Physiol. Res. 52: 341-346, 2003

Inhibin B, Follicle Stimulating Hormone, Luteinizing Hormone, and Estradiol and Their Relationship to the Regulation of Follicle Development in Girls during Childhood And Puberty

M. CHADA, R. PRŮŠA, J. BRONSKÝ, M. PECHOVÁ, K. KOTAŠKA, L. LISÁ[1]

Department of Clinical Biochemistry and Pathobiochemistry and [1]First Pediatric Department, Second Faculty of Medicine, Charles University, Faculty Hospital Motol, Prague, Czech Republic

Received April 5, 2002
Accepted June 24, 2002

Summary

Inhibin B, produced by granulosa cells in the ovary, is a heterodimeric glycoprotein suppressing synthesis and secretion of the follicle stimulating hormone (FSH). The aim of the present study was to determine hormone profiles of inhibin B, FSH, luteinizing hormone (LH), and estradiol in girls during childhood and puberty and to evaluate whether inhibin B is a marker of follicle development. We examined the correlation between inhibin B and gonadotropins and estradiol during the first two years and across the pubertal development. Using a specific two-side enzyme-linked immunosorbent assay (ELISA), inhibin B levels were measured in the serum of 53 healthy girls divided into 8 groups according to age. In addition, serum FSH, LH, and estradiol were measured by chemiluminescent immunoassay in all serum samples. A rise in serum levels of inhibin B (55.2±7.3 ng/l, mean ± S.E.M.) and FSH (1.78±0.26 UI/l), concomitant with a moderate increment of serum LH (0.36±0.09 UI/l) and estradiol (45.8±12.2 pmol/l) concentrations was observed during the first three months of life and declined to prepubertal concentrations thereafter. A strong positive correlation between inhibin B and FSH ($r = 0.48$, $p<0.05$), LH ($r = 0.68$, $p<0.001$) and estradiol ($r = 0.59$, $p<0.01$) was demonstrated during the first 2 years of life. A rise in serum levels of inhibin B, FSH, LH, and estradiol was found throughout puberty. Inhibin B had a strong positive correlation with FSH (stage I of puberty: $r = 0.64$, $p<0.05$; stage II of puberty: $r = 0.86$, $p<0.01$), LH (I: $r = 0.61$, $p<0.05$; II: $r = 0.67$, $p<0.05$), and estradiol (II: $r = 0.62$, $p<0.05$) in early puberty. From pubertal stage II, inhibin B lost this relationship to gonadotropins and estradiol. Serum inhibin B and FSH levels increased significantly during pubertal development, with the highest peak found in stage III of puberty (133.5±14.3 ng/l), and decreased thereafter. In conclusion, inhibin B is produced in a specific pattern in response to gonadotropin stimulation and plays an important role in the regulation of the hypothalamic-pituitary-gonadal axis during childhood and puberty in girls. Inhibin B is involved in regulatory functions in developing follicles and seems to be a sensitive marker of ovarian follicle development.

Key words

Inhibin B • Girl • Childhood • Puberty

Introduction

Hormones and growth factors produced by the hypothalamus, pituitary, and gonads maintain a normal reproductive cycle and regulate genital, gonadal, and sexual development in males and females. Growth and maturation of follicles and ovulation in the female depend on endocrine, paracrine, and autocrine acting hormones (de Kretser *et al.* 2000, Pezzani *et al.* 2001). Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are involved in the activation and regulation of the reproductive axis. Hormones produced by the gonads regulate FSH and LH synthesis and secretion in a feedback loop. Inhibin was first identified as a gonadal hormone that inhibits FSH synthesis by the pituitary and by pituitary cells in culture (Burger *et al.* 1988, Hayes *et al.* 1998). Inhibin is a disulfide-linked heterodimeric glycoprotein consisting of an α subunit and one of two possible β subunits, βA and βB, forming inhibin A (α-βA) and inhibin B (α-βB) and belonging to the TGF β superfamily, a group of structurally similar but functionally diverse growth factors. Only dimeric forms of inhibin are biologically active. Human body fluids contain forms of the α subunit not assembled with a β subunit, and lacking inhibin bioactivity. Additional gonadal-derived peptides, such as activin and follistatin, participate in the regulation of FSH and act on gonadal functions within the ovary (Ying 1988). Activin, a homodimeric glycoprotein composed of the β subunits, is a member of the TGF β superfamily and stimulates FSH secretion. Follistatin, a cysteine-rich monomeric activin binding glycoprotein, decreases FSH secretion by preventing the interaction of activin with its receptor (de Winter *et al.* 1996). The ovary is the major source of inhibin synthesis in the female. Granulosa cells in developing follicles, or lutein cells in the corpus luteum, secrete inhibin in response to gonadotropins and various other factors (Knight and Glister 2001).

