Characterization of responses to neurokinin A in the isolated perfused guinea pig heart

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Hoover, Donald B., Yingzi Chang, and John C. Hancock. Characterization of responses to neurokinin A in the isolated perfused guinea pig heart. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1803–R1811, 1998.—Goals of this study were to identify and characterize effects of neurokinin A (NKA) in isolated guinea pig hearts. Bradycardia, augmentation of ventricular contractions, and reduction of perfusion pressure were prominent responses to bolus injections of NKA (0.25–25 nmol). NKA was more potent than substance P (SP) in causing bradycardia but did not differ in potency for lowering perfusion pressure. Doses of SP of 25 nmol or less decreased ventricular force, whereas 100 nmol caused a biphasic response. The percent decrease in heart rate produced by 25 nmol NKA was reduced from 58.0 ± 4.8 to 39.6 ± 3.5% in the presence of 1 µM atropine (n = 5). The positive inotropic response to 25 nmol of NKA in spontaneously beating hearts was replaced by a negative inotropic response during pacing (22.5 ± 3.3% increase vs. 11.7 ± 1.7% decrease, n = 5). Reserpine pretreatment did not affect the positive inotropic response to NKA. Specific binding sites for 125I-labeled NKA were localized to intracardiac ganglia and coronary arteries but not to myocardium. It was concluded that 1) negative chronotropic responses to NKA involve cholinergic and noncholinergic mechanisms, and 2) the positive inotropic response is an indirect action.

Many cardiac afferent nerves contain the tachykinin substance P (SP) (30–33). These nerves are especially abundant in the intrinsic cardiac ganglia and adventitia of coronary arteries. The tachykinin neurokinin A (NKA) has also been detected in the heart (7). This tachykinin is encoded by the same gene as SP and is colocalized with SP in a large population of primary afferent nerve fibers (7, 24). Tachykinin-containing neurons and nerve fibers have been characterized as being sensitive to stimulation by the neurotoxin capsaicin (7, 19, 21, 24). It is now accepted that capsaicin-sensitive neurons have classical afferent functions through release of neuropeptides from central nerve processes and efferent functions through release from peripheral nerve processes (7, 21, 24). Studies with isolated guinea pig hearts have demonstrated that capsaicin evokes the release of SP and NKA from cardiac sensory nerves (7, 8, 12). Specific SP binding sites have also been detected in intracardiac ganglia and coronary arteries by autoradiography (14). Accordingly, there is an anatomic and neurochemical basis for expecting that tachykinins released from peripheral processes of sensory nerves will affect cardiac and coronary function.

Our previous studies with isolated guinea pig hearts have established that SP causes a dose-dependent bradycardia mediated by cholinergic nerves (13, 14). Other investigators have shown that SP has a direct stimulatory action on neurons in isolated cardiac ganglia from guinea pigs (10, 11, 18). Because the intrinsic cardiac ganglia are intact in isolated hearts, we proposed that the negative chronotropic response to SP occurs through ganglion stimulation (13). We also reported that SP causes endothelium-dependent dilation of resistance vessels of the guinea pig heart (17). This observation is consistent with reports that SP is a potent coronary vasodilator in several species (24). Additionally, SP has been shown to produce a negative inotropic response in isolated guinea pig hearts (4, 9) and to decrease left ventricular peak systolic pressure in humans (26). Nitric oxide (NO) has been implicated as a mediator of the effect of SP to inhibit ventricular function (9, 26). This indirect effect of SP on ventricular performance may have pathophysiological importance because a tachykinin receptor antagonist prevents most of the reduction of left ventricular developed pressure associated with reperfusion after global ischemia in isolated guinea pig hearts (5).

It has been reported that NKA causes bradycardia and decreases ventricular contractility in isolated guinea pig hearts (19). However, dose-response characteristics and mechanisms of its cardiac actions have not been evaluated. SP and NKA are known to differ in their relative affinities for the three subtypes of tachykinin receptor (24, 25, 28). Accordingly, there are often quantitative and qualitative differences in the response profile of these peptides in a tissue (25, 28). These differences depend in large part on the presence and abundance of specific receptor subtypes in the tissue. Because of these considerations, the present study was done to identify and characterize responses of the isolated guinea pig heart to NKA.

