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Indirect, CB_2 receptor and mediator-dependent stimulation of human whole blood neutrophils by exogenous and endogenous cannabinoids

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List of Abbreviations: Adenosine 3´5´cyclic monophosphate (cAMP), Anandamide (AEA), 2-Arachidonoylglycerol (2-AG), [(-)-*cis*-*3*-[2- hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol] (CP55 940), dimethylsulfoxide (DMSO), fetal calf serum (FSC), N-formyl-methionyl-leucyl-phenylalanine (fMLP), methanandamide (MethAEA), natural killer cell (NK cell), 3-[1-(p-chlorobenzyl)-5(isopropyl)-3-t-butylthioindol-2-yl]-2,2-dimethylpropanoic acid,Na (MK886), phosphate buffered saline (PBS), polymorphonuclear neutrophiles (PMN), [*N*[1*S*)-*endo*-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide](SR144 528), Δ9-tetrahydrocannabinol (THC), T-helper cell1 (Th1), T-helper cell2 (Th2).

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Abstract

Immunomodulatory effects of endogenous and exogenous cannabinoids have been investigated in numerous studies, mostly performed with isolated cells or transformed cell lines, but only sparse data exist on human polymorphonuclear neutrophils (PMN). We therefore investigated the respiratory burst reaction of human whole blood PMN under the influence of cannabinoids using flow cytometry. In their natural whole blood milieu, a CB_2 receptor-dependent stimulation of the PMN respiratory burst was found at nanomolar concentrations of CP55 940 and methanandamide after a 3 h incubation period, whereas the short living and rapidly hydrolyzed endogenous ligand anandamide did not alter the burst reaction of whole blood PMN under the same experimental conditions. The stimulatory cannabinoid effect was totally absent in isolated PMN, but could be transferred onto isolated PMN by adding the cell-free low molecular weight plasma fraction (<5000 D) of cannabinoid incubated blood, indicating an indirect mechanism depending on humoral products or mediators. Results of our further experiments suggest that products of the arachidonic acid metabolism are mediators of the cannabinoid-induced enhancement of the respiratory burst reaction of whole blood PMN.

Introduction

After detection of the "peripheral" cannabinoid receptor (CB_2R) on leukocytes, numerous in-vitro studies evaluated the potential effects of natural exogenous (Klein et al., 2003) and endogenous cannabinoid ligands on various immune functions, mainly in lymphocytes and macrophages. Suppression of NK cell cytotoxicity (Massi et al., 2000), B-lymphocyte activity (Klein et al., 1985) and impairment of macrophage functions (Lopez-Cepero et al.,1986; Baldwin et al., 1997) have been described as well as an enhancement of oxygen radical production in alveolar macrophages (Sarafian et al., 1999) and an increased B-cell proliferation response (Derocq et al., 1995). Some authors also found an altered cytokine production with a shift from Th1 to Th2 cytokines and an impairment of macrophage/T-cell cooperation induced by certain cannabinoids (Klein et al., 1991; Klein et al., 1998).

To date, cannabinoids are considered to act mainly as immunosuppressive agents in animals and humans because they are potent inhibitors of the adenylate cyclase activity and thus may alter leukocyte functions by reduction of intracellular cAMP levels (Schatz et al.,1997; Slipetz et al., 1997). Reduced intracellular cAMP levels in polymorphonuclear neutrophils have been shown to increase chemotaxis, lysosomal enzyme release and respiratory burst reaction (Wright CD et al., 1990). Therefore G_i-coupled receptors such as CB_2 would be expected to enhance rather than inhibit neutrophil function. However, not all effects elicited by cannabinoid receptor activation can be explained by cAMP-dependent mechanisms and most in-vitro studies were performed with either isolated cells from animals or transformed cell lines expressing the CB_2 receptor. The data reported from these in-vitro studies are often unequivocal and difficult to interprete,

depending on cell type, animal species, cannabinoid compound, concentration and cellular environment.

Even from in-vitro and animal studies, only sparse data exist about the influence of cannabinoids on the major leukocyte population of polymorphonuclear neutrophils (PMN), although these phagocytes are the first line defense against bacterial and fungal infections. Two independent previous reports showed a suppression of the oxygen radical production of isolated human PMN at high micromolar concentrations of the natural cannabinoid Δ^9-tetrahydrocannabinol (THC) and the synthetic THC analogue CP55 940 in vitro (Djeu et al., 1991; Kraft et al., 2004). In both studies, the concentrations necessary for this suppression were far above the range that can be reached in vivo, and no data are available about cannabinoid effects under more physiological conditions, i.e. in whole blood. Since dibenzopyrane cannabinoids such as THC (Marinol®) or nabilone (Cesamet®) are therapeutically used in immunocompromised HIV and cancer patients as antiemetics and to improve appetite, a potential impairment of the phagocytic and oxidative microbicidal activity of human PMN would be clinically relevant, and the conditions of its appearance should be known in more detail.

Therefore the objective of the present study was to investigate the effects of relevant concentrations of the synthetic THC-analogue CP 55 940, the endogenous cannabinoid anandamide and its more stable derivative methanandamide, on the respiratory burst of human PMN in the whole blood milieu.