It has been reported that inhibin exhibits different temporal profiles in the human menstrual cycle. Inhibin B is maximal in late follicular phase and luteal phase, whereas inhibin A is maximal in early follicular phase (Groome *et al.* 1996). Little is known about the possible biological role of inhibin in the regulation of the hypothalamic-pituitary gonadal hormonal axis in girls during childhood and puberty. It is well established that the hypothalamic-pituitary gonadal hormonal axis is postnatally activated in boys, exhibiting increased serum levels of inhibin B, gonadotropins, and gonadal steroids, and (re)activated with the onset of puberty, suggesting that both activations of the reproductive axis are important for Sertoli cell proliferation (Burger *et al.* 1991, Andersson *et al.* 1998a, b).

The aim of the present study was to determine secretion of the biologically active inhibin B, FSH, luteinizing hormone (LH), and estradiol and their changes in serum concentrations in girls during childhood and puberty. We studied the physiological consequence of the postnatal and pubertal activation of the hypothalamic-pituitary-gonadal hormonal axis in girls. We investigated the relationships between inhibin B and FSH, LH and estradiol in relation to age and pubertal development. This study was designed to evaluate inhibin B as a marker of ovarian function and to ascertain whether inhibin B along with changes in FSH, LH and estradiol are correlated with the pubertal development in girls.

Methods

We studied 53 girls divided into 8 groups according to age. Changes in inhibin B, gonadotropins and estradiol secretion were determined. Serum inhibin B, gonadotropins, and estradiol levels were measured from the day of birth to the final genital stage of puberty. The girls were healthy, with a normal growth pattern and pubertal development. None had any signs of acute or chronic disease. The pubertal developmental stage was recorded according to the method of Tanner (1975) (Table 1). The study was performed in accordance with the Helsinki Declaration.

Table 1. Stages of puberty according to Tanner (1975).

I	Preadolescent: elevation of papilla only (B1).
II	Breast bud stage: elevation of breast and papilla as small mound. Enlargement of areola diameter (B2).
III	Further enlargement and elevation of breast and areola with no separation of their contours (B3).
IV	Projection of areola and papilla to form a secondary mound above level of the breast (B4).
V	Mature stage: projection of papilla only, due to recession of the areola to the general contour of the breast (B5)

Venous blood samples were taken from all girls between 09:00 and 11:00. After clotting, the serum was separated by centrifugation and stored at –70 °C prior to analysis. Serum inhibin B was measured in duplicate by two-side enzyme-linked immunosorbent assays (ELISA) (Oxford

Bio-Innovation Inhibin-B Immunoassay Kit, Serotec Ltd, UK) using a monoclonal antibody raised against the inhibin βB subunit in combination with a labeled antibody raised against the inhibin α subunit as previously described (Groome *et al.* 1996). This assay was recently used in our laboratory to measure levels of serum inhibin B in boys (Chada *et al*. 2003). The limit of detection of the assays was 15 ng/l. The intra- and interassay coefficients of variation were below 7 %. FSH, LH and estradiol were measured in serum samples. Serum FSH, LH and estradiol levels were determined by chemiluminescent immunoassay technology using commercially available kits (Advia Centaur TM, Bayer, USA). The detection limit was 0.3 IU/l for FSH, 0.07 IU/l for LH and 37 pmol/l for estradiol.

Values were expressed as means ± S.E.M. Analysis of group means was performed using ANOVA analysis. Correlation coefficients were determined by regression. $P<0.05$ value was considered significant.

