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The Combined Effects Of 2-Arachidonoylglycerol 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

Bone is constantly being remodelled through the action of osteoclasts and osteoblasts, and research has shown endocannabinoids to be involved. Leptin is a satiety hormone that also has role in bone metabolism, however, the mechanisms involved are not clear, especially in human cells where stimulation and inhibition of osteoblast growth is reported. Research shows leptin may have a role in down-regulating the endocannabinoid system, including 2-Arachidonoylglycerol (2-AG), in the hypothalamus and reproductive system. The aim of this study was to investigate potential modulatory effects of leptin on 2-AG in human osteoblasts (HOBs).

HOBs (isolated from femoral head trabecular bone) were grown *in vitro* for 1, 4 and 7 days in osteogenic medium (containing 150µg/ml L-ascorbic acid, 10nM dexamethasone and 10mM sodium beta-glycerophosphate) and supplemented with 1nM-10µM 2-AG alone, or in combination with 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. Data were analysed using a two-way ANOVA and a one-way ANOVA with Dunnett's *post-hoc* test with GraphPad Prism software.

Treatment with 2-AG alone resulted in a decrease in DNA after 24 hours at 100nM and 10µM concentrations ($P<0.05$ and $P<0.001$ respectively, $n=4$) and an increase in ALP activity at 10nM and 1µM 2-AG ($P<0.05$ and $P<0.001$ respectively, $n=4$). A concentration dependant increase in ALP per cell was also observed after 24 hours at 10nM-10µM 2-AG ($P<0.05$-$P<0.001$, $n=4$). No effect on DNA or ALP activity was observed in cells treated with 2-AG only after 4 days. After 7 days, DNA was decreased in cells treated with 1nM-1µM 2-AG ($P<0.05$-$P<0.001$, $n=4$) but no effect on ALP alone or per cell was observed at this time-point. Treatment with 2-AG and 1nM leptin resulted in no significant changes to DNA or ALP activity at any time-point when compared to 2-AG alone. However, ALP activity per cell was increased in cells treated with 1nM leptin and 1nM-1µM 2-AG ($P<0.05$-$P<0.01$, $n=8$) compared to 2-AG only. There was no difference in ALP activity per cell after 4 days but an increase after 7 days in 1nM 2-AG and 1nM leptin treated cells compared to 1nM 2-AG alone ($P<0.001$, $n=8$). The addition of 1µM leptin and 2-AG resulted in no effect on DNA or ALP activity after 24 hours or 4 days. After 7 days, DNA was decreased in cells treated with 1µM and 10µM 2-AG and 1µM leptin ($P<0.001$ and $P<0.05$ respectively, $n=4$). ALP activity was decreased after 7 days in 10µM 2-AG and 1µM leptin treated HOBs ($P<0.05$, $n=4$), with no effect on ALP activity per cell observed at this time-point.

In conclusion, this study confirms a role for 2-AG and leptin in bone metabolism and provides further evidence for an interaction between these two compounds. Evidence was also shown for the concentration of leptin to affect the nature of the interaction, with 1nM leptin increasing ALP, a human osteoblast differentiation marker, implying an osteogenic role and 1µM leptin decreasing ALP and DNA, implying an anti-osteogenic role.