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Material and Methods

Cannabinoid compounds

Three different ligands of the two cannabinoid receptors, CB_1R and CB_2R, were tested: the synthetic dibenzopyrane cannabinoid CP55 940 as an analogue of the marijuana cannabinoid Δ^9-THC, the endogenous eicosanoid compound N-arachidonylethanolamide (anandamide, AEA) and its non-hydrolyzable and thus more stable derivative methanandamide (MethAEA) (Devane et al., 1992; Martin et al., 1999). CP55 940 (Tocris Inc., USA), and the specific CB_2-antagonist SR144 528 (kindly provided by Sanofi Inc., France) were dissolved in dimethylsulfoxide (DMSO, Sigma Chemicals, St Louis, USA) and further diluted with phosphate-buffered saline (PBS, Gibco Inc., UK) to the final working concentrations. AEA (Cayman Chemicals Co, Ann Arbor, MI, USA) and MethAEA (Cayman Chemicals Co, Ann Arbor, MI, USA) were dissolved in ethanol and also diluted with PBS. The sterile solutions were always freshly prepared for each experiment, and the final DMSO or ethanol concentration was 0.001% v/v in each test.

Inhibitors of the cyclooxygenase and lipoxygenase enzymes

To characterize the cannabinoid-induced mechanisms and the potential humoral mediators involved, three different inhibitors of cyclooxygenase (COX) or lipoxygenase (LOX) enzymes were used: meclofenamic acid (Sigma Chemicals, St. Louis, USA), an inhibitor of COX with some LOX-inhibitory effects at higher concentrations (IC50 = 47μM) (Conroy et al. 1991; Streefkerk et al. 2003), flurbiprofen (Aldrich Chemicals, USA), a COX-inhibitor without strong suppressive effects on the neutrophil respiratory burst (Parij et al., 1998), and MK886 (kindly provided by Merck Frosst, Canada), an inhibitor of the 5-lipoxygenase activator protein (FLAP) (Daniels et al., 1998). The three

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inhibitors were dissolved in DMSO and diluted with PBS to the respective working concentrations. The final concentrations in the tests were 50 and 100 µM for meclofenamic acid (Ramos et al, 1994), 10 and 25 µM for flurbiprofen and 4 and 40 µM for MK886, as previously described by Daniels et al. 1998.

In-vitro assessment of the respiratory burst reaction in human whole blood PMN

After approval by our institutional ethics committee, heparinized venous blood obtained from informed and consenting healthy volunteers (6 males, 2 females, median age 36 years) was incubated with CP55 940 at logarithmic concentration steps from 10^{-11}M to 10^{-4}M. After incubation, the respiratory burst reaction of the whole blood PMN was determined using the commercially available Bursttest® (Orpegen Inc., Germany) as described by Rothe et al. (1998). The synthetic PMN stimulant N-formyl-methionyl-leucyl-phenylalanine (fMLP, final concentration 10^{-7} M) or plain buffer were added to 100 µl triplicate aliquots of the whole blood samples to detect potential stimulatory effects, and the two strong and almost maximum stimulants, the phorbolester PMA (final concentration 8.1 x 10^{-7} M) and a suspension of *Eschericha coli* (10^9 bacteria/ml), were used for detection of a potential suppressive effect. The burst reaction was determined by the conversion of 123-dihydrorhodamine to the fluorescent dye rhodamine in the cytoplasm of activated PMN. Rhodamine fluorescence was measured using a FACSCalibur (Becton Dickinson, USA) flow cytometer with a 488 nm argon laser as previously described (Kraft et al., 2004). Other blood cells were excluded from analysis by a live gate on the PMN cluster in the SSC/FSC dot plot. For each sample 10,000 events were acquired, and aggregation artefacts or cell detritus were detected by the addition of 200 µl propidium iodide solution (125 mg/ml) after lysing the erythrocytes and washing (live gate on the Fl 2 histogram).

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Data were analyzed with the Cell Quest® software (Becton Dickinson, USA), and the mean cellular fluorescence (Fl 1), which is proportional to the amount of produced oxygen radicals, as well as the percentage of stimulated rhodamine positive cells were determined to assess the activation of the oxidative burst reaction in whole blood PMN (Kraft et al., 2004).

Discrimination of direct cellular from humorally mediated cannabinoid effects

Direct cellular effects on PMN

In order to further characterize the role of direct cannabinoid effects on PMN, heparinized venous blood from each healthy donor was divided into two aliquots: one for the whole blood incubation with the respective cannabinoids as already described under 2.3., and the other aliquot for the separation of PMN as described below (Kraft et al., 2004; Deusch et al., 2003). Briefly, from the plasma supernatant obtained by Ficoll-Hypaque (Pharmacia Inc., Sweden) sedimentation, PMN were separated by centrifugation (20°C, 25 min, 250 g) through a two-step Percoll (Pharmacia Inc., Sweden) density gradient (62% v/v and 73% v/v). Separated PMN (2.5×10^6 cells/ml) were resuspended in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS) and maintained at 37°C. Cell viability was monitored by trypan blue exclusion (>95%) and PMN enrichment was verified by differential count (> 95%).

Aliquots of the remaining whole blood sample from the same donor were incubated with vehicle as controls or the respective cannabinoid concentrations. After centrifugation (20ºC, 10 min, 250 g) the supernatants were removed and separated into a high (> 5,000 D) and a low (<5,000 D) molecular weight fraction by centrifugation through a molecular pore filter system (Centrisart I, Sartorius Inc., Germany) with a cut-off threshold of 5000 D. Aliquots of the isolated PMN were incubated (30 min, 37°C) either with the cannabinoid dilution alone or with the respective low

and high molecular plasma fractions derived from the whole blood incubation experiments, and thereafter the PMN respiratory burst reaction was examined as described before.