Materials and Methods

Isolated heart preparation. Male Hartley guinea pigs (350–550 g) were pretreated with 500 U of heparin ~20 min before undergoing decapitation while they were under pentobarbital sodium anesthesia (75 mg/kg ip). The heart was rapidly removed and placed into a petri dish containing ice-cold perfusion buffer to enable cannulation of the ascending aorta. After flushing the heart with 5 ml of cold buffer, we transferred it to an isolated heart apparatus for perfusion by a modification of the Langendorff technique (2). The perfusion solution was a modified Krebs-Ringer bicarbonate buffer (pH 7.35–7.4) of the following composition (in mM): 127 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 KH2PO4, 24.9 NaHCO3, 1.2 MgSO4, 2.0 sodium pyruvate, and 5.5 dextrose and 0.1% BSA. A Masterflex peristaltic pump (Cole Parmer, St. Louis, MO) was used...
Samples were stored in 50-ml plastic tubes at compound (Ted Pella, Redding, CA) and powdered dry ice. The heart was frozen on brass specimen plates using OCT through the middle of the ventricles. The upper one-half of the heart was surrounded by a glass jacket kept at the same temperature, and the heart was wrapped in an individual layer of aluminum foil. After the hearts were rinsed in saline, they were bisected to expose the ventricles. The first section of each set was stored at 80°C for maximum contractility. Output from the force or pressure transducer was sent to a Gould universal amplifier and a Gould BioTach amplifier to monitor ventricular contractions and rate, respectively, with a Gould 2400 recorder. Perfusion pressure was monitored using a transducer that was attached to the side arm of a three-way stopcock located at the proximal end of the aortic cannula. Because the rate of perfusion was held constant during each experiment, perfusion pressure would vary in direct proportion to coronary resistance. Experiments were started after a 20- to 40-min stabilization period.

Administration of drugs. NKA and SP were dissolved in sterile saline, and 100-µl aliquots (3.2 mM) were stored at −80°C. The tachykinins were diluted with saline that contained 0.1% BSA. ACh, adenosine, and norepinephrine (NE) were dissolved in saline. Each of these agonists was administered by bolus injection through a length of polyethylene tubing (Clay Adams) that emptied into the perfusion buffer at a point near the three-way stopcock attached to the aortic cannula. A volume of 100 µl was given over ~3 s. Injections of saline with 0.1% BSA were given as a control. Bolus injections of tachykinins were spaced at 10- to 15-min intervals to avoid receptor desensitization. To evaluate tachyphylaxis to NKA, the peptide was given by infusion (Harvard pump 22). Blockade of muscarinic and adenosine receptors was accomplished by perfusion with buffer that contained atropine (1 or 10 µM) and theophylline (100 µM), respectively. For experiments with antagonists, responses to responses of agonists were measured first during perfusion with drug-free buffer and again at least 30 min after switching to perfusion with buffer that contained blocker.

Pacing. To evaluate inotropic responses, we paced hearts in one group of experiments using a SD9 stimulator (Grass Instruments, Quincy, MA). One wire for pacing was connected to the apex of the heart with a needle electrode, and the other wire was attached to the perfusion cannula with an alligator clamp. Stimulation parameters were 2 V and 10 ms in duration, with a 2-ms delay at a frequency to clamp heart rate at ~50 beats/min above the spontaneous rate.

Autoradiography. Hearts were obtained from guinea pigs that were anesthetized with pentobarbital sodium (75 mg/kg ip). After the hearts were rinsed in saline, they were bisected through the middle of the ventricles. The upper one-half of the heart was frozen on brass specimen plates using OCT compound (Ted Pella, Redding, CA) and powdered dry ice. Samples were stored in 50-ml plastic tubes at −80°C. Serial 20-µm cross sections were cut at −20°C using a microtome cryostat. Sets of three adjacent sections were thaw mounted onto separate slides that had been coated two times with chrome alum-gelatin. Two to four sections were placed on each slide. After the sections dried, the slides were transferred to plastic slide boxes on dry ice. Slides containing the first section of each set were stored at −20°C until they were stained with hematoxylin and eosin. The remaining sections were stored at −80°C until use for autoradiography.

