Physiol. Res. 40: 419–425, 1991

Estradiol-Induced Adenohypophyseal Growth Reaction and Beta-Adrenergic Receptors

K. PACÁK, J. NEDVÍDKOVÁ*, P. ŠTOLBA*, V. SCHREIBER

3rd Department of Medicine, Faculty of Medicine, Charles University and
**Research Institute of Endocrinology, Prague*

Received February 15, 1990
Accepted December 14, 1990

Summary

The effect of administration of estradiol benzoate on β-adrenergic receptors of rat adenohypophyseal cells was studied. Twenty days' administration of estradiol benzoate was followed by an increase of adenohypophyseal weight and a decrease in specific binding of ^{3}H-dihydroalprenolol (^{3}H-DHA). In contrast to thyroid hormone treatment which induced an increase in ^{3}H-DHA binding, thyroid hormone treatment decreased both the growth reaction and the reaction of β-adrenergic receptors after estradiol. Although the relationship between the adenohypophyseal receptors and the growth reaction is unclear, changes in β-adrenergic receptors after hormonal therapy can be one of pathophysiological conditions that may influence this reaction.

Key words:
Beta-adrenergic receptors – Estradiol benzoate – Thyroid hormones – Adenohypophysis

Introduction

The anterior pituitary is the target tissue both for estrogens and thyroid hormones. Experimental hypertrophy of the rat adenohypophysis after administration of the estrogens was first described by Selye *et al.* (1935). Schreiber (Schreiber 1979, Dušková and Schreiber 1989) has shown that this growth reaction is potentiated by dopaminergic antagonists and inhibited by thyroid hormones and dopaminergic agonists. The mechanism of the adenohypophyseal growth reaction is not known but most studies have focused on changes at the molecular level including receptor mechanisms. Specific receptor sites for dopamine, noradrenaline, serotonin and other neurotransmitters have been identified and extensively characterized in the adenohypophysis (De Souza 1988).

In a previous study we found that the chronic treatment of rats with thyroid hormones was followed by an increase of specific ^{3}H-DHA binding (Pacák *et al.* 1990). The present study was designed to investigate the effect of chronic treatment with estrogens, thyroid hormones (T_3 or T_4) or both on the adenohypophyseal β–adrenergic receptors (βAR).

Sexual hormone-dependent changes of βAR have been demonstrated in the brain, e.g. hypothalamic and cortical βAR were increased after estrogen treatment (Wilkinson *et al.* 1979a,b). This is in contrast to studies showing an increase of these receptors in the cerebral cortex and hypothalamus both in ovariectomized and orchidectomized rats (Petrovic *et al.* 1985). With regard to the adenohypophysis, Petrovic *et al.* (1985) demonstrated an increased number of βAR in ovariectomized rats and their reduction after estrogen replacement. These contrasting results may be due to organ-specific differences or to the time of treatment or to the type and dosage of estrogens.

The available findings on the effects of thyroid hormones on βAR are not sufficiently consistent and detailed to provide a reliable explanation of the mechanism of the effect of these hormones at the molecular level. Williams *et al.* (1977) described changes in ^{3}H-DHA binding in the hearts of hyperthyroid rats. Clinical observation in euthyroid subjects after triiodothyronine treatment showed changes of leukocyte βAR even after one week (Ginsberg *et al.* 1981).

In the central nervous system Gross *et al.* (1980) found a decreased number of βAR in the cerebral cortex of hypothyroid rats. Perumal *et al.* (1984) showed that thyroxine administration induced an increased number of βAR in the cerebral cortex and a decreased number of these receptors in the midregion. Finally, Atterwill *et al.* (1984) demonstrated that hyperthyroidism in rats caused an increase in striatal βAR with no changes in the cerebral cortex or hypothalamus. In contrast, hypothyroidism reduced the number of striatal and hypothalamic βAR without changes in the cerebral cortex.

In this study we investigated by means of ^{3}H-DHA whether the administration of thyroid hormones (T), estradiol (E) or their combination (T+E) would influence the number and the affinity of β-adrenergic receptors on the adenohypophyseal cells.