COX- or LOX- dependent mediators

The unseparated whole blood samples were pre-incubated with meclofenamic acid, flurbiprofen or MK886 before adding CP55 940 (0.1 and 1.0 nM). Thereafter, the plasma supernatants were obtained by centrifugation (20°C, 10 min, 250 g) as described under 2.4.1 and the freshly isolated PMN from the same donor were incubated with the supernatants (30 min, 37°C), followed by the Bursttest®, as described in detail before.

Statistical analysis

Data were analyzed by means of Jandel Sigma Stat 2.0 software for Windows.Unless otherwise indicated, results are expressed as a percentage of the control measurements with vehicle alone. . For multiple comparisons, one-way ANOVA followed by Bonferroni´s post-hoc test was usedfor normally, ANOVA-on ranks followed by Dunn´s post hoc test for non-normally distributed values, respectively. Where appropriate, paired Student´s t-test was applied. $P < 0.05$ was considered significant.

Results

Pilot experiments were performed to determine the optimum incubation period for whole blood PMN using two high (10^{-6} and 10^{-5} M) and two low (10^{-10} and 10^{-9} M) concentrations of CP55 940. The micromolar concentrations as well as the long incubation periods of up to 180 min were chosen based on our previous report (Kraft et al., 2004). Because of the CB_2R dissociation

constants for the cannabinoids studied, the nanomolar concentrations were tested, because they are considered more relevant to determine CB receptor mediated effects.

The oxygen radical production of fMLP-stimulated whole blood PMN was activated at 10^{-10} and 10^{-9} M CP 55 940 , starting after a 90 min incubation period and reaching a maximum after a 120-180 min duration of cannabinoid exposure (Fig 1).

Therefore, a 180 min exposure period to CP55 940 was used in all further experiments. Resting (Fig. 2A) as well as fMLP-stimulated PMN (Fig. 2B) showed a statistically significant stimulation of the respiratory burst reaction at subnanomolar and nanomolar concentrations of CP55 940 in both, the percentage of activated rhodamine positive PMN as well as the oxygen radical generation expressed as mean fluorescence per cell (data not shown). The observed stimulation by CP 55 940 was completely abolished after pre-incubation of the whole blood aliquots with the specific CB_2R antagonist SR 144 528 (100 nM) (Fig. 2A, B).

In contrast to the experiments with the weak stimulus fMLP, the burst enhancement with E. coli was so strong that an additional stimulation with CP55 940 between 0.1 nM and 1 nM did not reach statistical significance. As expected, PMA produced a maximum stimulation that could not be enhanced by CP55 940 (Fig. 3).

To rule out an exclusive CP55 940-specific effect, the endocannabinoid anandamide (AEA) and its stable derivative methanandamide (MethAEA) were also investigated in our experimental setting. While anandamide, which is rapidly degraded by fatty acid amido hydrolase (FAAH) in cell containing media, had neither a stimulatory nor a suppressive effect (data not shown), its non-hydrolyzable derivative MethAEA showed an activation of resting (Fig. 4) and fMLP-stimulated whole blood PMN similar to CP55 940, and this effect was also CB_2R-dependent as shown by its antagonism with SR144 528 (Fig. 4).

To further characterize the mechanisms underlying this cannabinoid-induced burst stimulation in whole blood PMN, isolated human PMN were exposed under identical incubation conditions to concentrations of CP55 940 that produced stimulation in whole blood (Fig. 5). The cannabinoid did not exert any detectable effect on isolated neutrophils, in contrast to the significant enhancement of the respiratory burst in whole blood PMN.

To evaluate the involvement of humoral factors, aliquots of whole blood were incubated for 180 min with stimulatory concentrations of CP55 940 at 10^{-10} and 10^{-9} M as described before. The plasma supernatants of the aliquots were then added to isolated PMN of the same donor, and after a further 30 min incubation the respiratory burst was measured by flow cytometry.

The isolated PMN were stimulated by the plasma supernatants of the CP55 940-containing samples, but not by the CP55 940-free plasma controls, clearly indicating that inducible humoral factors were responsible for this effect (Tab.1).

When the supernatants were separated into a high molecular weight fraction expected to contain cytokines and a low molecular weight fraction containing the smaller mediator molecules such as prostaglandins and leukotrienes, isolated PMN showed a significant stimulation only after incubation (30 min) with the low molecular weight fraction, but not with the high molecular weight fraction of the cannabinoid-primed plasma supernatant (Fig. 6).

In order to clarify the potential involvement of other mediators in the cannabinoid-induced PMN stimulation, whole blood samples were incubated (37°C, 30 min) with three different inhibitors of the cyclooxygenase (COX) and/or lipoxygenase (LOX) enzymes prior to the incubation with CP55 940 at 10^{-10} (Table 2A and B) and 10^{-9} M (data not shown), respectively. After pre-incubation with meclofenamic acid (50 µM, 100 µM), the stimulatory effect of CP55 940-primed plasma supernatant was dose-dependently abolished. This was true for the percentage of

rhodamine positive PMN as well as the mean cellular fluorescence (Tab. 2A, B). In these experiments, meclofenamic acid alone did not significantly alter the burst activity of resting PMN, whereas the fMLP-stimulated PMN activity was insignificantly reduced by 10% to 20% (Tab. 2B).

