"Don't flog the heart!" — development of specific drug therapies for heart failure

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ABSTRACT

Understanding the cellular and molecular biology of heart failure is essential to developing targeted and effective treatment. Investigators are divided in their belief regarding the primary abnormality and whether it lies in dysregulation of neurohormonal signalling; nitric oxide synthesis and oxidative stress; cellular energy supply; or cellular ions. Our research demonstrates that these independently studied pathways are, in fact, closely interrelated. The Na^+–K^+ pump is critical in the determination of intracellular sodium levels, which are elevated in heart failure. Drug therapies have been developed targeting the neurohormonal abnormalities seen in the clinical syndrome of heart failure. We have examined the effect of many of these medications on the activity of the Na^+–K^+ pump and observed a perfect correlation between the ability of the treatment to stimulate the pump and its clinical outcome. This is illustrated by the stimulation of the pump by inhibition of the renin–angiotensin signalling pathway, and by aldosterone antagonists. We have also examined the role of reactive oxygen species as mediators of angiotensin and adrenergic regulation of the pump, demonstrating that intracellular pathways activated by β_1/β_2-adrenoceptors and the angiotensin II type 1 receptor converge, with both activating NAD(P)H oxidase and inhibiting the Na^+–K^+ pump via oxidative stress. In contrast, targeted stimulation of the β_3-receptor resulted in nitric oxide-dependent pump stimulation *in vitro*, and improvements in left ventricular function in a large-animal heart failure model. Further characterisation of the intricate pathways involved in the hormonal regulation of the myocyte and its response to heart failure may aid in specific targeting of therapy.

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Heart failure is characterised by systemic signs and symptoms resulting from reduced contraction and/or impaired relaxation of the heart muscle. It is associated with well-described neurohormonal abnormalities, including elevated levels of angiotensin II, catecholamines, aldosterone, endothelin and natriuretic peptides. These abnormalities have provided a focus for pharmaceutical research and development of drugs, which have had variable success in clinical trials.

Drugs that antagonise the effect of angiotensin II (receptor blockers) or prevent its synthesis (angiotensin-converting enzyme inhibitors), as well as antagonists of the aldosterone and β-adrenoceptor, significantly reduce morbidity and mortality. However, trials of endothelin-receptor blockers and of drugs that raise serum levels of natriuretic peptides have not shown benefit. While the development of pharmacological treatment has been empiric, important lessons about the molecular biology of heart failure can be learned from the outcome of the clinical trials. These lessons may, in turn, be used in a more rational design of new candidate treatments. Here, we review aspects of the molecular biology of heart failure that are likely to account for the efficacy, or lack thereof, of previous treatments, with implications for drug development.

Research into the cellular and molecular biology of heart failure has led to the development of four major hypotheses. Investigators are divided in their belief regarding the primary abnormality and whether it lies in:

- dysregulation of neurohormonal signalling;[1]
- nitric oxide synthesis and oxidative stress;[2]
- cellular energy supply;[3] or
- cellular ions (sometimes referred to as the "ionic hypothesis").[4]

These four options for the pathophysiology of heart failure are studied largely as independent entities. We propose that they are closely interrelated and by no means mutually exclusive. This may have important therapeutic implications. We shall take as our starting point the "ionic hypothesis": that the electromechanical phenotype of abnormalities in contraction and cardiac arrhythmias in heart failure is caused by dysregulation of myocyte cations, in particular Na^+ and Ca^{2+}. We shall then explore how this dysregulation may interact with neurohormonal abnormalities, oxidative stress and cellular energy metabolism.

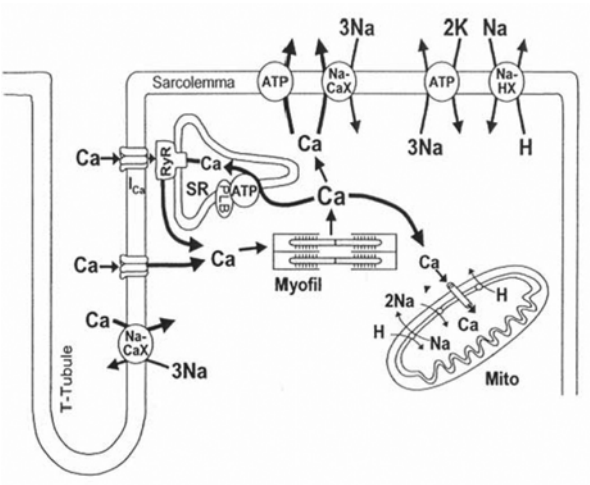
Contraction in the normal heart

It is important for our understanding of the "ionic hypothesis" to briefly review contraction in the normal heart. During the cardiac cycles, membrane potential undergoes cyclical changes to generate the action potential. The interaction between the cardiac action potential and contraction is referred to as excitation–contraction coupling. The change in membrane potential during the action

potential activates channels in the membrane to allow Ca^{2+} to enter the cell from an area of high concentration outside the cell to one of low concentration inside. The Ca^{2+} that enters "triggers" release of Ca^{2+} stored in the sarcoplasmic reticulum (SR). The Ca^{2+} that enters via channels or is released from the SR combines to interact with contractile proteins and initiate contraction.

