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SUBSTANCE P IN HEART FAILURE: THE GOOD AND THE BAD

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Abstract

The tachykinin, substance P, is found primarily in sensory nerves. In the heart, substance P-containing nerve fibers are often found surrounding coronary vessels, making them ideally situated to sense changes in the myocardial environment. Recent studies in rodents have identified substance P as having dual roles in the heart, depending on disease etiology and/or timing. Thus far, these studies indicate that substance P may be protective acutely following ischemia-reperfusion, but damaging long-term in non-ischemic induced remodeling and heart failure. Sensory nerves may be at the apex of the cascade of events leading to heart failure, therefore, they make a promising potential therapeutic target that warrants increased investigation.

Keywords

substance P; tachykinin; heart failure; neuropeptide; sensory nerve; myocardial remodeling

1.0 INTRODUCTION

Substance P is from the tachykinin family of sensory nerve neuropeptides. The other classic members of this family are neurokinin A (NKA) and neurokinin B (NKB). While NKB is encoded for by its own gene (*TAC2*) and is restricted to the central nervous system, substance P and NKA are both encoded by the *TAC1* gene and are found in the central nervous system and peripheral afferent sensory neurons (1;2). The *TAC1* gene expresses pre-mRNA that can generate four mRNA isoforms (α , β , γ , and δ). All four isoforms give rise to substance P, whereas only the β and γ isoforms encode for NKA. This means that substance P can be expressed without NKA, however, NKA will always be accompanied by substance P. However, since the β and γ isoforms appear to be the most common, substance P and

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Disclosures

None

NKA will often be synthesized, stored, and released together (1). Substance P acts primarily through the neurokinin (NK)-1 receptor, while NKA exerts its effects via the NK-2 receptor, although there is some overlap between the two (3). The actions of tachykinins are many, but include smooth muscle contraction, vasodilation, nociception, and modulation of inflammatory/immune cell function (4-8). Substance P and NKA have long been known to have negative inotropic and chronotropic effects on the normal heart (9) (10), but it is only recently that we are beginning to consider that sensory nerve neuropeptides may have key roles in regulating adverse myocardial remodeling and the subsequent development of heart failure. Outside of the aforementioned effects on heart rate and contraction, little has been published relating to NKA and myocardial remodeling. Accordingly, this article will focus on substance P. What makes substance P so interesting is that recent experimental studies have revealed two sides to this neuropeptide in myocardial remodeling and heart failure; the good and the bad. Accordingly, the purpose of this review is to draw attention to the role of substance P in adverse myocardial remodeling and heart failure, since to this point in time it's role in these events have not been studied in detail. As such, this review will: 1) describe the localization of substance P within the myocardium; 2) describe the beneficial role of substance P acutely following ischemia reperfusion; 3) describe the detrimental role of substance P in long-term remodeling of the heart; 4) describe the direct effects of substance P on cardiomyocytes, cardiac fibroblasts, and cardiac inflammatory cells; and 5) discuss the clinical implications of substance P in the heart.

2.0 SUBSTANCE P LOCALIZATION IN THE HEART

Before discussing the good and the bad of substance P, it is necessary to understand the localization of this peptide in the heart (Table 1). This is what makes it ideally placed to rapidly respond to changes in the myocardial environment.

2.1 Substance P-Containing Nerves in the Heart

Substance P is considered a neuropeptide, being produced primarily by C-fiber sensory nerves. Descriptions of the distribution of substance P-containing nerves in the heart are extensive, at least in rodents. The most extensive studies have been performed in guinea pig hearts.

2.1.1 Guinea Pig—Many studies have identified substance P-containing nerves in the guinea pig heart. The following studies are not exhaustive, but are meant to be representative of the overall findings. Reinecke et al. (11) were the first to describe substance P-containing nerve fibers in the hearts of mammals in 1980. They reported that the entire coronary arterial system of the guinea pig heart was innervated by substance P-containing nerves. Hougland and Hoover (12) subsequently found that abundant numbers of substance P-containing nerve fibers were also present in the endo-, epi-, and myocardial regions of the atria and ventricles, as well as the mitral and tricuspid valves. Nerve fibers in the myocardial regions tended to run in parallel with cardiomyocytes. Meanwhile, Wharton et al. (13) described identifying more endocardial than epicardial substance P-containing nerves, particularly around the trabeculae and papillary muscles of the ventricles. There are no apparent differences between the left and right ventricles, however, more fibers are

