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COMMENTARY

Divergence of hypertrophic growth and fetal gene profile: the influence of β-blockers

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While the expression patterns of cardiac hypertrophy-related genes have been well documented and widely used as markers for hypertrophy, recent research has revealed uncoupling of hypertrophy-related gene profiles and hypertrophic growth. The role of β-adrenergic signalling in the development of hypertrophy is incompletely understood. The finding of an upregulated expression of hypertrophy-related genes but a suppressed hypertrophy following β-blockade reveals previously unrecognized sympatho-adrenergic mechanisms of hypertrophic growth.
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Abbreviations: Akt, protein kinase B; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; GC, guanylate cyclase; GSK3β, glycogen synthase kinase-3β; MHC, myosin heavy chain; NFAT, nuclear factor of activated T cell; NPR, natriuretic peptide receptor; PI3K, phosphoinositide 3-kinase; PKG, cGMP-dependent protein kinase; SERCA, sarcoendoplasmic reticulum Ca^{2+} ATPase; α-SKA, α-skeletal actin; TAC, transverse aorta constriction

Activation of the sympathetic nervous system and myocardial hypertrophy occur in the setting of cardiovascular disease and precipitate progression of cardiac remodelling, dysfunction and heart failure. Although there has been no convincing evidence for a direct antihypertrophic effect of β-adrenoceptor antagonists (β-blockers), a prohypertrophic action of β-adrenergic signalling has been shown by experimental and clinical studies (Zahabi *et al*., 2003; Burns *et al*., 2007).

Pathological hypertrophy is associated with a well-documented pattern of gene expression, including reactivation of a set of fetal genes like atrial or B-type natriuretic peptides (ANP, BNP), β-myosin heavy chain (β-MHC) and α-skeletal actin (α-SKA), and downregulation of adult cardiac genes, most notably sarcoendoplastic reticulum Ca^{2+} ATPase (SERCA) and α-MHC. Such a transcriptional profile, particularly ANP upregulation, has been used as measure of hypertrophy *in vivo* and *in vitro*. Although poorly defined, there also exist intrinsic signal networks that counter-regulate hypertrophic growth.

In the current issue of the *BJP*, Patrizio *et al*. (2007) report an interesting finding; treatment with β-blockers in models of cardiac hypertrophy *in vivo* (transverse aortic constriction (TAC)) and *in vitro* (cardiomyocytes treated with phenylephrine or noradrenaline) suppressed hypertrophic growth even though expression of fetal genes was further upregulated. In the TAC model, sympatho-adrenergic signalling contributes to hypertrophic growth, as shown by a suppressed left ventricle hypertrophy in dopamine-β-hydroxylase-null mice, depleted of catecholamines (Esposito *et al*., 2002). Patrizio *et al*. (2007) took a good approach by investigating the effect of β-blockers both *in vivo* and *in vitro*. They tested propranolol, metoprolol (β_1-selective) and ICI-118551 (β_2-selective) with findings showing a class effect mediated by β_1-adrenoceptors.

This study (Patrizio *et al*., 2007) is the first to show such paradoxical combinations using β-blockers commonly prescribed to patients with heart disease. Actually, uncoupling of hypertrophy-related gene profile and hypertrophic growth has been noticed in recent years by studies using genetically engineered models or gene targeting. For instance, lack of fetal gene expression was reported in α_{1A}- and α_{1B}-adrenoceptor dual-knockout mice with severe pressure-overload hypertrophy (O'Connell *et al*., 2006). Conversely, α_{1A}-adrenoceptor transgenic mice had increased expression of ANP but did not develop hypertrophy nor exacerbated pathological hypertrophy (Lin *et al*., 2001; Du *et al*., 2006a). In cultured cardiomyocytes, inactivation of activating protein 1 function reversed hypertrophy-related gene profile evoked by phenylephrine, but hypertrophy remained unaltered (Jeong *et al*., 2005). Uncoupling of expression of individual fetal genes has also been reported. Cardiac overexpression of glycogen synthase kinase-3β

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(GSK3β) inhibited hypertrophy due to either calcineurin overexpression, isoproterenol administration or TAC, phenotypes associated with further elevation of ANP expression but downregulation of both BNP and β-MHC (Antos *et al*., 2002). Similarly, concomitant expression of modulatory calcineurin-interacting protein 1 markedly inhibited calcineurin-mediated hypertrophy, but expression of ANP was further activated and that of α-SKA inhibited (Hill *et al*., 2002). All these findings suggest that expression of individual fetal and adult genes in the hypertrophic myocardium is regulated by distinct signal mechanisms.

Signalling mechanisms responsible for the findings by Patrizio *et al*. (2007) remain unexplored. Studies using genetically engineered models targeting ANP or the natriuretic peptide receptor-A (NPR-A) have provided strong evidence for an antihypertrophic property of the ANP/NPR-A/PKG signalling pathway under basal or pathological conditions, as summarized in Table 1. This signal pathway counteracts multiple hypertrophic signal pathways including those involving nuclear factor-κB (NF-κB), p-38-mitogen-activated protein kinase (p38-MAPK), calcineurin/nuclear factor of activated T cell (NFAT) and protein kinase C (Figure 1). Inhibition of TAC-hypertrophy with a further elevation of ANP expression was observed in mice treated with 17β-estradiol (van Eickels *et al*., 2001), the effect mediated through the NPR-A/cGMP-dependent protein kinase (PKG) pathway (van Eickels *et al*., 2001; Du *et al*., 2006b).