NKA binding sites were labeled using 125I-labeled NKA (2,200 Ci/mmol, NEN Life Science Products, Boston, MA) according to an established protocol (22). Slides were preincubated in 50 mM Tris-HCl (pH 7.4) for 10 min at room temperature before incubation in buffer with radioligand. To determine total binding of radioligand, one slide in each set was incubated for 2 h at room temperature in 50 mM Tris·HCl buffer (pH 8.0) containing 3 mM MnCl2, 0.02% BSA, 40 mg/l bacitracin, 2 mg/l chymostatin, 4 mg/l leupeptin, and 0.1 mM 125I-labeled NKA. Slides containing adjacent sections were incubated in the same buffer, with the addition of 1 µM NKA to determine nonspecific binding. After incubation with radioligand, slides were rinsed four times for 5 min each in 50 mM Tris-HCl (pH 7.4) at 4°C and briefly dipped into cold deionized water. Excess water on the slides was carefully removed with gauze, and the sections were rapidly dried at room temperature with an electric fan. The slides were subsequently placed in X-ray cassettes with 125I-labeled microscales and Hyperfilm-1H (Amersham, Arlington Heights, IL). Films were exposed for 1 to 4 wk after exposure at 4°C for 4–5 wk, and the sections were stained with hematoxylin and eosin. A microcomputer-assisted imaging device (Image Research) was used for quantitative evaluation of the film autoradiograms. Stained sections were used to identify labeled sites in the films.

Data analysis. Heart rate (beats/min), force of ventricular contractions (g or mmHg), and diastolic perfusion pressure (mmHg) were measured before each bolus injection of agonist and at the times of maximum responses. Changes were calculated from the raw data and expressed as a percentage of baseline. Graphing of data and most statistical analyses were performed using GraphPad Prism version 2.0 (GraphPad Software, San Diego, CA). Group data are summarized as arithmetic means ± SE. Statistical comparisons were made using a paired or unpaired t-test, repeated-measures ANOVA, or three-factor ANOVA (NCSS 97 software; NCSS, Kaysville, UT). Post hoc comparisons after ANOVA were made using the Newman-Keuls procedure. A probability level of 0.05 or smaller was used to indicate statistical significance.

Drugs. NKA and SP were purchased from Peninsula (Belmont, CA) or Bachem California (Torrance, CA). ACh chloride, atropine sulfate, adenosine hemisulfate, NE bitartrate, theophylline, tyramine hydrochloride, and reserpine were purchased from Sigma (St. Louis, MO).

RESULTS

Dose-response study. NKA consistently evoked a negative chronotropic response (Fig. 1A) that increased in magnitude with dose of peptide (Fig. 2A). The maximum response occurred within 20 s after injection of NKA. Heart rate returned to baseline over a longer interval and rarely exceeded this value. The amplitude of ventricular contractions was also increased by NKA in most experiments (Figs. 1A and 2B). This effect occurred in hearts with ventricular function measured from a suture attached to the apex of the heart and in hearts with a left ventricular balloon (Fig. 3). Smaller decreases in force of contractions sometimes preceded and/or followed the positive inotropic response. Negative inotropic effects were not quantified in most experiments due to their variable incidence.

A dose-dependent decrease in perfusion pressure was evoked by NKA despite the low basal level for this
parameter (Figs. 1A and 2C). In several preparations, this initial decrease in perfusion pressure was followed by a pressor response (Figs. 1A and 2C).

After a recovery period of ~20 min, a second set of dose-response data was collected from some hearts. Most responses were similar during the second trial, but the pressor effect of NKA was occasionally less evident.

Comparison of NKA and SP. The dose-response characteristics of SP and NKA were evaluated in a second group of hearts with a left ventricular balloon used to monitor cardiac responses. Three doses of each peptide were evaluated in the same hearts, with the order varied between experiments. Results for NKA were comparable to those obtained in the initial dose-response study with ventricular function measured from a suture attached to the apex of the heart (compare Figs. 2 and 3). Responses to SP differed from those to NKA in several aspects. SP was less potent than NKA in causing bradycardia, and the negative chronotropic response to SP was generally followed by a slight tachycardia (Fig. 3A). The dominant effect of SP on ventricular contractility was inhibitory; a positive inotropic response only occurred at the highest dose tested (Fig. 3B). Lastly, SP produced only decreases in perfusion pressure rather than biphasic changes seen with NKA (Fig. 3C).

