The atrial natriuretic peptide regulates the production of inflammatory mediators in macrophages

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Abstract
The atrial natriuretic peptide (ANP), a member of the natriuretic peptide family, is a cardiovascular hormone which possesses well defined natriuretic, diuretic, and vasodilating properties. Most of the biological effects of ANP are mediated through its guanylyl cyclase coupled A receptor. Because ANP and its receptors have been shown to be expressed and differentially regulated in the immune system, it has been suggested that ANP has an immunomodulatory potency.

Much investigation of the effects of ANP on the activation of macrophages has been carried out. ANP was shown to inhibit the lipopolysaccharide (LPS)-induced expression of inducible nitric oxide synthase (iNOS) in macrophages in an autocrine fashion. ANP in this context was shown to reduce significantly the activation of NF-κB and to destabilise iNOS mRNA. ANP, furthermore, can significantly reduce the LPS-induced secretion of tumour necrosis factor α (TNFα) in macrophages. The relevance of these findings on a regulatory role for ANP on TNFα in humans was shown by the fact that ANP significantly reduces the release of TNFα in whole human blood. It was furthermore shown to attenuate the release of interleukin 1β (IL1β). Interestingly, ANP did not affect the secretion of the anti-inflammatory cytokines IL10 and IL1 receptor antagonist (IL1ra).

In summary, ANP was shown to reduce the secretion of inflammatory mediators in macrophages. Therefore, this cardiovascular hormone may possess anti-inflammatory potential.

ANP and ANP receptors: expression and function
It is now 20 years since de Bold and coworkers discovered that injection of atrial extracts into rats gave rise to a profound diuresis, natriuresis, and hypotension.1 The compound found to be responsible for this effect is a 28 amino acid, disulphide bonded, cyclic peptide named atrial natriuretic peptide (ANP).2 The finding that with ANP a hormone was produced in heart atria meant the first description of the heart as an endocrine organ. In the following years additional peptides related to ANP were discovered. The first one was isolated from porcine brain and was therefore named brain natriuretic peptide (BNP).3 In analogy to ANP and BNP the third natriuretic peptide (NP) was named CNP (C-type natriuretic peptide).4

The natriuretic peptides exert their biological actions through three receptors,5 6 two of which are membrane bound guanylyl cyclases (NPR-A and NPR-B; fig 1). The guanylyl cyclases NPR-A and NPR-B contain an extracellular, ligand binding domain whereby NPR-A binds ANP and BNP, and NPR-B binds CNP. The third receptor serves as a clearance receptor (C-receptor).7 NPR-C has the potency to internalise and clear the natriuretic peptides. Moreover, an increasing number of reports show that several biological effects of ANP are mediated through this “clearance” receptor (NPR-C).8 These effects seem to be related to a G-protein coupled inhibition of adenyl cyclase.9 10

The action of ANP in the cardiovascular system has been well studied and investigations have concentrated mainly on the diuretic, natriuretic, and vasodilating properties of ANP.11–13 However, it is increasingly recognised that the functions of ANP are not restricted to the regulation of volume homoeostasis. NP and their receptors have been shown to be expressed in diverse tissues besides the cardiovascular and renal system.14 Interestingly, ANP has been linked to the immune system, which provided new aspects of the biological profile of NP.15 ANP and its receptors are expressed and differentially regulated in thymus,16 spleen,17 18 lymph nodes, tonsils,19 20 as well as in macrophages.21–24 ANP was also shown to exert various effects in the immune system where it is known that ANP inhibits thymopoiesis and thymocyte proliferation.25 In macrophages ANP was shown to increase phagocytosis and respiratory burst.26

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Figure 1 Natriuretic peptide receptors (NPR). The guanylyl cyclases, NPR-A and NPR-B, contain an extracellular ligand binding domain. NPR-A binds ANP and BNP, whereas NPR-B binds CNP. NPR-C has the potency to internalise and clear the natriuretic peptides and exerts other biological effects by inhibiting the production of cAMP.
ANP as an autocrine regulator of iNOS

Macrophages represent a cell type with a crucial role in inflammatory processes. They were shown to produce ANP, and activated macrophages even express highly increased levels of ANP. Because macrophages were shown to express all three types of NPR, it was suggested that they represent target cells for NPs.

