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COMMENTARY

PDE4 in the human heart – major player or little helper?

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PDEs restrict the positive inotropic effects of β-adrenoceptor stimulation by degrading cAMP. Hence, PDE inhibitors sensitize the heart to catecholamines and are therefore used as positive inotropes. On the downside, this is accompanied by exaggerated energy expenditure, cell death and arrhythmias. For many years, PDE3 was considered to be the major isoform responsible for the control of cardiac force and rhythm. However, recent work in gene-targeted mice and rodent cells has indicated that PDE4 is also involved. Furthermore, selective PDE4 inhibitors augment catecholamine-stimulated cAMP levels and induce arrhythmias in human atrial preparations, which suggests that PDE4 has a more prominent role in the human heart than anticipated, and that PDE4 inhibitors such as roflumilast may carry an arrhythmogenic risk. In this issue of the journal, a team of researchers from three laboratories report on the effect of PDE3 and PDE4 inhibitors on ventricular trabeculae from explanted human hearts. The key result is that the PDE4 inhibitor rolipram does not affect the positive inotropic effects of β_1- or β_2-adrenoceptor stimulation. Given that the ventricle rather than the atria is the critical region in terms of arrhythmogenic consequences, this is an important and reassuring finding.

LINKED ARTICLE

This article is a commentary on the research paper by Molenaar *et al.*, pp. 528–538 of this issue. To view this paper visit http://dx.doi.org/10.1111/bph.12167

When I started to do research in the laboratory of Hasso Scholz in 1989, the prominence of PDE inhibitors for the treatment of heart failure had just reached its end. Although these compounds, which include milrinone, enoximone, saterinone and pimobendan, were effective at lowering peripheral resistance/BP and increasing cardiac inotropy ('the inodilatators') and improving the symptoms of heart failure, the mortality rate was increased when they were administered as a chronic oral treatment (Packer *et al.*, 1991). Some years earlier, Bristow and colleagues had shown that the density of β-adrenoceptors was lower in terminally failing hearts than in controls (Bristow *et al.*, 1982). Then, we and others showed that protein and mRNA levels of inhibitory G-proteins were increased under this condition (Neumann *et al.*, 1988; Eschenhagen *et al.*, 1992). These molecular and clinical data resulted in a new theory for the pathophysiology of heart failure and, consequently, its treatment. It was postulated that the chronically failing heart adapts to chronic stressors, such as the elevated sympathetic nervous drive (Cohn *et al.*, 1984), by down-regulating energy consuming mechanisms and up-regulating those that dampen the stimulatory input to the heart (Bristow, 1993). The most convincing evidence in support of this concept came from clinical trials – drugs that further stimulate the force or frequency of the heart tended to increase mortality (PDE inhibitors, catecholamines, partial β-adrenoceptor agonists and mixed compounds such as vesnarinone), whereas β-blockers, ACE inhibitors and aldosterone receptor antagonists, which all directly or indirectly reduce the heart's work, pre- and afterload, impressively improved the survival prognosis.

In the early 1990s, the knowledge about the role of PDEs in the heart was limited and its mechanism of action appeared to be relatively simple. PDE inhibitors increase heart rate and contractility by inhibiting the breakdown of the second messenger cAMP, which activates PKA and thereby promotes phosphorylation of proteins controlling the magnitude of intracellular Ca^{2+} transients and the response of the myofilaments to Ca^{2+}. cAMP is always synthesized at a low rate under basal conditions, which explains why non-specific PDE inhibitors such as IBMX exert positive chronotropic and inotropic effects on isolated heart muscles even in the absence of cAMP-stimulating agents. This effect is strongest in sinoatrial node cells, because basal cAMP production is particularly prominent in these cells (Vinogradova *et al.*, 2008). However, clinically, the main effect of PDE inhibitors is to inhibit the breakdown of cAMP, the synthesis of which is stimulated by catecholamines. In effect, the concentration–response curves for noradrenaline or adrena-