Effects of infused NKA. Infusion of NKA evoked initial cardiac and coronary responses that were identical to those produced by bolus injections of the peptide, but tachyphylaxis occurred. Within 5 min of the start of the infusion, all recorded parameters stabilized at values near the preinfusion baselines despite continued exposure to NKA. After tachyphylaxis developed to infused NKA, no responses occurred to bolus injections of NKA or SP when they were given during the infusion of NKA, but ACh remained effective (Fig. 4). Responses to bolus injections of the tachykinins returned when tested at least 30 min after the infusion of NKA was stopped (Fig. 4).

Effect of atropine and theophylline on chronotropic responses. A concentration of 1 µM atropine completely blocked the bradycardia evoked by 1 nmol ACh but only reduced the response to 25 nmol NKA by 32% (Table 1). No additional reduction in the negative chronotropic response to NKA occurred when the concentration of atropine was increased to 10 µM (not shown) or when 1 µM atropine and 100 µM theophylline were both present in the buffer (Table 1). The bradycardic response to 1 nmol adenosine was significantly attenuated by 100 µM theophylline.

Effect of pacing. The effects of pacing on responses to bolus injections of NKA, SP, and NE were evaluated in five isolated hearts. The positive inotropic response to NKA observed in spontaneously beating hearts was replaced by a small negative inotropic response in paced hearts (Fig. 5A and Table 2). Neither the positive inotropic response to NE nor the negative inotropic effect of SP were affected by pacing (Fig. 5, B and C, and Table 2).

Effect of reserpine pretreatment. Bolus injections of 100 nmol tyramine caused sympathetic stimulation in hearts from untreated guinea pigs but had no effect in hearts from reserpinized animals. Reserpine pretreatment did not affect baseline heart rate, ventricular contractility, or perfusion pressure (Table 3). Likewise, neither the positive inotropic response to 25 nmol NKA nor the percentage decrease in heart rate were significantly affected. However, the duration of the negative chronotropic response to NKA was increased. The

![Fig. 1. Recorder tracings showing changes in heart rate, ventricular contractions, and perfusion pressure evoked by bolus injections of neurokinin A (NKA, A), substance P (SP, B), and ACh (C) in an isolated guinea pig heart perfused with buffer at 8 ml/min. Ventricular contractions were recorded from a silk suture attached to apex of heart. Injections were separated by 10- to 15-min intervals.](http://ajpregu.physiology.org/)

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interval from the start of bradycardia until 80% recovery was 59 ± 6 s for the control group and 130 ± 31 s for the reserpine group (P = 0.0022, Mann-Whitney test). The vasodilator effect of NKA was augmented in hearts from reserpinized guinea pigs (Table 3), and none of the hearts in this group exhibited a pressor response to NKA.

Localization and quantification of specific 125I-labeled NKA binding sites. Specific binding of 125I-labeled NKA was associated with the intrinsic cardiac ganglia and coronary arteries at the base of the heart (Fig. 6 and Table 4). Labeling of large- and small-caliber vessels was detected when film autoradiograms were evaluated under magnification (Fig. 6, D–G). Over 80% of 125I-labeled NKA binding to the ganglia and large coronary arteries was specific, and the density of specific binding sites in these regions was similar (Table 4). Specific binding was not detected in the atrium or ventricles.

**DISCUSSION**

Functional and autoradiographic techniques were used in this study to characterize effects of NKA in the isolated guinea pig heart. Bradycardia, increased force of ventricular contractions, and decreases in perfusion pressure were consistently evoked by bolus administration of NKA. The initial depressor response to NKA was frequently followed by a brief increase in perfusion pressure, but the magnitude of this response was much less than the pressor response evoked by NE. Specific binding sites for NKA were localized to the intrinsic cardiac ganglia and coronary arteries. No NKA receptors were detected in the atrial or ventricular myocardium. These anatomic observations suggest that cardiac responses to NKA may occur primarily through stimulation of neural tissue present in the isolated heart, whereas vascular responses could result from stimulation of receptors in the coronary arteries.

