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Effect of 4-MeSer-GnRH on the release and synthesis of gonadotropins in the female rat *in vivo**

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ABSTRACT

The experiment was performed to confirm selective stimulation of FSH release by GnRH analog 4-MeSer-GnRH and to determine if analog can act on α, LHβ and prolactin gene expression. Our studies *in vivo* have shown that 4-MeSer-GnRH FSH releasing potency was less expressed than it was shown in our previous experiments and that this peptide did not affect LH release. GnRH analog inhibited both pituitary prolactin and LHβ mRNAs level but this effect was much more strongly expressed in case of prolactin mRNA. Such data point out a necessity of more detailed studies on the biological properties of 4-MeSer-GnRH.

KEY WORDS: 4-MeSer-GnRH, GnRH, LH, FSH, pituitary, gonadotropin release, gonadotropin biosynthesis, mRNA

INTRODUCTION

Gonadotropin releasing hormone (GnRH) plays a pivotal role in the regulation of reproduction through the stimulation of gonadotropin biosynthesis and secretion. Regulation of anterior pituitary function is mediated by the

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release of this neuropeptide into the primary portal capillaries in the median eminence and its delivery to the target via the hypophyseal portal veins. In mammals, the majority of GnRH neurons are situated in the hypothalamus and – functionally coupled – form a pulse generator that governs the pulse pattern of GnRH secretion and, in turn, gonadotropin secretion (Knobil, 1980). Among varied excitatory substances affecting GnRH release are: neuropepide Y, galanin, (Xu et al., 1996) amino acid glutamate (Brann, 1995) and gaseous diffusible messenger nitric oxide (Bonavera et al., 1996). Also an inhibitory neurotransmitters and neuromodulators are varied and they include opioids (Bonavera et al., 1993), tachykinins (Afione et al., 1990), GABA (Leonhardt et al., 1995). Feedback regulation on the gonadotrope can operate altering GnRH receptor number coupling to intracellular signal transduction pathways and the binding of GnRH to its receptors leads to stimulation of phospholipase activities in the plasma membrane of gonadotrope cell (Braden and Conn, 1993). The result of this activation is the cascade of physiological events leading to the relase of gonadotropins LH and FSH and to stimulation of their biosynthesis (Kochman and Gajewska, 1996).

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are pituitary glycoprotein hormones that regulate gonadal function including the production of sex-steroid hormones, maturation of ovarian follicles in the female and development of spermatocytes in the male. They consist of a common α subunit and an unique, structurally related β subunit. Each of those subunits (α, LHβ and FSHβ) result from post-translational maturation of precursors that are encoded by separate genes (Gharib et al., 1990; Counis and Jutisz, 1991). The expression of α and β subunit genes is regulated by a number of hormones including GnRH (Andrews et al., 1988; Starzec et al., 1989), gonadal steroids (Corbani et al., 1990) and – isolated from gonadal fluid structurally related dimeric peptides – such as inhibin and activin (Attardi et al., 1989; Carroll et al., 1989; Vale et al., 1990). At the pituitary level, activins stimulate FSH secretion and synthesis (Carroll et al., 1989) but inhibit GH (Billestrup et al., 1990; Bilezikjian et al., 1990), ACTH (Bilezikjian et al., 1991) and PRL (Kitaoka et al., 1988) secretion and synthesis. Inhibins were shown to suppress FSH secretion and synthesis (Carroll et al., 1989).

Relations between GnRH and activin action seem to be very interesting. Katayama and Conn (1994) showed that activin increases the sensitivity of the system that regulates gonadotropin release to increases in cytosolic Ca^{2+} concentration and PKC activation .

In our studies on petides selectively releasing LH and FSH from the anterior pituitary gland in female rats we found (Kochman and Gajewska, 1990) that 4-MeSer-GnRH expressed activin-like activity by very potent and selective stimulatory effect on the FSH release. This study was performed to confirm this

property of modified GnRH and to investigate how such modified peptide can act on gonadotropins and prolactin gene expression.

MATERIAL AND METHODS

Rats

Adult female Wistar rat of laboratory strain regularly cycling were used in the experiment. Both ovariectomy and injections into jugular vein and 3rd cerebral ventricle were performed under Narkamon anesthesia. Animal experiments were performed according to NIH regulations.

