Cannabidiol Enhances Vasorelaxation To Acetylcholine In Femoral Arteries From Zucker Diabetic Rats

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Incubation of aortae with cannabidiol (CBD) restores endothelial function in a model of type 2 diabetes (Zucker Diabetic Fatty (ZDF) rats) (Stanley & Wheal *et al*.). The aim of the present study was to establish the underlying mechanisms of this finding.

Male ZDF rats (340-405g, blood glucose = 25.1 ± 1.1 mM (mean ± SEM), n=11), and their lean control rats (276-315g, blood glucose = 7.6 ± 0.3 mM, n=8) were killed by cervical dislocation. Femoral arterial rings were isolated and the segments were bathed in warmed (37^oC) and gassed (95% O_2/5% CO_2) modified Krebs'-Henseleit solution in a myograph and set to a resting tension of 4.91mN. Arteries were incubated with CBD (10μM), or its vehicle (5μl ethanol) for 2h before contracting with methoxamine and/or U46619. Following contraction, cumulative concentration-response curves to the endothelium-dependent vasorelaxant acetylcholine (Ach, 1nM-100μM) were constructed. The involvement of cyclooxygenase products were investigated by the additional incubation with indomethacin (3μM), flurbiprofen (10μM) or nimesulide (10μM). The roles of nitric oxide (300μM NG-nitro-L-arginine methyl ester (L-NAME)), peroxisome proliferator-activated receptor gamma (PPARγ; 1μM GW9662), endothelium-dependent hyperpolarising factor (EDHF, inhibited by indomethacin, L-NAME, apamin (500nM) and TRAM-34 (10μM)), hydrogen peroxide (300U/ml PEG-catalase), fatty acid amide hydrolase (1μM URB597) and superoxide dismutase (300μM sodium diethyldithiocarbamate trihydrate (DETCA)) were examined. Involvement of cannabinoid CB_1, CB_2 and the endothelial cannabinoid receptors were also investigated, using their respective inhibitors, AM251 (1μM), AM630 (1μM) and O1918 (1μM). Comparison of maximal vasorelaxations between strains and treatments was done using one-way ANOVA followed by either Dunnett's or Bonferroni *post hoc* tests, with $P<0.05$ taken as significant.

The weights and blood glucose levels in ZDF rats were higher than in the lean control rats ($P<0.05$, Students' t-test). ACh caused concentration-dependent vasorelaxation in femoral arteries incubated with vehicle in both lean (n=8) and ZDF (n=6) rats, with the maximal relaxation in arteries from ZDF rats being blunted (R_{max}; Lean = 63.8 ± 2.2 %, ZDF = 40.5 ± 2.1 %) ($P<0.0001$). Incubation with CBD in arteries from lean rats had no effect, but CBD significantly enhanced vasorelaxation to ACh in the arteries taken from ZDF rats to achieve a maximal relaxation similar to that observed in lean rat femoral arteries (R_{max} 68.5 ± 5.7 %, n=6, $P<0.0001$). The enhanced vasorelaxation to ACh in CBD-treated vessels was still seen when the arteries were co-incubated with L-NAME, GW9662, EDHF inhibitors, PEG-catalase, AM251, AM630, O1918 and URB597. The presence of indomethacin, flurbiprofen or nimesulide abolished the enhancement of ACh responses by CBD in ZDF rats. Co-incubation with DETCA also abolished the effects of CBD incubation ($P>0.05$).

In conclusion, CBD restores endothelium-dependent vasorelaxation to normal levels in ZDF rats. This involves both a COX-2- and a superoxide dismutase-mediated mechanism, and supports the hypothesis that CBD may restore endothelial dysfunction in type 2 diabetes.

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Stanley C. & Wheal AJ. *et al*. (2011). British Pharmacological Society winter meeting poster P165.