Because NKA and SP are colocalized in many primary sensory neurons (7, 21, 24), we compared the response profiles and potencies of these peptides. Quantitative and qualitative differences were identified when responses to both tachykinins were determined in the same hearts. NKA was clearly much more potent than SP in causing bradycardia and increasing the force of ventricular contractions. At doses of 25 nmol and less, the effect of SP on ventricular contractility was exclusively inhibitory. A small positive inotropic effect was only noted after administration of 100 nmol SP, and even this effect was preceded by a decrease in left ventricular developed pressure. Another difference between SP and NKA was that SP frequently evoked biphasic changes in heart rate. In these cases, the initial bradycardia was followed by a small tachycardia. This effect was noted in an earlier study, but the mechanism is unknown (13). Lastly, although SP and NKA did not differ in potency for decreasing perfusion pressure, biphasic responses were often evoked by NKA.

Tachyphylaxis occurred to NKA when the peptide was administered by infusion. The most likely explanation for this effect is that continuous exposure to NKA caused desensitization of tachykinin receptors (24). A nonspecific effect on the isolated heart is unlikely because responses to ACh were unaffected by the infusion of NKA. Because infusion of NKA also caused...
cross-tachyphylaxis to SP, a common pool of tachykinin receptors may be activated by both peptides. However, these experiments do not eliminate the possibility that NKA can activate additional tachykinin receptors that are unaffected by SP.

A dose-dependent bradycardia was the most prominent response to NKA in the present study. Stimulation of cholinergic neurons present in the heart was considered the most likely mechanism for this response. The presence of NKA receptors in the intrinsic cardiac ganglia provided further support for a neural mechanism. Our results from experiments with atropine implicate cholinergic neurons but also indicate that other mechanisms must be operating. Over one-half of the negative chronotropic response to 25 nmol NKA persisted in the presence of atropine, even after the concentration of antagonist was increased to 10 µM. At these concentrations, atropine totally blocked the larger negative chronotropic response evoked by bolus injections of 1 nmol ACh in the same hearts. Doses up to 100 nmol ACh were tested in some hearts with atropine present and still evoked no bradycardia. Accordingly, the negative chronotropic response to NKA must occur through a combination of cholinergic and noncholinergic mechanisms. In hearts from reserpinized animals, the duration of the negative chronotropic response to NKA was significantly increased compared with control. This observation may indicate that NKA produces some sympathetic activation in control hearts that reduces the duration of the bradycardic response.

Purines were considered the most likely candidates for mediators of the noncholinergic component of the bradycardia. It is well known that adenosine and ATP can cause bradycardia (23), and purinergic neurons may be present in the heart (3). Negative chronotropic responses to purines are thought to occur entirely through activation of adenosine receptors in mammalian hearts (23). However, we were unable to detect an involvement of purines in the negative chronotropic response to NKA. The nonselective adenosine receptor antagonist theophylline had no effect on the noncholinergic component of the response to NKA but did inhibit negative chronotropic responses to adenosine. Because NKA receptors were not detected in the atrial myocardium, a direct negative chronotropic action is considered unlikely. Therefore, at present we can only speculate that the noncholinergic component of the bradycardia may involve some uncharacterized mediator released from nerves or a vascular element where NKA receptors are known to occur. Other investigators have provided evidence that noncholinergic mechanisms contribute to the bradycardia evoked by SP in isolated guinea pig hearts (4). The noncholinergic component of the response to SP was most evident in these