The results with the COX-inhibitor flurbiprofen (10 µM, 25 µM, data not shown) were similar to those obtained with meclofenamic acid. The pre-incubation with flurbiprofen also produced a dose-dependent and significant inhibition of the PMN burst activation mediated by the CP55 940-primed plasma. This effect could be observed in both the resting and fMLP-stimulated PMN for the percentage of rhodamine positive cells, as well as for the oxygen radical generation.

The pre-incubation with MK886 (4 µM, 40 µM), an inhibitor of the 5-lipoxygenase-activator-protein (FLAP) did not significantly inhibit the CP55 940-induced stimulation at 10^{-10} (Tab. 2A and 2B) and 10^{-9} M (data not shown).

Discussion

Our study demonstrated for the first time a significant stimulatory effect of the cannabinoids MethAEA and the THC analogue CP55 940 on human PMN when incubated in their natural whole blood milieu. In clear contrast to the previously described receptor-independent suppressive action of CP 55 940 (Kraft et al., 2004) and Δ^9-THC (Djeu et al., 1991) on isolated human PMN, this stimulation occurred at much lower and pharmacologically more relevant concentrations of the cannabinoid agents and showed a bell-shaped dose response relationship with a maximum stimulation between 0.1 nM and 1.0 nM. Although the 10^6-fold concentration range of CP55 940 covered more than only the pharmacologically relevant concentrations, those lower than 0.01 nM were not tested, and thus the dose response relationship was not determined

below half-maximum stimulation. However, at high CP55 940-concentrations, the decline in stimulatory activity probably reflects the counteracting receptor-independent, direct inhibitory effect of CP55 940 on PMN that has been shown to occur in isolated PMN at similar concentrations. The slight, statistically not significant suppression of the respiratory burst reaction of fMLP-treated PMN at concentrations above 100 nM also supports this interpretation and confirms data from the literature, reporting a concentration-dependent appearance of receptor-mediated versus receptor-independent cannabinoid actions on immune cells. Whereas the suppressive effect on isolated PMN was neither CB_1R nor CB_2R dependent, the stimulatory activity in whole blood PMN could be completely blocked by co-incubation with the specific CB_2R-antagonist SR144 528. In addition, the subnanomolar and low nanomolar concentrations of CP55 940 and the even lower concentrations of MethAEA sufficient to enhance the respiratory burst of resting as well as fMLP-stimulated whole blood PMN are consistent with their respective K_i-values for CB_2-receptors (Howlett et al., 2002; Rinaldi-Carmona et al.,1998).

Together with the SR144 528 antagonism, these findings indicate an involvement of CB_2R-mediated mechanisms. Since human PMN isolated from the blood of healthy individuals lack functional CB_2R (Deusch et al., 2003), the failure to detect any direct stimulatory effect of the two cannabinoids on isolated PMN in the present study confirms our previous results and fits the impression that – although mRNA for CB_2R was found by PT-PCR in PMN (Bouaboula et al., 1993; Galiegue et al., 1995) – human circulating PMN are not a direct target for cannabinoid actions. Instead they may be indirectly influenced by the interactions of the cannabinoid agents with other blood cells. The present results with unfractioned and fractioned cell-free plasma supernatants from whole blood exposed to cannabinoids argue for a humoral mechanism that is clearly dependent on a CB_2R activation of cannabinoid-sensitive blood cells, presumably

macrophages or other peripheral mononuclear leukocytes. The fact that the cannabinoid-induced burst stimulation in whole blood PMN did not show a rapid onset, but started slowly after an at least 90 min incubation gives further evidence for such an indirect, mediator-dependent mechanism.

Previous reports demonstrated the release of arachidonic acid (Diaz et al., 1994) and the modulation of cytokine production of mononuclear leukocytes (Klein et al., 2003; Zhu et al., 1994) by the marijuana cannabinoid Δ^9-THC. Both mediator pathways are known to be physiological and pathophysiological activators of PMN, but the fact that in the present study the stimulating activity was exclusively found in the low molecular weight fraction of the plasma incubation supernatants strongly argues in favor of an the involvement of arachidonic acid or its metabolites. Interactions between prostanoid metabolism and cannabinoids have already been described by various investigators (Burstein et al., 1988; Perez-Reyes et al., 1991) and illustrate the close relationship between arachidonic acid metabolism and endogenous cannabinoid ligands (Pestonjamasp et al., 1998; Edgemont et al., 1998). The activity of COX-enzymes, the release of PGE_2 and arachidonic acid were stimulated by cannabinoids in astrocytes (Shivachar et al., 1996), cortical slices (Reichmann et al., 1987), but also lymphocytes (Audette and Burstein 1990). Diaz et al. could demonstrate that THC increased the production of leukotriene B4 (LTB_4) and 12-HETE from mononuclear blood cells (Diaz et al., 1994). Arachidonic acid as well as LTB_4 are known to be potent chemoattractants, activating PMN-migration and oxygen radical generation (Liu et al., 2003). Thus, arachidonic acid and its metabolites are promising candidates for a potential involvement in the observed indirect, low molecular weight mediator-dependent stimulatory effect of cannabinoids on whole blood PMN.

The results with the COX and LOX-inhibitors, respectively, suggest mainly the involvement of COX-dependent pathways induced by a CB_2R-evoked interaction of the cannabinoids with blood-cells others than PMN. These findings further support the idea of a stimulation of eicosanoid synthesis by cannabinoids as suggested in former investigations by Hunter et al.(1997).