located at the base of the heart than at the apex (12;13). In the ventricular septum, substance P-containing nerve fibers were associated with branches of the bundle of His (13). Substance P-containing fibers are also associated with the ascending aorta and pulmonary trunk (12). Papka and Urban (14) also observed substance P-containing neurons in the epicardium and musculature of the atria, the atrioventricular valves, and pericellular baskets around intrinsic cardiac ganglia. They also identified numerous substance P-containing nerve fibers in the parietal portion of the pericardium. Consistent with these findings, Dalsgaard et al. (15) also found many substance P-containing fibers in the atria, with fewer in the ventricles and mainly associated with blood vessels. Radioimmunoassay analysis of frozen heart samples revealed that substance P levels were roughly four times higher in the right atria compared to the left ventricle (15). Interestingly though, in the left ventricle the levels of substance P were almost identical to the levels of epinephrine (4.2 ± 0.6 v 4.0 ± 0.4 pmol/g, respectively).

2.1.2 Rat—In contrast to the guinea pig, Hougland and Hoover (12) were unable to detect any substance P-containing nerve fibers in the rat heart. Subsequently though, Papka and Urban (14) were able to identify substance P-containing fibers in both the atrial and ventricular epicardium and myocardium of the rat heart. However, these were relatively few compared to the guinea pig heart. Radioimmunoassay analysis has confirmed this difference, with Holzer et al. (16) reporting 0.33 pmol/g of substance P in the rat heart, while Wharton et al. (13) and Dalsgaard et al. (15) reported 2.7 and 4.2 pmol/g respectively in the guinea pig left ventricle.

2.1.3 Mouse—To the authors knowledge there is only one published article describing substance P localization in the mouse heart. This is somewhat surprising considering the wide spread use of mice for studying cardiac disease. Rysevaite et al (17), evaluated substance P-containing nerves in the intrinsic cardiac neural plexus. They found substance P-containing nerves to be most abundant in the epicardium and in ganglia adjacent to the heart hilum (the portion of the heart bounded by the serous pericardium above the heart base, ascending aorta and pulmonary trunk). These nerves were mainly thin, mixed with choline acetyltransferase and tyrosine hydroxylase-containing nerve fibers, and located close to blood vessels. In addition to being identified within the ganglia, substance P-containing nerves were also found in the intrinsic nerve bundles and interganglionic nerves. Since this study was focused on the intrinsic cardiac ganglia, they did not explore substance P-containing nerves within the ventricles of the mouse heart. D'Souza et al. (18) reported 2,807 pg of substance P/mg of protein in mice with myocarditis, compared to 71 pg/mg in uninfected mice. This shows the extent to which substance P can be increased in the heart in a disease state. Similar substance P levels were also reported by Robinson et al. (19), also in mice with myocarditis. Since it was necessary to concentrate the samples for these assays, those values cannot be directly compared to those described for guinea pig and rat hearts.

2.1.4 Felines/Canines—Fewer studies have investigated the spatial location of substance P-containing nerves in the cat and dog heart, and in far less detail. Zhu and Dey (20) described substance P-containing nerves in the atrial and ventricular myocardium as well as

the endocardium in cats. In dogs, radioimmunoassay detection found that the left anterior descending coronary artery and circumflex coronary artery contain substance P (21).

2.1.5 Non-Human Primates—In the interatrial septum, both varicose and non-varicose nerve fibers have been identified as being substance P positive in the cardiac ganglia and musculature of the monkey heart (*Macaca fascicularis*) (22). Substance P-containing nerves were found to form perivascular networks around blood vessels and to traverse muscle fibers.

2.1.6 Humans—In the human heart itself, Wharton et al. (23) reported relatively few substance P-containing neurons, with some occurring mainly around neural cell bodies in intrinsic ganglia and in nerve trunks. Hoover et al. (24) reported that substance P was observed in nerve fibers from the right atrial ganglionated plexus from patients undergoing coronary artery bypass grafting. In endomyocardial biopsies from patients with congestive or hypertrophic cardiomyopathies, Weihe et al. (25) found that all patients had substance P-containing nerve fibers close to arterioles, capillaries and veins. Substance P-containing nerve fibers have also been found surrounding the adventitia of coronary vessels in atherosclerotic regions of human coronary arteries (26). In atrial biopsies taken from patients undergoing open-heart surgery (disease etiology not described), substance P-containing nerves were identified between cardiomyocytes and around blood vessels (27).

