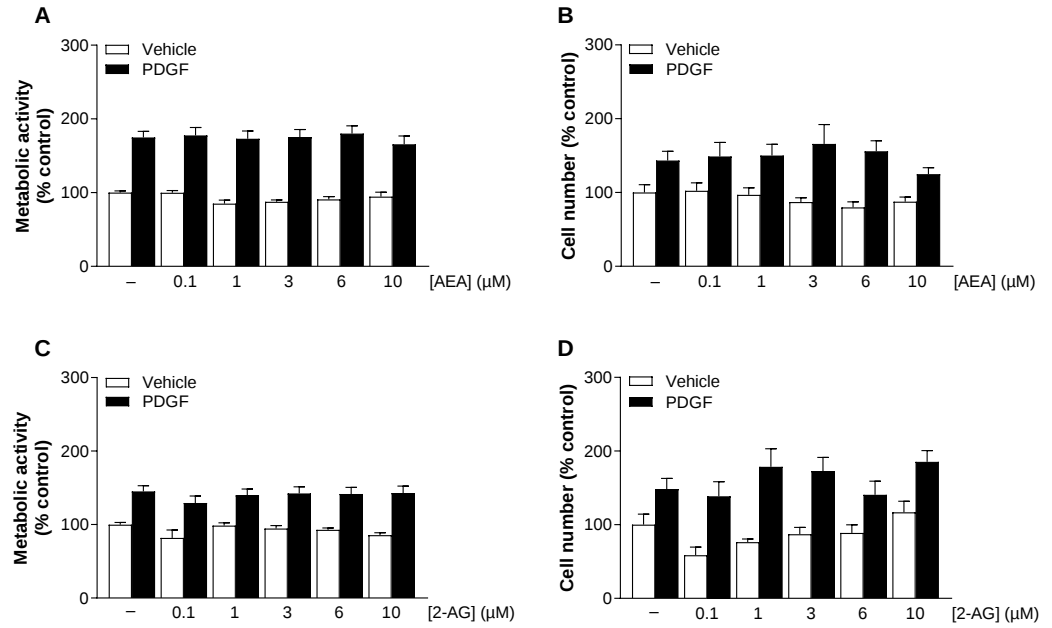


Anandamide Inhibits Vascular Smooth Muscle Migration, Endothelial Adhesion Protein Expression and Monocyte Adhesion of Human Coronary Artery Cells

