

EFFECTS OF INTRACEREBROVENTRICULARLY ADMINISTERED OCTREOTIDE ON GONADOTROPHIC CELLS IN FEMALE RATS

Nataša Nestorović, Mirjana Louren, Milka Sekulić, Nataša Negić, Branka Šošić-Jurjević,
Milica Manojlović-Stojanoski, Branko Filipović, Verica Milošević

Siniša Stanković Institute of Biological Research, Belgrade, Serbia and Montenegro

Summary: Effects of intracerebroventricularly (ICV) administered octreotide on gonadotrophic cells (FSH and LH) of adult Wistar female rats were examined by immunocytochemical and morphometric methods. The animals received ICV three 1.0 µg doses of octreotide dissolved in 10 µL saline every second day. The controls were treated with equivalent volume of physiological saline by the same schedule. FSH- and LH-producing cells were examined using peroxidase-antiperoxidase immunocytochemical procedure. Morphometric and stereologic examinations were performed to evaluate changes in the number, volume and volume densities of gonadotrophic cells. In females treated with octreotide, the gonadotrophic cells were smaller and often pycnotic, while the number of FSH- and LH-immunopositive cells *per unit area* (mm²) was significantly reduced. Octreotide also induced a significant reduction of the FSH- and LH-immunoreactive cells volume, as well as of their volume densities. On the basis of these results it can be concluded that octreotide, centrally administered to adult female rats provokes changes in immunocytochemical and morphometric features of both types of gonadotrophic cells.

Key words: rat females, somatostatin, octreotide, gonadotrophic cells, immunocytochemistry, stereology

Introduction

Somatostatin or somatotropin release inhibiting hormone (SRIH) was originally detected by Krulich et al. (1) during the studies of the distribution of growth hormone-releasing factor in rat hypothalamus. In 1973, it was characterized by Brazeau et al. (2) and since then attracted much attention because of its wide variety of biological functions in nervous and other tissues. At the periphery, SRIH is a modulator of endocrine and exocrine functions and regulates differentiation and proliferation of normal and tumour cells (3–6). Within the nervous system, SRIH acts as a neuromodulator with physiological effects on neuroendocrine, motor and cognitive functions (3, 7, 8). Because of its ability to inhibit many functions of various

organs, its therapeutic value in clinical conditions involving the hyperfunction of these organs, was expected. There are two important native bioactive somatostatins: a tetradecapeptide – somatostatin 14 (SRIH-14) and an amino-terminal-extended form somatostatin 28 (SRIH-28). Both of them have a short duration of action (a half-life in the circulation of less than three minutes) which made their clinical use difficult. Thus, octreotide, a synthetic somatostatin analogue consisting of 8 amino acids, was introduced for clinical use. It was shown to inhibit the release of growth hormone (GH), glucagon and insulin in monkeys 45, 11, and 1.3 times more powerfully, respectively, than SRIH-14. Furthermore, its elimination half-life after subcutaneous administration is two hours (9).

In the pituitary gland, SRIH dramatically inhibits GH secretion and also blocks the release of other adenohypophyseal hormones such as thyrotrophin (TSH; 2, 10) prolactin (PRL), and in some cases, adrenocorticotropin (ACTH) (11, 12). However, the effects of SRIHs on gonadotrophin release are still controversial. Several authors reported that SRIH does not affect gonadotrophin secretion (3, 13, 14). On the other hand, some others found that somatostatin induced reduction of gonadotrophin-producing tumours (15)

Address of correspondence

Nataša Nestorović, M.Sc.
Institute for Biological Research
29 Novembra 142
11060 Belgrade
Serbia and Montenegro
Telephone: # (381-11)-207-8323 ext. 123
Fax: # (381-11)-761-433
E-mail: mata@ibiss.bg.ac.yu

and their secretion (16). This prompted us to examine the effects of ICV administered octreotide on immunocytochemical and morphometric properties of gonadotrophic cells in female rats.

Material and Methods

Animals

Adult virgin Wistar female rats maintained at 22 ± 2 °C with a 12/12 h light/dark cycle and free access to water and food, were used.