Relaxation is just as important as contraction and depends on removal of Ca^{2+}. This occurs by an energy-dependent reuptake of Ca^{2+} into the SR, and by its extrusion from the inside of the cell to the surrounding milieu. Extrusion across the sarcolemma occurs via an energy-dependent Ca^{2+} pump and via an exchange mechanism that transports Ca^{2+} out of the cell in exchange for Na^+, which is transported into the cell. In most mammalian species, including humans, the Na^+–Ca^{2+} exchange is quantitatively by far the most important. The processes involved in excitation–contraction coupling are summarised in Figure 1.

Figure 1. Processes involved in excitation–contraction coupling in the cardiac myocyte



PLB = phospholamban. RyR = ryanodine. T-tubule = transverse tubule. Mito = mitochondrion. Myofila = myofilament. (Reproduced with permission from Bers.[5])

Contraction in heart failure

In heart failure, the processes involved in excitation–contraction coupling are deranged. Abnormalities in cellular handling of Ca^{2+} during excitation–contraction coupling are widely accepted as a hallmark of heart failure. Typically, there is a delay in the decline in intracellular Ca^{2+} after the transient increase that initiates contraction. The resulting raised cytosolic levels of Ca^{2+} result in impaired relaxation. The amplitude of the Ca^{2+} transient is also often reduced. Many mechanisms have been proposed, including abnormalities in the function or abundance of the membrane proteins that mediate reuptake of Ca^{2+} into the SR, or removal of cellular Ca^{2+} to the outside by exchange with Na^+ across the sarcolemmal membrane. The reduced amplitude of the Ca^{2+} transient may be due to diastolic Ca^{2+}-induced release of Ca^{2+} from the SR, and hence reduced storage in the SR.

Raised intracellular levels of Na^+ (and hence a decrease in the electrochemical driving force that extrudes Ca^{2+} in exchange for Na^+) is a pivotal abnormality in heart failure (see reviews by Pieske and Houser[6] and Pogwizd et al[7]). The raised intracellular Na^+ levels are believed to be a major cause of abnormal cellular Ca^{2+} handling, because of the role of the transmembrane Na^+ gradient in Na^+–Ca^{2+} exchange. They are also probably directly responsible for cardiac arrhythmias in heart failure, a common cause of death in the condition. The high Na^+ levels may also be responsible for abnormalities in contraction and may activate genes that contribute to abnormal cardiac myocyte growth and adverse cardiac remodelling.[6,7] While high intracellular Na^+ levels are now widely accepted to occur in heart failure, the mechanism for the increase is uncertain. Enhanced Na^+ influx, as well as diminished Na^+ efflux, have been proposed.

Many potential influx routes may be responsible for raised myocyte Na^+ levels. Specific inhibition of any route is difficult and is expected to be associated with adverse effects. Enhanced Na^+ influx is therefore a difficult therapeutic target. However, there is effectively only one efflux route, the membrane Na^+–K^+ pump. Historically, this pump has been a therapeutic target in heart failure, with cardiac glycosides used for more than two centuries. However, the glycosides also inhibit the Na^+–K^+ pump, do not improve survival, and are even harmful in subsets of patients.

Heart-failure treatments and the Na^+–K^+ pump

We systematically examined the effects of treatments that have been shown in clinical trials to have efficacy in heart failure. Our interest in this area dates from a series of experiments 10 years ago, in which we administered the angiotensin-converting enzyme (ACE) inhibitor captopril to normal rabbits as a control for other purposes. We isolated ventricular myocytes and measured their electrogenic Na^+–K^+ pump current (I_p, arising from the 3 : 2 Na^+ : K^+ exchange ratio) using the whole-cell patch clamp technique. We found that the I_p measured in myocytes from rabbits treated with captopril for 7 days was significantly higher than the I_p in myocytes from control rabbits, and, as expected, the intracellular Na^+ concentration in intact myocytes was reduced after treatment.[8] We subsequently found that the angiotensin II (Ang II) receptor blocker losartan had effects