Estimation of gene expression

RNA extraction and hybridization. Total RNA was prepared according to Counis et al. (1981) method. Pituitaries were solubilized by lysis in 4 M guanidine hydrochloride, 1% sodium sarcosinate, 0.1 M β-mercaptoethanol, 50 mM sodium acetate pH 5 and then centrifuged in a small tube (total volume 650 μl) on a 6 M CsCl cushion for 18 h in a Beckman ultra-centrifuge (113.000 x g, 22^{0}C). The RNA pellet (10 μg) was recovered in 0.1 M sodium acetate -0.5% sodium dodecyl sulfate (SDS) and ethanol precipitated for dot or Northern hybridization analysis. Samples were electrophoresed in 1.5% agarose after glyoxal denaturation (McMasters and Carmichael, 1977), RNA was transfered onto a nylon filter (Hybond N, Amersham, UK) and immobilized by baking according to the manufacture's recomendations. HindIII-digested lambda DNA and/or HinfI-digested pBR 322 DNA, ^{32}P-labelled by filing of the 5'-protruding ends with [^{35}S]dATP and Klenow enzyme, were co-electrophoresed in wells adjacent to mRNA samples and thus cotransfered onto the membrane, to serve as size markers.

Prehybridization and hybridization were achieved as previously described (Corbani et al., 1990) at 42^{0}C in the presence of 50% formamide, 5 x SSC (1 x SSC = 150 mM NaCl, 15 mM trisodium citrate), 0.1% bovine serum albumin (BSA), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% sodium sarcosinate, 50 mM phosphate buffer, pH 7, 125 μg/ml sonicated salmon sperm DNA (Sigma Chemicals, St. Lois, MO, USA).

The complementary DNA (cDNA) probes. The rats α, LHβ and FSHβ cDNA probes were fragments of cDNAs previously obtained in our laboratory subcloned in pUC vectors. Inserts excised from plasmids were labelled by random oligonucleotide priming to specific activities of approximately

2×10^9 cpm/mg using [α-^{32}P]dCTP (400 Ci/mmol) and the multiprime labelling kit (Amersham). The specific hybrids were revealed by autoradiography (X-OMAT-AR films, Kodak) and quantified by densitometry using a Hoefer GS 300 scanning densitometer.

LH and FSH assay

The concentration of LH and FSH in plasma samples was measured by RIA using reagents supplied by NIDDK with all samples from one experiment being assayed in triplicate in the same assay. The reference preparation used was rLH-RP-2 and rFSH-RP-3 and the minimum detectable concentration was 200 pg/ml. The intra- and interassay coefficients of variation were $<10\%$.

Statistical analysis

Data are expressed as mean $\pm$SD. Statistical analysis of the data was performed using the unpaired and paired Student's t-test, as applicable. A P value of less than 0.05 was considered to be statistically significant.

RESULTS

Experimental data show that 4-MeSer-GnRH analog injected into jugular vein did not result in increased level of mean plasma LH neither after time-dependent injections (Figure 1) nor after dose-dependent injection (Figure 3). Injection of native GnRH caused very rapid LH release (36.0 ± 4.5 ng/ml) compared to control (2.9 ± 0.4 ng/ml). When we measured the mean plasma FSH level an increasing effect of 4-MeSer-GnRH was observed both after time-dependent injections (Figure 2; $P<0.05$) and after dose dependent injections (Figure 4; $P<0.05$) but this analog was less potent (12.8 ± 0.7 ng/ml) in FSH releasing than GnRH alone (18.13.8 ng/ml; $P<0.05$). FSH releasing ability of 4-MeSer-GnRH was not dependent neither on the time after injections – as the mean plasma level of this gonadotropin in comparison to control was the same after 15, 30, and 60 min after i.v. injections – nor on administered dose of analog. There were no significant differences between FSH serum level after increasing dose of 4-MeSer-GnRH however the highest FSH concentration was found after 1 μg analog injection (17.5 ± 1.2 ng/ml) as compared to control (12.7 ± 0.7 ng/ml).

After 5 h following intraventricular 4-MeSer-GnRH infusions pituitaries were excised and α, LHβ, and prolactin mRNAs were estimated by Northern blot. We found that analog did not influence on subunit α mRNA expression but strongly lowered prolactin mRNA and LHβ expression in the rat pituitary (Table 1).