Whereas the short-living, rapidly hydrolyzed endogenous cannabinoid AEA (DiMarzo et al., 1999) had no stimulatory effect on the burst reaction of whole blood PMN, its stable, non-hydrolyzable derivative MethAEA enhanced the respiratory burst of PMN in whole blood, similar to the synthetic Δ^9-THC analogue CP55 940. Our observations confirm the CP55 940 data by using a second completely different CB_2 R-ligand, and suggest a potential physiological role of endocannabinoids as indirect regulators of PMN activity in humans by means of the COX- or LOX- dependent arachidonic acid pathways in other blood cells. Macrophages (Diaz et al., 1994) or mast cells (Samson et al., 2003) are possible candidates for the release of arachidonic acid into whole blood after cannabinoid incubation. Interestingly enough, the exposure to marijuana smoke was recently reported to increase the oxygen radical production from alveolar macrophages in humans, resulting in oxidative stress and inflammation (Baldwin et al., 1997).

Although the endogenous cannabinoid, 2-arachidonylglycerol (2-AG), also acts on CB_2 R and is supposed to play a role in immunomodulation (Sugiura and Waku, 2000), experiments with 2-AG would have been hampered by its significant instability in cell culture media (Rouzer et al., 2002). Even under cell-free conditions, 2-AG rapidly rearranges to 1- or 3-arachidonylglycerol (1(3)-AG) in a first order process with a half-life of only 2.3 min in RPMI medium containing 10% fetal calf serum. As AEA resembles 2-AG in its affinity to the CB_2 R (Gonsiorek et al., 2000) and – what is more important from a practical point of view - has the stable derivative

MethAEA available, only AEA and MethAEA were used as endocannabinoid substances in our experiments.

In conclusion, our results suggest that human circulating PMN are not a direct cellular target of endogenous or exogenous cannabinoids, but are nevertheless strongly activated by a CB_2R-evoked COX-dependent mediator pathway induced by cannabinoid interactions with other blood cells. Thus, there is no evidence for a potential cannabinoid-induced suppression of PMN functions in healthy human individuals, but in contrast even an enhancement of oxidative burst activity is to be expected.

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Footnotes:

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Legends for Figures

Fig. 1: *Time-dependent oxygen radical generation in fMLP-stimulated whole blood PMN under CP55 940 exposure. After more than 90 min incubation, only exposure to 10^{-10} and 10^{-9} M CP55 940 showed an significant increase. Values are means of the mean log fluorescence channel number (Fl1) (percent of control) ± SEM obtained from 5 independent triplicate tests.*

Fig. 2*: Stimulation of the respiratory burst reaction of human whole blood PMN by CP55 940 and antagonism by pre-incubation with 100 nM SR 144 528 (PMN/SR).*

A: Resting PMN (3.61% ±0.61 rhodamine positive cells): Significant activation of PMN at 0.1 and 1.0 nM CP55 940.

B: FMLP-stimulated whole blood PMN (fMLP/PMN: 13.70% ±1.11 rhodamine positive cells): Significant stimulation at 1.0 nM CP 55 940)

*Data are expressed as mean values (percent of control) ±SEM of 8 independent triplicate tests. *P<0.05, ANOVA.*

Fig. 3: *Oxygen radical production of whole blood PMN under exposure to CP55 940 and stimulated with PMA or E. coli.*

Data are shown as mean log fluorescence channel number (Fl1) ± SEM obtained from 8 independent triplicate tests.

Fig. 4: *Activation of resting whole blood PMN after incubation with MethAEA and complete antagonization by pre-incubation with 100 nM SR 144 528 (SR/MethAEA).*

*Bars represent the means ± SD (percent of control) of 8 independent triplicate tests. *P< 0.05, ANOVA.*

Fig. 5: *Differential effects of CP55 940 on isolated and whole blood PMN from the same blood sample. Data represent activated PMN after 180 min incubation with CP55 940 expressed as a percentage of unstimulated controls. Mean values ± SEM of 8 independent experiments. *P< 0.05, ANOVA.*

Fig. 6: Increase of the relative number of activated rhodamine positive PMN after incubation with the low molecular weight (MW) plasma fraction.

*Mean values ± SEM of 6 independent triplicate tests. *P< 0.05, ANOVA.*

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Tables

Table 1*: Activation of isolated PMN by CP 55 940-primed plasma supernatants. Relative number of activated rhodamine positive (Rhod. pos) PMN and mean cellular fluorescence (FL1) with or without fMLP-stimulation.*

	% of control	
[CP 55 940]	*0.1 nM Plasma*	*1.0 nM Plasma*
Rhod. pos. PMN (without fMLP)	272.66 ± 156.18	244.96 ± 123.51
Rhod. pos. PMN (with fMLP)	204.44 ± 141.55	170.58 ± 85.78
FL1 (without fMLP)	116.76 ± 21.42	117.52 ± 3.75
FL1 (with fMLP)	128.91 ± 34.60	121.32 ± 21.11

Data are presented as a percentage of the effects of CP55 940-free control plasma. Mean ± SD of 3 independent triplicate experiments.