Surgical procedures were performed under ether anaesthesia. A headset was implanted into the rats and used for intracerebroventricular (ICV) injections. A minimum recovery time of five days was allowed before the experiments. The headset consisted of Silastic-sealed 20-gauge cannula (17), implanted into the lateral cerebral ventricle, 1.0 mm posterior and 1.5 mm lateral to the bregma and 3 mm below the cortical surface. A small stainless cerebroventricular steel cannula and a screw were cemented in the skull with dental acrylic resin (Stigmall; ICN Galenika).

Treatment protocol

After recovery from the surgery, the rats were divided into two groups each consisting of five animals. Females of the first group received ICV three 1.0 µg doses of octreotide (Sandoz, Switz.) dissolved in 10 mL saline every second day. The second group serving as a control received the equivalent volume of physiological saline by the same schedule. All animals were killed by decapitation under deep ether anaesthesia five days after the last injection.

Immunocytochemistry

The pituitaries were excised, fixed in Bouin's solution, dehydrated and embedded in paraffin wax. Pituitary sections (5 µm thick) were immunocytochemically stained. For immunocytochemistry, series of seven sections of the pituitary cut through three tissue levels (dorsal, middle and ventral portion) of the *pars distalis* were used. The pituitary gonadotrophs were localized by the peroxidase-antiperoxidase (PAP) method. Non-specific background staining was reduced by incubation with non-immune porcine serum (1:10; 45 min). Anti-rat βLH serum (diluted with PBS 1:500) and anti-rat βFSH (diluted with PBS 1:300) kindly provided by Dr. A.F. Parlow, NIH, Bethesda, MD, USA, served as primary antibodies (45 min, ambient temperature). Subsequently, the sections were treated with secondary antibody (1:200 swine-anti-rabbit IgG; 45 min; a product of DAKO A/S, Glostrup, Denmark) and rabbit-antiperoxidase serum (1:100; 45 min; a product of DAKO A/S, Glostrup, Denmark). The antigen-antibody complex was visualized by incubating

the sections with chromogen substrate, 0.05% 3,3-diaminobenzidine (DAB; Serva, Heidelberg, Germany) and 0.03% H₂O₂. The incubated sections were counterstained with haematoxylin. Control sections were incubated with normal porcine serum without primary antisera.

Morphometry

Immunocytochemically stained sections of pituitaries cut through three tissue levels of the *pars distalis* were used for morphometric examinations of anti-rat βFSH and anti-rat βLH reactive cells with visible nuclei. The cell volume of gonadotrophic cells (V_c) and their volume densities (V_v) were estimated under the light microscope at 1000 Vv magnification on 5 µm thick sections, using the M_{42} multipurpose test system (18). The volumes of FSH- and LH-positive cells were expressed in µm³ and their volume densities as percentages of total pituitary cells in mm³. At the same time, the number of immunoreactive cells *per* unit area (mm³) in each section was analyzed.

Results

Gonadotrophic cells (FSH and LH) of control female rats were strongly immunostained, present throughout pituitary *pars distalis*, occasionally alone or in clusters and often in close contact with blood capillaries (Figs. 1a, b). They were large, polygonal, oval or polyhedral in shape, with prominent, often eccentrically located nuclei. In the group ICV treated with octreotide, gonadotrophic cells were smaller comparing to the controls and often pycnotic (Figs. 1b, c).

In pituitaries of octreotide-treated female rats, the number of FSH- and LH-immunoreactive cells *per* unit area (mm²) was decreased by 21.4% and 17.2%, respectively, and the difference in comparison with the control was statistically significant ($p < 0.005$) (Figure 2a). Stereological analysis showed that octreotide treatment resulted in a significant volume decrease in both types of gonadotrophic cells (Figure 2b). The volume of immunopositive FSH cells was reduced by 15.6% ($p < 0.01$), while the immunolabelled LH cell volume was decreased by 20.6% ($p < 0.005$). In octreotide-treated females the percentage of FSH- and LH-positive cells *per* volume unit (mm³) of total pituitary gland tissue, *i.e.* the volume density, was significantly reduced and the extent of this reduction was about equal in both types of gonadotrophic cells (Figure 2c). Namely, volume density of FSH-immunopositive cells was decreased by 30.6% ($p < 0.001$) and that of LH-reactive cells by 30.1%.

