C-REACTIVE PROTEIN-INDUCED INCREASE IN CYTOSOLIC FREE CALCIUM INVOLVES BOTH IP3 MEDIATED STORES AND CAPACITIVE ENTRY.

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INTRODUCTION

C-Reactive protein (CRP) is an acute-phase reactant that serves as a pattern-recognition molecule in the innate immune system. CRP has been traditionally thought of as a bystander marker for vascular inflammation and cardiovascular disease, without playing a direct role in the inflammatory process. However, recent evidence suggests that CRP may directly affect endothelial cells[1], decreasing nitric-oxide production and promote apoptosis[2].

Intracellular calcium concentrations were previously shown to modulate NOS activity and apoptosis in vascular endothelial cells[3]. Taken together, this data suggests a potential complex interaction between CRP, calcium, and NO within endothelial cells. To further elucidate the mechanisms by which CRP modulates endothelial cells function we studied intracellular calcium response to CRP stimulation.

MATERIALS AND METHODS

Cell culture

Human umbilical vein endothelial cells (HUVEC), passage 4-8, were grown to confluence in 96 wells plates. Cells were cultured in EBM-2 media supplemented with 15% fetal bovine serum (FBS, Gibco) and 1% penicillin-streptomycin (PS, Gibco), and kept at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Human recombinant CRP (Fitzgerald, Concord, MA), Dantrolene Sodium (Sigma), Heparin (Sigma), BAPTA (Sigma) were used.

Experiments were carried out at room temperature under continuous superfusion with Tyrode solution.

Fluorescence measurements

$[Ca^{2+}]_i$ measurements were performed using the fluorescent ratiometric Ca^{2+} indicator fura-2. Cells were loaded with the dye by exposure to Tyrode solution containing 5µM fura-2 AM (Molecular Probes) and 5µl of Plutonic F-127 (Molecular Probes) for 45 min at 37°C . Fura-2 was excited by alternately illuminating the cells at 340 nm (F_{340}) and 390 (F_{390}). Emitted cellular fluorescence was recorded at 510 nm. Fluorescence signals were measured and recorded using fluoroscan.

Changes in $[Ca^{2+}]_i$ were expressed as changes of the ratio $R= F_{340}/F_{390}$.

RESULTS AND DISCUSSION

CRP acutely increases intracellular calcium levels in a dose-response manner. The dose-effect was abrogated after >20 minutes (Fig.1)

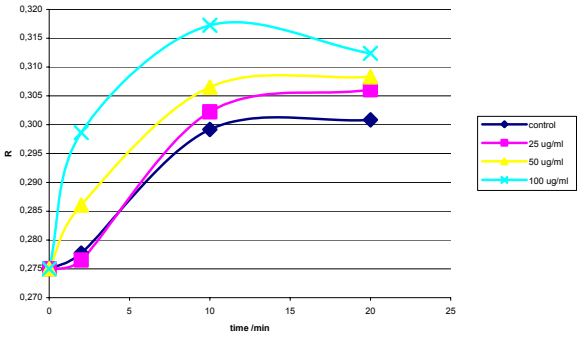

Figure 1: $[Ca^{2+}]i$ dose- and time-responses to CRP.

To determine the channels activated by CRP in the Endoplasmatic Reticulum (intracellular mechanism), dantrolene (D) and heparin (H) were used as Ryanodine and IP3 channels blockers, respectively.

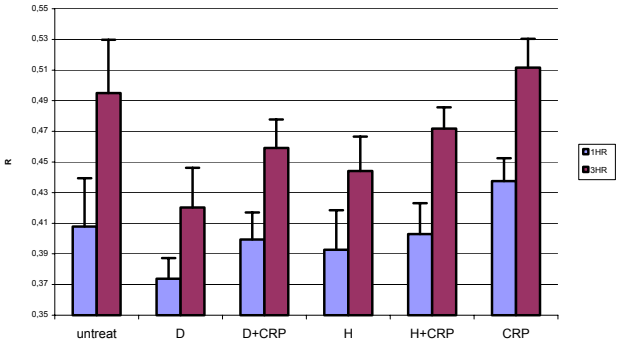

Figure 2: Effect of CRP on $[Ca^{2+}]i$ release from intracellular stores.

CRP-induced increase in [Ca $^{2+}$]i was reduced at 1 h in the presence of heparin to heparin-baseline levels suggesting a major role for IP3 channel that Heparin blocks (IP_3).

In order to asses whether CRP stimulates $[Ca^{2+}]_i$ via an extra-cellular pathway, an extra-cellular calcium chelation was applied.

Short chelation of extra-cellular calcium by BAPTA had no effect on basal $[Ca^{2+}]_i$. However, CRP-induced increase in $[Ca^{2+}]_i$ was substantially reduced in the presence of BAPTA indicating a major role for extra-cellular calcium and capacitive calcium entry in the CRP-induced effect (Fig.2).

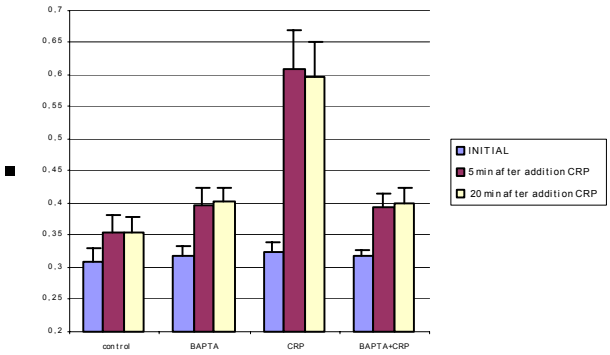

Figure 3: The effect of extracellular calcium chelation on $[Ca^{2+}]i$ response to CRP.

CONCLUSIONS

CRP acutely increases cytosolic free calcium concentrations in HUVEC in a dose-dependent manner.

These findings further support a direct effect of CRP on vascular endothelial cells that is mediated through calcium signaling. The increase in [Ca $^{2+}$]i is modulated through activation of both intracellular IP3-sensitive channels and through extra-cellular calcium entry.

REFERENCES

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