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Table 2A: *Inhibition of the CP 55 940-supernatant-induced stimulation of resting PMN by pre-incubation with the COX-inhibitor meclofenamic acid and the FLAP-inhibitor MK886.*

	Without CP	*0.1nM CP*
	Rhod.pos. PMN (% of control)	
No inhibitor	*100*	*155.69 ±23.79*
50µM meclo	*83.91 ±7.12*	*112.47 ±9.35*
100µM meclo	*78.75 ±6.93*	*80.46 ± 6.04**
4µM MK886	*104.84 ±5.14*	*135.34 ± 6.57*
40µM MK886	*109.31 ±6.07*	*140.80 ±7.96*
	Mean fluorescence	
No inhibitor	*100*	*149.72 ±8.47*
50µM meclo	*93.70 ± 6.52*	*105.01 ± 6.92**
100µM meclo	*91.77 ± 3.11*	*92.69 ± 6.49**
4µM MK886	*113.19 ± 3.26*	*131.64 ± 9.05*
40µM MK886	*118.44 ± 3.96*	*137.61 ± 7.51*

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Table 2B: *Inhibition of the CP 55 940-supernatant-induced stimulation of fMLP-treated PMN by pre-incubation with the COX-inhibitor meclofenamic acid and the FLAP-inhibitor MK886.*

	Rhod.pos. PMN (% of control)	
	without CP	*0.1nM CP*
No inhibitor	*100*	*241.85 ± 29.41*
50µM meclo	*93.84 ±6.41*	*139.40 ±4.84**
100µM meclo	*97.96 ±3.64*	*109.92 ±6.41**
4µM MK886	*101.62 ±18.34*	*149.99 ±20.19*
40µM MK886	*104.39 ±6.49*	*168.47 ±26.63*
	Mean fluorescence	
No inhibitor	*100*	*194.20 ± 63.71*
50µM meclo	*103.31 ±2.46*	*121.80 ± 7.64**
100µM meclo	*100.66 ±3.98*	*111.43 ±8.47**
4µM MK 886	*106.74 ± 6.42*	*136.91 ±11.68*
40µM MK 886	*109.21 ±5.35*	*142.25 ±11.48*

*Data are presented as a percentage of the cannabinoid-free control without COX and LOX inhibitors (resting PMN: 3.61% ±0.61 rhodamine positive cells; fMLP-stimulated PMN: 13.70% ±1.11 rhodamine positive cells). Mean ±SEM of 6 independent triplicate experiments. Concentrations are given as final concentrations in the tests. *P< 0.05, ANOVA. Rhod. pos. = rhodamine positive PMN.*

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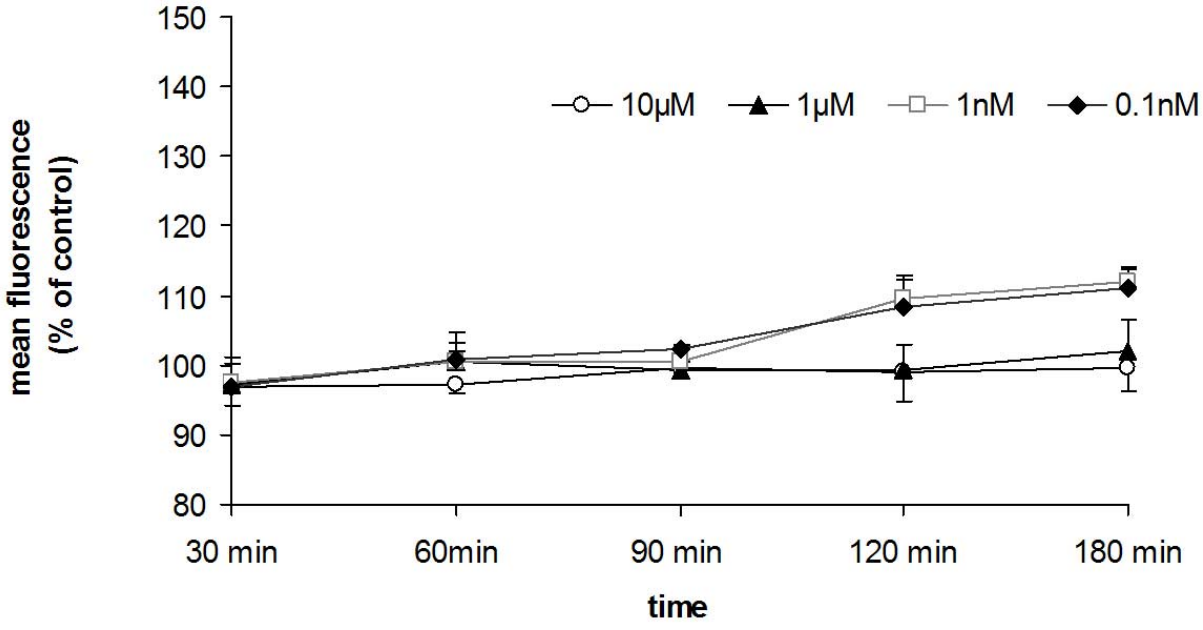
Figure 1



