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Circumventing a broken heart: cytokines and the subfornical organ

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Heart failure is a growing epidemic in developed nations. According to the most recent figures from the Centers for Disease Control and Prevention and the 2016 statistical update from the American Heart Association, it is estimated that approximately 1.8% of the US population, approximately 5.8 million adults, have heart failure (4, 8). This is reflected in other nations; for example, in Australia an estimated 1-2% of the population have heart failure (13). Worryingly, in the US alone, it has been forecast that a 46% increase in the prevalence of heart failure will occur during the period from 2012 to 2030. Thus, it is predicted that in the US more than 8 million adults will suffer from heart failure by 2030 (8). This increase in the prevalence of heart failure has been largely attributed to an aging population, but the increases in the rates of obesity and diabetes, as well as improvements in detection, monitoring and treatment of acute myocardial infarction, are significant factors contributing to this rise (14).

Although current therapies for heart have some benefit, they do not prevent the progression of the disease and prognosis remains poor. Thus, there is great interest in the identification of new pathways that can be targeted in heart failure to slow the disease process and thereby reduce morbidity and mortality. One focus of research has been the role of circulating pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukins (such as IL-1), the levels of which are elevated in patients with multiple forms of heart failure, including heart failure with reduced and preserved ejection fraction as well as acute decompensated heart failure (6). Increased circulating levels of proinflammatory cytokines (PIC) are associated with adverse outcomes in patients with heart failure; however, the mechanisms by which PICs have detrimental effects remain largely unknown. Over the past decade, several research groups, including that led by Robert Felder, have explored the roles and actions of PICs in the brains of animals with heart failure (12, 15, 16). They showed that peripheral administration of PICs activates the sympathetic nervous system, presumably via a direct central action (16). It is well established that sympathetic overactivity in heart failure is detrimental and contributes to peripheral vasoconstriction and extracellular fluid volume accumulation, which places additional stress on the failing heart leading to further cardiac damage (9). So how do large, hydrophobic peptides such as circulating inflammatory cytokines, which are too big to cross the blood-brain barrier, activate the sympathetic nervous system? Current theories suggest that cytokines, such as

TNF-α and IL-1β, may activate receptors on brain endothelial and perivascular cells and signal via COX-2 and prostaglandin E2 (5). Another suggested mechanism involves active transport of cytokines such as TNF-α and IL-1β across the blood-brain barrier (1, 2). The paper by Yu et al. investigates the role of another signalling pathway, which involves a direct action of TNF-α on the sensory circumventricular organs of the brain (18) (Figure 1).

Located along the wall of the third and fourth ventricle, the sensory circumventricular organs are specialised brain nuclei that lack an intact blood-brain barrier due to the presence of fenestrated capillaries. There are three of these specialised structures in the brain – the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis in the anterior wall of the third ventricle and the area postrema located on the floor of the fourth ventricle. In earlier work, the Felder group demonstrated that the SFO plays a key role in mediating the sympathoexcitatory effects of circulating TNF-α, because ablation of the SFO abolished the sympathoexcitatory effects of peripherally administered TNF-α (16). Furthermore, direct microinjections of TNF-α into the SFO upregulated key components of the renin-angiotensin system (RAS) and the excitatory effects of TNF-α on renal sympathetic nerve activity were attenuated by local microinjection of the angiotensin II receptor type-1 antagonist, losartan into the SFO (15).

In this issue of the *American Journal of Physiology: Heart and Circulatory Physiology*, Yu *et al*., (17) present a series of elegant experiments that shed additional light on the role of TNF-α and its cognate receptor TNF-α receptor type 1 (TNFR1) in activating central pathways that lead to increased sympathetic drive in rats with heart failure (17). Using a lentiviral vector to deliver shRNA specifically against the TNFR1 receptor in the SFO, they were able to show effective knockdown of TNFR1 mRNA and receptor protein in the SFO, but not in the hypothalamic paraventricular nucleus (PVN), in rats with heart failure. Heart failure rats injected with vehicle (artificial cerebrospinal fluid) or control (scrambled shRNA) did not have reduced levels of TNFR1 mRNA expression. Next, they determined the effect of TNFR1 knockdown on the expression of components of RAS (namely angiotensin converting enzyme and the angiotensin type-1 receptor), as well as the inflammatory mediators, TNF-α, IL-1β and COX-2 (but not COX-1) in the SFO. Interestingly, in rats with heart failure, where

TNFR1 was knocked down in the SFO (i.e. injected with lentiviral vector encoding the TNFR1 shRNA), all these excitatory mediators were significantly reduced not just in the SFO, but also in the PVN. The fact that the PVN also showed lower expression of RAS components and inflammatory mediators fits nicely as the PVN receives direct inputs from SFO neurons and mediates, in part, the increase in plasma norepinephrine seen in rats with coronary artery ligation induced heart failure (18).