2.2 Origins of Substance P-Containing Myocardial Nerves

Bilateral removal of the stellate ganglia resulted in a marked reduction in substance P in the right atria of the guinea pig heart (15). Vagus nerve depletion with capsaicin also decreased substance P in the right atria. Conversely, neither intervention affected substance P in the left ventricle, suggesting separate origins of ventricular substance P-containing nerves that lie outside the stellate ganglia (15). Occlusion of the left anterior descending coronary artery in rats, resulted in an increase in substance P in the T4 region of the spinal cord (28) as determined by microprobes coated in substance P antibody. Similarly, spontaneously hypertensive rats (SHR) were found by immunofluorescence to have more substance P in their dorsal root ganglia than the normotensive Wistar Kyoto rat (WKY) (29). In fact, sensory nerves from the dog ventricle have been traced to dorsal root ganglia in the T₃ region of the spinal cord, with approximately 13% of these dorsal root ganglia (DRG) containing substance P (30). Together, these studies indicate that substance P-containing neurons from the ventricles likely connect with the thoracic region of the spinal cord. Corbett et al. (31) traced cardiac afferent neurons to the nodose ganglion of the vagal nerve and nucleus of the solitary tract (NTS). They identified that approximately 17% of cells in the nodose ganglia were substance P positive, while only a small subpopulation of cardiac afferent axons could be found in the NTS that were substance P positive. Together, these studies serve to highlight the diverse origins of substance P-containing nerves from the heart and the complexity of the neural networks associated with the heart.

2.3 Coronary Artery Endothelial Cell-Derived Substance P

C-fiber sensory neurons are considered the predominant source of substance P. However, capsaicin, which causes selective degeneration of these C-fibers, causes a substantial (60%),

but not complete, loss of substance P in the rat heart (16). This suggests other sources of substance P. A small population (~5-10%) of coronary artery endothelial cells from rat hearts, also contain substance P (32). These cells were usually found in isolation, surrounded by non-substance P-containing endothelial cells. That release of substance P occurred rapidly (one minute) upon subjecting isolated rat hearts to hypoxic conditions suggests that substance P-containing endothelial cells were likely the source.

2.4 Neurokinin Receptors in the Heart

Thompson et al (33) took a pharmacological approach to determining the presence of neurokinin receptor subtypes in intrinsic cardiac neurons in canines. A selective NK-1 receptor agonist reduced activity of right atrial neurons in some animals, while it increased activity in others. A selective NK-2 receptor agonist reduced right atrial neuron activity, while selective stimulation of NK-3 receptors increased activity. Using autoradiography in the guinea pig heart, Hoover and Hancock (34) described substance P binding sites (i.e. receptors) in the parasympathetic ganglia contained within the epicardial connective tissue adjacent to the pulmonary trunk, ascending aorta and right atrium, as well as coronary arteries. They were unable to detect binding sites in the atria, ventricles, ascending aorta and pulmonary trunk. Walsh et al. (35) conducted a radiographic study in Wistar rats and found substance P binding site labeling on clusters of connective tissue cells in the adventitia of the great vessels and coronary arteries, as well as within the connective tissue skeleton of the heart and the cusps of the cardiac valves. They did not find any evidence of substance P binding sites on cardiomyocytes. However, isolated neonatal rat cardiomyocytes have been shown to express genes for the NK-1 and NK-3 receptors, but not NK-2 (36). We have detected the NK-1 receptor on isolated adult cardiac fibroblasts (unpublished data). Thus, while the distribution of substance P-containing neurons may be distinctly localized in the heart, many cells in the heart are capable of responding to substance P as it perfuses the heart.

In summary, there are differences in the number of substance P-containing nerves in the heart between species. Overall, it appears that substance P-containing nerve fibers are located in the intrinsic ganglia of the heart and around coronary vessels. Likely, there are also limited numbers of fibers in the ventricles themselves. Also, a small number of coronary endothelial cells contain substance P. However, it is important to realize that the vast majority of studies have been performed in normal hearts and there is evidence, including in humans, that substance P levels increase in disease. Localization of substance P-containing nerve fibers around the coronary arteries, as well as substance P in coronary artery endothelial cells, means that substance P is ideally placed to be released in response to changes in coronary arterial pressure/flow.