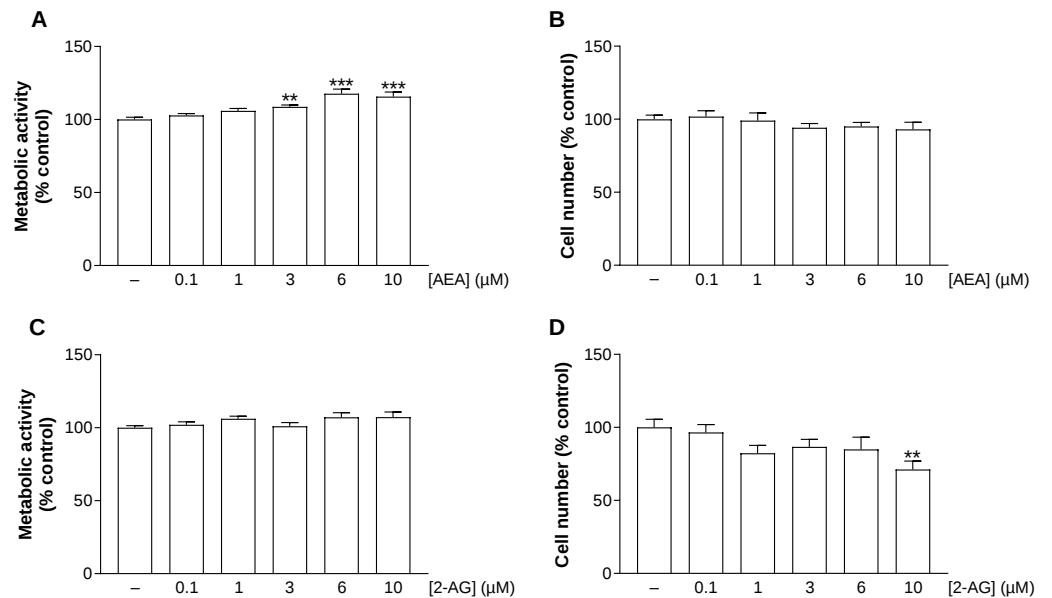
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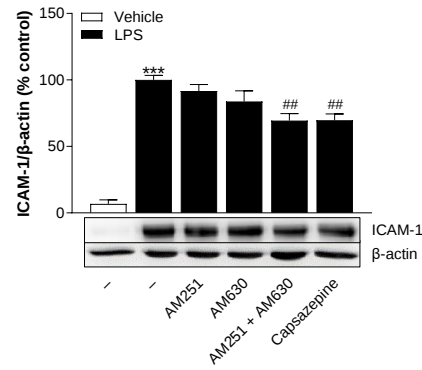
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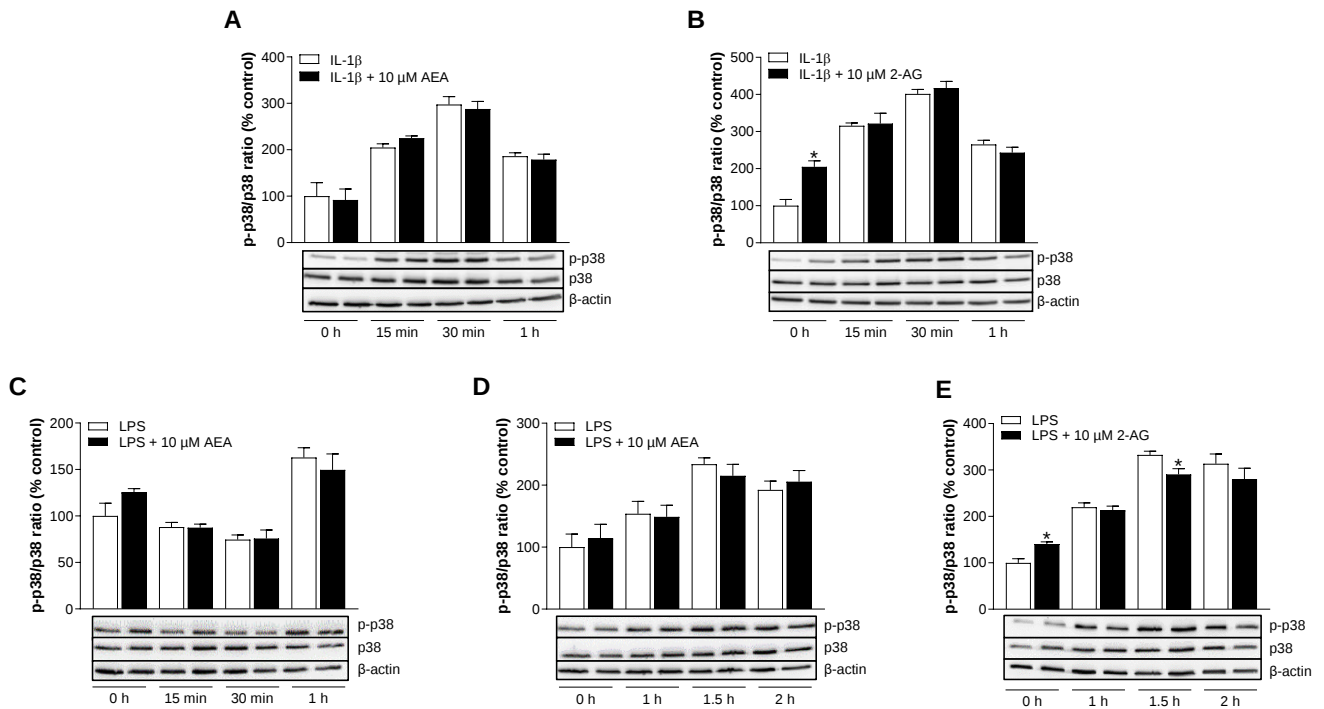
Supplementary Figure S1: Effect of AEA (A,B) and 2-AG (C,D) on metabolic activity and cell number of HCASMC. HCASMC were incubated with 25 ng/mL PDGF or its vehicle together with increasing concentrations of AEA or 2-AG or its vehicle. After 6 days of incubation (144 h), metabolic activity was analyzed using the WST-1 assay (A,C) and cell number was determined by crystal violet staining (B,D). Data are means $\pm$ SEM of $n = 12$ (4 independent experiments, (A)) or $n = 9$ (3 independent experiments, (B–D)). Significant effects of AEA and 2-AG under basal and PDGF-stimulating conditions were excluded by one-way ANOVA plus Dunnett post hoc tests.



