

## P16. Effects of melatonin on steroidogenesis in rat ovarian theca-interstitial cells: relevance to hormone therapy in polycystic ovary syndrome (PCOS)

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Context - Melatonin is a hormone and it plays a role in the reproductive system. Objective- to understand effects of melatonin on steroidogenesis in the ovarian theca-interstitial cells of rats and relevance to hormone therapy in polycystic ovary syndrome Methods- Twenty-five-day-old female Sprague-Dawley rats were injected subcutaneously with 1.5 mg of 17 ?-estradiol for three consecutive days. On the fourth day, the rats were euthanized by CO2 asphyxiation. In the first experiment, the T-I cells were divided into four groups :GI, (Ctr) - controls (n=10); GII, (hCG) - experimental animals receiving human chorionic gonadotropin (hCG) (50 ng/ml) (n=10); GIII, (hCG + Mel) - experimental animals receiving hCG (50 ng/ml) and melatonin (Mel) (1 nM/ml), (n=10); and GIV, (Mel) - experimental animals receiving Mel (1 nM/ml), (n=10). In the second stage of the experiment, the T-I cells were divided into five groups: GI,(Ctr) - controls (n=10); GII, - experimental animals receiving rapamycin (R) (20 nM), (n=10); GIII, (hCG) experimental animals receiving hCG (50 ng/ml), (n=10); GIV, (hCG + Mel) experimental animals receiving hCG (50 ng/ml) and Mel (1 nM/ml), (n=10); and GV, (hCG + Mel +R) - experimental animals receiving hCG (50 ng/ml), Mel (1 nM/ml), (n=10), and R (20 nM), (n=10). All of the T-I cells from the ovary of the first and second experiment were treated with hCG, Mel, and R.Statistical analysis was carried out with the analysis of variance (ANOVA) complemented with the Tukey-Kramer multiple comparisons test (p<0.05). Results- In the first experiment, the quantitative analysis of the gene expression (qRT-PCR) in the T-I cells from rat ovary when the latter were stimulated by hCG and melatonin showed hyperexpression of the StAr, Cyp17a1, Cyp11a1, and Hsd3b1 genes when set against the other groups. The StAr gene was also comparatively hyperexpressed in group hCG. In the second experiment, the quantitative analysis of the gene expression (gRT-PCR) in the T-I cells from rat ovary when stimulated by hCG, melatonin, and rapamycin showed the StAr, Cyp17a1, Cyp11a1, and Hsd3b1 genes were hyperexpressed in the group treated with hCG when compared to the other groups. The StAr gene was also comparatively highly expressed in groups hCG +Mel and hCG +Mel+ R. This was confirmed by the Western Blot technique. Conclusions- Finally, our data suggest that melatonin exerts a negative influence on steroidogenesis similarly to what occurs with rapamycin.

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