3.0 THE GOOD: ISCHEMIA-REPERFUSION

In 1995 Ustinova et al. (37), depleted rat hearts of sensory nerve neuropeptides with capsaicin and then subjected those hearts to global ischemia (20 minutes) followed by 30 minutes of reperfusion on the isolated heart apparatus. They found that in comparison to non-capsaicin pretreated hearts, capsaicin-treated hearts had reduced recovery of heart rate, coronary flow and left ventricular developed pressure. An important point to note is that in

addition to substance P, capsaicin will cause the depletion of other sensory nerve neuropeptides including calcitonin gene-related peptide (CGRP). Replacement of substance P (1 nM-1 μ M) restored contractile function and coronary flow; the beneficial actions of substance P could be prevented by NK-1 receptor antagonism. Conversely, just a year later, Chiao and Caldwell (38) subjected guinea pig hearts to 15 minutes of global ischemia followed by 60 minutes of reperfusion and found that pretreatment with NK-1 receptor antagonists or capsaicin both significantly improved left ventricular developed pressure and left ventricular end diastolic pressure (LVEDP) following reperfusion. These contradictory findings may relate to differences in the length of ischemia, the time of reperfusion, or to species differences. However, since those initial conflicting reports in the mid 1990's, ischemia-reperfusion models in mice have consistently found that substance P is an important factor in functional recovery of the myocardium during acute reperfusion. Stimulation of the transient receptor potential vanilloid type 1 (TRPV1) on sensory nerves, is responsible for their activation. Using the isolated heart preparation, mouse hearts deficient in the *TRPV1* gene were found to produce less substance P in comparison to the wild type under conditions of 40 minutes of global ischemia followed by 30 minutes of reperfusion (39). *TRPV1*^{-/-} hearts also had an increased LVEDP, reduced developed pressure, and reduced coronary flow. When TRPV1-deficient hearts were perfused with substance P (10⁻⁶ mol/L) initiated prior to ischemia, LVEDP, developed pressure, and coronary flow, were all improved. Conversely, addition of an NK-1 receptor antagonist to wild type mice caused a worsening of these functional parameters. In a follow-up paper, the Wang laboratory tested a preconditioning regimen on isolated perfused hearts from *TRPV1*^{-/-} mice (40). The protocol was three cycles of 5 minutes of ischemia followed by 5 minutes of reperfusion, before 30 minutes of global ischemia and 40 minutes of reperfusion. Preconditioning was less effective in the *TRPV1*^{-/-} hearts with reduced coronary flow, +dP/dt, and developed pressure, as well as an increase in LVEDP. Blockade of the NK-1 receptor in wild type mice subjected to the preconditioning protocol induced almost identical changes in coronary flow, +dP/dt, developed pressure, and LVEDP as had occurred in the *TRPV1*^{-/-} hearts.

Ren et al. (41) investigated the role of substance P in ischemia reperfusion of diabetic rat hearts. Using the isolated heart apparatus, they employed a post-conditioning protocol, whereby, hearts were exposed to 30 minutes of global ischemia, then 5 cycles of 10 seconds of reperfusion and 10 seconds of global ischemia, before a final 40 minute period of reperfusion. In non-diabetic rats, this post-conditioning protocol was effective in attenuating the negative effects of ischemia on LVEDP, +dP/dt and -dP/dt, coronary flow, and developed pressure. However, this protection was lost in the diabetic hearts, except when the post-conditioning was replaced with cycles of substance P infusion (10⁻⁶ mol/L) to mimic the post-conditioning protocol. Since substance P levels increased following post-conditioning in the normal heart, but did not in the diabetic hearts, this would suggest that the benefits of post-conditioning were lost because of the decrease in substance P in the diabetic heart. Infusion of substance P to mimic post-conditioning also led to a decrease in creatine kinase and cardiac troponin I, which indicates a possible reduction in cell death.

The protective effects of substance P in ischemia-reperfusion injury have been attributed to its potent coronary vasodilator actions, which would allow for improved reperfusion. If this is in fact true, then one would expect the infarct size to be reduced in experimental settings where the left anterior descending coronary artery is ligated and then released to allow reperfusion. However, to date, all of the ischemia reperfusion studies have used isolated heart models of global ischemia. We also do not know if substance P is protective only when there is reperfusion following ischemia. If reperfusion is critical then substance P should have no beneficial effects in myocardial infarction models of permanent coronary artery ligation where there is no reperfusion. What happens to substance P levels following permanent myocardial infarction is not clear. Wang et al. (42) reported that substance P levels progressively increase over the first hour in the rat heart following permanent ligation of the left anterior descending coronary artery. Zhang et al. (43) described that myocardial substance P was elevated at 3 hours post-occlusion in the rat, and had returned to normal by 6 hours. That infarct size was not changed by an NK-1 receptor antagonist may argue that reperfusion is required for the beneficial effects of substance P. However, this study was conducted after only 3 hours of infarction and did not measure any functional parameters, therefore, we do not know the acute effects on cardiac function or the long-term effects on both structure and function. Amadesi et al. (44) found elevated serum levels of substance P in the mouse 24 hours after ligation of the left anterior descending coronary artery. This variation in time-line may reflect differences between species, or that a biphasic response exists that includes an immediate release of substance P and then a more sustained up-regulation of the *TAC1* gene.