Table 1. Relationship between effect of heart failure treatments on Na^+–K^+ pump current (I_p) and efficacy

Experiment in rabbit myocytes	Na^+–K^+ pump current (I_p) (treatment v control)	Clinical trial equivalent	Implied treatment effect on I_p in HF	Clinical trial outcome
In-vivo ACE inhibitor/receptor antagonist[1-4]	158%–169%*	ACE inhibitors/receptor blockers in chronic HF	↑	+
In-vivo aldosterone v aldosterone + spironolactone[6]	60%* v 102%	Spironolactone or eplerenone in chronic HF	↑	+
In-vitro sodium nitroprusside[7]	180%*	Nitrates in chronic HF	↑	+
Cyclic AMP in pipettes,[8] + H-89	63%,* 102%	$Beta_{1,2}$-blockers in chronic HF	↑	+
Cyclic AMP in pipettes,[8] + H-89	63%,* 102%	$Beta_1$-agonist in chronic HF	↓	–
Milrinone in pipettes,[8] + H-89	74%,* 120%	PDE III inhibitors in chronic HF	↓	–
In-vitro "low" ANP, "high" ANP,[9] + H-89	154%,* 97%, 197%*	Neutral endopeptidase inhibitor in chronic HF	No effect	No effect
In-vitro endothelin[10]	143%*	Endothelin antagonist in HF	↓	–
In-vitro insulin[11]	135%*	Insulin in acute HF	↑	+
In-vivo cyclosporine[12]	57%*	Cyclosporine-induced HF*	↓	–*
In-vivo amiodarone[13,14]	67%*	Amiodarone in Class III HF	↓	–*

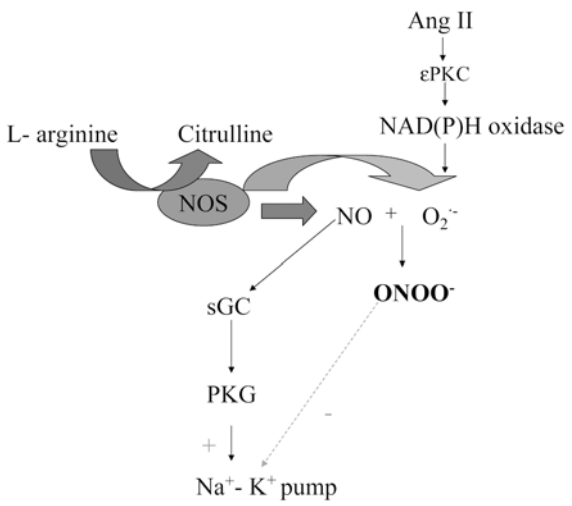
* Unpublished data, details available from the authors.
H-89 = a protein kinase A inhibitor. ANP = atrial natriuretic peptide. PDE = phosphodiesterase. HF = heart failure. ↑ = increase. ↓ = decrease. + = positive. – = negative. ACE = angiotensin converting enzyme.

similar to captopril, indicating that the effects of ACE inhibition on the Na^+–K^+ pump are mediated by Ang II.[9] We also identified a role for protein kinase C in the intracellular pathway coupling Ang II receptors to the pump.[10]

In other studies, we examined the effects of aldosterone and aldosterone antagonists,[11] and the nitric oxide (NO) donor sodium nitroprusside[12] on the Na^+–K^+ pump, and found that aldosterone antagonists and NO donors increase pump activity. Many other treatment modalities have been studied but cannot be described in detail here. The relationship between the effect of the treatment on the pump and the outcome of the associated clinical trials is summarised in Table 1. This table indicates that there is a perfect correlation between the ability of a treatment to stimulate the pump and the clinical outcome.

At first sight, none of the treatment modalities shown in Table 1 seem to have any commonalities in possible cellular mechanisms. However, subsequent studies have established a number of links. This is best illustrated by the cellular effects of Ang II. The Ang II type 1 receptor is known to be coupled to NAD(P)H oxidase, the main source of "superoxide" (O_2^-), a highly oxidising reactive oxygen species. In an entirely different role, NAD(P)H oxidase supplies the O_2^- used by phagocytes to kill bacteria; its absence causes chronic granulomatous disease. For optimal cellular oxidative capacity, O_2^- combines with NO to form peroxynitrate ($OONO^-$). We examined whether the effects of Ang II on the Na^+–K^+ pump may be mediated by O_2^-. Ang II induced a decrease in the I_p of voltage clamped myocytes. This decrease could be abolished by inhibition of NAD(P)H oxidase with a specific peptide blocker, by introduction of superoxide dismutase into the myocyte to eliminate O_2^-, and by a scavenger of $OONO^-$. In independent experiments using confocal microscopy, we showed that Ang II induced an increase in O_2^- production by isolated myocytes. The

Figure 2. Proposed mechanism mediating angiotensin II (Ang II) regulation of the Na^+–K^+ pump



εPKC = epsilon isoform of protein kinase C.
NOS = nitric oxide synthase. sGC = soluble guanylyl cyclase.
PKG = protein kinase G.

increase was abolished by superoxide dismutase, and by inhibiting protein kinase C or NAD(P)H oxidase.[13] The pathway involved in Ang II regulation of the Na^+–K^+ pump is summarised in Figure 2.