Discussion

The pituitary gland is a major target for the physiological actions of somatostatins. Although some

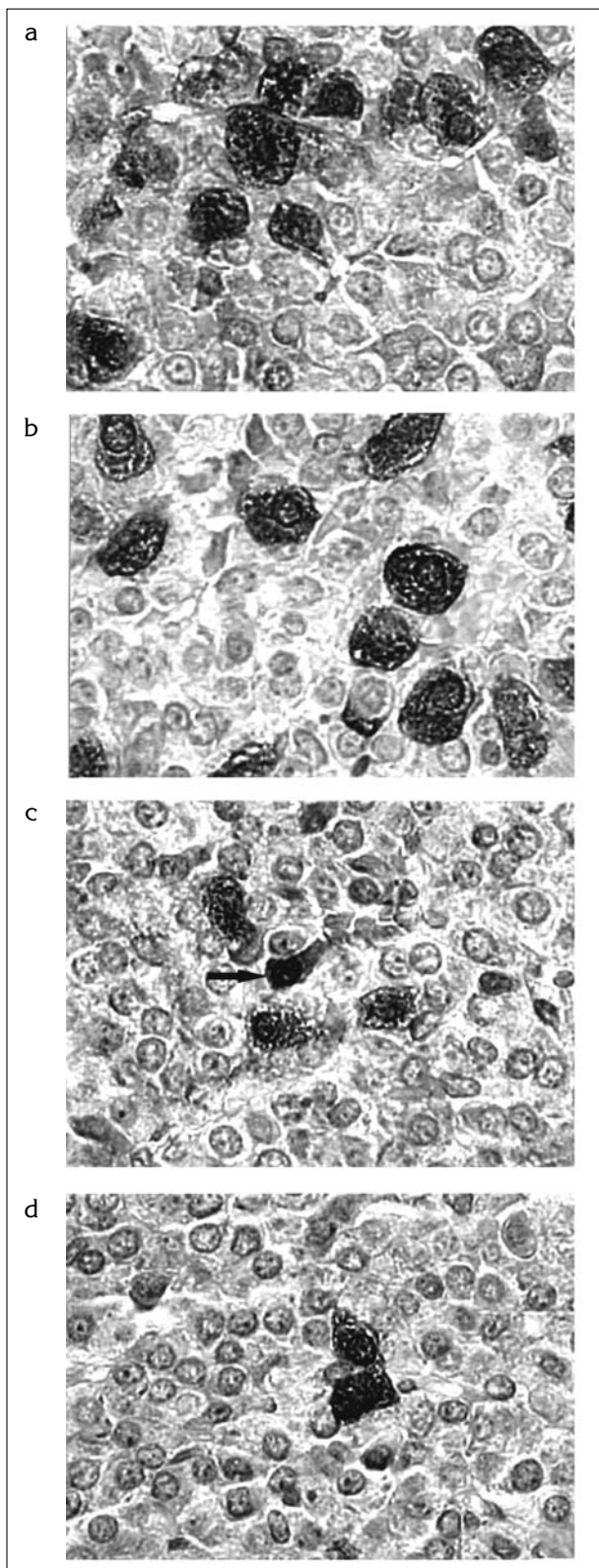


Figure 1. Parts of *pars distalis* of the adenohypophysis in control and octreotide-treated rat females. a) Immunoreactive β FSH and (b) β LH cells in the controls; c) Small FSH-immunopositive cells and (d) immunoreactive LH cells after octreotide treatment. Arrow indicates small pycnotic FSH-positive cell. Objective (Magnification 63 \times).

