Vagotonic Effects of Enkephalin Are Not Mediated by Sympatholytic Mechanisms

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This study examined the hypothesis that vagotonic and sympatholytic effects of cardiac enkephalins are independently mediated by different receptors. A dose-response was constructed by administering the δ-receptor opioid methionine-enkephalin-arginine-phenylalanine (MEAP) by microdialysis into the interstitium of the canine sinoatrial node during vagal and sympathetic stimulation. The right cardiac sympathetic nerves were stimulated as they exited the stellate ganglion at frequencies selected to increase heart rate approximately 35 bpm. The right cervical vagus was stimulated at frequencies selected to produce a two-step decline in heart rate of 25 and 50 bpm. A six-step dose-response was constructed by recording heart rates during nerve stimulation as the dose of MEAP was increased between 0.05 pmol/min and 1.5 nmol/min. Vagal transmission improved during MEAP at 0.5 pmol/min. However, sympathetically mediated tachycardia was unaltered with any dose of MEAP. In Study 2, a similar dose-response was constructed with the κ-opioid receptor agonist trans(−)-3-4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide-HCl (U-50488H) to illustrate an independent sympatholytic effect and to verify its κ-receptor character. U-50488H gradually suppressed the sympathetic tachycardia, with a significant effect obtained only at the highest dose (1.5 nmol/min). U-50488H had no effect on vagally mediated bradycardia. Surprisingly, the sympatholytic effect was not reversed by withdrawing U-50488H or by the subsequent addition of the κ-antagonist 17,17′-(dichloropropyl-methyl)-6,6′,7,7′-6,6′-imino-7,7′-binorphinan-3,4′,14,14′-tetroldi-hydrochloride (norBNI). Study 3 was conducted to determine whether the sympatholytic effect of U-50488H could be prevented by norBNI. NorBNI blocked the sympatholytic effect of the U50488H for 90 mins. When norBNI was discontinued afterward and U-50488H was continued alone, a sympatholytic effect emerged within 30 mins. Collectively these observations support the hypothesis that the vagotonic influence of MEAP is not dependent on a sympatholytic influence. Furthermore, the sympatholytic effect is mediated independently by κ-receptors. The sympatholytic effect of sustained κ-receptor stimulation appears to evolve gradually into a functional state not easily reversed. Exp Biol Med 231:387–395, 2006

Key words: enkephalins; opioids; dynorphins; heart rate; vagus nerves; sympathetic nerves

Introduction

The endogenous opioid peptides belong to one of several related peptide families, including the enkephalins, dynorphins, endorphins, and endomorphins. The opioids generally function as neuromodulators and exert the majority of their effects through μ-, δ-, and κ-opioid receptors. The opioids are active in a wide variety of physiologic systems, including the heart. Cardiac tissue contains both dynorphins (6, 10) and enkephalins (1–5, 8, 9, 11) and the messenger RNA (mRNA) for their respective precursors, preprodynorphin (6, 10) and preproenkephalin (4, 5, 7, 8). The three classes of opioid receptors have also been reported in heart through the δ-receptor, which is probably the predominate opioid receptor expressed (12–14). Although the mRNA for the opioid precursor peptides was easily demonstrated (4–6, 10), the yield of processed peptides (1–11) and receptor mRNA was limited (12–14). This disparity is consistent with a neuromodulatory function for the peptides and a primary location for cardiac opioid receptors on cardiac innervation.

Cardiac opioids are elevated in neonatal animals, decline during adulthood, and accumulate again late in life (4, 5, 8). Opioids are acutely elevated after myocardial infarction (15, 16), autonomic denervation (1, 4, 5), and hemorrhagic hypotension (17). Circulating and/or myocardial opioids are chronically elevated in genetic hypertension, in cardiomyopathic hearts, and after heart transplantation (18–20). Circulating opioids are elevated in congestive heart failure, and opioid receptor blockade improves cardiovascular function in experimental subjects with that condition (21, 22). Cardiac opioids and their receptors have recently become therapeutically more interesting because they have been implicated in protecting the heart from reperfusion injury, prevention of subsequent arrhythmias, and in extending the viable lifespan of harvested tissues (23–29). The physiologic function of

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opioids in the heart is less well understood. However, the ability of cardiac opioids to locally modify the autonomic innervation of the heart has become a productive area of inquiry. This study specifically focused on paracrine interactions between locally administered opioids and the autonomic innervation of the cardiac pacemaker.