![Fig. 3. Comparison of dose-response characteristics for effects of NKA and SP on heart rate (A), ventricular contractility (B), and perfusion pressure (C) in isolated perfused guinea pig hearts (n = 5). Left ventricular developed pressure was measured by means of a left ventricular balloon connected to a pressure transducer. Peptides were given by bolus injection in order of increasing dose. Doses of peptide were administered at 20-min intervals. Collection of dose-response data for SP and NKA was separated by 30 min, and sequence for administering SP or NKA was varied. A: SP and NKA both caused a dose-dependent bradycardia (repeated-measures ANOVAs). * P < 0.01, different from other means for same peptide. Magnitude of small positive chronotropic response to SP did not vary with dose. Maximum rates achieved after 2.5 and 25 nmol SP were different from baseline values (paired t-tests). Comparison of negative chronotropic responses to equal doses of tachykinins by 3-factor ANOVA revealed a significant difference between SP and NKA (F(1,4) = 162.9, P < 0.001). B: significant effect of dose on amplitude of ventricular contractions was detected by repeated-measures ANOVA for positive inotropic actions of NKA and SP but not for negative inotropic effect of SP. * P < 0.01, different from other means for same peptide. + P < 0.05, different from mean for lowest dose of same peptide. C: significant effect of dose was detected for depressor response to each tachykinin by repeated-measures ANOVAs. However, magnitude of depressor response to equal doses of SP and NKA did not differ (3-factor ANOVA, F(1,4) = 1.26, P = 0.32). Magnitude of depressor response to NKA did not vary with dose. Maximum perfusion pressure achieved after each dose of NKA was different from baseline (paired t-tests).]
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Fig. 4. Effect of NKA infusion on responses to bolus injections of NKA, SP, and ACh. Responses to bolus injections of 25 nmol NKA, 25 nmol SP, and 1 nmol ACh were obtained before, during, and after infusion of 25 nmol NKA 100 µl·min⁻¹. Responses to agonists before infusion of NKA are shown for 1 experiment in Fig. 1. Injections of agonists were spaced at 10- to 15-min intervals before and after infusion of NKA. Tachyphylaxis occurred to NKA when it was infused. Bolus injections during infusion of NKA were commenced after recorded parameters stabilized at values near preinfusion baselines. Injections were spaced at 1- to 2-min intervals during NKA infusion to conserve peptide. Interval between end of infusion and last series of bolus injections was at least 30 min. Responses to each agonist before, during, and after infusion of NKA were evaluated by separate repeated-measures ANOVAs. Four hearts were used for this study. A: infusion of NKA affected negative chronotropic responses to NKA (F₂,₆ = 15.1, P = 0.005) and SP (F₂,₆ = 18.7, P = 0.003) but not ACh (F₂,₆ = 1.93, P = 0.225). B: infusion of NKA affected positive inotropic response to NKA (F₂,₆ = 18.1, P = 0.003) and negative inotropic response to SP (F₂,₆ = 16.2, P = 0.004) but not negative inotropic response to ACh (F₂,₆ = 1.55, P = 0.286). C: infusion of NKA affected vasodilator responses to NKA (F₄,₁₂ = 13.9, P = 0.0002) and SP (F₂,₆ = 19.7, P = 0.002) but not ACh (F₂,₆ = 1.04, P = 0.409). * P < 0.05, different from control response. 

Table 1. Effect of atropine and theophylline on the negative chronotropic response to NKA, ACh, and adenosine

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Baseline</th>
<th>%Change</th>
<th>Group 2</th>
<th>Baseline</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>219 ± 4</td>
<td>-51.1 ± 3.9</td>
<td>Control</td>
<td>233 ± 3</td>
<td>-58.0 ± 4.8</td>
</tr>
<tr>
<td>No antagonist</td>
<td>213 ± 2</td>
<td>-49.3 ± 2.0</td>
<td>1 µM Atropine</td>
<td>221 ± 4</td>
<td>-39.6 ± 3.5</td>
</tr>
<tr>
<td>NKA</td>
<td>221 ± 4</td>
<td>-77.6 ± 2.4</td>
<td>ACh</td>
<td>236 ± 6</td>
<td>-72.6 ± 2.3</td>
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<tr>
<td>ACh</td>
<td>208 ± 12</td>
<td>-68.2 ± 4.1</td>
<td>Adenosine</td>
<td>205 ± 12</td>
<td>-64.3 ± 4.6</td>
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</tbody>
</table>

Values are means ± SE; n = 5 for each group. Ventricular contractions were recorded from apex of heart. NKA, neurokinin A. * P < 0.05, different from control response.

