REVIEW —Current Perspective—

Actions of Tachykinins Within the Heart and Their Relevance to Cardiovascular Disease

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ABSTRACT—Substance P and neurokinin A are tachykinins that are co-localized with calcitonin gene-related peptide (CGRP) in a unique subpopulation of cardiac afferent nerve fibers. These neurons are activated by nociceptive stimuli and exhibit both sensory and motor functions that are mediated by the tachykinins and/or CGRP. Sensory signals (e.g., cardiac pain) are transmitted by peptides released at central processes of these neurons, whereas motor functions are produced by the same peptides released from peripheral nerve processes. This review summarizes our current understanding of intracardiac actions of the tachykinins. The major targets for the tachykinins within the heart are the intrinsic cardiac ganglia and coronary arteries. Intrinsic cardiac ganglia contain cholinergic neurons that innervate the heart and coronary vasculature. Tachykinins can stimulate NK₃ receptors on these neurons to increase their excitability and evoke spontaneous firing of action potentials. This action provides a mechanism whereby tachykinins can indirectly influence cardiac function and coronary tone. Tachykinins also have direct effects on coronary arteries to decrease or increase tone. Stimulation of NK₁ receptors on the endothelium causes vasodilation mediated by nitric oxide. This effect is normally dominant, but NK₂ receptor-mediated vasoconstriction can also occur and is augmented when NK₁ receptors are blocked. It is proposed that these ganglion stimulant and vascular actions are manifest by endogenous tachykinins during myocardial ischemia.

Keywords: Tachykinin, Cholinergic neuron, Coronary artery, Cardiac afferent, Myocardial ischemia

Substance P (SP) and neurokinin A (NKA) are prominent members of the tachykinin family of neuropeptides (1, 2). Both of these peptides have a wide distribution in the periphery where they are co-localized with calcitonin gene-related peptide (CGRP) in a special class of nociceptive neurons that has both afferent and efferent functions (2–4). Afferent (i.e., sensory) functions are mediated by tachykinins and CGRP that are released from nerve processes terminating in the central nervous system, whereas efferent (i.e., motor) functions are exerted by the same neuropeptides released from peripheral processes. These neurons have also been implicated in axon reflex responses whereby activation of sensory nerve endings at one site evokes responses at a distant site in the same tissue (2, 4). Such axon reflexes occur because action potentials gener-

ated at the site of stimulation invade collateral nerve processes of the sensory-motor neurons as well as their central projections. This review summarizes our current understanding of tachykinin actions within the heart and the potential role of endogenous tachykinins in cardiac pathophysiology.

Localization of sensory neuropeptides and their receptors within the heart

SP/CGRP-containing sensory-motor nerve fibers within the heart derive from cell bodies located in the dorsal root ganglia and nodose ganglia. While dorsal root neurons are the primary source of sensory-motor innervation to the guinea pig heart (5), projections from the nodose ganglia are also important in monkeys (6). Peripheral processes of these SP/CGRP-containing neurons are localized to the intrinsic cardiac ganglia, coronary arteries and several regions of the heart including nodal tissue, the conducting

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Fig. 1. Photomicrograph demonstrating innervation of intracardiac ganglion by SP-immunoreactive nerve fibers. Whole mount preparation of ganglionated plexus from guinea pig heart was stained using rabbit anti-SP antibody (1:800, DiaSorin) and the ABC immunohistochemical technique (Vector) with diaminobenzidine as the chromogen. SP-immunoreactive nerve fibers are evident in a nerve bundle (N) and surrounding neurons in a ganglion (G). Scale bar = 40 μ m. (Unpublished data by authors)

system and cardiac contractile tissues (7–9). Within the ganglia, SP- and CGRP-immunoreactive nerve processes surround many of the principal neurons (Fig. 1). The latter cells include cholinergic neurons that innervate the heart and coronary arteries. Although a few intracardiac neurons contain SP and CGRP, most SP/CGRP-containing nerve fibers in the heart have a sensory-motor origin since they degenerate after animals are treated with the selective neurotoxin capsaicin (3, 5).

