and cultures of adipose tissue stromal cells have been found to be a useful model to study peripheral aromatase activity (8, 11, 17, 18). Dexamethasone has been noted to stimulate

Table I. Effect of Thyroid Hormones on Aromatization by Adipose Tissue Stromal Cells Incubated in the Absence or Presence of $(Bu)_2$ cAMP (1 m*M*)

	Thyroxine (220 n*M*)		Thyroxine (440 n*M*) (pmol/4 hr/mg protein)		Triiodothyronine (7.4 n*M*)	
	−	+	−	+	−	+
Medium	$0.15 \pm 0.01^{a,b}$	$0.11 \pm 0.03^{a,b}$	$0.13 \pm 0.03^{a,b}$	$0.15 \pm 0.01^{a,b}$	$0.25 \pm 0.05^{a,b}$	$0.21 \pm 0.06^{a,b}$
Medium + $(Bu)_2$ cAMP	$5.2 \pm 1.0^{a,c}$	$3.9 \pm 1.2^{a,c}$	$2.3 \pm 0.4^{a,c}$	$2.2 \pm 0.4^{a,c}$	$2.1 \pm 0.3^{a,c}$	$2.2 \pm 0.4^{a,c}$

Note. Adipose tissue stromal cells were incubated in medium (Eagle's minimum essential medium, modified) with and without thyroid hormones in the absence or presence of $(Bu)_2$ cAMP. A minimum of four experiments from two different cell lines were done for each set of values.
[a] Mean ± SE.
[b,c] Difference between *b* and *c* is significant at $P < 0.01$.

Table II. Effect of Thyroid Hormones on Aromatization by Adipose Tissue Stromal Cells Incubated in the Absence or Presence of Dexamethasone

	Thyroxine (220 n*M*)		Thyroxine (440 n*M*) (pmol/4 hr/mg protein)		Triiodothyronine (7.4 n*M*)	
15% fetal bovine serum	$0.02 \pm 0.005^{a,b}$	$0.02 \pm 0.005^{a,b}$	$0.03 \pm 0.005^{a,b}$	$0.05 \pm 0.02^{a,b}$	$0.02 \pm 0.01^{a,b}$	$0.005 \pm 0.005^{a,b}$
15% fetal bovine serum + dexamethasone (2.5×10^{-7} *M*)	13.5 ± 1.36^{c}	$13.5 \pm 0.67^{a,c}$	$9.7 \pm 1.8^{a,c}$	$10.1 \pm 0.9^{a,c}$	$4.15 \pm 0.15^{a,c}$	$4.41 \pm 0.07^{a,c}$

Note. Adipose tissue stromal cells were incubated in 15% fetal bovine serum with and without thyroid hormone in the absence or presence of dexamethasone. A minimum of four experiments from two different cell lines were done for each set of values.
[a] Mean ± SE.
[b,c] Difference between *b* and *c* is significant at $P < 0.01$.

Table III. Effects of Progesterone and Medroxyprogesterone Acetate on Aromatization by Adipose Tissue Stromal Cells Incubated in the Absence or Presence of $(Bu)_2$ cAMP

	Progesterone		Medroxyprogesterone acetate	
	15 ng/ml (47.7 n*M*)		10 ng/ml (25.9 n*M*)	
	(pmol/4 hr/mg protein)			
	−	+	−	+
Medium	$0.28 \pm 0.11^{a,b}$	$0.30 \pm 0.06^{a,b}$	$0.27 \pm 0.05^{a,b}$	$0.77^{a,b} \pm 0.18^{a,b}$
Medium + $(Bu)_2$ cAMP (1 m*M*)	$4.1 \pm 0.6^{a,c}$	$6.7 \pm 0.4^{a,c}$	$9.6 \pm 1.1^{c,d}$	$15.2 \pm 3.4^{c,e}$

Note. Incubations were carried out as described for Table I. A minimum of four experiments from two different cell lines were done for each set of values.
[a] Mean ± SE.
[b,c] Difference between *b* and *c* is significant at $P < 0.01$.
[d,e] Difference between *d* and *e* is significant at $P < 0.05$.

the aromatase activity in this system provided the incubations were done using 15% fetal calf serum, and $(Bu)_2$ cAMP has been noted to stimulate the activity if the cultures were incubated in medium alone without added fetal calf serum (10, 11). Our data are in keeping with these earlier observations and indicate that our cultures resppponded similarly to dexamethasone and $(Bu)_2$ cAMP.