How does β-blockade upregulate ANP expression in hearts of sham-operated and TAC animals? Recent studies have shown that ANP expression is controlled by signal pathways involving calcineurin, phosphoinositide 3-kinase (PI3Kγ) and protein kinase B (Akt)/GSK3β. Activation of nuclear Akt by viral or transgenic means, selectively increased ANP expression (Tsujita *et al*., 2006). Upon β-adrenoceptor activation, ANP expression is promoted via Ca^{2+}/calcineurin signalling but suppressed by inactivation of GSK3β following its phosphorylation by Akt or cAMP-dependent protein kinase (Figure 1) (Morisco *et al*., 2000). Thus, GSK3β suppresses hypertrophy while it activates ANP expression (Antos *et al*., 2002) (Figure 1). In addition, following β-adrenoceptor activation, PI3Kγ and β-adrenoceptor kinase-1 are recruited by β-arrestins to the ligand-activated β-adrenoceptors, a process necessary to free G$\beta\gamma$ and to induce β-adrenoceptor desensitization (Esposito *et al*., 2002; Nienaber *et al*., 2003). If this β-adrenoceptor/PI3Kγ colocalization is associated with a reduced nuclear PI3Kγ/Akt activity, one would expect a disinhibition of GSK3β by β-adrenoceptor blockade, as tested by Patrizio *et al*. (2007), thereby promoting ANP expression via calcineurin/NFAT signalling (Figure 1). This and other possibilities remain to be tested.

The 'contradictory' findings by Patrizio *et al*. (2007) reveal our incomplete understanding on the role of β-adrenoceptor in hypertrophic development and hence the effect of β-blockers. If β-blockade increases ANP expression, one would expect a suppressed expression of at least some hypertrophy-related genes by β-adrenoceptor activation. Clinical studies on patients with dilated cardiomyopathy showed that treatment with β-blockers inhibited the expression of ANP and β-MHC and restored that of α-MHC and SERCA (Lowes *et al*., 2002). Thus, caution is required when extrapolating the findings from the mouse TAC model to clinical situations.

The findings by Patrizio *et al*. (2007) would have been strengthened by providing measures of cardiomyocyte hypertrophy (such as cell size, protein synthesis), exploring potential signalling mechanisms and validating the results from pharmacological approaches by using genetically engineered models, such as β-adrenoceptor knockout mice. Actually, a recent paper from the same group found no difference between the β_1- and β_2-adrenoceptor dual-knockout and wild-type mice in the extent of TAC-induced hypertrophy, fetal gene expression and fibrosis (Palazzesi *et al*., 2006), findings contradictory to the current report (Patrizio *et al*., 2007). Furthermore, although hypertrophy was inhibited, β-blockade had no effect on the suppressed SERCA expression (Patrizio *et al*., 2007). It would be interesting to know the chronic impact of this phenomenon. Thus, further research with extended study periods or using different heart disease models would be worthwhile.

Table 1 Summary of findings from genetically engineered mice indicating antihypertrophic action of natriuretic peptide/GC signal pathway

Model	*Cardiac phenotypes*
ANP KO (Wang *et al*., 2003)	Hypertrophy at baseline and exacerbated hypertrophy and fibrosis under pressure-overload
Corin KO (Chan *et al*., 2005)	Hypertension and cardiac hypertrophy
NPR-A KO (Oliver *et al*., 1997; Knowles *et al*., 2001; Franco *et al*., 2004; Tokudome *et al*., 2005)	Cardiac hypertrophy and sudden death at baseline; exacerbated hypertrophy by calcineurin activation or by pressure-overload
Cardiac NPR-A KO (Holtwick *et al*., 2003)	Mild hypertrophy, hypotension at baseline; exaggerated pressure-overload hypertrophy
TG-DN-NPR-A (Patel *et al*., 2005)	Increased severity of pressure-overload hypertrophy and fibrosis
NPR-A TG (Kishimoto *et al*., 2001)	Reduced heart size
TG-CA-GC (Zahabi *et al*., 2003)	Inhibited hypertrophy by isoproterenol or pressure-overload

Abbreviations: ANP, atrial natriuretic peptide; CA, constitutively active; DN, dominant negative; KO, knockout; NPR-A, natriuretic peptide receptor-A; TG, transgenic.

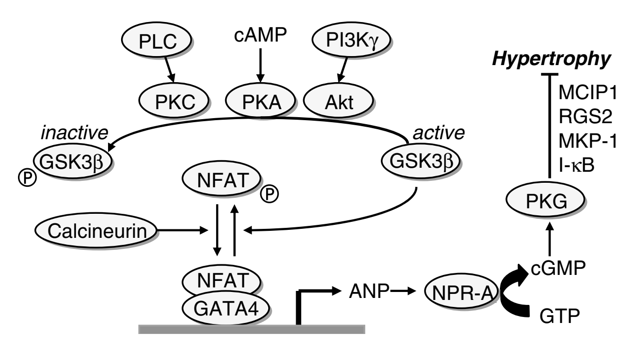

Figure 1 Signal pathways that promote ANP expression while inhibiting myocardial hypertrophy. ANP, atrial natriuretic peptide; I-κB, NF-κB inhibitor; MKP-1, MAPK phosphatase-1; RGS2, regulator of G-protein signalling 2.

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