Results

Serum inhibin B, FSH, LH and estradiol levels

Means and range of serum inhibin B, gonadotropins and estradiol concentrations are summarized in Table 2. The individual serum levels of inhibin B, FSH, LH and estradiol in relation to age and pubertal development are plotted in Figure 1 (A-D). During the first 3 months, serum inhibin B, LH, FSH, and estradiol levels were detectable. At this time, inhibin B reached approximately a third of the concentration (55.2±7.3 ng/l) that we observed at puberty, it fell significantly to prepubertal levels thereafter and remained low at similar concentrations until the onset of puberty. A moderate rise in serum FSH (1.78±0.26 UI/l), LH (0.36±0.09 UI/l) and estradiol (45.8±12.2 pmol/l) levels was found at this period. Thereafter, serum LH and estradiol levels declined and remained undetectable or very low until puberty. From 3 months of age onward, FSH decreased more gradually and was measurable in most samples throughout childhood. At the onset of puberty, serum levels of all four measured hormones increased with advancing pubertal stage. Serum inhibin B and FSH concentrations increased significantly between stage I and III of puberty, with a peak in stage III of puberty (133.5±14.3 ng/L, 5.41±0.3 UI/l, respectively), followed by a subsequent decrease. Serum LH and estradiol levels increased strong significantly throughout the pubertal progression ($p<0.01$ and $p<0.001$, respectively).

Table 2. Distribution of serum inhibin B, gonadotropins, and estradiol levels in girls during childhood and puberty.

Group (age)	Number	Inhibin B (ng/l)	FSH (UI/l)	LH (UI/l)	Estradiol (pmol/l)
A	n = 5	55.2 ± 7.3	1.78 ± 0.26	0.36 ± 0.09	45.8 ± 12.2
(0-3 months)		(35.8-76.5)	(1.12-2.56)	(0.15-0.65)	(<37-72.1)
B	n = 5	29.5 ± 5.5	1.08 ± 0.20	0.13 ± 0.05	<37
(4-6 months)		(19.3-49.3)	(0.45-1.63)	(<0.07-0.31)	(<37-50.9)
C	n = 4	22.0 ± 3.4	0.50 ± 0.09	<0.07	<37
(7-24 months)		(16.3-31.5)	(0.34-0.75)	(<0.07-0.11)	(<37)
D	n = 5	23.2 ± 2.7	0.32 ± 0.08	<0.07	<37
(25 months-puberty onset)		(15.6-31.8)	(<0.10-0.47)	(<0.07)	(<37-54.8)
E	n = 9	33.1 ± 2.3	0.53 ± 0.08	<0.07	<37
(Tanner stage I)		(20.2-41.1)	(0.32-1.02)	(<0.07-0.15)	(<37-66)
F	n = 8	60.6 ± 6.1	2.74 ± 0.41	1.65 ± 0.21	69.5 ± 6.8
(Tanner stage II)		(35.6-86.0)	(1.25-4.15)	(0.85-2.54)	(42.1-98.2)
G	n = 8	133.5 ± 14.3	5.41 ± 0.30	4.49 ± 0.34	133.9 ± 12.1
(Tanner stage III)		(85.6-196.0)	(4.53-6.80)	(3.45-5.78)	(98.4-199.1)
H	n = 8	66.9 ± 6.4	4.46 ± 0.39	7.21 ± 0.65	229.5 ± 24.1
(Tanner stages IV and V)		(45.5-93.1)	(3.30-6.06)	(4.80-10.73)	(143.0-324.3)

Data are given as means ± S.E.M. and ranges (min and max)