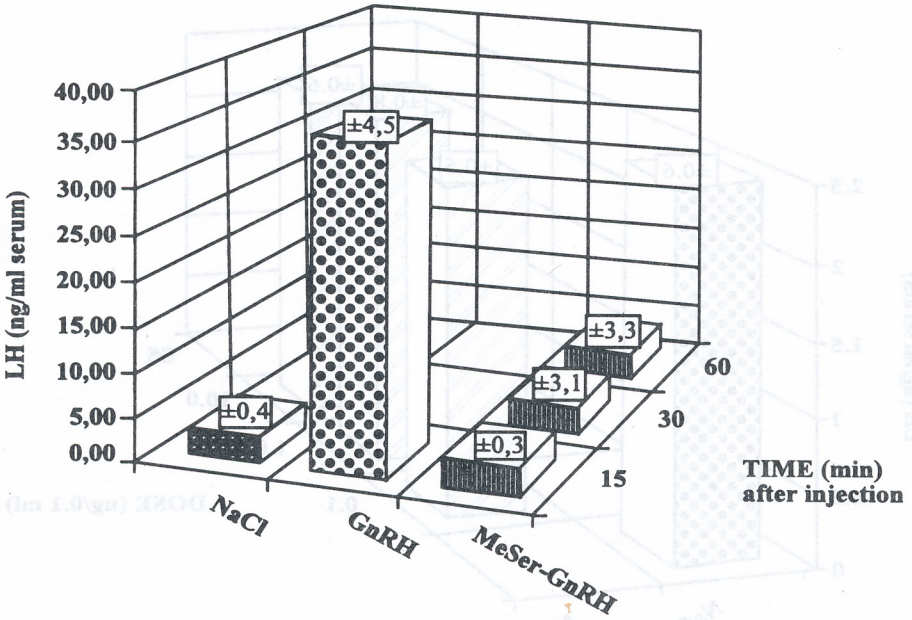

Figure 1. Serum LH release after 4-MeSer-GnRH i.v. injection to the female rat

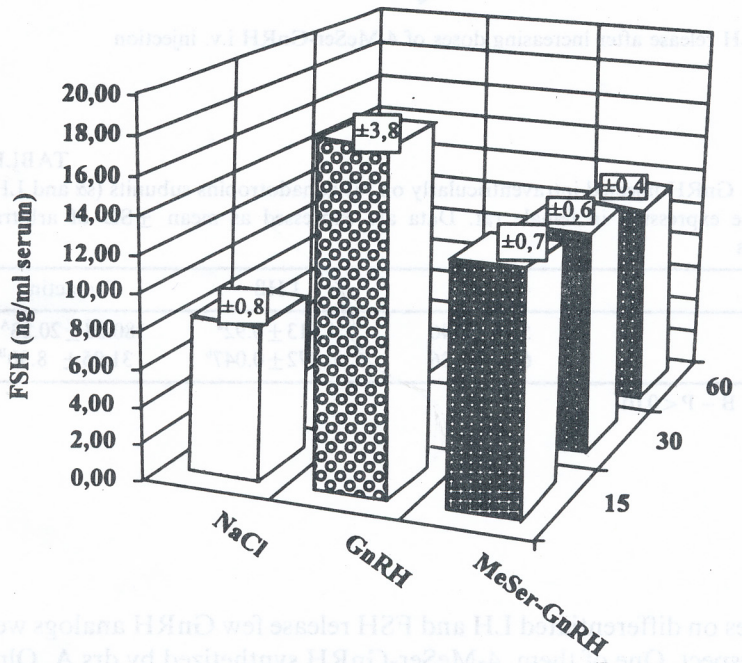

Figure 2. Serum FSH release after 4-MeSer-GnRH i.v. injections to the female rat

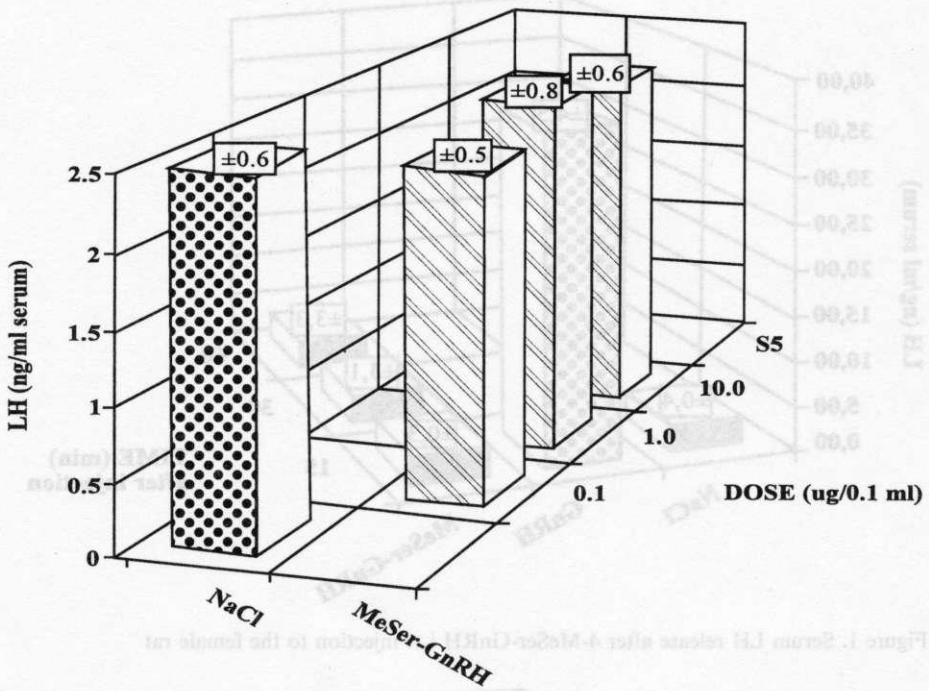

Figure 3. Serum LH release after increasing doses of 4-MeSer-GnRH i.v. injection

TABLE 1

Effect of 4-MeSer- GnRH injected intraventricularly on rat gonadotropins subunits (sα and LHß) and prolactin gene expression in female rat. Data are expressed as mean ±SD of arbitrary densitometric units

