Tachykinin Receptor Subtypes in the Isolated Guinea Pig Heart and Their Role in Mediating Responses to Neurokinin A

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ABSTRACT

Selective tachykinin agonists were used to identify cardiac and coronary responses mediated by specific tachykinin receptor subtypes in isolated, perfused guinea pig hearts. Receptor desensitization with selective agonists and blockade with selective antagonists were used to determine the role of specific subtypes in generating responses to neurokinin A (NKA). Dose-dependent cardiac and coronary effects were evoked by bolus injections of [Sar9, Met(O2)11]substance P ([Sar9, Met(O2)11]SP), GR64349, and [MePhe]neurokinin B ([MePhe]NKB) (selective agonists for NK1, NK2, and NK3 receptors, respectively). Each agonist caused bradycardia, but GR64349 was most effective (34 ± 4% decrease in heart rate with 32 nmol, n = 8). Prominent increases in ventricular contractility and perfusion pressure also occurred with 32 nmol of GR64349 (25 ± 6 and 33 ± 4%, respectively). [Sar9, Met(O2)11]SP was unique in having a high potency for decreasing ventricular contractility and perfusion pressure. Bolus injections of 25 nmol of NKA decreased rate (48 ± 2%, n = 51), increased contractility (26 ± 2%), and had biphasic effects on perfusion pressure (24 ± 1% decrease followed by 9.2 ± 1.4% increase). Desensitization with GR64349 or treatment with the NK2 antagonist SR48968 reduced the bradycardic response to NKA by greater than 75% and eliminated the positive inotropic response. The remaining bradycardia occurred through NK2 receptors. Desensitization with [Sar9, Met(O2)11]SP or NK1 blockade with FK888 eliminated the coronary relaxant action of NKA and enhanced the pressor response. It is concluded that three tachykinin receptor subtypes are present in the guinea pig heart and that each contributes to the overall response evoked by NKA.

The tachykinins, substance P (SP) and neurokinin A (NKA), are colocalized in a large subpopulation of primary afferent neurons that are capable of exerting effenter functions through release of these bioactive peptides from their peripheral processes (Maggi and Meli, 1988; Otsuka and Yoshioka, 1993). Many immunohistochemical studies have established that such tachykinin-containing nerve processes are present in the heart where they are localized to the intrinsic cardiac ganglia, adventitia of coronary arteries, and, at a lower density, to many myocardial sites (Wharton et al., 1981, 1988, 1990; Weihe et al., 1984). Within the intrinsic cardiac ganglia, tachykinins are localized to varicose nerve fibers that surround many of the principal neurons. Collectively, these observations suggest that tachykinins may have important effects on cardiac function through direct and neurally mediated mechanisms.

Administration of SP to isolated guinea pig hearts has been reported to cause bradycardia (Hoover and Hancock, 1988; Hoover, 1990), reduced ventricular contractility (Chiao and Caldwell, 1995; Hoover et al., 1998), and relaxation of coronary resistance vessels (Hoover and Hancock, 1988; Hoover, 1990; Vials and Burnstock, 1992; Hoover and Hossler, 1993). The negative chronotropic response to SP has been attributed to stimulation of cholinergic neurons of the intrinsic cardiac ganglia. Evidence supporting this conclusion includes the autoradiographic identification of specific SP binding sites in guinea pig cardiac ganglia (Hoover and Hancock, 1988) and the observations that negative chronotropic responses to SP are attenuated by muscarinic receptor blockade and potentiated by cholinesterase inhibition (Hoover, 1990; Chiao and Caldwell, 1995). There is also electrophysiological evidence that SP can directly activate neurons of guinea pig intracardiac ganglia in vitro (Konishi et al., 1985; Hardwick et al., 1995, 1997).

A recent evaluation of responses to NKA in isolated guinea pig hearts has revealed quantitative and qualitative differences compared with SP (Hoover et al., 1998). NKA had a greater potency for evoking bradycardia, and more than half of the negative chronotropic response to NKA was unaffected by treatment with atropine. The dominant effect of NKA on ventricular contractility was augmentation rather than sup-

ABBREVIATIONS: SP, substance P; ACh, acetylcholine; DMSO, dimethyl sulfoxide; NKA, neurokinin A; NKB, neurokinin B.
pression. Last, NKA had a biphasic effect on coronary vascular tone, whereas SP caused only relaxation. It is possible that some of these differences may be attributed to the presence of more than one subtype of tachykinin receptor in the heart.