Myocardial enkephalins and their receptors can function as potent regulators of heart rhythm. Though the primary cellular source of myocardial enkephalin remains unclear, enkephalins have been identified in cardiomyocytes and assorted autonomic structures, where they are well positioned to moderate myocardial contraction and heart rate (30–32). Opioid receptors within the SA node can both interrupt and facilitate vagal transmission (33–40). Picomolar infusions of methionine-enkephalin-arginine-phenylalanine (MEAP) inhibited vagally induced bradycardia through the activation of δ_2-opioid receptors. This vagolytic response was duplicated by the δ_2-agonist deltorphin and blocked by the δ_2-antagonist naltriben (34, 35). Ultra-low or femtomolar infusion rates produced the opposite response and increased vagal transmission through the activation of δ_1-opioid receptors. The vagotonic response was duplicated by the δ-1-agonist TAN-67 and blocked by the δ_1-antagonist BNTX (34, 35). Opioid-mediated preconditioning also appears to involve δ_1-receptors in that the δ_1-agonist TAN-67 mimics ischemic preconditioning and BNTX abrogates it (27, 28). The vagolytic opiate receptors are most likely located on postganglionic vagal nerve endings, where they presumably moderate the release of acetylcholine (33, 41). The functional location of the vagotonic receptors has not been investigated. The bimodal effects of the cardiac δ-receptors indicate a spectrum of potential roles in regulating the heart at rest and during stress.

The activation of κ-opioid receptors has also been reported to modify cardiac activity both *in vivo* and *in vitro* (26, 29, 42). The presence of prodynorphin mRNA within the myocardium indicates that cardiac κ-receptors may be activated by locally synthesized dynorphins (6, 10). Intracoronary dynorphin suppressed norepinephrine release and reduced contractile activity when this native κ-agonist was administered during sympathetic stimulation (43). Similar to δ-opioid receptors, κ-opioid receptor activation reduces infarct size following coronary ischemia (26, 29). Thus, similarities between the two opioid systems indicate that cross-talk between these two opioid receptor classes may produce cooperative or interactive responses.

Leucine-enkephalin (LE) administered into the canine SA node by microdialysis reduced tachycardia during electrical stimulation of the cardiac sympathetic nerves (42). The tachycardia following the infusion of norepinephrine was, however, unaltered by LE, indicating that the opioid receptor in question was localized prejunctionally on the sympathetic nerve terminals. The sympatholytic effect of LE was not reversed by the δ-antagonist naltrindole, indicating a non–δ-opioid receptor mechanism. This LE effect was surprisingly reversed by the κ-antagonist norBNI, indicating a κ-opioid receptor interaction. LE at this dose also produced a clear δ-mediated vagolytic effect similar to that observed with MEAP and ME (33–42).

Since cardiac LE concentrations are quite low, it seems likely that the sympatholytic effect of LE is normally mediated by a more potent or more selective κ-agonist like dynorphin. If enkephalins are sympatholytic, the more abundant MEAP might represent a better candidate. In fact, very low doses of MEAP were vagotonic, and this improved vagal transmission might be explained by the elimination of tonic opposition from sympathetically mediated tachycardia. Thus, the current study was designed to examine the hypothesis that the vagotonic effect of MEAP might result in part from a coincident sympatholytic effect mediated by κ-receptors. The study was also constructed to verify the κ-receptor character of the sympatholytic effect and to test whether a selective κ-agonist would produce a similar, more potent and/or more efficacious sympatholytic effect.

Materials and Methods

General Surgical Preparation. Mongrel dogs of either gender weighing 15–30 kg were assigned at random to the experimental protocols. All protocols were approved by the Institutional Animal Care and Use Committee and were in compliance with the *National Institutes for Health Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85–23, revised 1996). The animals were anesthetized with sodium pentobarbital (32.5 mg/kg), intubated, and initially ventilated mechanically at 225 ml/min/kg with room air. Fluid-filled catheters were inserted into the right femoral artery and vein and advanced into the descending aorta and inferior vena cava, respectively. The arterial line was attached to a pressure transducer to measure heart rate and arterial blood pressure continuously online (PowerLab; ADI Instruments, Colorado Springs, CO). The depth of anesthesia was regularly evaluated, and supplemental anesthetic was administrated as required. The acid-base balance and blood gases were determined with an Instrumentation Laboratories blood gas analyzer (Lexington, MA). The pO_2 (90–120 mm Hg), the pH (7.35–7.45), and the pCO_2 (30–40 mm Hg) were adjusted to normal by administering supplemental oxygen or bicarbonate or by modifying the tidal volume.

The right and left cervical vagus nerves were isolated from the carotid arteries through a ventral midline incision. The nerves were double-ligated with umbilical tape to eliminate afferent vagal nerve traffic and reflex vagal compensation during vagal and sympathetic stimulation, respectively. Anesthesia was carefully evaluated, and a single dose of succinylcholine (1 mg/kg) was administered intravenously to reduce muscle movement for the 10 mins required for electrosurgical incision of the chest. A thoracotomy was performed to expose the right heart and

the pericardium was opened and the pericardial margins were sutured to the body wall to support the heart. After the electrocautery was completed, the fluid-filled arterial catheter was replaced with a high-fidelity solid-state transducer (Millar Instruments, Houston, TX) to measure the arterial pressure and heart rate throughout the remainder of the protocol. The sympathetic cardiac nerves distal to the right stellate ganglion (right ansa subclavia) were isolated and stimulated to determine the frequency (0.5–1.5 Hz) needed to produce a 30–40-bpm increase in heart rate within 45 secs.