Increased force of ventricular contractions was another prominent response to NKA. This effect occurred regardless of the method used to record ventricular contractions (i.e., suture attached to apex of heart or left ventricular balloon). Two lines of evidence suggest that this response does not occur through direct stimulation of the ventricular myocardium. First, specific NKA binding sites were not detected in the right or left ventricular myocardium in our autoradiographic studies. Next, the positive inotropic response to NKA was reversibly eliminated and replaced by a small negative inotropic response during electrical pacing of the hearts. It is important to note that positive inotropic responses to NE were unaffected by pacing. The latter observations also suggest that NKA does not increase ventricular contractility through release of NE from sympathetic nerves in the heart. Further evidence against an adrenergic mechanism is provided by the results with reserpine. Pretreatment with this drug is known to deplete NE from sympathetic nerves (20). This was confirmed in our experiments by establishing a lack of response to tyramine, an indirectly acting sympathomimetic drug. Reserpine pretreatment had no significant effect on the positive inotropic response to NKA. Accordingly, this response to NKA appears to be an indirect effect related to the prominent bradycardia.

The negative inotropic response to 25 nmol NKA in paced hearts appears similar to the effect produced by the same dose of SP in paced or spontaneously beating hearts. Previous investigators have reported negative inotropic responses to SP in spontaneously beating guinea pig hearts perfused by the Langendorff method (4) and isolated ejecting guinea pig hearts that were paced (9). The negative inotropic response to SP in spontaneously beating hearts was partially blocked by atropine. Negative inotropic responses to SP in the
paced ejecting hearts were mediated by endogenous NO. It is noteworthy that bicoronal infusion of SP in humans also produces a negative inotropic response that is thought to be a paracrine effect of NO released from the coronary endothelium (26, 27). Because NKA is known to cause NO-mediated vasodilation in guinea pig hearts (17), the negative inotropic action of this peptide in paced hearts may likewise involve a paracrine influence of NO. The profile of the inotropic response to NKA in spontaneously beating hearts presumably reflects the relative magnitudes of the positive and negative influences.

We previously demonstrated that SP and NKA cause dose-dependent vasodilation in potassium-arrested guinea pig hearts (17). The one-half-maximal effective dose values for SP and NKA in these experiments were 0.33 and 14 pmol, respectively. Maximum responses to both peptides were achieved at a dose of 1 nmol or less. No difference in potency for vasodilation was detected for the tachykinins in the present study. This finding presumably reflects the fact that most doses were above the level required for maximum vasodilation.

NKA can also evoke a pressor response if the tachykinin receptors mediating vasodilation are desensitized (17). Results of the present study provide additional evidence that NKA can cause constriction of coronary resistance vessels in the guinea pig. However, this effect was difficult to analyze because of its small magnitude and variable occurrence. Both of these characteristics might derive from a relative dominance of the vasodilator action of NKA. Vasoconstrictor responses to NKA did not occur in hearts from reserpinized guinea pigs, whereas vasodilator responses were enhanced. These alterations may indicate a role for sympathetic nerves in generating coronary vasoconstrictor responses to NKA.

It is recognized that SP and NKA differ in their affinity for subtypes of the tachykinin receptor (24, 25, 28). SP has a higher affinity than NKA at NK1 receptors, whereas NKA has a higher affinity than SP at NK2 receptors.

### Table 2. Effect of pacing on responses to NKA, NE, and SP

<table>
<thead>
<tr>
<th>Change in Recorder Parameter, %Baseline</th>
<th>Before pacing</th>
<th>During pacing</th>
<th>After pacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 nmol NKA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular contractions</td>
<td>22.5 ± 3.3</td>
<td>-11.7 ± 1.7*</td>
<td>21.0 ± 4.0</td>
</tr>
<tr>
<td>Perfusion pressure</td>
<td>-17.0 ± 3.3</td>
<td>-8.6 ± 2.3*</td>
<td>-17.8 ± 3.1</td>
</tr>
<tr>
<td>0.32 nmol NE</td>
<td>50.5 ± 10.9</td>
<td>63.7 ± 8.5</td>
<td>61.4 ± 8.4</td>
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<tr>
<td>Ventricular contractions</td>
<td>56.6 ± 4.5</td>
<td>49.4 ± 4.5</td>
<td>44.9 ± 4.2</td>
</tr>
<tr>
<td>Perfusion pressure increase</td>
<td>-21.6 ± 2.7</td>
<td>-18.6 ± 2.0</td>
<td>-19.3 ± 1.9</td>
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<tr>
<td>Decrease</td>
<td>-13.4 ± 2.7</td>
<td>-12.1 ± 3.2</td>
<td>-11.8 ± 2.9</td>
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Table 3. Effect of pretreatment with reserpine on cardiac and coronary responses to a bolus injection of 25 nmol NKA