Our goals in this study were: 1) to identify cardiac and coronary responses evoked by stimulation of specific tachykinin receptor subtypes, and 2) to determine the role of tachykinin receptor subtypes in generating responses to NKA in the isolated guinea pig heart. Subtype-selective agonists were used to address the first aim. Desensitization with selective agonists and administration of selective tachykinin antagonists were two approaches used to address the second aim.

**Experimental Procedures**

**Isolated Heart Preparation.** Male Hartley guinea pigs (350–450 g) were pretreated with 500 U of heparin. Approximately 20 min later, they were deeply anesthetized with sodium pentobarbital (75 mg/kg i.p.) and decapitated. The heart was rapidly removed and placed in ice-cold perfusion buffer to enable cannulation of the ascending aorta. After being flushed with 5 ml of cold buffer, the heart was transferred to an isolated heart apparatus for perfusion by a descending aorta. After being flushed with 5 ml of cold buffer, the heart was transferred to an isolated heart apparatus for perfusion by a modification of the Langendorff technique (Broadley, 1979). The perfusion solution was a modified Krebs-Ringer-bicarbonate buffer that was most thoroughly studied in each class. Similar responses, so data are presented only for the drug that was most thoroughly studied in each class. Cardiac contractions were measured by attaching one end of a silk suture to the apex of the heart and the other end to an isometric force transducer. Tension on the heart during diastole was adjusted to approximately 1 g. Output from the force transducer was sent to a Gould Universal amplifier and a Gould Biotech amplifier to monitor ventricular contractions and heart rate, respectively, with a Gould 2400 recorder (Gould Instrument Systems, Valley View, OH). Because the perfusion rate was held constant, perfusion pressure was monitored as an indicator of coronary vascular resistance. This parameter was recorded using a pressure transducer that was attached to the sidearm of a three-way stopcock located at the proximal end of the aortic cannula. Experiments were started after a 40-min stabilization period.

**Preparation and Storage of Drugs.** NKA, [Sar8,Met(O2)11]SP, and GR64349 were dissolved in sterile saline. [MePhe7]NKB, FK888, SR48968, and SR142801 were dissolved in dimethyl sulfoxide (DMSO). NKA-[MePhe7]NKB and FK888 were diluted further with sterile saline to make stock solutions containing 15 and 60% DMSO, respectively. Aliquots of tachykinin receptor agonists (3.2 mM) and antagonists (1 mM) were diluted in saline that contained 0.1% BSA. Acetylcholine (ACh) was weighed and dissolved in saline before each experiment.

**Drug Administration.** The selective tachykinin agonists, NKA, and ACh were administered by bolus injection. Volumes of 100 μl were given over ~3 s. For dose-response studies, the selective agonists were given in order of ascending dose. Injections of the selective agonists were separated by 20- to 40-min intervals to avoid desensitization. In other experiments, we examined the effect of desensitization with selective agonists on responses to NKA and ACh. For these studies, a series of four bolus injections of selective agonist (32 nmol/100 μl) were given at 1-min intervals. Either 25 nmol of NKA or 1 nmol of ACh was given 30 s after the last injection of selective agonist. In experiments with selective antagonists, responses to 25 nmol of NKA and 1 nmol of ACh were determined in the absence of drug and again during exposure to the antagonist or a combination of antagonists. The NK1 receptor antagonist FK888 was diluted with saline and given by infusion (8 nmol/50 μl min -1) through a short length of polyethylene 20 tubing that emptied into the perfusion buffer at a point near the three-way stopcock attached to the aortic cannula. The infused solution contained 10% DMSO. The final concentration of FK888 at the heart was 1 μM. The NK2 and NK3 receptor antagonists SR48968 and SR142801, respectively, were included in the perfusion buffer when hearts were treated for 20 min before a second challenge with NKA. In later experiments, the treatment interval was extended to 60 min, and antagonists were given by infusion. The concentration of DMSO in the buffer was ~0.4%.