Supplementary Figure S2: Effect of AEA (A,B) and 2-AG (C,D) on metabolic activity and cell number of HCAEC. HCAEC were incubated with increasing concentrations of AEA or 2-AG or with vehicle for 24 h. Thereafter, metabolic activity was determined by WST-1 assay (A,C) and cell number by crystal violet staining (B,D). Vehicle-treated cells were used as controls (100%). Data are presented as means $\pm$ SEM of $n = 16$ (4 independent experiments, (A,B)) or $n = 11$ –12 (3 independent experiments, (C,D)). ** $p \leq 0.01$, *** $p \leq 0.001$ vs. vehicle control; one-way ANOVA plus Dunnett post hoc test.



Supplementary Figure S3: Influence of antagonists at cannabinoid receptors or TRPV1 on LPS-induced ICAM-1 protein expression in HCAEC. Cells were preincubated with antagonists of the cannabinoid receptors CB₁ (AM251) and CB₂ (AM630) or TRPV1 (capsazepine), each at a concentration of 1 μ M, or with vehicle for 1 h and then coincubated with 1 μ g/mL LPS or vehicle for another 24 h. Protein expression was determined by Western blot analysis and normalized to β -actin. Cells stimulated with LPS alone were set 100%. Data are presented as means $\pm$ SEM of $n = 3$ independent experiments. *** $p \leq 0.001$ vs. vehicle control; ## $p \leq 0.01$ vs. LPS control; one-way ANOVA plus Bonferroni post hoc test.



Supplementary Figure S4: Impact of AEA (A,C,D) or 2-AG (B,E) on IL-1 β - or LPS-induced phosphorylation of p38 MAPK in HCAEC. Cells were treated with 10 μ M AEA or 10 μ M 2-AG or vehicle and coincubated with 10 ng/mL IL-1 β . For analysis of LPS-induced p38 MAPK activation, cells were preincubated with 10 μ M AEA or 10 μ M 2-AG or vehicle 1 h prior to addition of LPS (1 μ g/mL). Protein samples were collected following stimulation for the indicated times. Western blotting was performed for p38 MAPK and its phosphorylated form (p-p38). Protein expression values were normalized to β -actin as equal loading control. Cells stimulated with LPS alone (0 h) were set 100%. Data are presented as means $\pm$ SEM of $n = 3$ independent experiments. * $p \leq 0.05$ vs. time-matched vehicle control; Student's unpaired two-tailed t test.

Supplementary Table S1: Effect of antagonists to cannabinoid receptors and TRPV1 on metabolic activity and cell number of HCASMC under PDGF-stimulated conditions. HCASMC were maintained in serum-reduced medium (0.5% FCS) for 24 h. Cells were then preincubated with antagonists of the cannabinoid receptors CB₁ (AM251) and CB₂ (AM630) or TRPV1 (capsazepine), each at a concentration of 1 μ M, or with a vehicle for 1 h, followed by the addition of 25 ng/mL PDGF or vehicle and 10 μ M AEA or vehicle and continuing the incubation for another 24 h. Subsequently, metabolic activity was analyzed by WST-1 assay and cell number by crystal violet staining. Cells treated with the vehicle were used as control (set as 100%). Data are presented as means $\pm$ SEM of $n = 6$ (3 independent experiments). Statistically significant effects of PDGF, AEA, and receptor antagonists were excluded using a one-way ANOVA plus Bonferroni post hoc test.

Treatment group	Metabolic activity (%)	Cell number (%)
Vehicle	100.0 $\pm$ 12.5	100.0 $\pm$ 8.8
PDGF	150.4 $\pm$ 18.1	128.4 $\pm$ 24.3
AEA + PDGF	140.9 $\pm$ 12.2	130.0 $\pm$ 14.4
AM251 + AEA + PDGF	154.8 $\pm$ 15.3	129.0 $\pm$ 13.4
AM630 + AEA + PDGF	158.9 $\pm$ 22.0	130.9 $\pm$ 12.7
Capsazepine + AEA + PDGF	159.3 $\pm$ 15.9	106.9 $\pm$ 15.1
AM251 + PDGF	144.3 $\pm$ 11.2	124.8 $\pm$ 23.0
AM630 + PDGF	146.8 $\pm$ 19.4	127.6 $\pm$ 19.8
Capsazepine + PDGF	160.8 $\pm$ 13.5	175.2 $\pm$ 36.6

Supplementary Table S2: Effect of LY294002 (PI3K/Akt inhibitor), Stattic (STAT3 inhibitor) or PP1 (Src inhibitor) on metabolic activity and cell number of HCAEC under basal, IL-1 β - or LPS-stimulated conditions. HCAEC were preincubated with the indicated inhibitors or vehicle for 1 h, followed by the addition of 10 ng/mL IL-1 β , 1 μ g/mL LPS or vehicle and continuing the incubation for another 24 h. Subsequently, metabolic activity was analyzed by WST-1 assay and cell number by crystal violet staining. Cells treated with vehicle were used as control (set as 100%). Data are presented as means $\pm$ SEM of $n = 8-9$ (3 independent experiments). ** $p \leq 0.01$ vs. vehicle control; # $p \leq 0.05$, ## $p \leq 0.01$, ### $p \leq 0.001$ vs. respective stimulated control; one-way ANOVA plus Bonferroni post hoc test.

