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THE ENDOCRINE HEART. FROM THE BENCH TO THE BEDSIDE AND BACK TO THE BENCH.

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From the bench…

Two polypeptide hormones are produced, stored and secreted in a regulated manner by cardiac muscle cells (cardiocytes) of the atria of the heart of mammals. These are atrial natriuretic factor (ANF)[1-3] and brain natriuretic peptide (BNP).[4] They form part of a family of hormones referred to as natriuretic peptides (NP). A third member: CNP, is not natriuretic and is not produced by cardiocytes.

Both ANF and BNP are co-stored in atrial cardiocytes in storage granules referred to as specific atrial granules **(Fig. 1)**. Some confusion has recently developed in the clinical literature as to the precise cell type where this secretory function resides, as well as to the peptide storage form and the location of the secretory function, which, of late, is often quoted as being the atria for ANF and the ventricular muscle for BNP. By Northern blot analysis, immunocytochemistry, in situ hybridization, radioimmunoassay and oligonucleotide array, ANF and BNP are far more abundant in the atria than the ventricles. In fact, the level of expression of NP in the normal ventricles is negligible compared to that of the atria.[5]

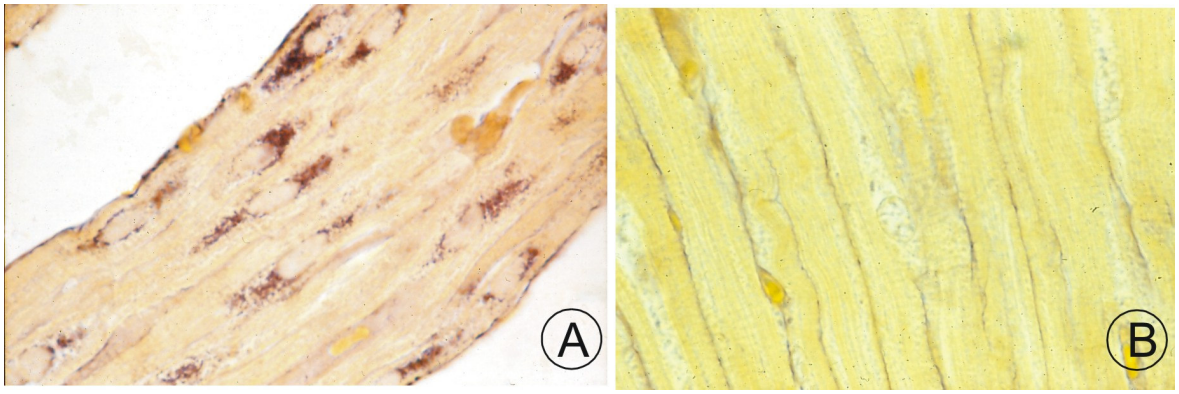

Figure 1. *Sections of atrial (A) and ventricular (B) muscle stained with aldehyde fuchsin after thiosulfation showing in "A" heavy concentration of atrial specific granules (deep red) mainly in paranuclear areas of atrial cardiocytes. These granules co-store ANF and BNP. Not such granules are found in ventricular muscle (B).*

ANF is stored in atrial granules mainly as the prohormone while BNP is stored as the mature hormone. In humans, the main, biologically active forms for these hormones are ANF_{99-126} and BNP_{77-108}. The numbering refers to the C-terminal portion of their respective prohormones **(Fig. 2)**.