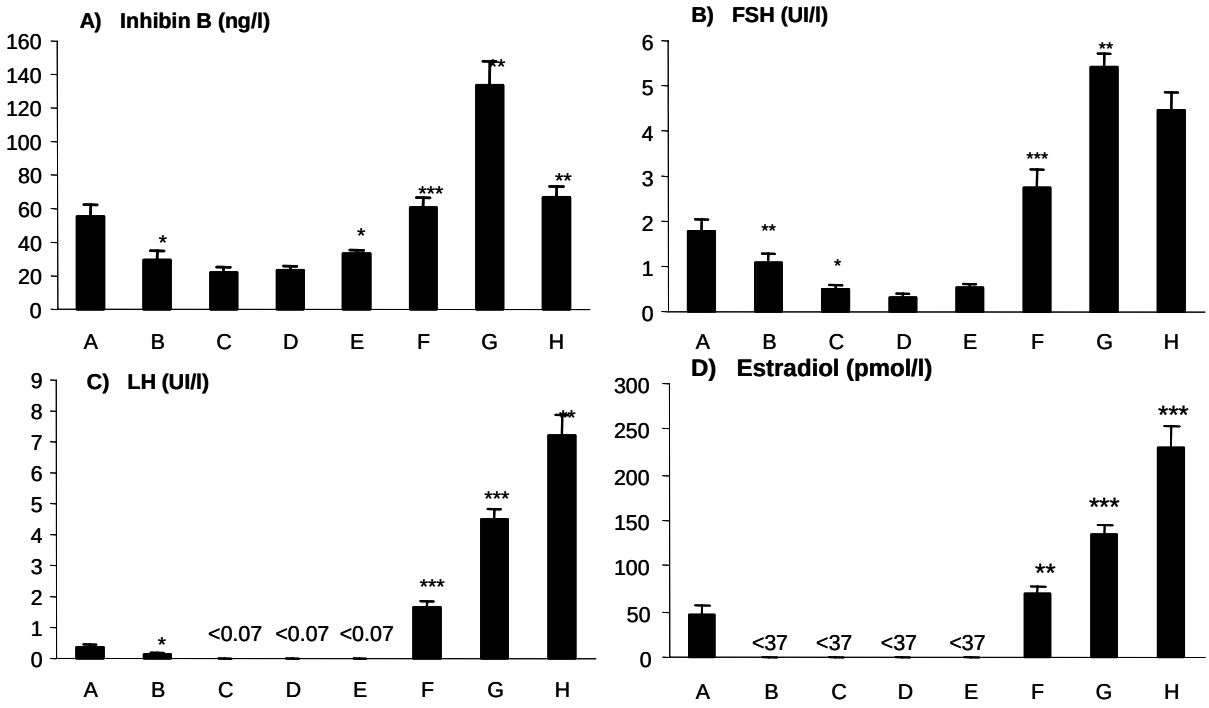

Fig. 1. *Individual serum inhibin B* ***(a)****, FSH* ***(b)****, LH* ***(c)*** *and estradiol* ***(d)*** *concentrations (mean ± S.E.M.) in girls during childhood and pubertal development. Age categories: A (n = 5), 0-3 months; B (n = 5), 4-6 months; C (n = 4), 7-24 months; D (n = 5), 25 months to the onset of puberty; E (n = 9), Tanner stage I; F (n = 8), Tanner stage II; G (n = 8), Tanner stage III, H (n = 8), Tanner stages IV and V. Asterisks above the bars indicate statistically significant differences of means with respect to previous age category (by Mann-Whitney U test): * p<0.05; ** p<0.01; *** p<0.001.*

Table 3. Correlation coefficients between inhibin B and gonadotropins and estradiol during the first two years and during pubertal development.

Age	Inhibin B vs. FSH	Inhibin B vs. LH	Inhibin B vs. Estradiol
0-2 years	0.48 *	0.68 ***	0.59 **
Stage I	0.64 *	0.61 *	0.38
Stage II	0.86 **	0.67 *	0.62 *
Stage III	0.44	0.52	0.46
Stage IV and V	0.22	0.46	-0.09

** p<0.05; ** p<0.01; *** p<0.001*

Correlation of inhibin B to gonadotropins and estradiol

Correlation coefficients of inhibin B to FSH, LH, and estradiol during the first 2 years of life and according to the stage of puberty are shown in Table 3. A positive correlation was demonstrated between inhibin B and FSH (r = 0.48, p<0.05), LH (r = 0.68, p<0.001) and estradiol (r = 0.59, p<0.01) during the first 2 years of life. At pubertal stage I and II, a significant relationship developed between inhibin B and FSH (I: r = 0.64, p<0.05; II: r = 0.86, p<0.01) and LH (I: r = 0.61, p<0.05; II: r = 0.67, p<0.05), but these relationships were lost thereafter. At pubertal stage II, inhibin B correlated strongly with estradiol (r = 0.62, p<0.05). From stage II

of puberty onward, no correlation between inhibin B and gonadotropins or estradiol was observed.