In summary, accumulated evidence suggests that substance P is protective following ischemia reperfusion, at least acutely. Missing are long-term studies to determine whether substance P continues to be protective after the initial reperfusion period. Also missing are studies of left anterior descending coronary artery ligation and reperfusion rather than global ischemia. Systematic study of myocardial infarction models with no reperfusion are required in order to determine whether reperfusion is critical to substance P protection.

4.0 THE BAD: NON-ISCHEMIC REMODELING

Disease etiology appears to be critical when it comes to the role of substance P in the heart, and the bad side of substance P is seen in long-term non-ischemic myocardial remodeling and heart failure.

4.1 Myocarditis

Parasitic infection of mice for 6 months by injection of *Taenia crassiceps* induces a dilated cardiomyopathy, with substance P and the NK-1 receptor both being increased in these hearts (18). Mice deficient in substance P were protected from adverse remodeling following infection. Individual cardiomyocytes from the substance P-deficient mice were protected against hypertrophy, unlike the wild type cells, which showed a 27% increase in cross-sectional area. Cardiomyocyte apoptosis also occurred in infected wild type hearts, but not in hearts from substance P-deficient mice. Production of pro-apoptotic cytokines by substance P was put forth by the authors as a possible mechanism leading to hypertrophy.

Substance P was also elevated (~60-fold) in hearts of mice infected with the encephalomyocarditis virus (EMCV) for 14 days (19). Similarly to parasite infected hearts, EMCV-infected hearts showed whole organ hypertrophy, increased cardiomyocyte cross-sectional area and apoptosis; hearts from substance P-deficient mice were protected against all of these. EMCV infected hearts exhibited obvious inflammation and necrosis that was also prevented in substance P-deficient mice. Importantly, while 14 day mortality was 51% in infected wild type mice, no deaths occurred in the substance P-deficient group. Thus, both parasite and viral infection of the heart results in dramatic increases in myocardial substance P levels that appears to regulate cardiomyocyte death, hypertrophy, necrosis and inflammation.

4.2 Volume Overload

Using a model of chronic volume overload-induced heart failure (28 days), our laboratory recently found that deletion of the gene for substance P (*TAC1*) protected mice from developing left ventricular hypertrophy in the form of ventricular dilatation (45). Furthermore, unlike the wild type, there was no increase in right ventricular mass or lung weight in the *TAC1*^{-/-} mice, indicating protection from heart failure. The mechanisms of protection involved prevention of matrix metalloproteinase (MMP) activation and subsequent collagen degradation. Unlike in myocarditis, cell death did not appear to be an important factor in our model. We were also able to determine in rats that substance P could activate cardiac mast cells via the NK-1 receptor following volume overload (3 days), and that TNF- α levels were prevented from increasing in these rats (45). Mast cells are important for initiation of MMP activation and collagen degradation in this model (46;47).

4.3 Magnesium Deficiency

Weglicki et al. (48) found that circulating substance P levels were elevated during magnesium deficiency in rats. They subsequently found that substance P was increased in the cardiac lesions of mice suffering from magnesium-deficiency (49), and that blockade of the NK-1 receptor significantly reduced TNF- α and IL-1 levels, but not IL-6 within the lesions (49). Further, blockade of the NK-1 receptor in hypomagnesemia rats improved diastolic and systolic function, as determined by the E/A ratio and fractional shortening, respectively (50). Unlike other ischemia reperfusion studies, antagonism of NK-1 receptors following 30 minutes of global ischemia and 30 minutes of reperfusion using the isolated heart apparatus, resulted in improved developed pressure and cardiac work in magnesium-deficient hearts (51). Lactate dehydrogenase and lipid hydrogen peroxide levels were decreased in the presence of the NK-1 receptor antagonist indicative of reduced tissue damage.

4.4 Hypertension/Pressure Overload

To date there are no studies that have investigated whether substance P plays a role in myocardial remodeling and heart failure due to hypertension or pressure overload. If, like we found in volume overload, substance P regulates MMP activation, could substance P be the stimulus for the transition from compensated remodeling to heart failure in the hypertensive heart? Or, given its presence in sensory nerves and endothelial cells, substance

P may be released early in response to increases in coronary pressure and thereby initiate the hypertrophic and fibrotic responses that occur in the hypertensive heart. We have preliminary data to show that *TAC1* is up-regulated in the SHR heart as blood pressure increases (unpublished data).