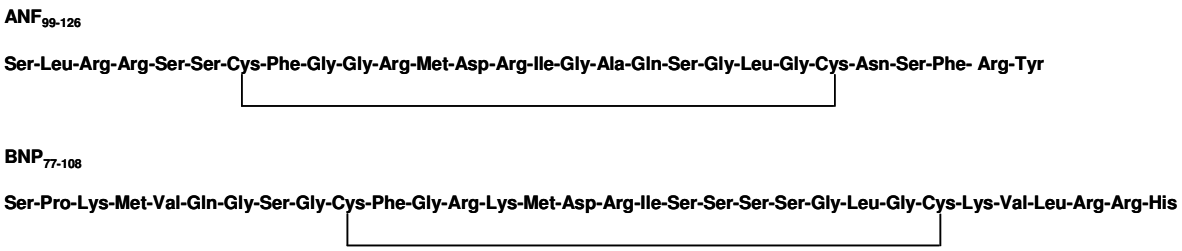

Figure 2. *Primary structure of the main circulating forms of human ANF and BNP numbered with the residue numbering derived from their respective prohormones. Both peptides posses a 17-amino acid loop that is essential for biological activity.*

ANF and BNP modulate intrinsic renal mechanisms, the sympathetic nervous system, the renin-angiotensin-aldosterone system (RAAS) and other vascular and renal parameters leading to the modulation of extracellular fluid volume and blood pressure. In addition, these hormones have potent growth-regulating properties hence making them of great interest in the study of the regulation of cardiovascular growth, including in cardiac remodeling following an acute myocardial infarction and in congestive heart failure.[2]

The properties of ANF and BNP are predominantly mediated through increases in cGMP in target cells through the activation of guanylyl cyclase-coupled receptors. Both ANF and BNP are agonists of the type A NP (NPRA) receptor. Intracellular cGMP receptors include cGMP-dependant protein kinases, cGMP-gated ion channels and cGMP-regulated cyclic nucleotide phosphodiesterases.[6]

Blockade or genetic disruption of NP genes or their receptors results in impairment of cardiorenal homeostasis.[7-9] ANF or NPRA gene disruption leads to salt-sensitive or salt-insensitive hypertension respectively, in homozygous null mice[10,11] and lack of a natriuretic response to volume expansion.[11] Absence of NPRA leads to cardiac hypertrophy with extensive interstitial fibrosis and, particularly in males, to sudden death.[12] BNP knockouts do not develop hypertension or cardiac hypertrophy but show cardiac fibrosis.[13]

Our understanding of the modulation of ANF and BNP secretion from the heart is based on the fact that an increase in blood volume in the low-pressure side of the circulation and the ensuing atrial muscle stretch triggers an increase in the rate of release of both ANF and BNP (stretch-secretion coupling)(reviewed in[14]). This is a very rapid secretory response that occurs independently of hormone neosynthesis.[15] We have recently reported on the molecular signaling mechanisms underlying this form of secretion and have implicated a pertussis toxin-sensitive mechanism that is further investigated at present.[16] In addition to mechanically-induced NP secretion, the rate of secretion and gene expression of these hormones can be modulated by neurohumoral stimuli.[17-20]

It is important to point out that both mechanical and neurohumoral stimuli referred to above cause an increase in gene expression and secretion of both ANF as well as BNP. This is thus referred to here as **coordinated** gene expression.

…to the bedside…

In pathophysiological situations that are associated with chronic hemodynamic overload on the heart, the expression of both ANF and BNP increases in the atria and in the ventricles. The cardiac upregulation of both ANF and BNP results in increased circulating levels of both hormones. This is seen as a compensatory mechanism to decrease hemodynamic load and to modify tissue remodelling.[21-24]

The increased circulating levels of ANF and BNP in hypertrophy and failure as well as the biological properties of ANF and BNP have led to new and exciting approaches in diagnosis and treatment in cardiology. Although too numerous to describe in detail here, the main clinical laboratory applications can be summarized as follows:

- Diagnostic aid to identify left ventricular systolic and diastolic dysfunction
- Prognosis or risk stratification of congestive heart failure and acute coronary syndrome patients
- Monitoring and guiding heart failure therapy
- Determining the effect of interventions

Therapeutically, both ANF (Carperitide®) and BNP (Nesiritide®) have found clinical indications for the treatment of acutely decompensated chronic congestive heart failure given their properties of unloading the heart and preventing rebound phenomena by the renin-angiotensin-aldosterone and sympathetic systems. Experimental studies in humans indicate that these compounds can also beneficially influence cardiac remodelling post myocardial infarction. Alternative routes of administration and longer acting analogues of these hormones are being developed for the treatment of chronic congestive heart failure and essential hypertension.

