Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue1abst053.pdf

The endocannabinoid-like mediator DHEA (docosahexaenoylethanolamide) modulates COX-2 activity

JOCELIJN MEIJERINK, MIEKE POLAND, MICHIEL BALVERS, RENGER WITKAMP. HUMAN NUTRITION/NUTRITION AND PHARMACOLOGY, WAGENINGEN, The Netherlands

Increasing evidence shows that DHEA (docosahexaenoylethanolamide), an endogenous metabolite of the *n*-3 fatty acid DHA (docosahexaenoic; 22 : 6*n*-3), possesses a diverse spectrum of biological activities [1]. Interestingly, DHEA concentrations in animal tissues and in human plasma have been found to parallel dietary intake levels of DHA and recently, we provided evidence for the anti-inflammatory effects of DHEA in macrophages and adipocytes [2, 3]. Taken together, these data point to a possible role of DHEA as endogenous mediator. However, not much is known about its mechanism(s) of action. To further elucidate the immune modulatory activities of DHEA, its effects on different key inflammatory mediators were investigated.

The following methods were used. RAW264.7 macrophages were pre-incubated with DHEA for 30 min. and subsequently stimulated with LPS (0.1 µg/ml) or poly-IC (1 µg/ml) and DHEA for 24 hr after which PGE2 and IFNβ levels were measured using EIA or ELISA, respectively (n=3). NF-κB activity (n=3) was determined with a HEK 293 NF-κB lacZ luciferase reporter assay and assessed at varying time-points (1.5 hr up to 48 hr). LC-MS/MS analysis was conducted in order to further explore effects of different DHEA concentrations on COX-2 and LOX derived eicosanoids (duplicate series performed on different days). Western blotting was used to asses COX-2 protein expression. Statistical analysis was performed using ANOVA followed by Bonferroni's post hoc test or, in case of eicosanoid analysis by Dunnett's t-test. $P < 0.05$ was considered as a significant difference.

It was found that DHEA dose-dependently reduced levels of PGE2 (EIA) in LPS stimulated RAW264.7 macrophages, with 1 µM and 10 µM inducing a significant reduction of 35% and 70% ($p < 0.001$), respectively. Further analysis with LC-MS/MS applying a targeted lipidomic approach [4] revealed that 10 µM DHEA significantly inhibited cyclooxygenase-2 (COX-2) generated prostaglandins ($p < 0.001$) by 80% - 93% (range for individual metabolites) and thromboxane B2 up to 40% ($p < 0.001$). By contrast, the parent compound DHA did not reduce levels of metabolites formed by COX-2 in that concentration range. The activity of NF-κB and IFNβ, both important players of the MyD88-dependent and the MyD88-independent pathway respectively, were not affected by DHEA. As expression of COX-2 protein was found not to be altered by DHEA, we hypothesize that the molecule acts at the level of the enzyme. This could be by substrate competition between DHEA, or its oxygenated metabolites, and arachidonic acid or via other forms of inhibition of COX-2 activity.

Taken together, it appears that the endocannabinoid-like mediator DHEA modulates COX-2 activity, which may have important consequences in relation to pain and inflammation.

[1] Meijerink J et al, Br J Pharmacol, in press

[2] Balvers MGJ et al, Biochim Biophys Acta 1801:1107, 2010

[3] Meijerink J et al, Br J Nutr 105:1798, 2011

[4] Balvers MGJ et al, Metabol.**:** 1-18, 2012