Material and Methods

Male rats (Wistar strain, Velaz, Prague) with an initial body weight of 180–200 g were given thyroid hormones *per os* in the food (T_3 or T_4 in a dose of 0.5 and 0.6 mg/kg) or estradiol benzoate as an aqueous microcrystal suspension (Agofolin-Depot, Biotika, 10 mg/kg i.m. twice a week). During the 20 days for which the hormones were administered, the rats were fed once a day, from 10–12 a.m., by a standard diet (Larsen diet, Velaz, Prague) with water *ad libitum*. The controls (C) were given the same diet, but without the hormones. The experimental animals were kept in an indirect daylight at a temperature of 24±2 °C. On terminating the experiment, the rats were decapitated and the adenohypophysis was quickly removed and weighed (within 2 min). Pooled adenohypophyseal samples from 7 animals in each experimental group were used for further examination. The adenohypophyseal membrane specimens were prepared according to Ali *et al.* (1985) and Bression *et al.* (1982) with some modifications. The adenohypophyseal tissue was homogenized in a Braun Potter-Elvehjem type all-glass homogenizer (10 strokes, 800 rpm) in ice-cold Tris-HCl buffer (50 mmol/l) at pH 7.4. The homogenate was then centrifuged 5 min at 1000 x *g* at 4 °C in a Beckman centrifuge and the supernatant was re-centrifuged 22 min at 16 000 x *g* at 4 °C in a Beckman ultracentrifuge. The sediment was resuspended in 2 ml buffer, incubated 10 min at 37 °C and centrifuged again in the relevant amount of incubation buffer (50 mmol/l Tris-HCl, 1 mmol/l $MgCl_2$, 0.01 % ascorbic acid, pH 7.4) so as to give a membrane protein concentration of about 200 μg protein per 100 μl sample. Protein was determined by Lowry's method (Lowry *et al.* 1951); β-adrenergic receptors in the membrane fraction of adenohypophyseal cells were determined by the binding of ^{3}H-DHA (74 Ci/mmol, Amersham, 0.1–5 nmol/l) in the presence and absence of unlabeled D,L-propranolol (Sigma, 10^{-5} mol/l) during 20 min

incubation at 30 °C. Incubation was stopped by adding 3 ml ice-cold buffer (50 mmol/l Tris-HCl, pH 7.4) and the contents of the tubes were filtered through Whatman GF/C glass filters. The filters were additionally washed through with 13 ml buffer and after adding SLD 41 scintillation liquid the activity was measured on the next day using B/F 5000 Berthold-Frieseke scintillation spectrophotometer. The dissociation constant (K_d, nM) and specific binding capacity (B_{max}, fmol/mg protein) were analyzed according to non-linear regression analysis.

Results are presented as mean values ± S.E.M. Statistical analyses were conducted with StatView II statistical software, including one factor analysis of variance (Anova) and Tukey post-hoc test (SuperAnova, BrainPower, Inc., Calabasas, CA). P value less than 0.05 was defined as statistically significant.

Results

Twenty days' administration of thyroid hormones (T), estradiol (E) or their combination (T+E) to male Wistar rats significantly reduced body weight (C: 305.5 ± 4.4 g, n = 92; T: 261.6 ± 3.8 g, n = 75; E: 231.1 ± 4.5 g, n = 86; T+E: 213.8 ± 9.8 g, n = 65 – $p<0.001$ with respect to the control group).