…and back to the bench.

The coordinated expression of ANF and BNP referred to above following physiological or pathological load on the heart is in sharp contrast to the unique specific upregulation of BNP observed clinically during cardiac allograft rejection first described by us.[25]

During studies concerning elevated NP plasma levels observed in heart transplant recipients, we identified that ANF did not normalize after transplantation from the elevated levels found in advanced heart failure prior to transplantation. The elevated circulating levels of ANF persisted even after intracardiac pressures and the renin-angiotensin-aldosterone system normalized. Further, we did not find any correlation between ANF levels and circulating epinephrine or norepinephrine levels. Altogether, these findings suggested that factors other than hemodynamic ones or activation of the renin-angiotensin-aldosterone system or sympathetic nerve activity were at play in the observed elevation of ANF circulating levels in transplant recipients.[26]

More recently, we found that circulating levels of BNP, but not ANF, increased in most patients that were about to or were suffering from an acute rejection episode.[25,27] Moreover, successful treatment of the rejection episodes with various immunosuppressive agents including OKT-3, a monoclonal antibody that inhibits T-lymphocyte activation,

results in a decrease of BNP plasma levels together with an improvement of the histopathological grade of endomyocardial biopsies suggesting that activated T-lymphocyte secretion products during a rejection episode modulate cardiocyte BNP secretion.[25] This effect was not merely due to an improvement in hemodynamic parameters because ANF plasma levels did not show the same changes.

We tested whether pro-inflammatory and immunoregulatory cytokines were capable of selectively upregulating BNP gene expression and secretion in neonatal cardiocyte cultures. We found that specific cytokines (TNFα and IL-1β) upregulate BNP at the transcriptional and translational levels through a specific (p38) signaling pathway.[28]

Even though we were able to reproduce the specific upregulation of BNP gene expression during acute cardiac rejection by exposing cardiocyte cultures to TNFα and IL-1β,[28] and elucidated the signalling mechanistic of this phenomenon, our clinical studies[27] do not show an increase in plasma in these cytokines during periods of acute (ISHLT grade 3) rejection. However, by RT-PCR as well as by immunocytochemistry, several observations indicate that there is an enhanced gene expression of both, the pro-inflammatory cytokines IL-1β, IL-6, TNF-β and the immunoregulatory cytokines IL-2, IL-4 and IFN-γ during the acute phase of cardiac allograft rejection.[29] Hence, there are two possibilities that may help explain our findings. *Firstly*, cytokines are local mediators and plasma levels do not accurately reflect *in situ* levels at the graft (i.e. lack of sufficient cardiac spill over to be reflected peripherally).[30,31] *Secondly*, cytokines other than or in addition to TNFα and IL-1β participate in the observed upregulation of BNP during allograft rejection. To our knowledge, these possibilities have never been tested and are the subject of current research in our laboratory. In all, these latest developments point to an exciting new area of inquiry that will provide insights into the relationship between the natriuretic peptide system and the immunological system.

In summary, the study of the natriuretic peptide system has provided much new knowledge that may be group as follows:

1. Discovery of a regulatory mechanism of the systems that increase blood pressure and volume such as the RAAS.

2. Discovery of a new class of cellular receptors.

3. Development of a new class of therapeutic agents: vasopeptidase inhibitors and others under development.

4. Development of an objective, simple and inexpensive test for the diagnosis of heart failure.

5. Development of criteria to guide heart failure treatment.

6. Diagnosis and prognosis of cardiac allograft rejection.

7. Numerous new avenues of research into the physiology, pathophysiology, biochemistry and molecular biology of the cardiovascular and other systems (>15,000 publications since 1981)

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