**Materials.** NKA and selective tachykinin agonists were purchased from Peninsula Laboratories (Belmont, CA) or Research Biochemicals International (Natick, MA). FK888 was obtained from Research Biochemicals International. SR48968 (saredutant; [S]-N-methyl-N-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butylbenzamide) and SR142801 (osanetant; (R)-(N)-(1-(3-1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl-N-methylacetamide) were generously given by Dr. Xavier Emonds-Alt (Sanoﬁ Recherche, Montpellier, France). ACh chloride was purchased from Sigma Chemical (St. Louis, MO).

**Data Analysis.** Heart rate (beats/min), diastolic perfusion pressure (mm Hg), and ventricular contractility (g) were measured before each injection and at the times of maximum responses. Changes were expressed as a percentage of baseline. Group data are presented as mean ± S.E. GraphPad Prism version 2.01 (GraphPad Software, San Diego, CA) was used for analysis of dose-response data and preparation of graphs. Statistical comparisons were made by a two-tailed, paired t test or ANOVA using GraphPad Prism or NCSS 97 software (NCSS, Kaysville, UT). In the case of significant F values, post hoc comparisons following ANOVA were made using the Newman-Keuls procedure. A probability level of .05 or less was used to indicate statistical significance.

**Results**

**Effects of Selective Agonists.** The presence and function of tachykinin receptor subtypes in the isolated guinea pig heart was evaluated using the NK1 agonists [Sar8,Met(O2)11]SP and GR73632, NK2 agonists GR64349 and [βAla]NKA(4–10), and NK3 agonists [MePhe7]NKB and senktide. For each receptor subtype, both agonists produced similar responses, so data are presented only for the drug that was most thoroughly studied in each class.

Each of the selective agonists caused a concentration-dependent, short-lasting bradycardia (Figs. 1 and 2A). [Sar8,Met(O2)11]SP was more potent than GR64349, or [MePhe7]NKB (ED50 of 71 pmol versus 4.4 and 3.8 nmol for NKA and 1 nmol of ACh were determined in the absence of drug and again during exposure to the antagonist or a combination of antagonists. The NK1 receptor antagonist FK888 was diluted with saline and given by infusion (8 nmol/50 μl min -1) through a short length of polyethylene 20 tubing that emptied into the perfusion buffer at a point near the three-way stopcock attached to the aortic cannula. The infused solution contained 10% DMSO. The final concentration of FK888 at the heart was 1 μM. The NK2 and NK3 receptor antagonists SR48968 and SR142801, respectively, were included in the perfusion buffer when hearts were treated for 20 min before a second challenge with NKA. In later experiments, the treatment interval was extended to 60 min, and antagonists were given by infusion. The concentration of DMSO in the buffer was ~0.4%.

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[MePhe$^7$]NKB produced biphasic changes in the force of ventricular contractions (Figs. 1C and 2B).

GR64349 caused a dose-dependent increase in perfusion pressure (ED$_{50}$ = 0.95 nmol; Figs. 1B and 2C). All of the selective agonists evoked small decreases in perfusion pressure in spite of low basal levels for this parameter (Figs. 1 and 2C). A depressor response preceded the pressor effect of GR64349 at doses of 10 and 32 nmol.

Desensitization to each of the selective tachykinin agonists occurred when bolus doses of 32 nmol were given repeatedly at 1-min intervals. After the third injection, hearts no longer responded to these agents.

**Effect of Tachykinin Receptor Desensitization with Selective Agonists on Responses to NKA.** NKA caused bradycardia, an increase in the force of ventricular contractions, and either a decrease in perfusion pressure or a biphasic coronary vascular response (Figs. 3–6), as reported previously (Hoover et al., 1998). Biphasic vascular responses consisted of an initial decrease in perfusion pressure, followed by an increase above the previous baseline; this type of response occurred in 30 of 51 hearts.