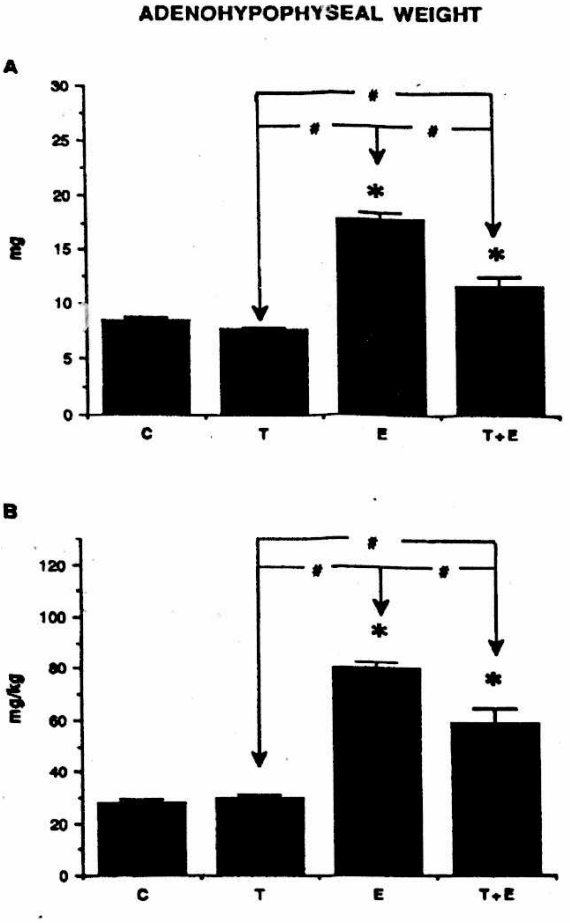

Fig. 1
Absolute (A) and relative (B) adenohypophyseal weight (mg or mg/kg) in controls (C) and in rats treated with either thyroid hormones (T) or estradiol (E) or their combination (T+E). Values are means ± S.E.M. * $p<0.001$ compared to controls, # $p<0.001$ between groups.

With regard to adenohypophyseal weight (Fig. 1) there was a significant increase in both estradiol-treated groups ($p<0.001$) in both relative and absolute adenohypophyseal weight (mg/kg body weight) which was inhibited by thyroid hormones. Using simple regression analysis we found a weak correlation between body and adenohypophyseal weight (C: $r^2 = 0.086$, E: $r^2 = 0.031$, T: $r^2 = 0.018$, T+E: $r^2 = 0.088$). Thyroid hormones alone did not influence adenohypophyseal weight. Tab. 1 shows the results of experiments for the pooled adenohypophyseal samples in each group. Rats treated with thyroid hormones or T+E showed a significant increase of B_{max} of βAR in the adenohypophysis ($p<0.05$) in contrast to estradiol treatment which induced a significant decrease in number of βAR compared to controls ($p<0.05$). Thyroid treatment induced a decrease in βAR affinity when compared to estradiol or T+E treated groups ($p<0.01$). However, no significant difference was found in the affinity when experimental groups were compared with the controls.

Table 1

Binding characteristics of beta-adrenergic receptors of anterior pituitary membranes in control rats (C) and in animals treated with thyroid hormones (T), estradiol (E) or their combination (T+E)

Pool No.	B_{max} (fmol/mg protein)				K_d (nM)			
	C	T	E	T+E	C	T	E	T+E
1	1.8	3.7	1.8	2.9	1.7	3.0	1.6	1.5
2	1.7	3.2	1.3	3.7	2.0	4.6	0.7	1.3
3	1.8	4.3	1.3	3.6	2.8	1.6	1.0	1.4
4	2.7	4.8	0.8	3.6	2.6	3.4	0.5	1.5
5	2.5	3.0	1.5	4.2	4.5	2.5	1.4	1.7
6	1.6	3.9	1.3	3.5	1.4	3.0	1.0	1.5
7	1.7	–	–	–	1.0	–	–	–
8	2.2	–	–	–	1.4	–	–	–
9	2.0	–	–	–	1.4	–	–	–
Means	2.0	3.8*	1.3*	3.6*	2.1	3.0	1.0	1.5
±SEM	±0.1	±0.3	±0.1	±0.2	±0.4	±0.4	±0.2	±0.1

*Pools No. 1, 2, and 3 (T and T+E) – animals treated with T_4. Values are means ± S.E.M. for each group. * $P<0.05$ compared to the controls.*

Discussion

The present study demonstrated that chronic administration of thyroid hormones and estradiol alone or in combination significantly reduced body weight. Apparently, the main mechanism of body weight reduction was the fall of food

