Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue1abst029.pdf

The Effects Of Anandamide And Leptin On Human Osteoblasts *In Vitro*

Marie Smith, Saoirse O'Sullivan, Susan Anderson. Department of Vascular Medicine, School of Graduate Entry Medicine and Health, University of Nottingham, Derby, UK

The endocannabinoid system is reported to play a role in bone metabolism with their synthesis and degradation enzymes, associated receptors and production being reported in bone. Leptin is a protein which has also been linked to bone metabolism and acts both centrally (via the hypothalamus and sympathetic nervous system) and peripherally. Both osteogenic and anti-osteogenic properties have been attributed to leptin. A regulatory relationship between anandamide (AEA), a widely studied endocannabinoid, and leptin has been reported in the reproductive system and hypothalamus. The aim of this study was to establish whether leptin modulates the effects of AEA on human osteoblasts (HOBs) *in vitro*.

HOBs (previously isolated from femoral head trabecular bone) were grown for 1, 4 and 7 days in an osteogenic medium (containing 150µg/ml L-ascorbic acid, 10nM dexamethasone and 10mM sodium beta-glycerophosphate) and supplemented with a range of concentrations (1nM-10µM) of AEA alone, or in the presence of 1nM or 1µM leptin. DNA concentration (using Hoechst 33258) and relative alkaline phosphatase activity, ALP, (using p-nitrophenyl phosphate) were measured as markers of proliferation and differentiation respectively. Collagen production, another marker of differentiation, was measured using the Biocolor Sircol collagen assay kit. Statistical analysis was conducted using a two-way ANOVA and a one-way ANOVA with Dunnett's *post-hoc* test with GraphPad Prism software

Treatment with AEA alone had no effect on DNA at any concentration or time-point. ALP activity was not affected after 24 hours or 4 days but was increased in cells treated with 10µM AEA after 7 days ($P<0.01$, $n=8$). A decrease in ALP per cell was observed in cells treated with 10µM AEA after 24 hours ($P<0.001$, $n=4$) with no effects observed after 4 or 7 days. Addition of 1nM leptin and AEA resulted in no differences in DNA or ALP activity after 24 hours and 4 days compared to AEA treatment alone. After 7 days, DNA was increased in cells treated with 1nM leptin and 10µM AEA compared to 10µM AEA only ($P<0.05$, $n=8$). No changes in ALP activity alone or per cell were observed after 7 days. Treatment with 100nM AEA and 1µM leptin resulted in a decrease in DNA ($P<0.05$, $n=4$) after 24 hours compared to 100nM AEA only. ALP activity alone and per cell was increased after 24 hours in cells treated with 10nM and 1µM AEA in combination with 1µM leptin ($P<0.05$- $P<0.01$ and $P<0.001$ respectively, $n=4$) in comparison to these AEA concentrations alone. There was no effect on DNA or ALP activity observed in cells treated with 1µM leptin and AEA compared to AEA only after 4 or 7 days.

In conclusion, this study confirms a role for both AEA and leptin in bone metabolism and provides further evidence for an interaction between these compounds. There is also evidence for the results of these interactions to be leptin concentration dependant, with the high concentrations having a greater effect on the proliferation and differentiation of HOBs in the presence of AEA.