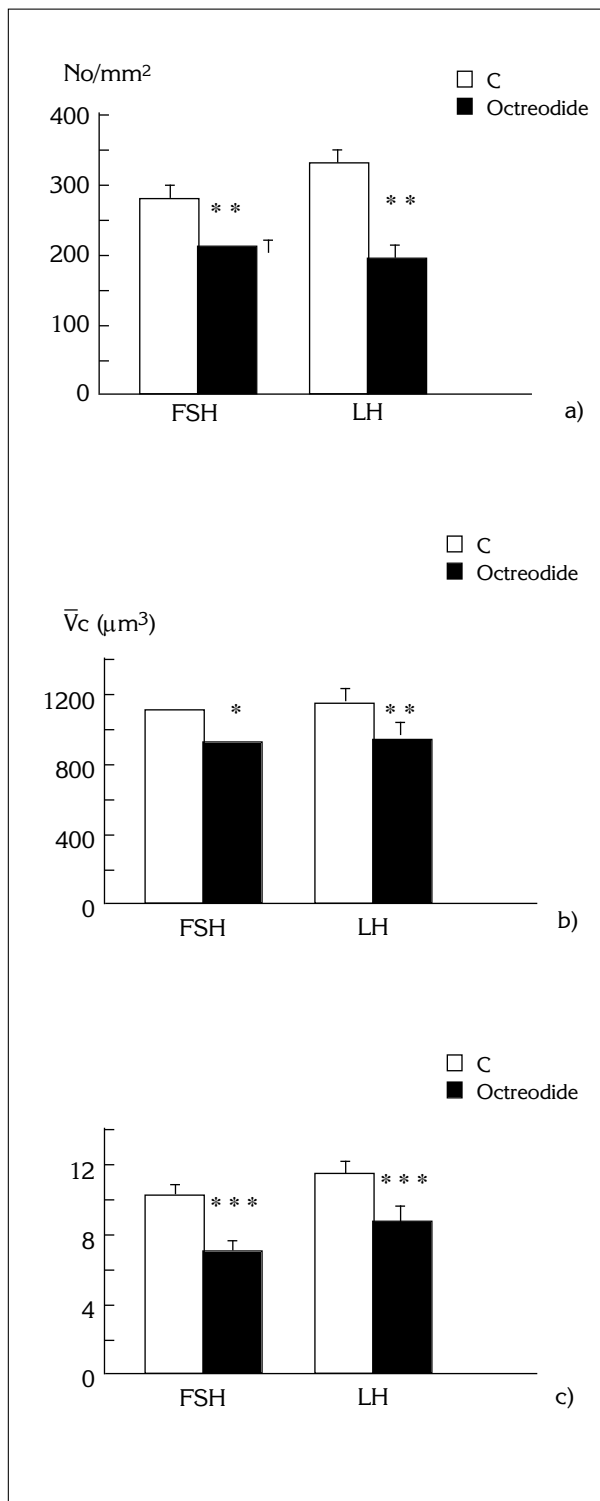


Figure 2. a) The number (No) of immunoreactive FSH and LH cells *per* unit area (mm^2) in control (C) and octreotide-treated rat females; b) Cellular (\bar{V}_c) volume (μm^3) of FSH- and LH-immunopositive cells in control and octreotide-treated animals; c) Volume density (\bar{V}_v) of immunoreactive FSH and LH cells expressed in percentages (%) of total adenohypophyseal cells in mm^3 in control (C) and octreotide-treated rats. All values are presented as means \pm SD. * $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$.

studies indicate that SRIH does not affect FSH and LH secretion (3, 13, 14), our results clearly demonstrate that centrally used somatostatin analogue octreotide acted inhibitory on immunocytochemical and morphometric characteristics of both types of gonadotrophic cells. FSH- and LH-immunoreactive cells were often pycnotic after three bolus ICV injections of octreotide, what is a clear sign of its inhibitory influence. The number of gonadotrophic cells *per* unit area of pituitary *pars distalis* was lower in octreotide-treated females rats than in the controls that received physiological saline by the same route. Furthermore, stereological analysis showed a significant decrease in volume and volume densities of FSH- and LH cells after octreotide treatment.

The inhibitory action of somatostatin was also detected under *in vitro* conditions. It suppressed LH secretion in cultured human foetal cells (19) and significantly inhibited LH but not FSH release in the presence of luteinizing hormone releasing hormone (LHRH) in the culture of rat pituitary cells (20). It should also be mentioned, that octreotide efficiently lowered high LH levels in women with polycystic ovary syndrome (PCOS; 21). We have previously demonstrated *in vivo* inhibition of pituitary gonadotrophs by somatostatin. Intracerebroventricularly administered SRIH-14 and -28 acted inhibitory on pituitary FSH and LH cells of adult rat females, as judged by morphometric parameters (22). Furthermore, both these somatostatins, ICV administered to male rats, not only induced a decrease in LH cells size, but also reduced LH serum levels (23).