Discussion

In this study, age-related changes in levels of serum inhibin B, gonadotropins and estradiol occurring during childhood were determined during pubertal development in girls. Changes in correlation between inhibin B and the other reproductive hormones were examined during the first 2 years after birth and through the pubertal stages. High serum inhibin B concentrations in newborn girls reached levels observed in pubertal girls (Bergada *et al*. 2002). In girls under 6 months of age, Bergada *et al.* (1999) reported that serum inhibin B attained half the concentration seen at puberty and dropped to prepubertal levels after this period. We demonstrated a strong positive correlation between inhibin B and gonadotropins and estradiol during the first 2 years of life. A significant positive correlation of inhibin B with LH was described by Bergada *et al.* (2002), whereas no significant correlation with FSH from birth to 2 months of age was found. There was a significant positive relationship between inhibin B and FSH, LH and estradiol from birth to puberty (Bergada *et al.* 1999). Burger *et al.* (1991) observed a positive correlation between immunoreactive inhibin and LH which was better than that with FSH during the first months of life. These data supported biological role of inhibin B and gonadotropins in the regulation of early postnatal activation of the hypothalamic-pituitary-gonadal hormone axis in girls leading to growth and development of follicles in the ovary during the neonatal period. Inhibin B produced by developing follicles is influenced by gonadotropins, whereas the wide variability in gonadotropins secretion makes difficult to estimate time sequence between inhibin B and gonadotropins production in early infancy. A higher gonadal endocrine activity during the first months after birth appears to be important for folliculogenesis. At the onset of puberty, the hypothalamic-pituitary-gonadal hormone axis is (re)activated. In agreement with earlier studies (Foster *et al.* 2000, Sehested *et al.* 2000), we found a rise in serum levels of inhibin B, FSH, LH, and estradiol throughout puberty in girls. Serum inhibin B and FSH levels increased markedly with pubertal development, with the highest peak being found during stage III of puberty, and decreasing thereafter. Serum inhibin B levels in girls during puberty attained less than half the concentrations found in previous findings in boys (Andersson *et al.* 1997, Crofton *et al.* 1997). LH concomitantly with estradiol increased progressively during pubertal development. We proved a strong positive correlation between inhibin B and FSH, LH, and estradiol in early puberty. From pubertal stage II, inhibin B lost this relationship to gonadotropins and estradiol. Growth and development of follicles associated with oocyte maturation depend on differential regulations by extrinsic and intraovarian factors (Knight and Glister 2001). A rise in serum inhibin B levels during puberty seems to be a consequence of a higher activity of follicle development which is induced by FSH in early puberty. A positive correlation betwen inhibin B and FSH in early puberty suggests that FSH is able to regulate inhibin B secretion. It has been established that inhibin B is secreted from developing preantral and small antral follicles from the beginning of the follicular phase, with a peak following the FSH increase and a progressive fall during luteal phase. In contrast, inhibin A levels are low in the early follicular phase and maximal in the late follicular phase and luteal phase (Groome *et al.* 1996). It suggests that inhibin B is produced during follicle development and is a marker of follicle growth.

In conclusion, inhibin B is produced in a specific pattern in response to gonadotropin stimulation and plays an important role in the regulation of the hypothlamic-pituitary-gonadal axis during childhood and puberty in girls. Inhibin B is involved in regulatory functions in developing follicles and appears to be a sensitive marker of ovarian follicle development. Further knowledge of changes in serum levels of gonadal hormones occurring during childhood and puberty may be useful in understanding the pathophysiology of gonadal diseases.

Acknowledgements

The study was supported by research grant MŠMT ČR No. 111100003/12.1.

References

ANDERSSON AM, JULL A, PETERSON JH, MÜLLER J, GROOME NP, SKAKKEBAEK NE: Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone and estradiol levels. *J Clin Endocrinol Metab* **82**: 3976-3982, 1997.

ANDERSSON AM, TOPPARI J, HAAVISTO AM, PETERSON JH, SIMELL T, SIMELL O, SKAKKEBAEK NE: Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab* **83**: 675-681, 1998a.

ANDERSSON AM, MÜLLER J, SKAKKEBAEK NE: Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. *J Clin Endocrinol Metab* **83**: 4451-4458, 1998b.

BERGADA I, ROJAS G, ROPELATO MG, BERGADA C, CAMPO S: Sexual dimorphism of monomeric and dimeric inhibins in normal boys and girls from birth to puberty. *Clin Endocrinol* **51**: 455-460, 1999.

