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COMMENTARY

Stimulation of cardiac β-adrenoceptors targets connexin 43

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Connexin 43 (Cx43) is the major protein of cardiac ventricular gap junctions and is crucial to cell–cell communication and cardiac function. The protein level of Cx43 is reduced in patients with heart failure or dilated cardiomyopathy (DCM), pathophysiological conditions often associated with arrhythmias. As catecholamines are often increased in cardiac diseases, Salameh *et al.*, in this issue of the *BJP*, investigated the effect of β-adrenoceptor stimulation of neonatal cardiomyocytes on Cx43 expression and found increased Cx43 mRNA and protein levels following 24 h stimulation. Up-regulation of Cx43 was associated with phosphorylation of mitogen-activated protein kinases and translocation of transcription factors into the nucleus. In patients with DCM, a situation often associated with desensitization of the β-adrenoceptor system, Cx43 expression was reduced. The characterization of the signal transduction pathways involved in Cx43 expression and intracellular localization in human myocardium *in vivo* is a promising target for the development of new anti-arrhythmic strategies.
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Abbreviations: Cx 43, connexin 43

Commentary

In an adult myocardium, connexin 43 (Cx43) is expressed in the ventricles and is predominantly localized at the sarcolemma (see van Veen *et al.*, 2001). Six connexins assemble into a so-called connexon or hemichannel, and two opposing connexons form a pore, which is central to electrical cell–cell coupling. Clusters of these pores assemble into gap junctions. Although most of Cx43-formed connexons are located at the terminal intercalated discs, some are also present at the lateral sides of cardiomyocytes. Within cardiomyocytes, Cx43 is also present in the nucleus (Dang *et al.*, 2003) and in mitochondria (Boengler *et al.*, 2005). Synthesis, transport, half-life and degradation of Cx43 determine the number (Saez *et al.*, 2003), whereas intracellular pH, calcium and/or adenosine triphosphate concentrations regulate the conductance and permeability of Cx43-formed channels (Delmar *et al.*, 2004). Cx43 phosphorylation/dephosphorylation further contributes to the regulation of gap junction conductance and permeability. Cx43 is phosphorylated by several protein kinases such as the mitogen-activated protein (MAP) kinases p38, extracellular signal regulated kinase (ERK) 1/2 (also termed 'p42/44') or c-Jun N-terminal kinase (JNK). Dephosphorylation of Cx43 is observed with ischaemia in pig myocardium (Schulz *et al.*, 2003), in a rabbit model of heart failure and in patients with non-ischaemic heart failure (Ai and Pogwizd, 2005). Apart from dephosphorylation, reduced expression and enhanced lateralization of Cx43 have also been reported in patients with heart failure or dilated cardiomyopathy (DCM), and such disturbances may contribute to enhanced arrhythmogenicity (Severs *et al.*, 2008). Indeed, most patients in heart failure New York Heart Association (NYHA) class II and III die due to ventricular arrhythmias. The anti-arrhythmic effect of the peptide rotigaptide was attributed to Cx43-expressing cells (Clarke *et al.*, 2006). In heart failure, noradrenaline concentration is increased, initially leading to stimulation of β-adrenoceptors. However, with sustained increases in noradrenaline concentration, β-adrenoceptor density becomes down-regulated, and functional uncoupling of remaining receptors occurs.

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In this issue of the *BJP*, Salameh *et al.* (2009) report their studies of Cx43 expression during β-adrenoceptor stimulation in neonatal rat cardiomyocytes. In their carefully performed study, isoprenaline stimulated Cx43 mRNA and protein levels as well as phosphorylation of the MAP kinases p38, ERK1/2, JNK and of their target Cx43. Furthermore, isoprenaline induced translocation of the transcription factors activator protein 1, cAMP response element binding protein and nuclear factor of activated T-cells into the nucleus, and this translocation was abolished by MAP kinase inhibition. In a second part of the study, these authors found decreased Cx43 levels in patients with DCM and increased Cx43 levels in patients with hypertrophic cardiomyopathy (HCM), although the lateralization of Cx43 was enhanced in both groups.

This article provides interesting data on the signalling cascades regulating Cx43 expression in a model of prolonged β-adrenoceptor stimulation and thereby extends our knowledge of the regulation of Cx43 content. The understanding of the relationship between β-adrenoceptor stimulation and Cx43 expression and localization in human myocardium under different pathophysiological conditions is of great importance for new treatment modalities related to cell–cell coupling such as new treatments for ventricular arrythmias.

The authors provide convincing evidence for the dose-dependent effect of isoprenaline on the Cx43 expression. Augmentation of *de novo* Cx43 synthesis requires the assembly of regulatory proteins with their respective promoter region. Indeed, Salameh *et al.* showed that isoprenaline did enhance nuclear translocation of transcription factors with DNA-binding consensus sequences in the Cx43 promoter region. Demonstration of an enhanced transcription factor binding to the Cx43 promoter in response to isoprenaline would strengthen the conclusion that Cx43 transcription was directly mediated by the proteins concerned.

In addition to transcription and translation, post-translational modifications such as phosphorylation are important for Cx43 trafficking and content at gap junctions (Beardslee *et al.*, 2000; Schulz *et al.*, 2003) and may affect the Cx43 level in a transcription-independent way. Stimulation with isoprenaline in the presence of an inhibitor of transcription would allow us to discriminate more clearly between the contribution of *de novo* synthesized and post-translationally modified Cx43 to the overall enhanced protein content.

