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EDITORIAL

Natriuretic peptide receptors and heart failure: to B or not to B blocked?

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The role of natriuretic peptides in the dynamic regulation of blood volume and pressure is now well established and comprises the complementary endocrine actions of atrial (ANP) and brain (BNP) natriuretic peptides, with the more local, paracrine bioactivity of C-type natriuretic peptide (CNP). This family of mediators also coordinates to maintain cardiac function and integrity (Potter et al. 2009). However, much of the information pertaining to the physiological functions of these peptides has not been garnered via the use of selective pharmacological tools. Indeed, there are a paucity of such reagents and this has been the bane of researchers in the field for their inception with the identification of ANP in 1981(de Bold et al. 1981). Several selective agonists and antagonists at natriuretic peptide receptors (NPRs), the membrane spanning proteins that transduce signals conveyed by natriuretic peptides, have been described, but the vast majority are high molecular weight, often peptide-based, molecules with suboptimal pharmacodynamic and pharmacokinetic properties.

Perhaps the most useful tool to date has been HS-142-1, a polysaccharide extracted from *Aureobasidium* sp. (Morishita et al. 1991). This reagent inhibits activation of both guanylate cyclase-coupled NPRs (i.e. NPR-A and NPR-B) through an allosteric interaction, but does not bind NPR-C (Poirier et al. 2002). It has therefore been an ideal intervention to define mechanisms reliant on the activation of particulate guanylate cyclases (and to differentiate between these membrane-bound isoforms and the NO-sensitive soluble guanylate cyclase). Regrettably, the production of HS-142-1 has now been halted and is no longer available. Several additional molecules have been reported to selectively inhibit NPR-A (the cognate receptor for ANP and BNP, e.g. A71915 (Delporte et al. 1992), S-28-Y (Minamitake et al. 1990), anantin (Weber et al. 1991; Wyss et al. 1991) and PL-3994 (Edelson et al. 2013)) or NPR-B (the cognate receptor for CNP, e.g. a monoclonal antibody 3G12 (Drewett et al. 1995) and P19 (Deschenes et al. 2005)). A truncated, modified natriuretic peptide, $cANF^{4-23}$, has been established as a useful pharmacological tool for dissecting the biology of NPR-C. Originally, it was used to identify NPR-C binding sites in vivo and subsequently shown to block the clearance of natriuretic peptides from the circulation (Maack et al. 1987). However, this peptide also has positive signalling roles, courtesy of the Gi coupling of NPR-C (Anand-Srivastava et al. 1996); thus, $cANF^{4-23}$ may well represent a partial agonist at this receptor. Additional molecules described as selective NPR-C antagonists have been developed more recently (e.g. AP-811, M372049 (Veale et al. 2000)) and used to establish the importance of endothelium-derived CNP in regulating vascular reactivity in vitro (Chauhan et al. 2003).

As a consequence of the various shortcomings of these molecules (e.g. source, cost, solubility, selectivity and route of administration), they have either been unavailable or not readily amenable for use in vivo, and many of the physiological roles of natriuretic peptides have only been established unequivocally with the recent development of global and tissue-specific transgenic animals. Good examples of this are the cardiac hypertrophy and fibrosis in ANP- and BNP-deficient mice (Kuhn 2009) and the perturbed bone homeostasis and dwarfism in mice lacking the CNP (*Nppc*) gene (Chusho et al. 2001). However, whilst pharmacological tools have not been used avidly to dissect the physiological and pathological roles of natriuretic peptides, the administration of native or synthetic natriuretic peptides has been far more successful in terms of therapeutic intervention. ANP (carperitide) and BNP (nesiritide) are used in the treatment of heart failure, but with limited efficacy,

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predominantly due to reductions in systemic blood pressure and renal perfusion. Novel chimeric peptides, which harness several favourable actions by combining portions from two or more native natriuretic peptides, perhaps hold greater promise (Lisy et al. 2008; Dickey et al. 2008). For example, CD-NP (cenderitide) consists of CNP with an additional 15aa corresponding to the C-terminal tail of DNP. This peptide activates both NPR-A and NPR-B and promotes the venodilatory, antifibrotic properties of CNP in tandem with the natriuretic properties of DNP (the peptide is also more resistant to enzymatic degradation) without the therapeutically limiting hypotensive response. CD-NP had a favourable pharmacodynamic profile in phase I (Lee et al. 2009), and in a small phase IIa safety and tolerability study (Lieu et al. 2011), and is currently under phase IIb evaluation. A similar favourable pharmacodynamic profile has also been established for a mutant ANP consisting of the native peptide with a C-terminal 12aa extension (McKie et al. 2009)