consumption during the first week in all three experimental groups compared to controls. With regard to absolute and relative adenohypophyseal weight, thyroid hormones inhibited the growth reaction of the adenohypophysis to estrogens. Since there was only a weak correlation between adenohypophyseal and body weight, there is probably no effect of body weight on the reduction of adenohypophyseal weight. The antagonistic effect between estrogen and thyroid action on the adenohypophyseal receptors has long been demonstrated. Schreiber *et al.* (1970) described the increase of thyroxine binding by adenohypophyseal proteins after estrogen treatment and showed that estrogens increase the affinity or the number of the adenohypophyseal binding sites for thyroxine. The role of the adenohypophyseal receptors was emphasized by De Lean *et al.* (1977) who discovered an increased number of pituitary TRH receptors in hypothyroid rats, which increased after estrogen administration. Moreover, Altschuler *et al.* (1988) showed that hypothyroid rats had a significant decrease in the concentration of estrogen receptors in the cytosol and nuclei of pituitary cells and in the nuclei of hypothalamic cells. On the basis of these and other studies, a new mechanism of the action of steroid and thyroid hormones may include changes in the binding characteristics of adrenergic and dopaminergic receptors of the hypothalamo-hypophyseal system. In our case we found that the inhibition of the adenohypophyseal growth reaction to estradiol, given simultaneously with thyroid hormones, was accompanied by a significant increase of β-adrenergic receptors (but not their affinity) as compared to controls and estradiol-treated groups. A similar change of these receptors was also found in the thyroid-treated group. In experimental models of hypo- and hyperthyroidism, specific brain-regional changes in noradrenaline turnover and enzymes of catecholaminergic synthesis were demonstrated (Atterwill *et al.* 1984). It cannot therefore be excluded that the changes of these receptors and other receptor classes may reflect such alterations. Krieg *et al.* (1988) showed that the β-adrenergic system can inhibit GH secretion by means of somatostatin release and Schwartz *et al.* (1977) described that β-NGF content and secretion rate of neuroglial cells were increased by isoproterenol activation of βAR. Recently Riss *et al.* (1989a,b) described that GH_3C_{14} rat pituitary tumor cells required both thyroid hormones and estrogens for optimum tumor formation and that a 56 °C labile serum factor(s), most likely a member of the insulin-like family, was required for the estrogen-induced growth response. Huang *et al.* (1982) demonstrated high cAMP levels in regressing rat mammary tumors compared to low levels in rapidly growing tumors. With regard to α- and β-adrenergic receptors, Zierhut *et al.* (1989) observed that the rapid development of cardiac hypertrophy in the norepinephrine model seems to be directly mediated by stimulation of myocardial α- and β-adrenergic receptors rather than by haemodynamic changes. On the basis of these results and recent discoveries of growth factors in the adenohypophysis it cannot be ruled out that thyroid hormones influence the growth reaction of the adenohypophysis (? *via* β-adrenergic receptors) through these factors or *vice versa*.

The changes in pituitary receptor binding characteristics described in the present study show that the inhibitory effect of thyroid hormones on the estrogen-induced growth reaction of the adenohypophysis is accompanied by an increase of β-adrenergic receptors which can be one of the causes or a consequence of pituitary growth.

References

ALI S.F., PECK J. Jr.: Modulation of anterior pituitary dopamine receptors by estradiol 17-β: dose-response relationship. *J. Neurosci. Res.* **13**: 497–507, 1985.

ALTSCHULER L.R., CEPPI J.A., RITTA M.N., CALANDRA R.S.: Effects of thyroxine on estrogen receptor concentration in anterior pituitary and hypothalamus of hypothyroid rats. *J. Endocrinol.* **119**: 383–387, 1988.

ATTERWILL C.K., BUNN S.J., ATKINSON D.J., SMITH S.L., HEAL D.J.: Effects of thyroid status on presynaptic α_2-adrenoceptor function and β-adrenoceptor binding in the rat brain. *J. Neural. Transm.* **59**: 43–55, 1984.

BRESSION O., BRANDI A.M., NOVSBAUM A., LE DAFNIET M., RACADOT J., PEILLON F.: Evidence of dopamine receptors in human growth hormone (GH)-secreting adenomas with concomitant study of dopamine inhibition of GH secretion in a perifusion system. *J. Clin. Endocrinol. Metab.* **55**: 589–593, 1982.

DE LEAN A., FERLAND L., OROVIN J., KELLY P. A., LABRIE F.: Modulation of pituitary thyrotropin releasing hormone receptor levels by estrogens and thyroid hormones. *Endocrinology* **100**: 1496–1504, 1977.

DE SOUZA E.B.: Localization and modulation of brain and pituitary receptors involved in stress responses. *Psychopharmacol. Bull.* **24**: 360–364, 1988.

DUŠKOVÁ, J., SCHREIBER V.: Nucleolar inhibition after combined thyroid hormone plus lisuride treatment in estrogenized rats. *Endocrinol. Exp.* **23**: 185–195, 1989.