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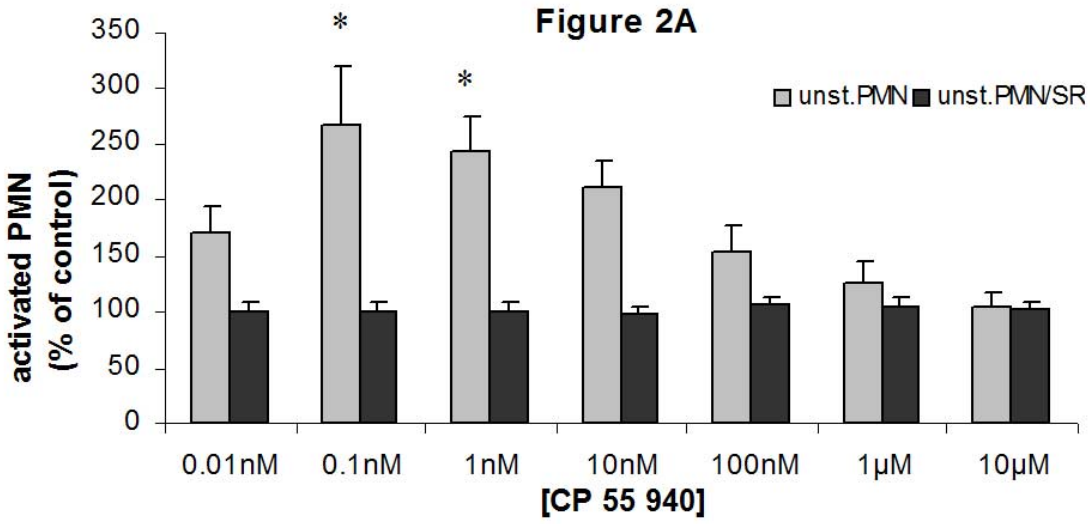

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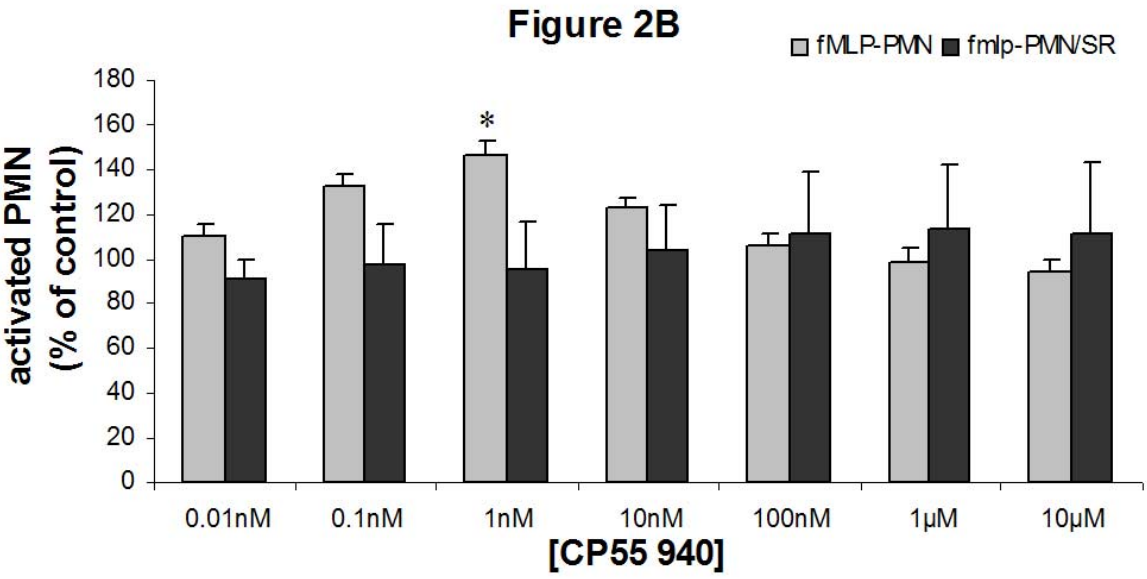

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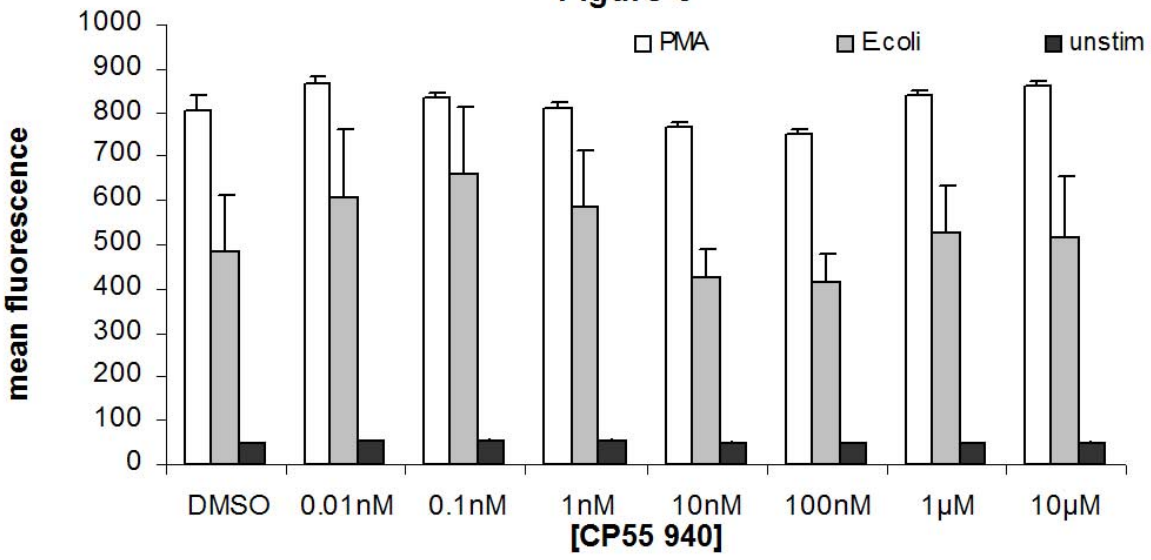
Figure 3