Negative chronotropic responses to NKA were reduced by 82% after desensitization with GR64349 and by 41% after desensitization with [MePhe$^7$]NKB (Fig. 3A). Desensitization with [Sar$^9$, Met(O$_2$)$_{11}$]SP did not affect the bradycardic action of NKA, but the positive inotropic response was increased by 92% (Fig. 3B). Positive inotropic responses to NKA were abolished after desensitization with GR64349 and reduced by about half after desensitization with [MePhe$^7$]NKB (Fig. 3B). Vasodilator responses to NKA were reduced by 75% or more during desensitization with each of the selective agonists (Fig. 4A). The pressor effect of NKA was abolished by desensitization with GR64349 and enhanced after desensitization with [Sar$^9$, Met(O$_2$)$_{11}$]SP (Fig. 4B). Desensitization with a combination of GR64349 and [MePhe$^7$]NKB eliminated all responses to NKA. Pretreatment with subtype selective agonists did not affect cardiac or coronary responses to 1 nmol of ACh (not shown).

**Effect of Subtype-Selective Tachykinin Receptor Antagonists on Responses to NKA.** Negative chronotropic responses to NKA were attenuated by treatment with 100 nM SR48968 for 20 min (75% decrease; Figs. 5 and 7A) or 100 nM SR142801 for 60 min (48% decrease; Fig. 5A). Treatment with SR142801 (100 or 320 nM) for 20 min was ineffective (not shown). Combined treatment with NK$_2$ and NK$_3$ antagonists (100 nM each for 60 min) nearly eliminated the chronotropic response to NKA without affecting the bradycardia evoked by 1 nmol of ACh (76 ± 1% decrease for control versus 73 ± 5% decrease in presence of antagonists; n = 4; P = .39). Minor reductions in bradycardic responses to NKA (Fig. 5A) and ACh occurred during treatment with 1 μM FK888. Positive inotropic responses to NKA were abolished by 100 nM SR48968 and reduced by 100 nM SR142801 (Fig. 5B). Ventricular contractile responses to NKA were more variable in the presence of 1 μM FK888 (Fig. 5B), so a reduction in the mean response was not statistically significant.

Treatment with FK888 eliminated coronary vasodilator responses to NKA, whereas SR48968 and SR142801 caused reductions (Fig. 6A). Pressor responses to NKA were not observed in the presence of 100 nM SR48968 or SR142801 (Fig. 6B). The pressor response to 32 nmol of GR64349 was unaffected by treatment with 100 nM SR142801 (19.0 ± 9.8% increase in perfusion pressure for control versus 17.2 ± 4.1% increase in presence of NK$_3$ antagonist; n = 5; P = .60). In marked contrast, pressor responses to NKA were enhanced by treatment with FK888 (Figs. 6B and 7B).
Cardiac and coronary responses to 1 nmol of \([\text{Sar}^9,\text{Met(O2)}^{11}]\text{SP}\) could be blocked by 1 mM FK888. Negative chronotropic and pressor responses to 32 nmol of GR64349 were abolished by 100 nM SR48968, and the positive inotropic response was reversed (19.7 ± 3.6% increase in contractility for control, compared with 10.1 ± 3.7% decrease in the presence of NK2 antagonist). The negative chronotropic response to 32 nmol of GR64349 was attenuated by SR142801 (30.0 ± 6.5% decrease in rate for control versus 20.9 ± 7.5% with NK3 antagonist present; \(n = 5; P = .006\)) but positive inotropic responses were unaffected. During treatment with 100 nM SR48968, baseline ventricular contractility was reduced by 26% and perfusion pressure was elevated by 12% (Table 1). Treatment with 100 nM SR142801 increased baseline perfusion pressure by 12% and decreased baseline heart rate by 10%.

**Discussion**

Results from this study provide the first evidence that all three subtypes of the tachykinin receptor are present in the guinea pig heart. The overall response evoked by administration of NKA to isolated hearts comprises elements gener-
ated through activation of each receptor subtype. Bradycardia occurs through activation of NK2 and NK3 receptors, whereas positive inotropic responses are mediated primarily by NK2 receptors. The dominant effect of NKA on coronary resistance vessels is NK1 receptor-mediated vasodilation, but a vasoconstrictor action becomes prominent following suppression of the vasodilator response. Last, this study provides the first evidence that NK2 receptors mediate coronary vasoconstriction.

