105. CYTOSENSOR MONITORING OF MICROVASCULAR ENDOTHELIAL CELLS

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The cytosensor is a silicon-based device capable of measuring minute alterations in extracellular pH by monitoring the changes in μvoltage of a low-buffered medium. It is used to measure the metabolic response of cells to a variety of ligands specific for membrane receptors. Microvascular endothelial cells (MEC) from the human uterus have been implicated in a number of clinical problems including contraception-associated break-through bleeding. The aim of this study was to investigate the direct and indirect effect of estrogens and progestins on primary cultures of MEC, including modification of an inflammatory response by progestins. Myometrial MEC (MMEC) were isolated from six hysterectomies from premenopausal women not taking any hormones. MMEC were purified using *ulex*-coated Dynabeads™ and cultured for 3 passages. MMEC culture purity was monitored using flow cytometry for the expression of CD31. Eighteen hours prior to the experiment, cells were plated at 5×10^6 or 8×10^6 cells per well and kept under low serum media conditions. In the cytosensor the cells were exposed to varying concentrations of histamine (for 120 s) or TNFα (for 360 s). Pilot work has been conducted exposing MMEC to physiological concentrations of estrogen (10 nM), progesterone (100 nM) and levenorgestral (1 nM) for 60 min. The MMEC culture purity was 98 ± 2% (mean ± SD). The optimal cell number per well was 8×10^6 providing a basal voltage of ≥60 μV. Histamine induced an immediate spiked response in acidification rate and produced a non-continuous dose response due to receptor saturation. Maximal stimulation occurred at 10 ± 2 nM with an EC_{50} of 1 ± 0.1 nM. TNFα produced an unusual acidification response, with an initial small receptor response, followed by a second larger response peaking 90 min after the TNFα solution was removed. Furthermore, TNFα had its greatest effect at 10 ng/mL as determined from the initial response. However, 20 ng/mL produced the largest second phase acidification increase. Preliminary data showed that MMEC exposed to estrogen and the progestins did not significantly alter the acidification rate compared the vehicle controls. The interactions of progestins and inflammatory cytokines are still under investigation.

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