Proceedings of the Nutrition Society (2012), **71 (OCE1)**, E10 doi:10.1017/S0029665112000389

Scottish Section Meeting, 26–27 March 2012, Future food and health

The effect of 17-β oestradiol, resveratrol, and genistein on Na^+/H^+ exchange in breast cancer cells lines

J. L. Wallace, D. Foy, H. Yousuf, M. Warnock and I. F. Gow
Department of Dietetics, Nutrition, and Biological Sciences, School of Health Sciences, Queen Margaret University, Edinburgh, EH21 6UU

The tumour environment is more acidic than normal (pH 6.8 vs 7.3)[1], and this may aid tumour progression or affect the uptake of drugs. The extracellular pH may be partly-regulated by cellular sodium/hydrogen exchangers (NHEs), and NHE activation may in turn be regulated by hormones such as oestradiol[2]. Some breast cancers possess receptors for oestradiol, and stimulation or blockade of these receptors may modulate the cell's ability to regulate pH. We studied this by investigating the effect of oestradiol on the ability of breast cancer cell lines to regulate pH by NHE. We used MCF-7 (expresses oestrogen receptors α and β) and MDA-MB-231 (expresses only oestrogen receptor β) cell lines. Given the interaction of phytoestrogens with the oestrogen receptor[3], we also looked at the effect of resveratrol (RSV) or genistein (Gen).

MCF-7 and MDA-MB-231 cells were used either as suspensions, or on coverslips, in the absence of added oestradiol. Cells were loaded with H^+-sensitive fluorescent probe BCECF, then washed to remove excess dye. Suspended cells were allowed to adhere to the glass bottom of the 35 mm perfusion chamber, coverslips with cells were added to the chamber directly. Cells were perfused (1 ml/min) at 37°C with HEPES-buffed Tyrode (pH 7.4) to which drugs were added as required. Acidification was induced by the NH_4Cl pre-pulse technique, recovery was achieved by returning to normal Tyrode. Cells were illuminated by alternating wavelengths of 440 and 503 nm light (2 Hz sample rate), and fluorescent light (>535 nm) was captured by a CCD camera. The fluorescence ratios are directly proportional to the H^+ concentration. Drugs were added directly to the Tyrode, and since RSV and Gen were dissolved in DMSO, DMSO (0.01% final) was added to the pre-perfusing Tyrode in those experiments, and used as a control in the absence of drugs.

Results are mean ratio values ± SEM or slopes of rate of change of ratio/min ± SEM for three separate experimental days, except for the DMSO controls where $n = 6$. Differences were assessed by Student's paired or unpaired t tests. A brief (4 min) exposure of breast cancer cells to NH_4Cl (20 mM) caused a transient alkalisation (decrease in 440/503 ratio, i.e. decrease in $[H^+]_i$) followed by an acidification and recovery after ammonium removal. In MDA cells, addition of 17β-oestradiol (E2; 1 pM final concentration) had no effect on the rate of recovery from acidification (Control: −3.44E-04 ± 1.34E-04; MDA: −2.26E-04 ± 0.69E-04). In MCF-7 cells, perfusing with Tyrode containing 1 pM E2 significantly increased the rate of recovery from acidification (Control: −1.61E-04 ± 0.22E-04; MCF-7: −3.66E-04 ± 0.18E-04; $p<0.05$). Inclusion of DMSO alone in the medium perfusing MCF-7 cells significantly increased the basal ratio compared with cells in Tyrode alone (Control: 1.24 ± 0.18; DMSO: 1.71 ± 0.09; $p<0.05$), and also increased the rate of recovery (Control: −1.61E-04 ± 0.22E-04; DMSO: −3.97E-04 ± 0.52E-04; $p<0.02$). Inclusion of RSV (1 μM) in the perfusate with MCF-7 cells also caused an increase in the rate of recovery relative to the DMSO-only control (DMSO: −3.97E-04 ± 0.52E-04; RSV: −6.84E-04 ± 0.56E-04; $p<0.02$). Gen (1 μM) had no significant effects in MCF-7 cells, though all three recovery rates were numerically higher than the DMSO control.

In summary, we have shown that E2 and RSV at physiological levels can increase the rate of recovery from acidification in a breast cancer cell line expressing oestrogen receptors α and β. Given the potential importance of cytosolic and extracellular pH regulation in both the ability of cytotoxic drugs to cross the membrane or the cell to proliferate, the physiological effects of foodborne phytohormones on pathways regulating cell pH clearly requires further investigation.

1. Raghunand N, He X, van Sluis R, Mahoney B, Baggett B, Taylor CW, Paine-Murrieta G, Roe D, Bhujwalla ZM, Gillies RJ (1999) *Br J Cancer* **80**: 1005–1011.
2. Kilic A, Jaradov S, and Karmazyn M (2009) *J Mol Cell Cardiol* **46**:360–36.
3. Hwang CS, Kwak HS, Lim HJ, Lee SH, Kang YS, Choe TB, Hur HG & Han KO (2006) *J Steroid Biochem Mol Biol* **101**: 246–253.