line are shifted to the left, that is in the presence of a PDE inhibitor, smaller concentrations of catecholamines are needed to exert inotropic and chronotropic effects. At the time, 4 PDE isoforms were known and could be distinguished by chromatographic techniques and inhibitor profiles: a Ca^{2+}-dependent PDE1, a cGMP-stimulated PDE2, a cGMP- and cilostamide-inhibited cAMP-selective PDE3 and a cGMP-insensitive, rolipram-sensitive cAMP-selective PDE4. PDE3 was known to be associated with the sarcoplasmic reticulum and the dominant PDE isoform involved in cAMP breakdown and regulation of inotropy in human and dog hearts (Weishaar *et al.*, 1987; Movsesian *et al.*, 1991; Lugnier *et al.*, 1993). Accordingly, the clinically used PDE inhibitors milrinone and enoximone were widely presented as selective inhibitors of PDE3, despite the fact that they also inhibit PDE4 with similar potency (Bethke *et al.*, 1992). Studies in explanted human heart samples showed similar levels and biochemical properties of PDE3 and PDE4 in failing and non-failing hearts, suggesting that the reduced effect of PDE inhibitors (Bohm *et al.*, 1988) was not due to differences in cAMP degradation, but to reduced cAMP production (Movsesian *et al.*, 1991). Importantly, the PDE3 inhibitor pimobendan normalized the reduced contractile and phosphorylation response to isoprenaline in isolated muscle strips from failing hearts (Bartel *et al.*, 1996). However, because the chronic use of PDE inhibitors was associated with an increased risk of mortality in clinical studies, the era of PDE inhibitors for the treatment of chronic heart failure ended, at least provisionally, in the mid-1990s.

So, why now, 20 years later, is there renewed interest in the role of PDEs in the heart? Firstly, molecular cloning has revealed the existence of many more PDEs; at least 11 families have been documented, some with numerous members (Conti and Beavo, 2007). Moreover, many more subtype-selective inhibitors have been developed and have an important therapeutic role. Selective PDE5 inhibitors are blockbuster drugs today, established not only as a treatment for patients with erectile dysfunction, but also for those with pulmonary hypertension. Studies in patients with heart failure are ongoing. Secondly, the selective PDE4 inhibitor roflumilast was recently licensed for the treatment of chronic obstructive lung disease (Rabe *et al.*, 2005), a clinical scenario typically associated with increased cardiovascular risk. This calls for a fresh look at the role of PDE4 in the heart.

In mice, PDE4D was found to be associated with the cardiac ryanodine receptor, and PDE4D knockout mice developed heart failure and severe arrhythmias (Lehnart *et al.*, 2005). However, rodents differ from humans when it comes to PDEs. PDE4 represents ~30% of total PDE activity in mouse heart (Leroy *et al.*, 2011) and conclusive evidence suggests that PDE4 is the dominant isoform to control effects of catecholamines on I_{Ca}, force and rhythm in mouse, rat and rabbit heart (Verde *et al.*, 1999). In contrast, PDE4 constitutes only a small (~10%) fraction of the total PDE activity in human heart (Richter *et al.*, 2011; Johnson *et al.*, 2012; Molina *et al.*, 2012), which appears to be due to much higher activity of non-PDE4 subtypes (Richter *et al.*, 2011; Molina *et al.*, 2012). In earlier studies, PDE4 was not detected in the sarcoplasmic reticulum (SR) fraction of the human ventricle (Movsesian *et al.*, 1991), the compartment most likely to be critically involved in the regulation of contractile force and rhythm. However, in a more recent study PDE4 activity was found to be co-precipitated with an antibody directed against phospholamban (Richter *et al.*, 2011), suggesting that there is also SR-associated PDE4 in human hearts.

In this issue, Molenaar and colleagues (2013) describe the effects of PDE3 and PDE4 inhibition on β_1-adrenoceptor-mediated (noradrenaline in the presence of a β_2-antagonist) and β_2-adrenoceptor-mediated (adrenaline in the presence of a β_1-selective antagonist) effects on contractile function of isolated left and right ventricular trabeculae from terminally failing human hearts. The key results and also title of this study were that the selective PDE3 inhibitor cilostamide potentiated the effect of the catecholamines ($\beta_2 > \beta_1$) in both chambers (of hearts from patients treated with metoprolol), but the selective PDE4 inhibitor rolipram did not. Therefore, the data suggest that PDE4 does not control the contractile responses to catecholamines in the human heart. Although this is the first systematic study of these effects in human ventricular muscle, similar conclusions were reached from a study on human atrial trabeculae by the same group (Christ *et al.*, 2006). Given the great relevance of ventricular function for the human body and the logistical difficulties in performing a statistically meaningful series of experiments on explanted human hearts, the results of this study are of obvious interest.

