ROLE OF NEUROMEDIN B IN REGULATING FOLLICULAR DEVELOPMENT AND STEROIDGENESIS

Authors

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Abstract Body

The bombesin–related peptide Neuromedin B (NMB) has various physiological effects including the regulation of exocrine and endocrine secretions. This study investigated whether: (1) NMB is expressed in the bovine ovary; (2) NMB or NMB antagonist can modulate ovarian steroidogenesis or proliferation by bovine theca (TC) and granulosa (GC) cells; (3) expression of NMB by cultured ovarian cells is regulated by gonadotropins; (4) NMB can modulate follicular angiogenesis. GC (n=40) and TC (n=44) samples from bovine antral follicles (2-18 mm) were categorized into five size classes. Early, mid and regressing corpora lutea (CL) were also collected (n=17). Total RNA was harvested for qPCR analysis and data were analysed using the ΔΔCT method using β-actin for normalization. For functional studies bovine TC and GC were cultured under both non-luteinized (±LH or FSH) and luteinized (± forskolin) conditions and treated for 5 days with NMB (10^{-10}-10^{-6}M), NMB antagonist (BIM 23042; 10^{-10}-10^{-6}M) or a combination of the two. Steroid secretion (androstenedione, oestradiol, progesterone) was measured by ELISA; viable cell number was determined by neutral red uptake assay. For angiogenesis studies dissociated theca interna layers were treated for 6 days with angiogenic factors (±VEGF and FGF) and NMB. Results are based on 3-8 independent cultures. Significant effects of follicle cell-type ($P<0.01$) and cell-type x follicle category interaction ($P<0.05$) on NMB expression were observed. NMB expression in CL also varied according to CL stage ($P<0.05$). Neither NMB nor NMB antagonist affected TC/GC steroid secretion. However, NMB dose-dependently increased viable cell number by non-luteinized GC (~ 2.3-fold; $P<0.05$). LH/FSH had no effect on TC/GC NMB expression. Forskolin increased NMB expression by luteinized TC without affecting luteinized GC. Preliminary results from the follicular angiogenesis model indicate that NMB enhances VEGF/FGF-induced capillary network formation. Collectively, the results support intrafollicular roles of NMB in modulating cell proliferation and angiogenesis.