Although there are differences in the aromatase activity from various parts of the body (16), the aromatase activity of adipose tissue from the breast and the abdomen are not different (19). There are differences in aromatase activity of adipose tissue obtained from women of varying age (20) but we obtained adipose tissue from young women, and we could find no differences in the basal or stimulated rate of aromatization between samples from men and women. Thus, our results are not dependent on the source of the adipose tissue.

We were unable to find any stimulation of aromatase activity either in the basal or stimulated state by thyroid hormones, either l-thyroxine or triiodothy-

Table IV. Effects of Progesterone and Medroxyprogesterone Acetate on Aromatization by Adipose Tissue Stromal Cells Incubated in the Absence or Presence of Dexamethasone

	Progesterone		Medroxyprogesterone acetate	
	15 ng/ml (47.7 n*M*)		10 ng/ml (25.9 n*M*)	
	(pmol/4 hr/mg protein)			
	−	+	−	+
15% fetal bovine serum	$0.12 \pm 0.05^{a,b}$	$0.20 \pm 0.02^{a,b}$	$0.10 \pm 0.04^{a,b}$	$0.30 \pm 0.10^{a,b}$
15% fetal bovine serum + dexamethasone (2.5×10^{-7} *M*)	$10.9 \pm 1.4^{a,c}$	$10.7 \pm 2.0^{a,c}$	$9.0 \pm 0.5^{a,c}$	$10.1 \pm 0.5^{a,c}$

Note. Incubations were carried out as described for Table II. A minimum of four experiments from two different cell lines were done for each set of values.

[a] Mean ± SE.

[b,c] Difference between *b* and *c* is significant at $P < 0.01$.

ronine. Thus, the increase in aromatization which has been demonstrated to be present in some (3, 4) hyperthyroid subjects appear not to be due to a direct effect of thyroid hormone on the aromatase complex. Although it is possible that other circulating factors may cause the increased aromatase activity, it is tempting to speculate that the increased tissue blood flow which occurs in hyperthyroidism is primarily responsible for the increased aromatization (21). That the thyroid hormones do not affect the aromatase complex directly is also suggested by our recent observation that peripheral aromatization is increased in hypothyroidism (22).

Progesterone has been noted to inhibit aromatase activity in rat granulosa cells (5) and the progestin, (17,21-diemthyl-19-nor-4, 9-pregnadiene-3,20-dione; R5020), has been noted to inhibit aromatase activity in cultured rat granulosa cells previously stimulated by several factors including $(Bu)_2$ cAMP (6). However, we found that progesterone inhibited neither the basal aromatase activity nor that stimulated by dexamethasone or $(Bu)_2$ cAMP. Conversely, we found that progesterone caused an increase in the aromatase activity stimulated by $(Bu)_2$ cAMP. The progestogen, medroxyprogesterone acetate, appeared to be more active than progesterone in stimulating the aromatase complex and stimulation by medroxyprogesterone acetate was seen in the basal state as well as in the $(Bu)_2$ cAMP-stimulated state. Like progesterone, medroxyprogesterone acetate did not stimulate the aromatase activity already stimulated by dexamethasone. The failure to stimulate after dexamethasone may be due to the fact that stimulation of the stromal cell culture by progesterone is via the glucocorticoid receptor which is already maximally stimulated by dexamethasone.

Tseng et al. (23) and others (24) also noted an increase in the aromatase activity in cultured rabbit endometrial stromal cells exposed to progesterone or medroxyprogesterone acetate. The extraglandular aromatase complex may react differently than the glandular aromatase complex to progesterone and other perturbing factors. Osawa and Higashiyama (25) have suggested that aromatases may differ according to their tissue site, but this view has been questioned (18). Schreiber et al. (6) noted that the inhibition of aromatase activity in cultured rat granulosa cells by R5020 occurred as a post-cAMP action. The stimulation that we found with progesterone and medroxyprogesterone acetate would also appear to be post-cAMP, since increasing the amounts of $(Bu)_2$ cAMP caused no greater stimulation (unpublished data).

It would thus appear that the increased peripheral aromatization seen in postmenopausal women compared with younger women is not initiated by a change in progesterone concentration. However, it should be noted that ours was an *in vitro* study and the conclusions drawn from it may not necessarily be extrapolated to the *in vivo* situation.

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