5.0 DIRECT EFFECTS ON CARDIOMYOCYTES

Interactions between DRG containing substance P and cardiomyocytes certainly can occur. In co-cultures of isolated rat DRG and neonatal rat cardiomyocytes, DRG projections made connections with cardiomyocytes and more substance P (and CGRP)-containing neurons were present than in cultures without cardiomyocytes (52). Interestingly, capsaicin caused the release of more substance P (and CGRP) when cardiomyocytes were present in co-culture than when they were absent. While we have discussed the possible effect of substance P on myocardial hypertrophy *in vivo* (see section 4.0), to our knowledge only one study has investigated the direct effects of substance P on cardiomyocytes. Church et al. (36) demonstrated that neonatal rat cardiomyocytes express genes for both the NK-1 and -3 receptors, but not the NK-2 receptor. Further pharmacologic studies supported the presence of functional NK-1 and -3 receptors. In these studies, substance P (NK-1 receptor) and neurokinin B (NKB; NK-3 receptor) both induced synthesis of atrial natriuretic peptide (ANP), an established marker of pathological cardiomyocyte hypertrophy. In the case of substance P, PKC mediated the release of prostaglandins, which were necessary for ANP release. It is important to keep in mind that these experiments were performed in neonatal cardiomyocytes, which may not be fully reflective of adult cardiomyocyte characteristics. However, coupled with the *in vivo* findings in adult mice described in section 4.0, these findings suggest that substance P can induce hypertrophy via direct actions on cardiomyocytes.

6.0 DIRECT EFFECTS ON CARDIAC FIBROBLASTS

Substance P is well known as a mediator of inflammation and fibroblasts are capable of producing chemokines and adhesion molecules critical to this process. To this end, Sapna et al. (53) found that 1 and 10 μM of substance P was capable of inducing soluble ICAM-1 (sICAM-1) production by adult rat cardiac fibroblasts, via a p42/44 MAPK and PKC mechanism. sICAM-1 is a product of cleavage of ICAM-1 and may have anti-inflammatory properties since cleavage of ICAM-1 to form sICAM-1 may affect the amount of membrane bound ICAM-1 available for interaction with ligands (54). Further, inhibitors that blocked ICAM-1 shedding increased monocytic cell adhesion to stimulated endothelial cells (54). These anti-inflammatory effects would seemingly be in opposition to substance P established role as a pro-inflammatory neuropeptide, however, sICAM-1 can also be pro-inflammatory in nature, capable of inducing macrophage inflammatory protein-2 synthesis by astrocytes (55) and alveolar macrophages (56), as well TNF- α synthesis by alveolar macrophages (56). Substance P can also induce PGE₂ production by cardiac fibroblasts (53). COX-2-generated PGE₂ is elevated following myocardial infarction (57), and PGE₂ induces proliferation in rat neonatal fibroblasts (58).

Adult rat cardiac fibroblasts proliferate via a process involving generation of superoxide in response to substance P (10 μ M), however, substance P reportedly does not induce collagen synthesis by these cells (59). No studies have evaluated the effects of substance P on MMP production by cardiac fibroblasts. However, substance P, acting via the NK-1 receptor, has recently been shown to cause reduced collagen synthesis, increased collagen degradation and increased levels of MMP-1 in cultured human lung fibroblasts (60), and gingival fibroblasts from healthy humans have been shown to increase mRNA and protein levels for MMP-1, -2, -3, 7 and -11 as well as TIMP-2 in response to substance P (61). Further studies are required to better investigate the ability of substance P and the NK-1 receptor to regulate cardiac fibroblast function.

