

## MET-ENKEPHALIN ENHANCES STEROIDOGENESIS OF CULTURED GRANULOSA CELLS

F. Facchinetti, M. Ruspa, A. Turci, F. Petraglia, M. Abrate, A. Forabosco and A. R. Genazzani  
 Clinica Ost. e Ginecol. e Cattedra di Istol. ed Embriol. Generale, Università degli studi di Modena

Peptides related to the three opioid-systems have been detected in human follicular fluids. Since opiate receptors have been demonstrated on oocytes, we tested the effects of various opioid agonists and antagonists on FSH-dependent progesterone (P) secretion from cultured granulosa cells. Follicular fluids were aspirated in periovulatory period and the size of the follicles was at least 15mm. After centrifugation, the pellet was washed three times with culture medium (RPMI 1640, Flow, UK). In order to establish a primary culture, they were incubated in Petri dishes at 37°C in humidified 95% air / 5% CO<sub>2</sub> atmosphere for 48 hours. At that time the medium was collected and renewed adding 100mM/ml purified FSH (Metrodin, Serono, I) and various opioid agonists and antagonists. Incubation lasted other 48 hours.

µM to nM Naloxone hydrochloride (Nalx) inhibit FSH-induced P-secretion, independently from the line (Met-Enk) stimulated P production in both µM (160.3 ± 39.8, %) on control and nM concentration (82.3 ± 44.2%). This effect was reversed by adding Nalx. On the contrary, µM Leucine-Enkephalin (Leu-Enk) reduced P production (-51 ± 13.8%). This effect disappeared in nM range. K-receptor agonist U-50,488H, seemed to induce an inhibition, which was reversed by k-antagonist MR 2266.

In conclusion, almost everyone of the opioid peptides fisiologically present in follicular fluid are able to interfere with steroidogenesis of granulosa cells. Interestingly enough, Met-Enk stimulation is Nalx reversible and dose dependent, suggesting the mediation of an opiate-receptor.

## B-EP changes in pharmacologically induced-menstrual cycles

G. Di Meo, F. Petraglia\*, C. Nappi, F. Facchinetti\*, U. Montemagno, A. R. Genazzani\*

Dept. of Obstetrics and Gynecology, University of Napoli, V. Pansini 5, Napoli, Italy

Dept. of Obstetrics and Gynecology, University of Modena, Italy\*

Several studies suggest the involvement of the opioid system in the physiological cyclic regulation of gonadotropin release. The arcuate nucleus is the main source of B-endorphin (B-EP) in the brain. Pituitary represents the most important source of plasmatic B-EP and it has been shown that in the pre-and/or post-ovulatory days a significant peak of plasma B-EP levels occurs. The aim of this study was to evaluate the effects of menstrual cyclicity on plasma B-EP levels, measuring the concentrations of circulating B-EP in women affected by primary or secondary amenorrhea and in control healthy subjects. 15 nulliparous women (23-31 yrs) affected by hypothalamic primary (7) or secondary amenorrhea (6) or anovulatory oligomenorrhea (2) before and during treatment with purified FSH+hCG (9) or with hMG+hCG (6) at doses related to the ovarian response, evaluated by estradiol levels and ecographic evaluation of follicular maturation. A group of 12 healthy women (22-26 yrs) were also studied for the regularity of their menstrual cycles. Amenorrheic patients' basal levels were similar to healthy control women. In spontaneous and pharmacologically-induced ovulatory cycles a significant preovulatory peak of plasma B-EP levels occurs while it does not in oligomenorrheic patients and in unsuccessfully treated amenorrheic subjects. In the other period of the cycle B-EP levels don't show significant variations. These results suggest that when ovulation occurs, plasma B-EP levels show a relevant rise the physiological significance of which remains to be scanned.