Nitroxyl gets to the heart of the matter

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Heart failure (HF) is a progressively disabling and ultimately fatal disease, which is characterized by a decline in the heart's ability to pump blood efficiently enough to meet the body's metabolic demands. Despite substantial advances in our understanding of the underlying pathophysiology (1) and the therapeutic management of acute and chronic HF (2) in recent years, the outlook of patients with these conditions remains poor. Not only are mortality and morbidity discouragingly high, but also the patients' quality of life remains impaired because of a substantial symptom burden. In the United States alone, HF is responsible for almost 1 million hospital admissions (more than for all forms of cancer combined) and >50,000 deaths each year, with estimated annual costs exceeding $20 billion (3, 4). Despite improved patient information, beneficial changes in lifestyle and better treatment options, HF remains to be a major public health problem in industrialized nations and the leading cause of hospitalization in people older than 65 years. At a time when other cardiovascular diseases are on the decline, HF is rising and likely to further escalate over the coming decades because of an aging population and increased survival from the underlying causes such as coronary heart disease and hypertension. A broad spectrum of different drugs and various guidelines for the treatment of HF exist (5, 6). In the past, HF was mainly viewed as a problem of diminished cardiac output. Maximization of the latter with positive inotropic (contractility enhancing) agents led to therapies that initially improved functional capacity, but increased mortality. Today, the therapeutic focus is on reducing elevated filling pressures that lead to the symptoms of congestion (7). Although most recommendations agree on the major drug classes for the first-line and adjunct therapy of HF, there is considerable controversy about the role of positive inotropic agents (8). Despite a documented negative impact on survival, these agents are still widely used, often combined with vasodilators, to limit severe episodes of HF or as a bridge to transplantation. The rationale for combining vasodilatation with positive inotropic intervention lies in the possibility to "unload" the heart, i.e., to reduce its preload and afterload by venous and arterial dilatation, allowing to stimulate cardiac output without increasing oxygen consumption. Although conceptually ideal compounds, currently used inodilators (compounds with positive inotropic and vasodilatior properties) tend to increase myocardial oxygen demand at higher doses, precipitating ischemia in patients with coronary artery disease. Clearly, there is room for improvement in HF management, in particular with regard to quality of life and survival.

In this issue of PNAS, Paolocci *et al.* (9) describe the beneficial cardiovascular effects of nitroxyl (HNO)[†] in the failing heart. A well characterized canine model of chronic heart failure was used in which cardiac dysfunction is produced by rapid ventricular pacing over a period of weeks. The authors used sophisticated hemodynamic analyses suited to discriminate direct cardiac effects from indirect effects secondary to changes in preload and afterload to demonstrate that nitroxyl increases myocardial contractility and enhances relaxation (positive lusitropic effect) in failing hearts. These effects were accompanied by arterial and venous dilation. Paolocci's finding that the cardiotonic action of HNO was unaffected by β-receptor blockade and additive to that of dobutamine is therapeutically significant not only because the action of dopaminergic agonists and phosphodiesterase inhibitors are often attenuated in HF, but also in view of the recent advent of β-blockers and their negative inotropic effects in certain clinical settings. In contrast to nitric oxide (NO)-generating compounds, HNO production was not associated with increased plasma levels of the second messenger, cGMP. Instead, enhanced concentrations of calcitonin gene-related peptide (CGRP) were detected during HNO, but not NO administration, suggesting that the former may exert its favorable action, at least in part, via this endogenous neuropeptide. Although the same group had previously observed positive inotropic effects of HNO in healthy hearts (10), the study outcome with this compound in the setting of HF was not obvious. Numerous experimental and clinical studies in the past have demonstrated that the same pharmacological principle capable of increasing contractility in the normal heart can produce negative inotropy in the failing heart due to unfavorable effects on cardiac oxygen consumption and energetics. Taken together, these results suggest that nitroxyl donors represent a novel class of inodilator with potential for the treatment of HF.

In most cases, not a single cause but a combination of systolic dysfunction (inability to contract and eject blood normally) and/or diastolic dysfunction (inability to relax and fill normally), energetic and vascular loading factors, contributes to the manifestation of HF. The type of cardiac dysfunction prevailing and the accompanying hemodynamic situation of the patient have an obvious impact on the choice of pharmacological treatment. The analysis of pressure-volume loops obtained at different loading conditions is among the best of all current approaches to assess the contractile behavior of the heart *in vivo*. This approach, which also formed the basis of the present studies by Paolocci *et al.* (9), has a long-standing history in experimental physiology, but its diagnostic power in animal studies and clinical investigations has only been realized in the last two decades. In the past, effects on myocardial contractility have been difficult to evaluate because of the load dependence of conventional measures of ventricular function. Since a couple of years ago, left ventricular end-systolic pressure-volume relationships can be assessed in the setting of a routine cardiac catheterization procedure. Using a conductance catheter with a micromanometer tip for continuous measurement of intraventricular volume and pressure in combination with an occluding device to rapidly vary venous inflow, a largely load-independent measure of cardiac contractility can be obtained without altering the status of the heart (11). In addition to the assessment of systolic and diastolic function, additional parameters allow estimation of the relative effects of vasodilation on cardiac performance. In their studies, Paolocci *et al.* (9) used a nitrate (nitroglycerin) and a NONOate (DEA/NO) to generate NO and Angelis' salt to generate HNO. Although all three compounds were used at equieffective doses as

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[†]Not to be confused with nitroxyl radicals, which are spin traps for the detection of radical species by EPR spectroscopy.

