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P505

ANAKINRA, AN INIBITOR OF THE INTERLEUKIN-1 RECEPTORS, PREVENTES THE NADPH OXIDASE TRIGGER BY INTERLEUKIN-1β

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Introduction. We have previously suggested that a pro-inflammatory environment may represent a factor contributing to endothelial dysfunction in the rat mesenteric vasculature. Indeed, the endothelium impairment induced by interleukin-1β (IL-1β) could be potentiated in a high D-glucose medium, suggesting that hyperglycaemia may modulate the endothelial dysfunction induced by pro-inflammatory cytokines. Interestingly, all these effects could be prevented by the antagonism of IL-1 receptors anakinra (AK). Furthermore, the microvessels pre-incubated with NADPH oxidase inhibitor apocynin showed a significant reduction of the endothelial dysfunction (P<0,001). In the present study, to gain insight into the mechanisms mediating the impaired vasodilation elicited by IL-1β, we explored the potential role of NADPH-oxidase in cultured human umbilical vein endothelial cells (HUVEC) and rat microvascular preparations, as well as its possible interference by AK and apocynin.

Methods: Confluent HUVEC were treated with different concentrations of D-glucose (5.5 and 22 mmol/l) either alone or in combination with IL1-β, (2,5 ng/ml), AK (150 µg/ml) or apocynin (30 µmol/l) for 20-30 min; rat microvascular preparations were treated with AK (100 µg/ml) or apocynin (10 µmol/l) as the same conditions. The activity of NADPH oxidase was measured in both HUVEC and rat microvascular preparations by lucigenin-derived chemiluminiscence. All the experiemnts were in vitro, then, drug concentrations were directly added to the incubation medium. Results are expressed as mean±SEM. Statistical analysis was performed using Student's *t* test, with the level of significance chosen at $P<0.05$.

Results: In cultured HUVEC, the IL-1β preincubation increased NADPH-oxidase activation by 317.60% (P<0,001 vs basal), which was potentiated in a high D-glucose medium by an additional 106.16% (P<0,001 vs IL-1β 5,5 mml/l D-glucose activation). Indeed, the preincubation with apocynin (P<0,001 vs IL-1β) or AK (P<0,001 vs IL-1β) completely prevented both the activation of NADPH oxidase triggered by IL-1β and its enhancement by high D-glucose (Results from 6 independent experiments performed in triplicate). In rat mesenteric microvascular preparations, IL-1β preincubation increased NADPH-oxidase activation by 510.76% (P<0,001 vs basal), which was potentiated in a high D-glucose medium by an additional 180.64% (P<0,001 vs IL-1β in 5.5 mmol/l D-glucose). The preincubation with apocynin (P<0,001 vs IL-1β) or AK (P<0,001 vs IL-1β) prevented the activation of NADPH oxidase triggered by IL-1β and its enhancement by high D-glucose (Results form 5 independent experiments).

Conclusions: IL-1β stimulates vascular NADPH oxidase activity. This effect is enhanced by high D-glucose concentrations and prevented by the preincubation with AK or apocynin. We suggest that the mechanisms underlying the impairment of the endothelium-dependent relaxations by IL-1β include the activation of the vascular NADPH-oxidase and the increase of superoxide anions. Furthermore, high glucose concentrations can synergize with the endothelial dysfunction evoked by a pro-inflammatory environment.