adhesive contact with the skin in heavily perspiring patients. The six episodes of detachment all occurred with the use of a flexible plastic version of the sensor. This problem seems to have been solved with the use of a new cloth sensor. Conversely, the Capnomask™ was removed on numerous occasions in three patients, which required the intervention of a healthcare provider to reposition it. These periods combined with those of recalibration of the capnograph resulted in the absence of RR measurement for nearly 12% of the duration of the recordings, which could jeopardize the routine use of this technique. These results support the use of RRa when accurate and continuous RR monitoring is desired.

**Declaration of interest**

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Long-term activation of polymorph cannabinoid receptors does not affect receptor gene transcription

Editor—The role of cannabinoids in anaesthesia has received renewed interest with activation of the cannabinoid system capable of producing analgesia, anxiolysis, muscle relaxation, and immunomodulation.1 2 There have been major developments in cannabinoid receptor classification to possibly include the orphan G-protein-coupled receptor GPR55 along side the classical CB1 and CB2,3 although this is highly controversial. Therapeutic activation is likely to be long term and there are no in vitro data after long-term exposure on expres
tion of the genes encoding these receptors. We have therefore examined the effects of long-term treatment of human polymorphonuclear cells with the CB2 agonist L-5796564 and the GPR55 agonist L-α-lysophosphatidylinositol (LPI)5 on mRNA encoding for CB1, CB2, and GPR55 receptors.

Human neutrophils isolated by density gradient separation from 12 consented, healthy volunteers, and HL60 cells (a neutrophil pre-cursor cell) were treated with DMSO (1.75%; control), LPI (10 μM), LPI (1 μM), or L-579656 (10 μM) at 37°C for 66 h. HL60 cells were differentitated with 120 mM dimethylformamide (DMF) for 72 h,6 and then immediately treated with LPI or L-579656.

mRNA was extracted (mirVANA® System), cleaned to remove genomic DNA (Turbo DNA-free® kit), then reverse transcribed using a high-capacity cDNA reverse transcription kit. cDNA samples were probed using TaqMan® probes for CB1, CB2, and GPR55. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeper gene and quantitative PCR was performed using the Step-one machine, with non-template controls.7 PCR data are presented as cycle thresholds (Ct) [mean (SEM)], where ΔCt is the difference between GAPDH and the gene of interest. Statistical analysis was by analysis of variance (ANOVA) followed by Dunnett’s post hoc testing when P<0.05 or paired t-test as appropriate.

Human neutrophils expressed CB2 and GPR55 receptor mRNA with ΔCt values of ~7.3 and ~10.6 (n=12), respectively. CB1 receptor mRNA was present at very low levels (Table 1). Relative to CB1, there was ~25- and ~2.5-fold more CB2 and GPR55 mRNA, respectively. Pretreatment with either L-579656 or LPI (1 or 10 μM) for 66 h did not affect the transcription of CB1, CB2, or GPR55 (Table 1). Pretreatment with LPI at 10 but not 1 μM significantly reduced GPR55 mRNA by ~1 cycle or ~2-fold.

HL60 cells expressed CB2 and GPR55 receptor mRNA with ΔCt values of 8.65 (0.25) and 16.63 (0.55), respectively (n=8). CB1 receptor mRNA was not detected (n=8). In view of the low expression of GPR55, we differentiated cells with DMSO and observed an increase in the expression of GPR55 and CB2 by 73.88 (22.7) (n=6) and 7.31 (4.22) fold at 72 h (n=11). Subsequent treatment (n=3) with L-579656 and LPI (1 or 10 μM) was detrimental to cell survival.

In this study, we have shown that human neutrophils express the classical cannabinoid receptors along with the putative receptor GPR55 (rank order CB2>GPR55>CB1).
This pattern is mirrored in the HL60 cell and levels of expression can be increased with differentiation. Pretreatment of human neutrophils with either CB2 or GPR55 agonists did not produce any marked effects on gene transcription, but a reduction was observed with the higher concentration of LPI (10 μM).

**Declaration of interest**

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