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judged by the degree of reduction in systolic pressure, the cardiac effects of NO were dramatically different from those of HNO. This is most likely due to differences in the chemical properties of these two species, which dictates their reactivity with endogenous biomolecules and the signaling pathways affected.

NO is a ubiquitous endogenous messenger and modulator of cell function, which is produced from L-arginine by a family of isoenzymes, the NO synthases (NOS) (12). HF is associated with reduced expression of endothelial NOS and increased vascular oxidative stress, which translates into diminished NO availability, endothelial dysfunction and reduced vasodilator capacity (13). NO is also the pharmacological principle of a number of drugs collectively termed nitrovasodilators, which are used clinically to control hypertensive crises, protect patients from attacks of angina pectoris and to unload the heart during acute HF. Numerous other compounds, including NONOates are available to experimentally generate NO (14). Notwithstanding the principal difference that nitroglycerin requires tissue metabolism to generate NO whereas DEA/NO releases it spontaneously, the cardiac effects of both compounds were similar (9), indicating that their action was mediated by the same signaling mechanism. Nitroxyl anion (NO^-) is the one-electron reduction product of NO. Its chemistry is not very well understood and complicated by the fact that it exists in two electronic forms, a singlet and a triplet state (15). A recent reevaluation of its pK_a value revealed that at physiological pH it exists largely in its protonated form, HNO (16), which can readily cross cell membranes. Whether nitroxyl is formed *in vivo* is currently unclear. Nevertheless, it may be formed from nitrosothiols (17), which are found to be present in a variety of biological systems (18). In experimental settings, nitroxyl can be conveniently generated by using Angelis's salt (14). In fact, it was the spontaneous decomposition of this inorganic salt that led chemists to postulate the existence of HNO at the turn of the century (19). Angeli's salt has been shown to induce vasorelaxation and to lower blood pressure (20, 21). Unlike NO, which does not directly react with sulfhydryl groups, HNO is a potent thiol oxidant (22) and possesses a high affinity for ferric heme proteins (23). The physiological significance of these orthogonal properties of NO and HNO are not entirely clear, but may offer an explanation for the discrete effects of these two redox congeners in the failing heart. Although it has been suggested that NO^- and NO are redox-interconvertable species (24), NO^- may not be readily oxidized to NO under all conditions. Select nitroxyl donors, but not Angeli's salt, have been shown to undergo facile oxidative conversion to relax vascular tissue and inhibit platelet aggregation in a manner indistinguishable from NO (25). The clear-cut dichotomy between the pharmacological profile of Angeli's salt and that of NO donors observed by Paolocci *et al.* (9), however, indicates that HNO to NO conversion does not take place in every tissue. This conclusion is further supported by their cGMP and CGRP measurements, which suggest that the cardiac effects of HNO and NO are mediated by different signaling pathways. Besides the significance of these findings for HF, Paolocci's study also offers a novel pharmacological avenue for the modulation of CGRP levels.

CGRP is a 37-aa peptide that is synthesized by alternative splicing of the primary RNA transcript of the calcitonin gene in sensory neurons. Blood vessels of all vascular beds are surrounded by a dense network of CGRP-containing nerve fibers, and most of the CGRP circulating in plasma is thought to originate from perivascular nerves (26). CGRP is the most potent vasodilator known to date and thought to be involved in the regulation of resting blood pressure and regional blood flow (27), particularly in the coronary circulation (28). In addition, it is a cardiotonic agent with positive inotropic and, in normal subjects, positive chronotropic (heart rate increasing) effects (26). CGRP interacts with specific cellular receptors that are coupled via G proteins to adenylyl cyclase. The consecutive increase in cAMP is considered the principal mechanism responsible for CGRP-mediated smooth muscle relaxation, although NO-dependent effects (29) and opening of ATP-sensitive potassium channels (30) have been described as well. In addition to cAMP, phospholipase C may be involved in the stimulation of intracellular Ca^{2+} concentrations in the heart (31). In patients suffering from HF, CGRP has been shown to reduce pulmonary and systemic pressures and increase cardiac performance without producing tachycardia (32). This is consistent with the absence of a change in heart rate with HNO in Paolocci's study and suggests a possible inhibitory modulation of sympathetic nervous activity (33) at the sinoatrial and/or the arterial baroreceptor reflex level, which clearly distinguishes nitroxyl donors from other positive inotropes, including levosimendan (34).