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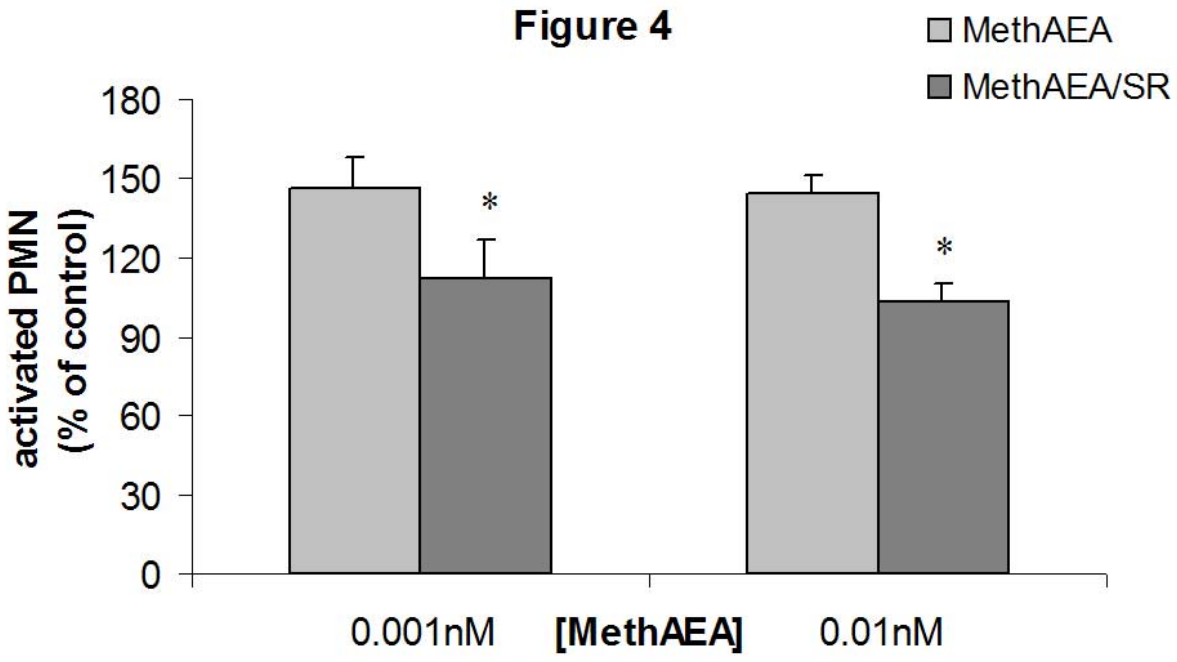

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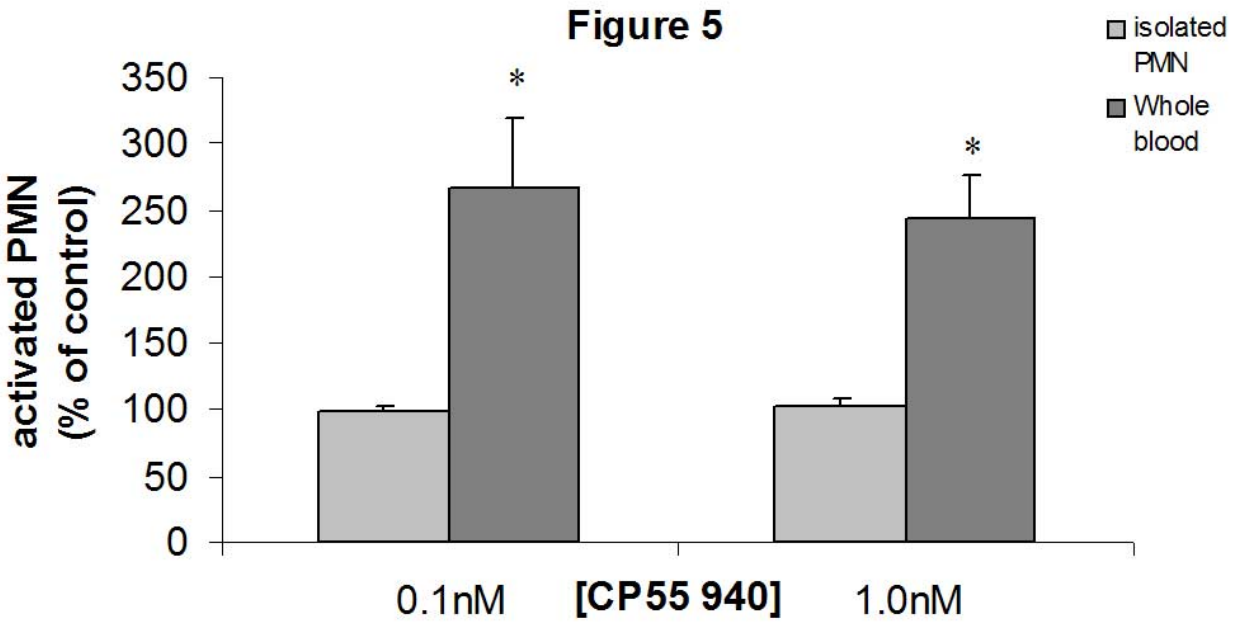

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