The above findings are interesting and shed additional light on the possible mechanisms by which TNF-α and TNFR1, acting in the SFO, drive increased sympathetic activity in rats with heart failure. Reducing TNFR1 expression in the SFO of heart failure rats led to significantly lower plasma levels of norepinephrine compared with vehicle injected rats (i.e. no knockdown of TNFR1 in the SFO). This suggests a lowering of sympathetic nerve activity, but as direct sympathetic nerve recordings were not made it is unknown if this reflects a generalised increase in sympathetic tone or a selective increase to individual organs such as the kidney. Importantly, TNFR1 knockdown led to improved cardiac hemodynamics, including an increase in LV contractility, reduced pulmonary congestion and decreased right ventricular remodelling. Such improvements in cardiac hemodynamics may reflect the effects of reduced renal sympathetic nerve activity. Despite these improvements, TNFR1 shRNA treated heart failure rats did not show reduced deterioration in echocardiographic indices of cardiac function, left ventricular ejection fraction or left ventricular end-diastolic volume, suggesting that autonomic balance to the heart may not have improved. It would be interesting to follow treated animals for longer periods of time to determine if the progressive decline in cardiac function that occurs in heart failure was prevented. Knockdown of TNFR1 in sham control rats had no adverse effects on hemodynamics or cardiac function.

Despite significant improvements over the past several decades in the treatment for patients with heart failure, morbidity and mortality remain high. There have been few new clinical breakthroughs in the past 10 years. The most recent advance, the first in many years, reported by the PARADIGM-HF trial, showed significant benefit of sacubitril/valsartan treatment over an angiotensin-converting enzyme inhibitor irrespective of previous coronary revascularisation or dose of β-blocker (10). The findings presented by Yu et al,

offer us a glimpse of a possible new target for heart failure treatment, the brain, via the knockdown of TNFR1 in the SFO. While it is early days and much work is still needed with regard to improving safety and efficacy of viral vector use to treat intractable diseases, viral vector mediated gene delivery is currently being trialled to treat a number of diseases ranging from Leber hereditary optic neuropathy (3) to Alzheimer's disease (11). Despite this rather hopeful outlook, we are nevertheless cognisant of the considerable barriers that need to be surmounted before findings such as these can be translated to the clinic. For example, we need to carefully consider the timing of interventions. Will this treatment be effective in patients with advanced heart failure and can we achieve stable knockdown of the TNFR1 receptor long-term? Do we need cell specific knockdown of TNFR1, perhaps to those cells that project directly to the PVN and are sympathoexcitatory? Would precise targeting be possible given that the SFO is an extremely small structure in humans (7)? Despite these challenges, the sensory circumventricular organs in the brain are attractive sites for targeted therapies due to the absence of a blood-brain barrier. Clearly more animal studies need to be performed to add to our understanding of the brain mechanisms leading to the central generation of increased sympathetic tone in heart failure. We have much work ahead of us; however, work such as that reported by Yu et al., will no doubt drive the future development of novel treatments to reduce morbidity and mortality in heart failure.

Figure Legend

Figure 1. The damaged heart releases pro-inflammatory cytokines such as tumor necrosis-α (TNF-α), which can activate neurons in sensory circumventricular organs such as the subfornical organ (SFO). The SFO has projections to the hypothalamic paraventricular nucleus (PVN), which project directly, or indirectly via the rostral ventrolateral medulla (RVLM), to the preganglionic sympathetic neurons in the intermediolateral cell column (IML) in the spinal cord to increase sympathetic nerve activity. The increases in sympathetic nerve activity to the heart and kidney have detrimental actions that enhance the progression of the disease. Abbreviations: COX-2, cyclooxygenase-2; IL-1β, interleukin-1β; RAS, renin-angiotensin system; shRNA, short hairpin ribonucleic acid; TNFR1, tumor necrosis factor receptor 1.