It would be interesting to study whether or not isoprenaline not only increases the Cx43 content but also alters the distribution of Cx43 at the sarcolemma, especially as enhanced lateralization of Cx43 contributes to slowing of the longitudinal conduction velocity (Severs *et al.*, 2008). The finding that the localization of Cx43 is not restricted to the sarcolemma makes it more likely that β-adrenoceptor stimulation affects Cx43 at intracellular sites other than gap junctions, for instance, within nuclei or within the mitochondria. The mitochondrial Cx43 content is particularly important for cardiac function, as a decrease of mitochondrial Cx43 reduces oxygen consumption (Boengler *et al.*, 2006).

Salameh *et al.* (2009) investigated Cx43 expression and lateralization in patients with DCM and confirmed the previously described reduced expression and enhanced lateralization of Cx43 (DuPont *et al.*, 2001). In contrast, in patients with HCM, both Cx43 content and lateralization were increased. As Salameh *et al.* demonstrated a stimulation of Cx43 by isoprenaline *in vitro*, it would be interesting to see whether or not alterations in Cx43 level and lateralization correlate with β-adrenoceptor stimulation *in vivo*, using noradrenaline content and treatment with β-blockers. As the present study nicely demonstrates that Cx43 expression is enhanced via MAP kinase activation, it would also be helpful to address the question of whether or not MAP kinases were differentially activated in patients with DCM or HCM. The finding that activation of JNK in isolated cardiomyocytes reduced Cx43 expression (Petrich *et al.*, 2002), suggests that JNK activation may result in a decreased, rather than increased, Cx43 expression. Furthermore, in patients with DCM and HCM, Cx43 lateralization was increased, independent of Cx43 expression. Possibly, the protein–protein interaction between Cx43 and zonula occludens 1, which is important for the regulation of gap junction size, is affected in patients with DCM or HCM (Bruce *et al.*, 2008).

Thus, the article by Salameh *et al.* (2009) provides data on the mechanism by which β-adrenoceptor stimulation regulates Cx43 expression *in vitro* and opens up a field of interesting research. The characterization of the signal transduction pathways involved in Cx43 expression and lateralization in human myocardium *in vivo* are highly relevant for the development of new anti-arrhythmic strategies.

References

Ai X, Pogwizd SM (2005). Connexin 43 downregulation and dephosphorylation in nonischemic heart failure is associated with enhanced colocalized protein phosphatase type 2A. *Circ Res* **96**: 54–63.

Beardslee MA, Lerner DL, Tadros PN, Laing JG, Beyer EC, Yamada KA *et al.* (2000). Dephosphorylation and intracellular redistribution of ventricular connexin 43 during electrical uncoupling induced by ischemia. *Circ Res* **87**: 656–662.

Boengler K, Dodoni G, Rodriguez-Sinovas A, Cabestrero A, Ruiz-Meana M, Gres P *et al.* (2005). Connexin 43 in cardiomyocyte mitochondria and its increase by ischemic preconditioning. *Cardiovasc Res* **67**: 234–244.

Boengler K, Ruiz-Meana M, Miro E, Cabestrero A, Rodriguez-Sinovas A, Dodoni G *et al.* (2006). Connexin 43 controls mitochondrial respiration. *Circulation* **114**: II_243 (abstract).

Bruce AF, Rothery S, Dupont E, Severs NJ (2008). Gap junction remodelling in human heart failure is associated with increased interaction of connexin43 with ZO-1. *Cardiovasc Res* **77**: 757–765.

Clarke TC, Thomas D, Petersen JS, Evans WH, Martin EM (2006). The antiarrhythmic peptide rotigaptide (ZP123) increases gap junction intercellular communication in cardiac myocytes and HeLa cells expressing connexin 43. *Br J Pharmacol* **147**: 486–495.

Dang X, Doble BW, Kardami E (2003). The carboxy-tail of connexin-43 localizes to the nucleus and inhibits cell growth. *Mol Cell Biochem* **242**: 35–38.

Delmar M, Coombs W, Sorgen P, Duffy H, Taffet SM (2004). Structural bases for the chemical regulation of connexin43 channels. *Cardiovasc Res* **62**: 268–275.

Dupont E, Matsushita T, Kaba RA, Vozzi C, Coppen SR, Khan N *et al.* (2001). Altered connexin expression in human congestive heart failure. *J Mol Cell Cardiol* **33**: 359–371.

Petrich BG, Gong X, Lerner DL, Wang X, Brown JH, Saffitz JE *et al.* (2002). c-Jun N-terminal kinase activation mediates downregulation of connexin43 in cardiomyocytes. *Circ Res* **91**: 640–647.

Saez JC, Berthoud VM, Branes MC, Martinez AD, Beyer EC (2003).

Plasma membrane channels formed by connexins: their regulation and functions. *Physiol Rev* **83**: 1359–1400.

Salameh A, Krautblatter S, Karl S, Blanke K, Rojas Gomez D, Dhein S *et al*. (2009). The signal transduction cascade regulating the expression of the gap junction protein connexin43 by ß-adrenoceptors. *Br J Pharmacol* **158**: 198–208.

Schulz R, Gres P, Skyschally A, Duschin A, Belosjorow S, Konietzka I *et al*. (2003). Ischemic preconditioning preserves connexin 43 phosphorylation during sustained ischemia in pig hearts in vivo. *FASEB J* **17**: 1355–1357.

Severs NJ, Bruce AF, Dupont E, Rothery S (2008). Remodelling of gap junctions and connexin expression in diseased myocardium. *Cardiovasc Res* **80**: 9–19.

van Veen TAB, Van Rijen HVM, Opthof T (2001). Cardiac gap junction channels: modulation of expression and channel properties. *Cardiovasc Res* **51**: 217–229.