Autoradiographic studies from this laboratory have localized and quantified receptors for tachykinins and CGRP within the guinea pig heart (10–12). Specific binding sites for [125 I]Boulton Hunter SP and [125 I]NKA are restricted to the intrinsic cardiac ganglia and coronary vessels. No tachykinin binding sites were detectable in either atrial or ventricular myocardium. In marked contrast, specific binding sites for CGRP have a broader distribution including atrial and ventricular myocardium, the conducting system, coronary arteries and intracardiac ganglia.

Cardiac responses to SP and NKA

The pharmacologic actions of SP and NKA on the heart have been most thoroughly studied in the guinea pig. SP decreases heart rate and ventricular contractility in isolated, spontaneously beating hearts from this species (10, 11, 13, 14). Negative chronotropic and inotropic effects have likewise been reported for SP in experiments with the isolated right atrial preparation (14). We found that bradycardic responses of isolated hearts to SP were blocked by atropine and potentiated by treatment with a cholinesterase inhibitor (13). Based on these observations, it has been concluded that cholinergic neurons mediate the negative

chronotropic response to SP. Other investigators have strengthened this concept by demonstrating that bradycardic responses to SP were likewise attenuated after endogenous acetylcholine had been depleted by vagal stimulation in the presence of hemicholinium-3 (14). Negative inotropic responses were also attenuated following muscarinic receptor blockade or treatment with hemicholinium, suggesting that tachykinins can likewise stimulate cholinergic neurons that influence ventricular contractility. Thus, there is clear functional evidence that SP can affect cardiac function indirectly by stimulating cholinergic neurons.

Recent work has demonstrated that NKA has a greater potency than SP for producing bradycardia in isolated guinea pig hearts, but a major portion of this response was unaffected by atropine (11). Accordingly, cholinergic neurons play a much smaller role in chronotropic responses to NKA. The mechanism responsible for the non-cholinergic component of the negative chronotropic response to NKA is unresolved at present. Adenosine has been eliminated as the mediator since the response was unaffected by adenosine receptor blockade with theophylline. Infusion of NKA causes desensitization to NKA and SP without affecting the negative chronotropic response to acetylcholine. These observations support the conclusion that NKA produces non-cholinergic bradycardia by a mechanism involving tachykinin receptors rather than by a non-specific suppression of the cardiac pacemaker. Although tachykinin receptors were undetectable in the myocardium in autoradiographic experiments, it remains possible that they are present at a low density below the limit for detection. If so, then non-cholinergic bradycardia may be a direct effect of NKA at the sinoatrial node.

NKA also differs from SP by augmenting ventricular contractions in spontaneously beating guinea pig hearts (11). Since this effect was replaced by a small negative inotropic response when hearts were paced to keep rate constant, it was concluded that positive inotropic responses to NKA occur secondarily to the large bradycardia normally evoked by the same dose of this peptide. Thus, SP and NKA have similar effects to decrease ventricular contractility in paced hearts. Since autoradiographic studies have not detected tachykinin receptors in the ventricular myocardium, such negative inotropic responses probably occur indirectly. Stimulation of cholinergic neurons is one mechanism already mentioned for tachykinins to decrease ventricular contractility. It is also feasible that tachykinins might affect ventricular contractile function by a paracrine mechanism involving peptide-evoked release of nitric oxide from microvascular endothelial cells. The latter mechanism has been proposed to explain ventricular contractile responses to SP in isolated ejecting guinea pig hearts (15) and in humans (16). According to this scheme, nitric oxide diffuses to nearby myocytes where it acts to facilitate relaxation. In humans, this action is manifest as a decrease in left ventricular peak systolic pressure.