Treatment group	Metabolic activity (%)	Cell number (%)
Vehicle	100.0 $\pm$ 2.0	100.0 $\pm$ 9.4
IL-1 β	102.6 $\pm$ 1.1	107.6 $\pm$ 19.8
3 μ M LY294002	99.9 $\pm$ 6.1	69.7 $\pm$ 4.6
3 μ M LY294002 + IL-1 β	99.2 $\pm$ 4.2	92.1 $\pm$ 9.4
10 μ M LY294002	85.8 $\pm$ 5.8	83.1 $\pm$ 8.1
10 μ M LY294002 + IL-1 β	87.1 $\pm$ 5.1	65.4 $\pm$ 8.1
0.3 μ M Stattic	124.5 $\pm$ 6.5 **	125.6 $\pm$ 17.1
0.3 μ M Stattic + IL-1 β	130.6 $\pm$ 6.9 ###	97.7 $\pm$ 9.3
3 μ M PP1	102.9 $\pm$ 3.9	105.7 $\pm$ 12.7
3 μ M PP1 + IL-1 β	96.8 $\pm$ 1.6	89.8 $\pm$ 11.3
10 μ M PP1	85.1 $\pm$ 4.2	60.1 $\pm$ 10.8
10 μ M PP1 + IL-1 β	64.9 $\pm$ 5.9 ###	45.9 $\pm$ 6.9 ##
Vehicle	100.0 $\pm$ 1.9	100.0 $\pm$ 6.8
LPS	90.8 $\pm$ 4.9	99.3 $\pm$ 19.5
3 μ M LY294002 + LPS	94.9 $\pm$ 5.8	89.4 $\pm$ 12.0
10 μ M LY294002 + LPS	82.1 $\pm$ 3.8	71.9 $\pm$ 10.1
0.3 μ M Stattic + LPS	132.7 $\pm$ 8.3 ###	102.5 $\pm$ 9.5
3 μ M PP1 + LPS	93.9 $\pm$ 2.6	80.4 $\pm$ 6.7
10 μ M PP1 + LPS	65.9 $\pm$ 5.5 ##	52.0 $\pm$ 9.4 #

Supplementary Table S3: Effect of siRNA treatment on metabolic activity and cell number of HCAEC under IL-1 β - or LPS-stimulated conditions. Opti-MEM™ I Reduced Serum Medium, Lipofectamine™ RNAiMAX and the desired siRNA were mixed in a solution and incubated for 20 min at room temperature to form a complex. HCAEC were transfected with a final siRNA concentration of 10 nM each. The cells were allowed to adhere for 24 h. Stimulation of 10 ng/mL IL-1 β or 1 μ g/mL LPS or vehicle was carried out for 24 h. Subsequently, metabolic activity was determined by WST-1 assay and cell number by crystal violet staining. Cells treated with vehicle were used as control (set as 100%). Data are presented as means $\pm$ SEM of $n = 9$ (3 independent experiments). ## $p \leq 0.01$, ### $p \leq 0.001$ vs. corresponding stimulated control; one-way ANOVA plus Bonferroni post hoc test.

Treatment group	Metabolic activity (%)	Cell number (%)
Vehicle + non siRNA	100.0 $\pm$ 3.3	100.0 $\pm$ 7.3
IL-1 β + non siRNA	112.2 $\pm$ 5.2	105.6 $\pm$ 10.0
IL-1 β + VCAM-1 siRNA	92.1 $\pm$ 3.2 ##	94.8 $\pm$ 6.2
IL-1 β + ICAM-1 siRNA	105.5 $\pm$ 5.6	96.5 $\pm$ 9.4
Vehicle + non siRNA	100.0 $\pm$ 2.3	100.0 $\pm$ 3.4
LPS + non siRNA	106.8 $\pm$ 2.1	164.8 $\pm$ 27.8
LPS + VCAM-1 siRNA	86.7 $\pm$ 3.7 ###	143.2 $\pm$ 16.1
LPS + ICAM-1 siRNA	100.0 $\pm$ 4.8	156.2 $\pm$ 24.4