The biological effects of somatostatin are mediated through its five distinct receptor subtypes (SSTR 1–5). The presence of all five SSTR's mRNA in every type of hormone-secreting cells (GH, TSH, PRL, LH, FSH and ACTH) of rat pituitary gland was documented by O'Carroll and Krempels (24). According to these authors the majority of LH cells was colocalized with SSTR-2 mRNA, while FSH cells expressed low but similar amounts of all five SSTR mRNAs. In a parallel study, Day et al. (25) detected colocalization of SSTR-2 and -5 mRNAs in FSH-secreting cells, while among these two, SSTR-5 was dominant. Octreotide primarily interacts with SSTR-2 and SSTR-5 and binds with a moderate affinity at SSTR-3 (26). Therefore, this somatostatin analogue can affect both types of gonadotrophic cells. The ability of octreotide to reduce the size and secretion of gonadotropinomas (15, 16) and to lower pathologically high LH levels in women with PCOS may be of a very important clinical relevance.

In conclusion, our results indicate that ICV administered somatostatin analogue octreotide exerts significant inhibitory effects on the immunocytochemical and morphometric characteristics of both types of gonadotrophs.

Acknowledgement. This work was supported by the Ministry for Science, Technology and Development of Serbia, Contract #1710. The authors are grateful to Dr. A.F. Parlow of the National Institute of Health, Bethesda, MD, USA, for a kind donation of antisera.

EFEKTI INTRACEREBROVENTRIKULARNOG TRETMANA OKTREETIDOM NA GONADOTROPNE ĆELIJE ŽENKI PACOVA

Nataša Nestorović, Mirjana Lovren, Milka Sekulić, Nataša Negić, Branka Šošić-Jurjević, Milica Manojlović-Stojanoski, Branko Filipović, Verica Milošević

Institut za biološka istraživanja »Siniša Stanković«, Beograd, Srbija i Crna Gora

Kratak sadržaj: Ispitivani su efekti oktreotida davanog intracerebroventrikularno (ICV) odraslim ženka pacova na gonadotropne ćelije (FSH i LH). Životinje su tretirane sa ukupno tri doze od 1 µg oktreotida rastvorenog u 10 µL fiziološkog rastvora svakog drugog dana. Kontrole su na isti način primile odgovarajuću zapreminu fiziološkog rastvora. FSH i LH ćelije su ispitivane imunocitohemijskim postupkom peroksidaza-antiperoksidaza. Da bi se ustanovile promene u broju, zapremini i zapreminskoj gustini gonadotropnih ćelija primenjen je morfometrijski kao i stereološki pristup. Kod ženki tretiranih oktreotidom, gonadotropne ćelije su bile manje i često piknotične. Broj imunopozitivnih FSH i LH ćelija po jedinici površine (mm²) je bio statistički značajno smanjen. Oktreotid je, takodje, izazvao značajno smanjenje zapremine FSH i LH imunoreaktivnih ćelija, kao i njihove zapreminske gustine. Na osnovu ovih rezultata, može se zaključiti da je oktreotid, dat ICV odraslim ženka pacova doveo do promena imunocitohemijskih i morfometrijskih osobina oba ispitivana tipa gonadotropnih ćelija.

Ključne reči: ženke pacova, somatostatin, oktreotid, gonadotropne ćelije, imunocitohemija, stereologija