	α	LHß	Prolactin
Control	5.74±0.46	1.013±0.92[a]	180.08±20.23[A]
4-MeSer-GnRH	6.00±0.26	0.372±0.047[b]	31.95± 8.90[B]

a, b – $P<0.05$; A, B – $P<0.01$

DISCUSSION

In our studies on differentiated LH and FSH release few GnRH analogs were tested in this respect. One of them, 4-MeSer-GnRH synthetized by drs A. Olma and M. Leplawy (Technical University, Łódź) was very potent in evoking *in vivo*

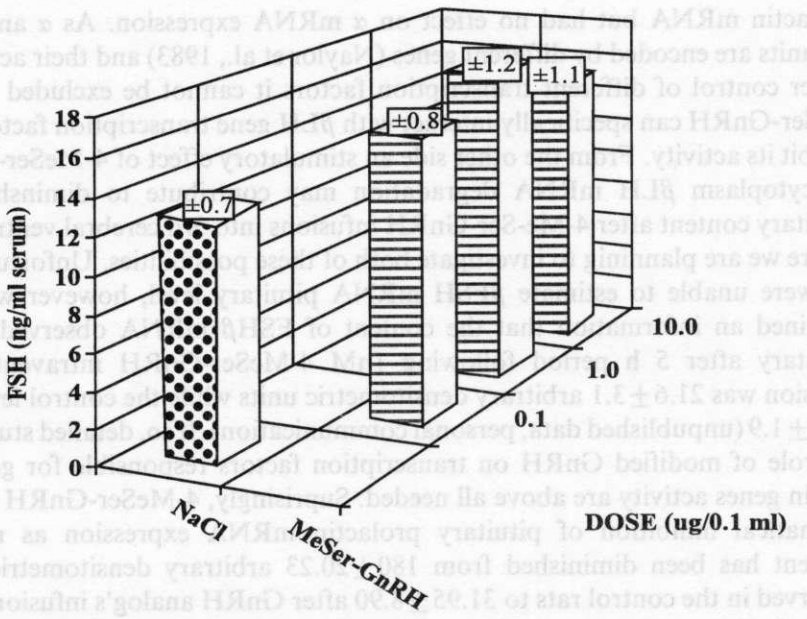

Figure 4. Serum FSH release after increasing doses of 4-MeSer-GnRH i.v. injection