Stimulation of intracardiac neurons by tachykinins

Several studies have demonstrated that tachykinins can directly affect neurons in intracardiac ganglia from guinea pigs (17–20). Application of SP or NKA to these cells evokes a dose-dependent slow depolarization by activation of a non-specific cation conductance (19). Tachykinins also produce an increase in neuronal excitability that occurs independent of the membrane depolarization. It is particularly noteworthy that SP can trigger bursts of action potentials from intracardiac neurons (17, 18). In a recent study (20), we found that a maximally effective dose of SP excited all intracardiac neurons evaluated and evoked bursts of greater than 20 action potentials from about 40% of these cells (Fig. 2). These observations support the conclusion that SP causes bradycardia by stimulating cholinergic neurons located in the intracardiac ganglia. It is also possible that SP can facilitate ganglionic transmission in the heart, as it does in sympathetic ganglia (2), but evidence for such a neuromodulator action is lacking at present.

Effects of SP and NKA on the coronary vasculature

SP has potent coronary vasodilator actions in many species including dogs (21), guinea pigs (13, 22), pigs (23), rabbits (24) and humans (25, 26). This effect occurs with conduit and resistance vessels. Coronary vasodilator responses to SP are endothelium-dependent and mediated by nitric oxide (22, 23, 25). NKA produces coronary vasodilation as well but its potency is significantly lower than that of SP (22, 23). Additionally, NKA also has the ability to produce

Fig. 2. Intracellular recording from guinea pig intracardiac neuron that fired multiple action potentials after pressure ejection of SP (100 μ m, 1-s pulse). **A:** Slow depolarization and burst of action potentials evoked by application of SP (arrow). **B:** Burst of action potentials shown with expanded time scale. (Unpublished data by authors)

constriction of coronary resistance vessels in the guinea pig (11, 22). Bolus injections of NKA often evoke biphasic changes in perfusion pressure in isolated beating guinea pig hearts. In such cases, a small pressure increase, indicating constriction of resistance vessels, follows a larger depressor response. Potassium-arrested guinea pig hearts normally yield pure vasodilator responses to NKA, but these are replaced by a prominent vasoconstriction after desensitization with SP (22). This observation suggests that NKA produces vasoconstriction of coronary resistance vessels through stimulation of different tachykinin receptors from those which mediate vasodilation. Nevertheless, vasodilation is the dominant effect of NKA when the peptide is delivered to the luminal surface of resistance vessels (11) and the only response evoked by application of tachykinins to epicardial coronary arteries (23).

Tachykinin receptor subtypes

Three subtypes of the tachykinin receptor have been identified using classic pharmacological techniques as well as molecular cloning methods (1, 2). These subtypes are designated NK₁, NK₂ and NK₃. Although SP and NKA can activate each of these receptors, they differ in their affinity for specific subtypes. The rank order of affinity for SP at tachykinin receptors follows the pattern NK₁ > NK₂ ≈ NK₃, whereas that for NKA is NK₂ > NK₁ ≈ NK₃. Functional studies with subtype-selective agonists and antagonists have provided evidence that each of these tachykinin receptors is present within the heart.

Dose-dependent cardiac and coronary effects occur with selective agonists for NK₁, NK₂ and NK₃ receptors in the isolated guinea pig heart preparation (27). Although each tachykinin agonist produced some degree of bradycardia, responses to NK₂ and NK₃ agonists were more prominent. The NK₂-selective agonists also increased the amplitude of ventricular contractions and caused dose-dependent constriction of coronary resistance vessels. Positive inotropic responses to NK₂ agonists in the isolated heart probably occur secondarily to bradycardia, as demonstrated in previous experiments for NKA (11). In contrast, vasoconstriction could be a direct effect mediated by NK₂ receptors on coronary smooth muscle. NK₁ agonists were the most potent in causing coronary vasodilation and often caused a small decrease in the amplitude of ventricular contractions as observed with SP (11, 14).