A number of conditions and stimuli can cause the release of CGRP, including ischemia, nicotine, capsaicin (35), nitroglycerin, and a nitroxyl donor (but not other NO generating compounds) (36). An alternative way to increase plasma CGRP is to slow down its enzymatic breakdown by inhibiting neutral endopeptidase, but this is bound to affect numerous other pathways. It appears fair to assume that the HNO-induced CGRP increase in Paolocci's study (9) originated from perivascular nerves. It would be interesting to see whether the inotropic effects of HNO are blunted in dogs depleted of endogenous CGRP by prolonged infusion of capsaicin (37). Further insight may also be gained from studies in CGRP-deficient mice (33). Such investigations would not only confirm the cardiotonic mechanism proposed for nitroxyl, but may also shed new light on the role of CGRP in HF. Both increased (38) and reduced (39) plasma CGRP concentrations have been reported in HF. Whether these changes are reflections of a counterregulatory mechanism or causally involved in disease progression is not known. Physiologically, it would make sense to increase CGRP early during HF as it would complement other compensatory systems aimed at improving cardiac efficiency. Prolonged stimulation of CGRP release may lead to gradual peptide depletion, offering an explanation for the lower plasma levels observed in this and other studies (9, 39).

CGRP-related immunoreactivity is often found either together with NOS in the same neuronal structures or in close proximity to NOS-containing nerves. Interestingly, NOS activity appears to be involved in capsaicin-induced CGRP release (40), and there is mounting evidence to believe that NOS is capable of producing NO^- under certain conditions (41, 42). Although admittedly speculative, the mechanism of CGRP stimulation by HNO may not just be a peculiar pharmacological phenomenon, but in fact represent an endogenous pathway involved in the fine-tuning of CGRP release. Whether all cardiovascular effects of HNO are due to CGRP remains to be investigated. In addition to the use of knockout animals this issue could be addressed by administration of a CGRP receptor antagonist. Should the response to HNO be only partially blunted by CGRP receptor blockade it might be of interest to investigate whether inhibition of Na^+/K^+-ATPase (digitalis-like activity) or a Ca^{2+}-sensitizing component are involved in addition. Considering the reactivity of HNO with thiols one possible site of action might reside at the level of the ryanodine receptor, which plays an important role in the regulation

of intracellular Ca^{2+} transients (hence contraction) by controlling Ca^{2+} release from the sarcoplasmic reticulum and whose channel opening probability is modulated by oxidation (43) and nitrosation (44) of critical thiols. Alternatively, protein oxidation may shift the association/dissociation equilibrium of the regulatory protein FKBP12 with the channel (45). Interestingly, the CGRP-related peptide, adrenomedullin has been shown to enhance cardiac contractility via cAMP-independent mechanisms including Ca^{2+} release from ryanodine-sensitive stores (46).

Clearly, there is much more to learn about the biological chemistry of HNO/NO^-. Nevertheless, it looks as if there is clear potential for therapeutic exploitation of nitroxyl donors, and it appears timely to consider intensifying research efforts in this relatively new field. Notwithstanding the fact that Angelis' salt is nothing more than an investigational tool, the studies by Paolocci *et al.* (9) are nothing less than a proof-of-principle for a potentially promising new class of inodilator. Additional studies will be required to address whether nitroxyl donors are subject to tolerance development, which often limits the effectiveness of organic nitrates. Tolerance to HNO might develop as a result of CGRP depletion from peripheral nerves or desensitization of signaling pathways downstream of CGRP receptor activation, albeit there is no indication for this to occur from infusion studies with CGRP in man (47). To come up with a drug candidate for commercial development that was sufficiently stable, orally available, and amenable to optimization of its pharmacokinetic properties, structures are required that offer a variety of possibilities for chemical derivatization. Further aspects that demand investigation include the frequency of unwanted side effects of nitroxyl donors such as hypotension, headache, and gastrointestinal symptoms, which limits the usefulness of other vasodilators, and the risk of triggering ventricular arrhytmias.

Because of the overall hemodynamic complexity of the different forms of HF there is no single, straightforward approach for the therapeutic management of all patients. Hence, we will continue to require several individual agents with distinct pharmacological profiles to correct specific hemodynamic abnormalities. CGRP has shown potential for HF management in clinical studies, but lacks oral availability, is rapidly metabolized and has thus to be given by continuous infusion. With no selective CGRP-mimetic on the horizon and a recently renewed interest in inodilators (34), this may be a unique chance for nitroxyl donors. As with any new pharmacological principle at this early discovery stage, many obstacles have to be overcome before a new lead compound can eventually enter the developmental phase. Should nitroxyl donors pass these hurdles in the next couple of years, HF may become the key indication for such compounds in the future and HNO-based inodilators a potentially useful addition to the therapeutic arsenal available for treatment of this life-threatening syndrome.

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