7.0 SUBSTANCE P REGULATION OF INFLAMMATORY CELLS IN THE HEART

There is extensive literature demonstrating that substance P is a mediator of neurogenic inflammation and up-regulator of pro-inflammatory cytokines. However, while Robinson et al. (19) found that deletion of the *TAC1* gene prevented the infiltration of inflammatory cells into the heart in their mouse viral myocarditis model (see section 4.1), very little work has investigated the direct effects of substance P on inflammatory cells in the heart. Roberto Levi's group demonstrated that substance P is released together with renin following 20 minutes of global ischemia and 30 minutes of reperfusion in the isolated guinea pig heart (62). The release of renin could be prevented by an NK-1 receptor antagonist or a mast cell stabilizer, suggesting that substance P stimulates the release of renin from cardiac mast cells. Levi's group went on to show that human mastocytoma cells (a cell line model of mast cells) are activated by substance P (30 nM) and release renin in response to substance P (300 and 1000 nM), via the NK-1 receptor. While it is important to remember that this cell line may not be totally representative of primary cardiac mast cells, our findings in volume overload-induced heart failure (see section 4.2), as well as those of Bot et al. (63) in atherosclerosis, support substance P activation of cardiac mast cells *in vivo*. Bot et al. demonstrated that substance P increased mast cell activation in the perivascular region of coronary arteries of mice and promoted intraplaque hemorrhages via this interaction, while we found that mast cell density did not increase in rats treated with an NK-1 receptor blocker (45); mast cell activation is necessary for mast cell density to increase (64). In further support of the Levi groups findings that substance P induces mast cell release of renin, we found that substance P (100 μ M) caused production of angiotensin II by a mixed population of isolated rat cardiac inflammatory cells (T cell, mast cells and macrophages) (65). We have also shown that substance P (100 μ M) can induce TNF- α production by this mixed population of cardiac inflammatory cells (45).

In an interesting paper, Weglicki's group found that substance P activation of the NK-1 receptor regulated the oxidative stress response and neutral endopeptidase (NEP) levels by neutrophils in the hearts of magnesium-deficient rats (50). More specifically, they found that NK-1 receptor blockade dramatically reduced superoxide production by neutrophils isolated from hearts of magnesium-deficient rats. Additionally, NEP was decreased in neutrophils from magnesium-deficient hearts, however, activity was partially restored by NK-1 receptor

blockade. Regulation of NEP by substance P provides an interesting mechanism by which substance P can regulate its own levels since NEP degrades substance P. Therefore, by down-regulating NEP substance P can sustain its own increased levels.

Taking from studies not related to the heart, murine peritoneal macrophages have the NK-1 receptor and both IL-4 and IFN- γ increase mRNA and protein for this receptor (66). Interestingly, we recently found that both IL-4 and IFN- γ are increased in the hearts of SHR (67), thus, it is tempting to speculate that the increased levels of these cytokines would provide an environment whereby NK-1 receptor density is increased in the hypertensive heart. Li et al. (68) proposed that substance P initiates mast cell activation via the NK-1 receptor in dermal tissue. However, a study reported by Lorenz et al. (69) suggests that substance P uptake in mast cells is rapid and independent of the NK-1 receptor, resulting in exocytosis of inflammatory compounds. The discrepancy may be due to the concentration and the mode of substance P treatment by each group; Lorenz et al. applied substance P directly to mast cells in culture whereas Li et al. looked at mast cell activation in vivo. We have shown that cardiac mast cell activation by substance P can be blocked by NK-1 receptor antagonism in vitro (45), despite high concentrations of substance P (30-100 μ M) being required to cause histamine release. It is likely that substance P-induced degranulation of mast cells (histamine release) may require higher concentrations than secretion of other products. For example, we have unpublished data showing that substance P causes tryptase release at 100 nM in bone marrow-derived mast cells. It is unambiguous though that substance P can initiate calcium signaling in mast cells via the PLC pathway. Yet it is unclear what signaling events occur downstream of PLC resulting in mast cell activation by substance P in the heart. In macrophages, substance P activates the ERK1/2, P38 MAPK, and PI3K-AKT pathways downstream of PKC resulting in NF- κ B transactivation and release of chemokines (70;71). Additionally, NF- κ B transactivation and subsequent up-regulation of cytokines by substance P is dependent on the NK-1 receptor in astrocytoma cells (72).

Despite a wealth of strong animal data supporting a role for substance P in neurogenic inflammation in many pathologies, whether this occurs in humans is less clear. Thus far, several clinical trials investigating NK-1 receptor antagonism in inflammation type pathologies have found little success. For example, a dual NK-1/NK-2 receptor antagonist was ineffective in preventing allergen-induced airway responses in patients with asthma, although no clear assessment of effects on inflammatory cells was able to be made (73). In a small trial, NK-1 receptor blockade failed to lower incidence of post-endoscopic retrograde cholangiopancreatography pancreatitis (74).