Responses of the isolated guinea pig heart to the NK₂ agonist GR64349 are unaffected by atropine, whereas treatment with atropine prevented negative chronotropic and

coronary vasodilator responses to the NK₃ agonist senktide (Fig. 3). The latter findings provide clear evidence that cholinergic neurons mediate the negative chronotropic response triggered by NK₃-receptor activation, while the NK₂-receptor-mediated bradycardia occurs by a non-cholinergic mechanism. Other investigators had previously demonstrated that NK₃-receptor blockade with SR142801 markedly reduced the amplitude of slow depolarization produced by SP in guinea pig intracardiac neurons (19). Our results with senktide (Fig. 3B) also provide the first evidence that NK₃-receptor stimulation causes coronary vasodilation by a cholinergic mechanism. Direct parasympathetic effects on the coronary vasculature have been demonstrated for several species and can influence both conduit and resistance vessels (28). Cholinergic vasodilator responses, as evoked by senktide, result from activation of muscarinic receptors on the vascular endothelium.

Most of the negative chronotropic response to NKA occurs through stimulation of NK₂ receptors that may be localized to the atrial myocardium. The remainder occurs through stimulation of NK₃ receptors on cholinergic neurons. Vasodilator responses to NKA are mediated by NK₁ receptors since they are eliminated by treatment with the NK₁ antagonist FK888 or desensitization with an NK₁-receptor agonist (27). These treatments also cause augmentation of the coronary vasoconstrictor response to NKA. The latter effect is mediated by NK₂ receptors that are probably localized to coronary smooth muscle. However, autoradiographic studies using the selective ligand [³H]SR48649 have not revealed NK₂ sites in the guinea pig heart or coronary vasculature (our unpublished observations). Accord-

Fig. 3. Effect of atropine on chronotropic and coronary vascular responses to selective NK₂ and NK₃ receptor agonists in isolated perfused guinea pig hearts. **A:** Responses to bolus injections of 32 nmol GR64349 were determined in the same hearts before (control) and during treatment with 1 μ M atropine ($n = 7$). Atropine did not affect the bradycardic, coronary vasoconstrictor or positive inotropic (not shown) response to GR64349. **B:** Since senktide (32 nmol) causes a prolonged desensitization of NK₃ receptors, control responses and responses in the presence of atropine were determined in separate hearts ($n = 6$, each). Atropine prevented negative chronotropic and vasodilator responses to senktide ($*P < 0.01$). Values are mean $\pm$ S.E.M. (Unpublished data by authors)

Although there is clear evidence that endogenous CGRP can evoke tachycardia and increase atrial contractility (3, 30, 34), less is known regarding effects of endogenous tachykinins on cardiac function. Investigation of this problem has been difficult since the tachykinins are co-localized with CGRP and effects of this peptide generally dominate when pharmacological agents are used to stimulate cardiac afferents. At the intrinsic cardiac ganglia, however, the excitatory action of tachykinins is much greater than that evoked by CGRP (35). Accordingly, early work in this field established that a SP antagonist could suppress slow depolarization of intracardiac neurons evoked by repetitive nerve stimulation (17). More recently, we were able to demonstrate that endogenous tachykinins can cause bradycardia in isolated guinea pig hearts by stimulating cholinergic neurons (36). For this study, capsaicin was used to activate sensory-motor nerve fibers in the heart and CGRP₈₋₃₇ was used to block responses to neurally-released CGRP. Under these conditions, capsaicin evoked a small bradycardia. The negative chronotropic response to capsaicin

Fig. 4. Diagram demonstrating proposed actions of endogenous tachykinins released from intracardiac sensory-motor neurons. Chemicals released during myocardial ischemia stimulate sensory-motor nerve endings to cause firing. Substance P (SP) and neurokinin A (NKA) are released at the site of ischemia and at central and collateral processes on the same neuron. Tachykinins released from collateral fibers mediate intracardiac axon reflexes. Collateral nerve fibers could supply intrinsic cardiac ganglia (shown) and coronary arteries. ACh, acetylcholine; M₂ and M₃, muscarinic receptor subtypes; N, nicotinic receptor; NK₁, NK₂ and NK₃, tachykinin receptor subtypes.

