## CVS-15 The Cytokine response of rat macrophages to lipopolysaccharide is modulated by adrenomedullin

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**Introduction:** Adrenomedullin (AM) is now recognised to be involved in inflammation. We investigated whether AM is an inflammatory or anti-inflammatory cytokine.
**Method:** Rat macrophages (NR8383) were incubated at $10^6$ cells/well in 3.5-cm tissue culture plates in RPMI 1640 medium containing 1% fetal bovine serum in the absence or presence of 10ng/mL to 1μg/mL AM. Conditioned media of parallel cultures were removed at 0, 3, 6, and 24-hours after AM stimulation. Secretion of cytokines including TNF-α and IL-6 were measured using ELISA. To test whether AM is anti-inflammatory, macrophages were activated by 1ng/mL to 1μg/mL lipopolysaccharide (LPS) in the absence or presence of 1_g/ml AM. The cytokine response was measured in the conditioned media at 0, 3, 6, and 24-hours.
**Results:** Basal secretion of TNF-α and IL-6 were 29.2±2.0 pg/mL and 58.7±2.8 pg/mL respectively. AM increased TNF-α concentration by 115.4±41.4% but reduced IL-6 concentration by 56.3±4.9 % at 24 hours. LPS at 1 ng/mL enhanced TNF-α by 45-fold and IL-6 production by 11-fold. The presence of AM reduced the TNF-α and IL-6 response to 66% and 49% respectively.
**Conclusion:** Our results suggest that AM modulates cytokine secretion from rat macrophages and may thus have a regulatory role in inflammation.

## CVS-16 Increased adrenomedullin expression in the heart, the lung and the mesenteric artery by endotoxin

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Previous studies have shown that the circulating levels of adrenomedullin (AM) are elevated during inflammation. The levels of AM and its messenger RNA (mRNA) in various tissues during the time course of inflammation remain to be determined. To study this, inflammation was induced in rats by intraperitoneal injection of lipopolysaccharide (LPS, 10mg/kg). The tissues were harvested at 0, 1, 3 and 6 hours after LPS administration. Tissue levels of AM were determined by radioimmunoassay. The gene expression levels of AM were determined by solution hybridization-RNase protection assay of preproAM mRNA levels. The preproAM mRNA levels were increased in mesenteric artery and right atrium at 1 hour after LPS injection, in the left ventricle and the lung at 3 and 6 hours after LPS injection and in the right ventricle at 6 hours after LPS injection. These results suggest that AM synthesis increases in these tissues by LPS at the time intervals specified. In addition, AM contents increased in the lung at 3 and 6 hours after LPS injection. From these data and the mRNA result, it is concluded that there is an increase in AM release in the lung. As the plasma AM levels were elevated at 3 and 6 hours, the results show that the lung may be an important organ for AM secretion in the septic state. However, the AM levels in the mesenteric artery were increased at 1, 3 and 6 hours whereas the preproAM mRNA were only elevated at 1 hour after LPS injection. The results would indicate an increase in AM release only at 1 hour after LPS injection and a decrease in AM release at 3 and 6 hours. The responses of the lung and the mesenteric artery in terms of AM secretion are different.
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