We previously reported that NKA and SP cause a dose-dependent bradycardia in the isolated guinea pig heart (Hoover and Hancock, 1988; Hoover, 1990; Hoover et al., 1998). The present findings demonstrate that bradycardia can occur through stimulation of each subtype of the tachykinin receptor, but activation of NK2 receptors produces the most prominent decrease in heart rate. Two lines of evidence suggest that negative chronotropic responses to NKA occur primarily through stimulation of NK2 receptors. First, desensitization with GR64349 caused an 80% decrease in the magnitude of bradycardia evoked by NKA. Second, blockade of NK2 receptors reduced the chronotropic response to NKA by 75%. Neither of these treatments affected the negative chronotropic response to ACh. Results from analogous experiments using [MePhe7]NKB and SR142801 also implicate NK2 receptors in the chronotropic response to NKA but suggest a smaller contribution. Neither desensitization with an NK1 agonist nor blockade of NK1 receptors with FK888 affected the bradycardic response to NKA. Thus, NK1 receptors have no role in mediating this effect.

Stimulation of intracardiac cholinergic neurons is one mechanism by which tachykinins can produce bradycardia. Binding sites for SP and NKA are localized to intracardiac ganglia (Hoover and Hancock, 1988; Hoover et al., 1998), and both peptides increase the excitability of intracardiac neurons (Konishi et al., 1985; Hardwick et al., 1995, 1997). Negative chronotropic responses to SP are also attenuated by treatment with atropine or hemicholinium-3 (Hoover, 1990; Chiao and Caldwell, 1995). Electrophysiological evidence suggests that intracardiac neurons express NK2 and NK3 receptors (Hardwick et al., 1995), but the slow depolarization evoked by SP occurs through the NK3 subtype (Hardwick et al., 1997). Our previous work established that more than half of the response to NKA occurs through a noncholinergic mechanism because it is resistant to blockade by atropine (Hoover et al., 1998). Accordingly, it is possible that tachykinin receptors could be present at the sinoatrial node in a concentration below the limit for detection by autoradiography.

**Fig. 4.** Effect of desensitization by selective tachykinin agonists on coronary vasodilator (A) and coronary vasoconstrictor (B) responses to bolus injections of NKA. Effects of 25 nmol of NKA were determined before desensitization, 30 s after desensitization, and after at least 30 min for recovery from the last injection of selective agonist. Values are means ± S.E.; n = 6 for each group. NK1, NK2, and NK3 signify desensitization by [Sar9, Met(O2)11]SP, GR64349, and [MePhe7]NKB, respectively. Data for each group were evaluated by ANOVA for repeated measures. Asterisk (*) indicates significant difference from other values in group. Plus sign (+) indicates significant difference from value before desensitization.

**Fig. 5.** Effect of selective tachykinin antagonists on chronotropic (A) and inotropic (B) responses to bolus injections of NKA. Responses to 25 nmol of NKA were determined in the absence and presence of an antagonist to NK1 (FK888), NK2 (SR48968), or NK3 (SR142801) receptors. The duration of treatment was 20 min for FK888 and SR48968, whereas hearts were exposed to SR142801 for 60 min before challenge with NKA. Values are means ± S.E. (n = 5–6 per group). Asterisk (*) indicates significant difference from control response (paired t test).
Ventricular contractility was decreased by NK₁ agonists and increased by NK₂ agonists in this study. Because tachykinin receptors are not present in the ventricular myocardium (Hoover and Hancock, 1988; Hoover et al., 1998), such inotropic responses must occur secondarily to other actions of these drugs. In this regard, we found that the positive inotropic response to NKA was replaced by a small inhibitory effect when isolated guinea pig hearts were paced to prevent changes in heart rate (Hoover et al., 1998). Based on this finding and our current results, we conclude that positive inotropic responses to NK₂ agonists probably occur secondarily to bradycardia. Other investigators have presented evidence that SP can attenuate ventricular contractions by a paracrine mechanism involving nitric oxide released from vascular endothelial cells (Grocott-Mason et al., 1994; Paulus et al., 1995). It is likely that this mechanism underlies negative inotropic responses to NKA in paced hearts and NK₁ agonists in this study because coronary vasodilator responses to NKA and SP are mediated by nitric oxide (Vials and Burnstock, 1992; Hoover and Hossler, 1993). The enhanced positive inotropic response to NKA in the presence of FK888 could be explained by the loss of such a paracrine effect due to blockade of endothelial NK₁ receptors.