BERGADA I, BALLERINI GM, AYUSO S, GROOME NP, BERGADA C, CAMPO S: High serum concentrations of dimeric inhibins A and B in normal newborn girls. *Fertil Steril* **77**: 363-365, 2002.

BURGER HG, IGARASHI M: Inhibin: definition and nomenclature, including related substances. *Endocrinology* **122**: 1701-1702, 1988.

BURGER HG, YAMADA Y, BANGAH ML, MCCLOUD PI, WARNE GL: Serum gonadotropin, sex steroids and immunoreactive inhibin in the first two years of life. *J Clin Endocrinol Metab* **72**: 682-686, 1991.

CHADA M, PRŮŠA R, BRONSKÝ J, KOTAŠKA K, ŠÍDLOVÁ K, PECHOVÁ M, LISÁ L: Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone during childhood and puberty in males: changes in serum concentrations in relation to age and stage of puberty. *Physiol Res* **52**: 45-51, 2003.

CROFTON PM, ILLINGWORTH PJ, GROOME NP, STIRLINGS HF, SWANSTON I, GOW S, WU FCW, MCNEILLY A, KELNAR CJH: Changes in serum inhibin A and B during normal early puberty in boys and girls. *Clin Endocrinol* **46**: 109-114, 1997.

DE KRETSER DM, MEINHARDT A, MEEHAN T, PHILLIPS DJ, O'BRYAN MK, LOVELAND KA: The roles of inhibin and related peptides in gonadal function. *Mol Cell Endocrinol* **161**: 43-46, 2000.

DE WINTER JP, TEN DIJKE P, DE VRIES CJ, VAN ACHTENBERG TA, SAGINO H, DE WAELE P, HUYLENBROECK D, VERSCHUEREN K, VAN DEN EIJNDEN-VAN RAAIJ AJ: Follistatin neutralize activin bioactivity by inhibin inhibition of activin binding to its type II receptors. *Mol Cell Endocrinol* **116**: 105-114, 1996.

FOSTER CM, PHILLIPS DJ, WYMAN T, EVANS LW, GROOME NP, PADMANABHAN V: Changes in serum inhibin, activin and follistatin concentrations during puberty in girls. *Hum Reprod* **15**: 1052-1057, 2000.

GROOME NP, ILLINGWORTH PJ, O'BRIEN M, PAI R, RODGER FE, MATHER JP, MCNEILLY AS: Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* **81**: 1401-1405, 1996.

HAYES FJ, HALL JE, BOEPPLE P, CROWLEY WF JR: Differential control of gonadotropins secretions in the human: endocrine roles of inhibins. *J Clin Endocrinol Metab* **83**: 1835-1841, 1998.

KNIGHT PG, GLISTER C: Potential local regulatory functions of inhibins, activins and follistatin in the ovary. *Reproduction* **121**: 503-512, 2001.

PEZZANI I, REIS FM, DI LEONARDO C, LUISI S, SANTUZ M, DRIUL L, COBELLIS L, PETRAGLIA F: Influence of non-gonadotrophic hormones on gonadal function. *Mol Cell Endocrinol* **181**: 37-42, 2000.

SEHESTED A, JULL A, ANDERSSON AM, PETERSEN JH, JENSEN TK, MÜLLER J, SKAKKEBAEK NE: Serum inhibin A and inhibin B in healthy prepubertal, pubertal, and adolescent girls and adult women: relation to age, stage of puberty, menstrual cycle, follicle-stimulating hormone, luteinizing hormone, and estradiol levels. *J Clin Endocrinol Metab* **85**: 1634-1640, 2000.

TANNER JM: Growth and endocrinology of the adolescent. In: *Endocrine and Genetic Diseases of Childhood and Adolescence*. GARDNER L (ed), WB Saunders, Philadelphia, 1975, pp 16-64.

YING SY: Inhibins, activins, and follistatin: gonadal proteins modulating the secretion of follicle-stimulating hormone. *Endocr Rev* **9**: 267-293, 1988.

Reprint requests

MUDr. Martin Chada, Department of Clinical Biochemistry and Pathobiochemistry, Second Faculty of Medicine, Charles University, Faculty Hospital Motol, V Úvalu 84, 150 06 Prague 5, The Czech Republic, Fax: +420 2 2443 5320, E-mail: martinchada@hotmail.com