Nitric oxide (NO) is an important regulator of diverse cell functions. In the organism nitric oxide is synthesised by nitric oxide synthases (NOS) from the amino acid L-arginine. Two constitutive nitric oxide synthases, the endothelial NOS (eNOS; NOS I) and the neuronal NOS (nNOS; NOS III), produce NO that mainly serves as vasodilator and neurotransmitter, respectively. NO produced by the inducible isoform of the enzyme (iNOS) is an important mediator of host defence. Induction occurs after exposure of cells to cytokines and bacterial products, such as lipopolysaccharides (LPS). However, NO produced in high amounts by activated macrophages may cause damage in host cells and contribute to the pathogenesis of several inflammatory diseases, such as septic shock or arthritis. Therefore, knowledge about the regulation of iNOS is of the highest importance for an understanding of pathomechanisms of respective immunological diseases. For this reason a regulatory effect of the endogenous ANP on iNOS seemed of special interest.

ANP has been shown to inhibit LPS-induced iNOS in macrophages in concentrations as low as $10^{-9}$ mol/l. The effect was shown to be mediated through the guanylcyclase coupled NPR-A. The ANP analogue, CNP, showed no effect on iNOS. Investigation of the mechanisms underlying this inhibitory action disclosed an involvement of both transcriptional and post-transcriptional processes. The post-transcriptional regulation of iNOS involves a destabilisation of iNOS mRNA. The stability of iNOS mRNA represents an important step in the induction of the enzyme owing to destabilising AU-rich sequences in the 3'-untranslated region of iNOS mRNA. ANP might influence AUUUAA binding proteins or induce RNases, which then specifically interact with respective regions in iNOS mRNA. Because raised intracellular calcium levels reduce iNOS mRNA stability it seems interesting to know that ANP increases intracellular calcium levels in macrophages. Increased calcium levels were shown to contribute significantly to the inhibitory action of ANP on NO production.

Besides this post-transcriptional regulation, ANP was shown to inhibit markedly the activation of the transcription factor NF-$\kappa B$, which is crucial for the induction of iNOS in murine macrophages. Owing to the fact that activated macrophages produce markedly raised levels of ANP, a potential autocrine mechanism was investigated. In fact, when ANP binding to the NPR-A was blocked by the addition of a specific antagonist to activated macrophages, the cells produced significantly raised levels of NO. This knowledge leads to the suggestion that ANP may be an autocrine regulator of iNOS (fig 2).

ANP as a regulator of cytokine production

Knowledge about the influence of ANP on the activation of NF-$\kappa B$ led to the hypothesis that ANP influences tumour necrosis factor $\alpha$ (TNF$\alpha$) as another NF-$\kappa B$ regulated gene. TNF$\alpha$ is a central proinflammatory cytokine and is regulated transcriptionally; the two transcription factors NF-$\kappa B$ and API1 are involved. The inhibitory action of ANP on the interferon $\gamma$ mediated activation of the p38

Figure 2  The inhibitory action of ANP on the induction of iNOS. The LPS induction of iNOS is inhibited by ANP through binding to the NPR-A. The inhibitory action involves the destabilisation of iNOS mRNA and inhibition of the activation of NF-$\kappa B$.

Figure 3  The inhibitory action of ANP on the induction of TNF$\alpha$. The LPS-induced expression of TNF$\alpha$ is inhibited by ANP through binding to the NPR-A. The inhibitory action involves transcriptional processes with a reduced activation of NF-$\kappa B$ and API1.
mitogen activated protein kinase was suggested to be the upstream step responsible for the attenuated activation of NF-κB in macrophages. Besides the reduced activation of NF-κB, 24, 25 ANP markedly inhibited AP1 activity in LPS activated macrophages. 40 The inhibitory action of ANP on the activation of both NF-κB and AP1 led to significantly lower expressed TNFα mRNA and to a significantly reduced release of TNFα 16 (fig 3). Investigations aimed at determining the receptor specificity of the inhibitory action of ANP on TNFα expression showed that the inhibition of TNFα production by ANP was mediated through the NPR-A. 41 A cell permeable cGMP analogue, dibutyryl-cGMP mimicked the ANP effect, and the microbial polyoxaccharide HS-142-1, which selectively blocks NPR-A and cGMP production, 42 reversed the ANP effect.

The relevance of these findings for the human system was investigated by determination of the effects of ANP on cytokine production in whole human blood. Importantly, ANP in this cellular system also exerted an inhibitory action against TNFα and additionally attenuated IL1β secretion. The production of interleukin 10 (IL10) and IL1 receptor antagonist (IL1ra) was not altered by ANP. These interesting findings suggest an anti-inflammatory potential for ANP in humans as well.

Summary and conclusion

In summary, ANP is supposed to be an important endogenous compound regulating the production of inflammatory mediators in macrophages. The modulation of macrophages by ANP may have broad implications in inflammatory states, such as arthritis or sepsis, where increased ANP plasma levels have been reported. 43, 44 The ability of ANP to inhibit the induction of iNOS 24, 36 and TNFα 16, 40 may represent two important aspects supporting an anti-inflammatory action of this cardiovascular hormone.

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