8.0 CLINICAL IMPLICATIONS

The concept that substance P may be at the tip of controlling the entire adverse myocardial remodeling response in a variety of cardiac pathologies, is an exciting new line of investigation. Conceptually it makes sense in relation to its location in sensory nerves at the coronary vasculature and endothelial cells in coronary arteries and arterioles. With experimental animal models indicating a good and bad side for substance P when it comes to cardiac remodeling and function, what could this mean for human patients? One is tempted

to envisage a scenario where treatment could be tailored specifically to etiology or stage of remodeling, where administration of substance P, or substance P analogues, may be beneficial during reperfusion therapy immediately following ischemic injury, and blockade of the NK-1 receptor could be beneficial longer term and in heart failure. Certainly support of a role for substance P can be found in humans, where plasma levels are elevated in patients with congestive heart failure (NYHA class I-IV) (75). Also, Kambam et al. (76) found that patients with angina pectoris had higher levels of substance P than patients without angina, however, they could only detect substance P in pericardial fluid and not plasma, indicating that in some instances plasma may not be a good marker of substance P due to its localized release. The targeting of substance P is very exciting, however, it is early days and many more studies both in animals and humans are needed to fine tune this concept. Even ischemic injury eventually leads to remodeling of the remote region of the myocardium and at that stage substance P may no longer be beneficial, but may in fact become detrimental. Because none of the ischemia reperfusion studies in experimental animals have gone beyond 60 minutes of reperfusion, we just don't know the long-term effects of substance P in this setting. Timing may be everything. Further, these studies have all used models of global ischemia rather than ligation of coronary arteries. Also, it is unclear if reperfusion is what induces the beneficial actions of substance P since we do not yet have clear experiments with myocardial infarction without reperfusion. The complexities of substance P were demonstrated in patients with non-ischemic dilated cardiomyopathy. While intracoronary infusion of substance P did cause a drop in peak systolic pressure (121 ± 8 to 111 ± 7 mmHg) and also end diastolic pressure (25 ± 3 to 18 ± 2 mmHg) (77), those patients with elevated end diastolic pressures >16 mmHg responded to substance P infusion with increased end diastolic volumes, stroke volume, and stroke work. Nonetheless, what makes this new paradigm so exciting is the availability of NK-1 receptor antagonists, which are currently in clinical use for chemotherapy-induced nausea. Further, they continue to be trialed clinically with promising results for numerous other uses including, prevention of pruritus related to cancer treatment (78), the prevention of post-operative nausea and vomiting (79;80), and depression (81). NK-1 receptor antagonists have also been trialed with little success for migraine (82;83) and painful diabetic neuropathy (84). We would argue that there is a need to aggressively pursue this avenue of research, and should the experimental evidence continue to demonstrate the importance of sensory nerves and substance P in particular, modulation of this pathway may represent a treatment strategy for the prevention of heart failure in the foreseeable future.

9.0 SUMMARY

The role of substance P in adverse myocardial remodeling and heart failure has been understudied to date. However, the cumulative evidence that has been collected so far suggests that substance P has two distinct effects in response to insult or overload on the myocardium. Short-term, substance P provides important vasodilatory effects that presumably appear to be protective initially by increasing myocardial reperfusion, as demonstrated by ischemia reperfusion studies (Figure 1 and 2). Conversely, long-term up-regulation of substance P appears to induce detrimental responses in the form of inflammation, apoptosis, MMP activation, and changes to the extracellular matrix, as

observed in myocarditis, volume overload, and magnesium-deficiency (Figure 1 and 2). Thus, the effects of substance P in the heart are very complex and it is still not clear if it is as simple as short-term versus long-term responses, or if the type of effect is related to disease etiology (i.e. ischemic versus non-ischemic). Clearly substance P is an important player in adverse remodeling of the heart, however, a more intense effort is required to unravel the intricacies of its actions as well as the mechanisms behind these actions.

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Figure 1. Schematic indicating the cardiac pathologies in which substance P is protective (ischemia reperfusion) and detrimental (myocarditis, volume overload, hypomagnesemia) and the possible mechanisms behind these responses. MMP, matrix metalloproteinase.

Figure 2. Schematic indicating the short-term and long-term substance P/TAC1 responses and subsequent outcomes in response to altered coronary flow/ventricular wall stress.

Table 1

Localization of substance P in the heart by species.

Species	Substance P Localization	References
Guinea Pig	Blood vessels, atria, ventricle (endocardium, epicardium, musculature), valves, papillary muscle, bundle of His, intrinsic cardiac ganglia	11, 12, 13, 14, 15
Rat	Atria, left ventricle (epicardium, musculature), blood vessels	14, 16
Mouse	Intrinsic cardiac ganglia, intrinsic nerve bundles	17
Feline	Atria, ventricle (epicardium)	20
Canine	Left anterior descending coronary artery, circumflex artery	21
Non-Human Primate	Atria, intrinsic cardiac ganglia, blood vessels, musculature	22
Human	Intrinsic cardiac ganglia, blood vessels, musculature	23, 24, 25, 26, 27

The major areas of localization of substance P in the heart by species.