References

1. Krulich L, Dhariwal APS, McCann SM. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary *in vitro*. *Endocrinology* 1968; 83: 783–90.
2. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 1973; 179: 77–83.
3. Reichlin S. Somatostatin. Parts 1 and 2. *N Engl J Med* 1983; 309: 1495–1501; 1556–63.
4. Raynor K, Reisine T. Somatostatin receptors. *Crit Rev Neurobiol* 1992; 6: 273–89.
5. Patel YC. Somatostatin and its receptor family. *Front Neuroendocrinol* 1999; 20: 157–98.
6. Ferjoux G, Bousquet C, Cordelier P, Benali N, Lopez F, Rochaix P, Buscail L, Susini C. Signal transduction of somatostatin receptors negatively controlling cell proliferation. *J Physiol Paris* 2000; 94: 205–10.
7. Epelbaum A. Somatostatin in the central nervous system: physiology and pathological modifications. *Prog Neurobiol* 1986; 27: 63–100.
8. Schindler M, Humphrey PP, Emson PC. Somatostatin receptors in the central nervous system. *Prog Neurobiol* 1996; 50: 9–47.
9. Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P et al. SMS 201–995: A very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci* 1982; 31: 1133–40.
10. Sekulić M, Lovren M, Milošević V. Somatostatin acts by inhibiting pituitary TSH cells in female rats. *Acta Vet* 2000; 50: 3–12.
11. Milošević V, Brkić B, Velkovski S, Sekulić M, Lovren M, Starčević V, Severs WB. Morphometric and functional changes of rat pituitary somatotropes and lactotropes after central administration of somatostatin. *Pharmacology* 1998; 57: 28–34.
12. Starčević V, Milošević V, Brkić B, Severs WB. Effects of centrally applied somatostatin on pituitary adrenocorticotropes in female rats. *Pharmacology* 2000; 60: 203–7.
13. Samson WK, Aguila MC, Lumpkin MD, McCann SM. Somatostatin: Recent studies on hypothalamic secretion and local bioaction. In: Negro-Villar A, Conn, PM. eds. *Peptide hormones: effects and mechanisms of action*. Boca-Raton: FL, CRC Press, 1988: 67–77.
14. Schusziarra V. Somatostatin: Biological actions and pathophysiology. In: Scarpignato C. ed. *Octreotide: From Basic Science to Clinical Medicine*, *Prog. Basic. Clin. Pharmacol.*, Basel: Karger, 1996: 35–53.
15. Sy RA, Bernstein R, Chynn KY, Kourides IA. Reduction in size of thyrotrophin- and gonadotrophin-secreting pituitary adenomas treated with octreotide acetate (somatostatin analog). *J Clin Endocrinol Metab* 1992; 74: 690–4.
16. Klibanski A, Alexander JM, Bikkal HA, Hsu DW, Swearingen B, Zervas NT. Somatostatin regulation of glycoprotein hormone and free subunit secretion in clinically nonfunctioning and somatotroph adenomas *in vitro*. *J Clin Endocrinol Metab* 1991; 73: 1248–55.
17. Starčević VP, Morrow BA, Farner LA, Keil LC, Severs WB. Long-term recording of cerebrospinal fluids pressure in freely behaving rats. *Brain Res* 1988; 462: 112–7.
18. Weibel ER. *Stereological methods: Practical Methods of Biological Morphometry*, Vol. 1. London: Academic Press, 1979: 1–415.
19. Shimon I, Melmed S. Structure and function of somatostatin receptors in growth hormone control. *J Endocrinol* 1997; 155: S3–S6.
20. Yu WH, Kimura M, McCann SM. Effect of somatostatin on the release of gonadotropins in male rats. *Proc Soc Exp Biol Med* 1997; 214: 83–6.
21. Prelević MG, Wurzbürger IM, Balint-Perić Lj, Nešić JS. Inhibitory effect of sandostatin on secretion of luteinizing hormone and ovarian steroids in polycystic ovary syndrome. *Lancet* 1990; 336: 900–1003.
22. Lovren M, Sekulić M, Milošević V, Radulović N. Effects of somatostatins on gonadotrophic cells in female rats. *Acta Histochem* 1998; 100: 329–35.
23. Starčević V, Milošević V, Brkić B, Severs WB. Somatostatin affects morphology and secretion of pituitary luteinizing hormone (LH) cells in male rats. *Life Sci* 2002; 70: 3019–27.
24. O'Carroll AM, Krempels K. Widespread distribution of somatostatin messenger ribonucleic acid in rat pituitary. *Endocrinology* 1995; 136: 5224–7.
25. Day R, Dong W, Panetta R, Kraicer J, Greenwood MT, Patel YC. Expression of mRNA for somatostatin receptor (sstr) types 2 and 5 in individual rat pituitary cells. A double labeling *in situ* hybridization analysis. *Endocrinology* 1995; 136: 5232–5.
26. Patel YC, Srikant CB. Somatostatin receptors. *Trends Endocrinol Metab* 1997; 8: 398–405.

Received: November 14, 2003

Accepted: December 18, 2003