was markedly enhanced by the further addition of a cholinesterase inhibitor. Such responses were prevented by treatment with atropine or prior desensitization of tachykinin receptors with NKA. Thus, both endogenous tachykinins and cholinergic neurons were implicated in production of the bradycardia. Since SP-immunoreactive nerve fibers supply most ganglia within the heart, it is likely that endogenous tachykinins also have the ability to stimulate cholinergic neurons that regulate cardiac conductile and contractile tissue as well as those affecting coronary vascular tone.

Conclusions and clinical implications

Our current model for the function of tachykinins within the heart is based on the assumption that cardiac afferents containing these peptides exhibit a branching pattern that would enable axon reflexes. According to this scheme (Fig. 4), afferent nerve fibers within the myocardium and around coronary vessels are stimulated to fire action potentials when exposed to chemicals released during myocardial ischemia. This causes local release of SP and NKA at the site of stimulation. Both of these tachykinins could activate NK₁ receptors on vascular endothelial cells, causing the release of nitric oxide and relaxation of adjacent coronary smooth muscle. There is some evidence, however, that coronary vasodilator responses to SP can be impaired in patients with cardiovascular disease (25, 26). Under this condition, the NK₂-receptor-mediated coronary vasoconstrictor action of NKA might be manifest. If NK₂ receptors are localized to coronary smooth muscle cells, as expected, then vasoconstriction would also be favored by the closer proximity of these cells to the sensory-motor nerve endings. Nitric oxide might also diffuse from the microvasculature to act on adjacent cardiac muscle. This mechanism is supported by experimental evidence that NK₁-receptor activation contributes to myocardial stunning that occurs in isolated guinea pig hearts after ischemia and reperfusion (37).

Action potentials initiated in afferent processes at the ischemic region would travel to the central nervous system where SP and NKA are likewise released. Since tachykinins have been implicated as neurotransmitters for pain (2), it is possible that this pathway plays a role in signaling cardiac pain. Lastly, action potentials in sensory-motor fibers would also invade collateral processes within the heart. The distribution pattern for these collaterals would dictate the profile of tachykinin-mediated responses. Since tachykinin receptors are localized to coronary vessels and intrinsic cardiac ganglia, the occurrence of axon reflex responses at these sites is likely. Vascular responses evoked at collateral sensory motor nerve endings would presumably be identical to those occurring at the site of stimulation. Tachykinins would have an excitatory effect within the intrinsic cardiac ganglia by stimulating NK₃ receptors on the post-

ganglionic neurons. Depending on the amount of tachykinins released, they could enhance responses to vagal stimulation or directly evoke neuronal firing. Activation of cholinergic neurons within specific ganglia could cause bradycardia, decreased cardiac conduction velocity and reduction in ventricular contractility (38).

It is known that a significant number of patients experience severe bradycardia and hypotension after myocardial infarction or during coronary occlusion (39). Such responses have been associated with sudden death. Activation of intracardiac neurons by a short-loop reflex involving endogenous tachykinins could play an important role in such cases of extreme bradycardia. Furthermore, activation of cholinergic neurons that innervate coronary vessels could have detrimental effects on cardiac perfusion. It is well documented that intracoronary injection of acetylcholine can produce vasospasm in patients with coronary artery disease (40). This effect occurs because endothelium-dependent vasodilator responses are impaired while the direct constrictor action of acetylcholine on coronary smooth muscle persists. Thus, intracardiac axon reflexes involving tachykinins provide a neural mechanism whereby ischemia could trigger vasospasm. Since the excitatory effect of tachykinins on intracardiac cholinergic neurons occurs through activation of NK₃ receptors, selective antagonists for this receptor subtype may be useful for blocking cholinergic responses evoked by tachykinins without affecting vagal efferent control of the heart.

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