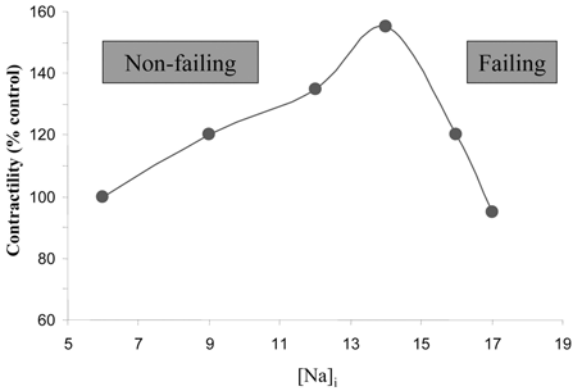
In an independent series of studies, we examined the effect of β_1/β_2-adrenergic activation on the Na^+–K^+ pump. In summary, cross-talk between the intracellular pathways activated by β_1/β_2-adrenoceptors and the Ang II type 1 receptor converges, with both activating NAD(P)H oxidase and inhibiting the Na^+–K^+ pump via oxidative stress. Blockade of the renin–angiotensin or α-adrenergic pathway may therefore relieve such Na^+–K^+ pump inhibition and the associated cardiac myocyte Na^+ overload, and hence be therapeutically beneficial in heart failure.

Beta$_3$-adrenoceptors

While the importance of β_1/β_2-adrenoceptors is widely appreciated, a third β-adrenoceptor, β_3, has received much less attention. Identified over 20 years ago and expressed in a wide variety of tissues, the β_3-receptor belongs to the family of G protein-coupled receptors. It shares limited amino acid sequence homology with the other β-receptors, and is *not* coupled to an inactivating β-adrenergic receptor kinase (as reviewed by Arch[14]). There was widespread interest in the β_3-receptors about 10 years ago, because selective agonists had been found to induce weight loss in rodents by enhancing lipolysis. Orally active agonists selective for the human β_3-receptor were developed by most major pharmaceutical companies as potential weight-loss agents, but subsequent human trials for that indication were disappointing.

Beta$_3$-adrenoceptors are up-regulated in the myocardium in heart failure. As this up-regulation mediates a catecholamine-induced decrease in contractility, it has been universally regarded as maladaptive in heart failure in the published literature. Efforts have therefore been directed at developing clinically useful β_3-receptor *antagonists* for the treatment of heart failure. However, as the receptor is coupled to endogenous NO synthesis in cardiac myocytes, and as NO activates the Na^+–K^+ pump, we speculated that the β_3-receptor might activate the Na^+–K^+ pump and perhaps be useful in heart failure. We tested this hypothesis by exposing voltage clamped isolated rabbit ventricular myocytes to the selective synthetic β_3-receptor agonists, BRL 37344 and CL 316,243, and to noradrenaline, the naturally occurring catecholamine with preferential β_3-receptor affinity. All three substances induced a large increase in I_p, even at very low, nanomolar, concentrations. There was no effect of blockade of β_1/β_2- or α-adrenoceptors, or of blockade of their intracellular messenger pathways. However, blockade of pathways known to be coupled to the β_3-receptor via NO abolished Na^+–K^+ pump stimulation.

Figure 3. Relationship between intracellular Na^+ concentration and cardiac performance in the failing and non-failing heart

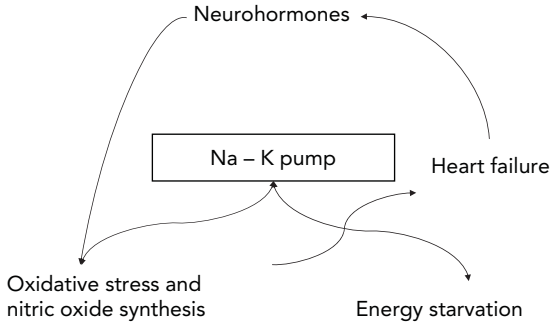


In the "non-failing" heart, a positive slope describes the relationship between intracellular Na^+ concentration and cardiac performance. In the "failing" heart, in the context of higher levels of intracellular Na^+, the positive slope is replaced by a negative slope.