GINSBERG A.M., CLUTTER W.E., SHAH S.D., CRYER P.E.: Triiodothyronine-induced thyrotoxicosis increases mononuclear leucocyte β-adrenergic receptor density in man. *J. Clin. Invest.* **67**: 1785–1791, 1981.

GROSS G., BRODDE O.E., SCHUMANN H.J.: Decreased number of β-adrenoceptors in cerebral cortex of hypothyroid rats. *Eur. J. Pharmacol.* **61**: 191–194, 1980.

HUANG F.L., CHO-CHUNG Y.S.: Dibutyryl cyclic AMP treatment mimics ovariectomy: new genomic regulation in mammary tumor regression. *Biochem. Biophys. Res. Commun.* **107**: 411–415, 1982.

KRIEG R.J., PERKINS, S.N., JOHNSON J.H., ROGERS J.P., ARIMURA A., CRONIN M.J.: Beta-adrenergic stimulation of growth hormone (GH) release in vivo, and subsequent inhibition of GH-releasing factor-induced GH secretion. *Endocrinology* **122**: 531–537, 1988.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265–275, 1951.

PACÁK K., NEDVÍDKOVÁ J., ŠTOLBA P., SCHREIBER V.: Increased number of beta-adrenergic receptors on the rat adenohypophyseal cells after thyroid hormone administration. *Physiol. Bohemoslov.* **39**: 14–17, 1990.

PERUMAL A.S., HALBREICH U., BARKAI A.J.: Modification of β-adrenergic receptor binding in rat brain following thyroxine administration. *Neurosci. Lett.* **48**: 217–221, 1984.

PETROVIC S.L., McDONALD J.K., BEDRAN DE CASTRO J.C., SNYDER G.D., MCCANN S.M.: Regulation of anterior pituitary and brain β-adrenergic receptors by ovarian steroids. *Life Sci.* **37**: 1563–1570, 1985.

RISS T.L., STEWART B.H., SIRBASKU D.A.: Rat pituitary tumor cells in serum-free culture. I. Selection of thyroid hormoneresponsive and autonomous cells. *In Vitro Cell. Dev. Biol.* **25**: 127–135, 1989a.

RISS T.L., STEWART B.H., SIRBASKU D.A.: Rat pituitary tumor cells in serum-free culture. II. Serum factor and thyroid hormone requirements for estrogen-responsive growth. *In Vitro Cell. Dev. Biol.* **25**: 136–142, 1989b.

SCHREIBER V.: Dopaminergic control of adenohypophyseal growth. *Voprosy biochimi mozga (Yerevan)* **13**: 221–225, 1979 (in Russian).

SCHREIBER V., PŘIBYL T., ROHÁČOVÁ J.: Adenohypophyseal growth after oestrogens in rats binding of ^{125}I-thyroxine by adenohypophysial proteins. *Physiol. Bohemoslov.* **19**: 501–506,1970.

SELYE H., COLLIP J.B., THOMSON D.L.: Effect of oestrin on ovaries and adrenals. *Proc. Soc. Exp. Biol.* **32**: 1377–1381, 1935.

SCHWARTZ J.P., COSTA E.: Regulation of nerve growth factor content in C_6 glioma cells by β-adrenergic receptor stimulation. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **300**: 123–129, 1977.

WILKINSON M., HERDON H., PEARCE M., WILSON C.A.: Precocious puberty and change in α- and β-adrenergic receptors in the hypothalamus and cerebral cortex of immature female rats. *Brain Res.* **167**: 195–199, 1979a.

WILKINSON M., HERDON H., PEARCE M., WILSON C.A.: Radioligand binding studies on hypothalamic noradrenergic receptors during the estrous cycle or after steroid injection in ovariectomized rats. *Brain Res.* **168**: 652–655, 1979b.

WILLIAMS L.T., LEFKOWITZ R.J., WATANABE A.M., HATAWAY D.R., BESH H.J.: Thyroid hormones regulation of β-adrenergic receptor number. *J. Biol. Chem.* **252**: 2787–2791, 1977.

ZIERHUT W., ZIMMER H.G.: Significance of myocardial α- and β-adrenoceptors in catecholamine-induced cardiac hypertrophy. *Circ. Res.* **65**: 1417–1425, 1989.

Reprint Requests

Dr. K. Pacák, 3rd Department of Medicine, Faculty of Medicine, Charles University, CS–128 21 Prague 2, U nemocnice 1.