It contains a number of noteworthy findings. Unexpectedly, cilostamide did not affect adrenergic effects on heart muscles from patients *not* treated with a β-blocker, but did so on those from pretreated patients, suggesting that treatment with a β-blocker increases the control of force induced by PDE3. This is an interesting and potentially important finding, but caveats should be noted. Firstly, up-regulation by β-blockers of a signalling element, such as PDE3, that dampens the responsiveness of β-adrenoceptors is opposite to what we know from β-adrenoceptors or inhibitory G-proteins. The first are up-regulated, the latter down-regulated under chronic β-blockade, leading to resensitization of the signalling cascade (Lohse *et al.*, 2003). This analysis was based on five hearts explanted at a time when β-blockers were not standard treatment for terminal heart failure. Thus, it is difficult to exclude the possibility that historical factors confound the conclusion. Finally, the lack of effect of the PDE3 inhibitor contrasts with earlier data (also prior to the use of β-blockers for heart failure era) showing potentiation of isoprenaline's effects by pimobendan in very similar preparations from terminally failing human hearts (Bartel *et al.*, 1996). One could argue that pimobendan also has Ca^{2+}-sensitizing effects that may come into play here (Hasenfuss *et al.*, 1989), but the potentiation extended to phosphorylation, suggesting that it was indeed due to PDE3 inhibition.

Rolipram alone did not affect basal or catecholamine-stimulated force, leading the authors to conclude that PDE4 has no role in the regulation of force in the human heart. This conclusion is in line with an earlier study that failed to detect PDE4 in the sarcoplasmic reticulum fraction of failing human ventricles (Movsesian *et al.*, 1991) and, as stated earlier, with that of the same authors in human atria (Christ *et al.*, 2006). However, it is in apparent contrast to the results of a recent study by the Fischmeister group, who concluded that PDE4 plays a major role in controlling arrhythmias in human atria

(Molina *et al.*, 2012). In this paper, significant levels of PDE4 were detected in human atria (protein, activity) and its inhibition by rolipram, besides exerting a minor effect on basal cAMP, markedly augmented the effect of cilostamide on intracellular cAMP levels measured in living myocytes, as assessed by fluorescence resonance energy transfer imaging techniques (Molina *et al.*, 2012). Furthermore, experiments on isolated atrial muscle strips suggested that rolipram potentiates the pro-arrhythmic effects mediated by stimulation of both β_1- and β_2-adrenoceptors. On the basis of these experiments, PDE4 was suggested to be a risk gene not only for stroke as shown previously, but for atrial fibrillation [a conclusion at odds with large genome wide association studies (Ellinor *et al.*, 2012)].

How can this apparent discrepancy between the findings of these two very experienced groups be explained? In my view, the data are not as disparate as they seem. Firstly, they were obtained in preparations from different regions of the heart that may well differ biologically. Secondly, all studies on human atrial or ventricular trabeculae concur that rolipram (=selective PDE4 inhibition) does not (significantly) affect basal or catecholamine-stimulated force (Christ *et al.*, 2006; Molina *et al.*, 2012). The new information in the Molina paper was that rolipram prolonged the cAMP effects mediated by a short pulse of isoprenaline. Also it provided evidence that rolipram enhances the pro-arrhythmic actions of catecholamines. Unfortunately, arrhythmias were not reported in the earlier study on human atria (Christ *et al.*, 2006). Thirdly, all the data consistently showed that PDE3 is the dominant isoform in human heart, which is in line with an abundance of earlier data as discussed previously. Fourthly, somewhat in contrast to the strong conclusion of the study by Molenaar and colleagues (2013), rolipram did in fact shift the concentration–response curves to adrenaline and noradrenaline further to the left when given together with cilostamide (Figure 3). The difference between cilostamide and cilostamide + rolipram was apparently not significant, but at the relatively low number of trabeculae studied this does not exclude a small, but relevant effect. Furthermore, the results depicted in Figure 6 suggest that the combination of rolipram and cilostamide stimulated the basal force of contraction and this would be in line with effects on force seen in the Molina paper. On the other hand, in a recent study on isolated human ventricular cardiac myocytes, it was also found that rolipram had no inotropic effects, either alone or in combination with milrinone (Johnson *et al.*, 2012).

Taken together, my conclusion is a relatively simple one. PDE3 is the major PDE in human heart controlling basal and adrenergically-stimulated contractility (whereas it is PDE4 in rodents). This is probably related to the higher overall expression of PDE3 and its localization at the sarcoplasmic reticulum (Movsesian *et al.*, 1991). However, when PDE3 is inhibited, PDE4 comes into play and dampens the effects of catecholamines on cAMP and L-type Ca^{2+} currents (Kajimoto *et al.*, 1997) with relatively small inotropic and potentially larger arrhythmic consequences. The largely ignored fact that enoximone and milrinone inhibit both PDE3 and PDE4 may explain why they have such pronounced effects on the heart. On the other hand, pure PDE4 inhibitors such as roflumilast are unlikely to exert cardiac side effects in patients as long as they are not administered concomitantly with PDE3 inhibitors. This is very unlikely to happen in practice, which may explain the absence of evidence that roflumilast exerts pro-arrhythmic risks (Food and Drug Administration document). The study by Molenaar and colleagues (2013) has thus provided important data for the elucidation of the role of PDEs in the human heart.

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