However, the dearth of small molecule, receptor-selective NPR tools (and ultimately therapeutics) may have come to an end with the report by Bach et al. in this issue. This work describes the development of a high-throughput screen to identify non-peptidic, small-molecule antagonists at NPR-B. Using HEK293 cells transfected with human NPR-A or NPR-B cDNA, and cGMP production as functional read-out, the authors have identified a series of compounds that block NPR-B and exhibit discernible selectivity over NPR-A. The ex vivo pharmacology of the novel small molecules was confirmed using rat myocardial muscle strips in which C10, one of the compounds with greatest antagonist potency at NPR-B, was shown to inhibit the CNP-driven potentiation of inotropic responses to β1-adrenoceptor activation. The novel NPR-B antagonists exert a non-competitive, reversible antagonism (little or no change in the EC_{50} to CNP but a signification reduction in E_{max}), suggesting allosteric modulation of the receptor, conceivably akin to that produced by HS-142-1. Further work is now needed to define this binding site. A crystal structure for NPR-B has not been reported, but co-crystals with these new molecules, in tandem with molecular data, might reveal a new allosteric site on NPR-B (and possibly mirrored by other NPRs) that is tractable for therapeutic benefit. One important unknown for the utility of these molecules as tools and therapeutics is binding and activity at NPR-C. Whether they can act as NPR-C agonists and mimic some of the beneficial effects of CNP in the cardiovascular system we have reported (Chauhan et al. 2003) remains to be determined. Nonetheless, even if these novel compounds bound tightly to NPR-C and prevent clearance of endogenous natriuretic peptides, this might also be construed as a beneficial activity, certainly in disorders such as pulmonary hypertension in which augmenting NP bioactivity has been proven to be beneficial in experimental models and humans (Baliga et al. 2008; Klinger et al. 2006).

In addition, this work has identified molecules that exhibit antagonist activity at NPR-B, but potentiate BNP-triggered activation of NPR-A (although the molecules do not appear to be NPR-A agonists per se). This is an intriguing profile of activity since activation of NPR-A is established to result in a positive effect in preclinical models and patients with different cardiovascular diseases, including heart failure, renal I/R injury and pulmonary hypertension, and may therefore offer an even greater therapeutic effect. Moreover, the structure–activity relationship (SAR) data from this study intimates that relatively minor and readily achievable modifications to this series of molecules can generate compounds with very different activity and selectivity at NPRs. Thus, this work should be an excellent starting point for the development of small molecules that can target specific NPRs that might be useful in a plethora of cardiovascular disorders. Interestingly, one of the molecules shown to modulate NPR-B activity, C37, was subsequently identified as loperamide (an opioid receptor agonist with antidiarrhoeal activity). Since this compound was shown to block NPR-B activation by CNP, it may well interact with the intestinal epithelium guanylate cyclase C (GG-C), which regulates salt and water excretion. This raises the fascinating possibility that part of the therapeutic effect of loperamide might be mediated via GC-C and that this work might stimulate the development of small molecules with therapeutic potential outside the cardiovascular system.

The rationale for the development of NPR-B antagonists is derived from previous work by the authors exploring the cardiac contractility effects of CNP in normal versus failing hearts. Using isolated myocardial strips, these authors have shown that CNP causes an increase in cGMP, via NPR-B, that leads to inhibition of PDE3 thereby promoting the actions of catecholamine-driven cAMP signalling, which exerts positive inotropic and chronotropic activities (Qvigstad et al. 2010). This is considered detrimental to the failing heart, with pharmacological inhibition of PDE3 associated with increased mortality (Packer et al. 1991; Amsallem et al. 2005). This fits with other reports in ex vivo systems showing that CNP can exert a positive inotropic activity (Pierkes et al. 2002). Thus, blockade of NPR-B may be a favourable approach in heart failure. However, the net effect of NPR-B antagonism in vivo may not be so clear cut. In rats over-expressing a dominant negative form of NPR-B in cardiomyocytes, development of cardiac hypertrophy, fibrosis and contractile dysfunction is accelerated compared to WT animals (Langenickel et al. 2006). Moreover, there appears to be a shift from NPR-A to NPR-B signalling in pressure-overload-induced cardiac hypertrophy, suggesting that CNP takes on the principal role as the natriuretic peptide guardian of cardiac integrity (Dickey et al. 2007). This concept is reinforced by the observations that cardiomyocyte-specific over-expression of CNP protects against MI-induced hypertrophy (Wang et al. 2007) and that CD-NP exerts a potent salutary effect in a preclinical

model of cardiac fibrosis (Martin et al. 2012). Finally, systemic administration of a NPR-B targeted drug would have to be evaluated carefully for effects on bone morphology (Chusho et al. 2001).

Regardless, the work reported by Bach et al. is an exciting and welcome addition to the pharmacology of natriuretic peptides and their receptors and should pave the wave for the design and development of more selective molecules that have new, unique activities across the NPR spectrum and should prove vital in advancing knowledge of the role of these receptors in mediating the biological activity of natriuretic peptides and in the pursuit of better therapeutics for cardiovascular disease.

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