Previous studies implicated NK₁ receptors in the coronary vasodilator action of tachykinins based on the greater potency of SP compared with NKA (Gulati et al., 1987; Hoover and Hossler, 1993). In accord with this concept, we observed that NK₂ agonists exhibited the highest potency for decreasing perfusion pressure and that vasodilator responses to NKA were eliminated by treatment with the NK₁ antagonist FK888. Vasodilator responses to NK₂ and NK₃ agonists might be attributed to a decrease in selectivity of these agents at higher doses. Alternatively, they might occur indirectly through stimulation of NK₂ and NK₃ receptors on cholinergic neurons. This mechanism could also account for our observation that vasodilator responses to NKA were attenuated after treatment with an NK₂ or NK₃ antagonist.

This study provides evidence that NK₂ receptors mediate coronary vasoconstriction in the guinea pig heart. Support for this conclusion comes from observations that NK₂-selective agonists caused a dose-dependent increase in perfusion pressure, whereas selective agonists for other tachykinin receptor subtypes only caused depressor responses. A majority of our data also indicates that NK₂ receptors can mediate coronary vasoconstrictor responses to administered NKA. Although NK₁ receptor-mediated vasodilation normally dominates, vasoconstrictor responses to NKA were unmasked or enhanced during NK₁ receptor blockade or receptor desensitization with an NK₁ agonist. The only discordant result comes from experiments with SR142801 because this NK₂ antagonist appeared to block pressor responses to NKA without affecting those evoked by the NK₂ agonist GR64349. It is unclear at present whether this result is a real effect of the NK₂ blocker or a consequence of the variability and small magnitude of the NKA-evoked pressor responses under normal conditions.

Although the compounds used to evaluate tachykinin receptor subtypes in this study are classified as selective agonists or antagonists, it is recognized that their selectivity is concentration-dependent (Advenier et al., 1992; Fujii et al., 1992; Petitet et al., 1993; Regoli et al., 1994; Wang et al., 1994b; Emonds-Alt et al., 1995; Patacchini and Maggi, 1995). Furthermore, SR48968 and SR142801 can produce nonspecific effects through binding to ion channels (Wang et al., 1994a). We have attempted to minimize or monitor the influence of these factors in the design of this study by the following procedures. Two selective agonists for each tachykinin receptor subtype were evaluated, and response profiles within each class were identical. Responses to NKA were evaluated by two distinct but complementary approaches. The first of these was to desensitize tachykinin receptors using selective agonists. Although this approach undoubtedly affected more than a single tachykinin receptor subtype, the results suggest that the dominant effect was on the targeted receptor. The other approach was to block receptors with selective antagonists. Desensitization and receptor blockade produced similar results in most experiments. Last, we determined responses to ACh before and after tachykinin receptor desensitization or treatment with tachykinin receptor blockers and observed that responses to ACh were generally unaffected.

In conclusion, this study has provided functional evidence for the presence of NK₁, NK₂, and NK₃ receptors in the guinea pig heart and identified receptor subtypes that mediate responses to the native tachykinin NKA. Although this study has used pharmacologic doses of NKA and synthetic...
tachykinins, it is likely that some or all of these effects could likewise be triggered by endogenous tachykinins under pathophysiological conditions. Local release of tachykinins from cardiac afferents has been demonstrated in response to various chemicals released within the myocardium during ischemia (Franco-Cereceda et al., 1987, 1994; Geppetti et al., 1988). Accordingly, these findings provide important insights regarding effects that endogenous tachykinins might produce within the heart and coronary vasculature during ischemic heart disease.

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