Heart failure is characterised by raised levels of cardiac myocyte Na^+. At this higher level of intracellular Na^+, the positive slope describing the relationship between intracellular Na^+ concentration and cardiac performance, which is seen in the normal heart, is replaced by a negative slope[6] (Figure 3). A decrease in cell Na^+ might therefore improve the performance of the heart, and Na^+–K^+ pump stimulation mediated by the β_3-receptor might be useful.

To examine the effect of acute activation of the β_3-receptor, we used a model of ischaemic cardiomyopathy with severe heart failure induced in sheep by repeated coronary microembolisation over several weeks. The model, which is characterised by stable heart failure, reflects the human condition well.[15] We administered BRL 37344 by intravenous infusion over 8 minutes, starting with the lowest dose of the protocol chosen. After an additional 5 minutes, we determined pressure–volume relationships. The procedure was then repeated using a larger dose of BRL 37344. We used the left ventricular end-systolic pressure–volume relationship (ESPVR) as an index of cardiac performance. ESPVR is relatively independent of confounding factors, such as pre- and after-load, as well as heart rate, and is widely accepted as a good index of left ventricular function. There was a dose-dependent negative effect of BRL 37344 on ESPVR before the induction of heart failure. This was converted to a positive effect after heart failure had been induced, confirming our prediction that Na^+–K^+ pump stimulation mediated by the β_3-receptor might be useful.

Figure 4. Proposed mechanisms of interaction of the four chief hypotheses about heart failure



Our sheep study showed an acute haemodynamic benefit of β_3-receptor stimulation. A subsequent study on a dog model of heart failure has shown more long-term benefits.[16] The model used in the study is known to be stable and of particular relevance to our research, as it is associated with high cardiac myocyte Na^+ levels. It is characterised by biventricular dilatation/hypertrophy and frequent ventricular tachycardia. Actively treated dogs were given a low dose of BRL 37344 by infusion for 30 days. The primary outcome of ventricular tachycardia was reduced in the treatment group approximately sixfold. The drug was also associated with shortened QT interval, reduced heart weight and down-regulation of the membrane Na–Ca exchanger protein (an excellent molecular/cellular marker of hypertrophy/failure). Furthermore, β_3-receptor stimulation resulted in up-regulation of β_3-receptor expression, with implications for improved specificity with continued treatment.[16]

The β_3-adrenoceptor-mediated Na^+–K^+ pump activation that we demonstrated in isolated myocytes sheds new light on the interpretation of an important study with a puzzling, unexpected adverse outcome. The centrally acting sympatholytic agent moxonidine was expected to reduce the high levels of plasma noradrenaline typically encountered in heart failure. This should achieve the same, or a more beneficial, effect than blocking the action of noradrenaline with receptor blockers. The drug induced up to a 50% decrease in serum levels of noradrenaline (and presumably much larger relative reduction in tissue levels). However, this was not beneficial — there was a large *excess* mortality in moxonidine-treated patients.[17] The rationale for the trial was sound, according to contemporary understanding of pharmacotherapy of heart failure, and there was no satisfactory explanation for the adverse outcome (reviewed by Coats[18]). Beta$_3$-receptors are activated at a higher level of endogenous catecholamines than β_1/β_2-receptors. The reduction in noradrenaline levels induced by moxonidine may therefore have reduced β_3-receptor stimulation more than β_1/β_2-stimulation and hence eliminated the beneficial effect of noradrenaline-induced Na^+–K^+ pump activation mediated by β_3-receptors. Indirectly, the MOXCON study therefore provides support for the use of β_3-receptors agonists in heart failure.

Conclusions

So far, we have described an interaction between the ionic, the neurohumoral and the oxidative hypotheses for the molecular biology of heart failure. The energy-deprivation hypothesis is likely to be linked to these, as it has been recognised that glucose uptake into myocytes is partly mediated by a glucose transporter that depends on the transmembrane concentration gradient for Na^+. As there is a switch towards glucose-dependent energy metabolism in heart failure, any reduction in the Na^+ gradient would be expected to reduce an already limited fuel supply. Conversely, restoration of the gradient with Na^+–K^+ pump stimulation should improve the energy metabolism. Figure 4 shows a proposal as to how the mechanisms we have discussed may interact. Further characterisation of intricate pathways involved in the hormonal regulation of the myocyte and its response to heart failure may provide important therapeutic insights and aid in the specific targeting of therapy.

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