FSH release while its LH releasing potency was minimal as it was estimated after 15 min following i.v. injection (Kochman and Gajewska, 1990). In this experiment we used next peptide's preparation vial and our results have shown the same as before tendency concerning 4-MeSer-GnRH activity: no effect on LH release and the stimulation of FSH release though such specific FSH release observed in this experiment was not so much expressed as before. It seems that modification in primary GnRH structure (MeSer in position 4) results in loosing its native properties to release both gonadotropins but an unique ability to selective FSH release is then manifested. Lack of LH stimulation release supports the idea that such modified GnRH analog does not exert its influence through pituitary GnRH receptor. If so, it is possible that 4-MeSer-GnRH can posses a partial binding property to several pituitary membrane receptors including activin receptor. But it must be stressed out that such suggestion needs further and detailed studies on conformational structure of the analog as well as systematic studies on interactions between various pituitary receptors' and 4-MeSer-GnRH *in vivo* and *in vitro* are needed to precise explanation both its ability to provoke specific FSH releasing potency as well as recognizing which pituitary receptor systems can be involved in that process. We have also investigated how 4-MeSer-GnRH can act on genes' expression in pituitaries *in vivo*. This peptide exerted an inhibitory action both on LHβ and very strongly on

prolactin mRNA but had no effect on α mRNA expression. As α and βLH subunits are encoded by different genes (Naylor et al., 1983) and their activity is under control of different transcription factors it cannot be excluded that 4-MeSer-GnRH can specifically interact with βLH gene transcription factors and inhibit its activity. From the other side an stimulatory effect of 4-MeSer-GnRH on cytoplasm βLH mRNA degradation may contribute to diminshing its pituitary content after 4-Me-Ser-GnRH infusions into 3rd cerebral ventricle. In future we are planninig to investigate both of these possibilities. Unfortunately, we were unable to estimate βFSH mRNA pituitary level, however we have obtained an information that the content of FSHβ mRNA observed in the pituitary after 5 h period following 1nM 4-MeSer-GnRH intraventricular infusion was 21.6 ± 3.1 arbitrary densitometric units while the control level was 20.8 ± 1.9 (unpublished data, personal communication). If so, detailed studies on the role of modified GnRH on transcription factors responsible for gonadotropin genes activity are above all needed. Suprisingly, 4-MeSer-GnRH caused dramatical inhibition of pituitary prolactin mRNA expression as mRNA content has been diminished from 180 ± 20.23 arbitrary densitometric units observed in the control rats to 31.95 ± 8.90 after GnRH analog's infusion. Such result indicate that this peptide can be involved in repression of PRL gene transcription. It was shown (Cao et al., 1987; Nelson et al., 1988) that Pit-1 factor is required for activation of PRL gene expression by binding with DNA elements within the PRL promoter region and Day and Day (1994) reported that altenatively spliced form of Pit-1 represses prolactin gene expression. It would be very interesting to determine if modified GnRH can influence on Pit-1 expression and its splicing and if this is the way through which 4-MeSer-GnRH exerts its inhibitory role on prolactin gene expression.

CONCLUSIONS

We suggest that MeSer-GnRH does not interact with GnRH receptor but partially binds to the activin receptor on the pituitary plasma membranes and cause slight selective FSH release.

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STRESZCZENIE

Wpływ 4-MeSer-GnRH na uwalnianie i syntezę gonadotropin *in vivo*

Celem obecnego doświadczenia było potwierdzenie selektywnego uwalniania FSH przez analog natywnego GnRH (4-MeSer-GnRH), a także określenie czy ten analog działa na ekspresję genów gonadotropin i prolaktyny. W badaniach *in vivo* wykazano, że aktywność uwalniająca 4-MeSer-GnRH była mniejsza niż poprzednio w stosunku do FSH i że ten peptyd nie wpływał na uwalnianie LH. Po raz pierwszy wykazano, iż analog GnRH hamował syntezę mRNA zarówno prolaktyny jak również LHβ, lecz ten efekt był większy w stosunku do mRNA prolaktyny. Obecne doświadczenia wskazują, że należy podjąć dalsze szczegółowe badania nad własnościami biologicznymi 4-MeSer-GnRH.