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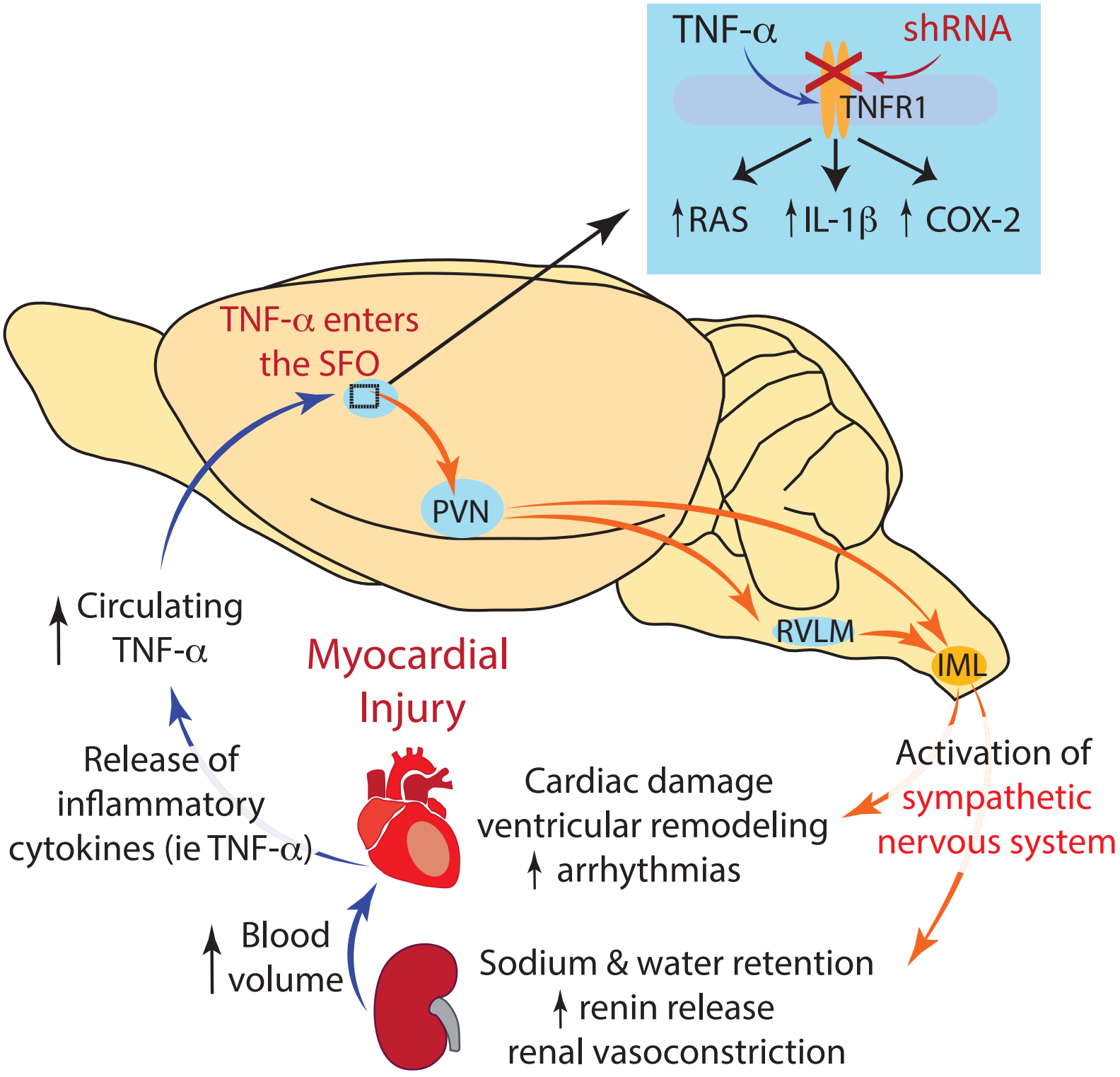
TNF-α
shRNA
TNFR1
↑RAS
↑IL-1β
↑COX-2
TNF-α enters
the SFO
PVN
RVLM
IML
↑ Circulating
TNF-α
Myocardial
Injury
Release of
inflammatory
cytokines (ie TNF-α)
Cardiac damage
ventricular remodeling
↑ arrhythmias
Activation of
sympathetic
nervous system
↑ Blood
volume
Sodium & water retention
↑ renin release
renal vasoconstriction