<table>
<thead>
<tr>
<th>Control</th>
<th>Baseline</th>
<th>%Change</th>
<th>Reserpine</th>
<th>Baseline</th>
<th>%Change</th>
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</thead>
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<tr>
<td>Ventricular contractions, g</td>
<td>5.6 ± 0.3</td>
<td>27.2 ± 3.6</td>
<td>6.6 ± 0.5</td>
<td>20.6 ± 5.6</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>268 ± 5</td>
<td>42.2 ± 4.9</td>
<td>255 ± 5.6</td>
<td>55.6 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>Perfusion pressure, mmHg</td>
<td>27 ± 2</td>
<td>17.9 ± 2.4</td>
<td>30 ± 2.6</td>
<td>31.3 ± 2.8*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 for each group. Ventricular contractions were recorded from apex of heart. Significant differences were not found for the other groups by repeated-measures ANOVA or paired t-test.
and NK3 receptors. Preliminary results with subtype-selective tachykinin receptor agonists suggest NK1 receptors may mediate negative inotropic and vasodilator responses in the isolated guinea pig heart (15, 16). Bradycardia occurs with selective agonists for all three receptor subtypes but is largest with NK2 agonists and of intermediate size with NK3 agonists. Lastly, positive inotropic responses and coronary vasoconstriction occurred only with NK2 agonists. Experiments with selective tachykinin receptor antagonists are currently underway to determine the role of tachykinin receptor subtypes in producing responses to NKA.

**Perspectives**

Recent anatomic and functional studies have revealed that the intracardiac nervous system has an unexpected degree of complexity (1, 29). Intracardiac reflex loops have been proposed as a mechanism for rapid fine tuning of cardiac performance. The dual sensory-motor function of tachykinin-containing afferent neurons has also been recognized (21, 24), and these nerves have been identified as a component of the intracardiac nervous system (30–33). Varicose processes of tachykinin-containing nerves are particularly abundant in the adventitia of coronary arteries and in the intrinsic cardiac ganglia, where they surround

<table>
<thead>
<tr>
<th>Region</th>
<th>Total</th>
<th>Nonspecific</th>
<th>Specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic cardiac ganglia</td>
<td>0.186 ± 0.024</td>
<td>0.032 ± 0.014</td>
<td>0.154 ± 0.017</td>
</tr>
<tr>
<td>Coronary arteries</td>
<td>0.184 ± 0.011</td>
<td>0.026 ± 0.007</td>
<td>0.158 ± 0.016</td>
</tr>
<tr>
<td>Right atrium</td>
<td>0.015 ± 0.004</td>
<td>0.010 ± 0.003</td>
<td>ND</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.018 ± 0.004</td>
<td>0.010 ± 0.003</td>
<td>ND</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>0.017 ± 0.004</td>
<td>0.009 ± 0.003</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 for ganglia and arteries, n = 5 for atria and ventricles. For each animal, total and nonspecific binding to a region were determined by calculating the average of multiple readings. Specific binding sites were identified by applying the criterion that total binding must be at least 3 times nonspecific binding. ND, none detectable. mg. Estimated tissue equivalent (dry weight) provided with 125I-labeled microscales.
many of the principal neurons. Release of tachykinins (i.e., NKA and SP) from fibers at these locations may occur through direct activation or by means of an axon reflex. The present findings demonstrate the diversity of responses that could be triggered by locally released tachykinins. Beneficial effects such as modulation of ganglion transmission might occur under physiological conditions. However, it is also possible that excessive stimulation of cardiac afferents and release of tachykinins during pathological conditions such as myocardial infarction could contribute to adverse clinical sequelae.

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