Nodal Microdialysis. The probe fabricated with dialysis fiber from a Clirans TAF 08 (Asahi Medical, Northbrook, IL) artificial kidney was positioned with the dialysis window completely within the nodal tissue. The probe (1 cm x 200 μm i.d. x 220 μm o.d.) restricts the transmural passage of molecules with a mass greater than 36 kDa. The glass-fiber inflow line was then attached to a micro infusion pump and perfused with vehicle (saline) at 5 μl/min and allowed to equilibrate for 1 hr.

Protocol 1. MEAP Dose-Response During Vagal and Sympathetic Stimulation. After equilibration for 1 hr, the right cervical vagus nerve was stimulated at a supramaximal voltage (15 V) for 15 secs at low (1–2 Hz) and high (3–4 Hz) frequencies selected empirically in each animal to produce 10–20-bpm and 30–40-bpm decreases in heart rate, respectively. The system was allowed 2 mins to recover between the successive vagal stimuli. Two minutes after completing the vagal stimulations, the cardiac sympathetic nerves (ansa subclavia) were stimulated at a frequency (0.5–1.5 Hz) selected empirically to increase heart rate by 30–40 bpm. Once these control measurements were recorded, a cumulative six-step MEAP dose-response (0.05–1500 pmol/min) was conducted. Each dose was delivered by microdialysis and allowed to equilibrate during 5 mins of infusion. After 5-min exposure at each dose, the vagal and sympathetic stimulations were repeated and then the next dose of MEAP was introduced. After completing the dose-responses, the enkephalin was discontinued and the dialysis line was flushed with saline for 10 mins. Nerve functions were reevaluated after 10 mins to verify complete recovery of control function and the absence of a time- or treatment-dependent change in the baseline response to nerve stimulation.

Protocol 2. U-50488H, (κ-Agonist) Dose-Response Curve During Vagal and Sympathetic Stimulation; Attempted Reversal by norBNI (κ-Antagonist). The dose-response described in Protocol 1 was repeated while substituting the κ-agonist trans(±)-3–4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide-HCl(U-50488H) for MEAP. After completing the dose-response, the κ-agonist was discontinued and the probe was perfused with saline for 10–30 mins. Nerve functions were reevaluated to determine whether control values could be reestablished. The κ-antagonist, 17,17′-(dichloropropylmethyl)-6,6′,7,7′-6,6′-imino-7,7′-binorphinan-3,4′,14,14′-tetroldihydrochloride (norBNI) was next perfused into the nodal probe at a dose rate equimolar to the maximal dose of U-50488H (1.5 nmol/min) to test for an effect of norBNI independent of the added agonist. After 5 mins of norBNI, the stimulation sequence was repeated. NorBNI was washed out with saline for 20 mins and the heart rate responses during nerve stimulation were evaluated once again to determine whether the control responses had been reestablished. Finally, equimolar concentrations of U-50488H and norBNI were combined and introduced together (1.5 nmol/min) to verify that the sympatholytic effect was mediated by a κ-receptor interaction.

Protocol 3. NorBNI Pretreatment Prevents the Sympatholytic Effect of U-50488H. This protocol was conducted to determine whether pretreatment with norBNI was more effective than adding norBNI after sustained exposure to the agonist U-50488H. The thoracic sympathetic nerves were isolated as described above. After 1 hr of equilibration, the sympathetic nerve was stimulated at 0.5 and 1 Hz for 45 secs each, with 3 mins of recovery between stimulations. An excess of the κ-antagonist norBNI (5 nmol/min) was perfused alone for 10 mins followed by a second pair of sympathetic stimulations to evaluate for a drug effect independent of the agonist. Then equimolar doses (1500 pmol/min) of norBNI and U-50488H were perfused for 90 mins. The duration was selected to exceed the duration required to complete the previous dose-response for U-50488H described in Protocol 2. The sympathetic nerve was restimulated every 30 mins at the two frequencies. Following the stimulations at 90 mins, the norBNI was discontinued and U-50488H (1500 pmol/min) was continued alone for another 60 mins. Additional nerve stimulations were tested every 15 mins to monitor for the emergence of the sympatholytic effect.

Materials. MEAP was synthesized by American Peptide (Sunnyvale, CA). NorBNI, Dynorphin A 1–13, and U-50488H were obtained from Sigma RBI (St. Louis, MO).

Data Analysis. The data were expressed as mean ± SEM. Differences were evaluated by analysis of variance, and multiple post-hoc comparisons were made with Tukey's test (GB-STAT, Dynamic Microsystems, Silver Springs, MD). Repeated measures comparisons were made where appropriate, and $P < 0.05$ was accepted as a statistically significant difference.

Results

The resting cardiovascular indices for all protocols are listed in Table 1. No differences were observed in the initial values for animals assigned to each protocol, and there were no changes in resting function during any of the intranodal treatments.

Study 1. MEAP Improves Parasympathetic Transmission Without Modifying Sympathetic Transmission. The lower and higher frequency vagal stimulation produced, respectively, mean decreases in heart

Table 1. Cardiovascular Indices

	MEAP (pmol/min)						
	Control	0.05	0.5	5	50	500	1500
Study 1 ($N = 6$)							
HR(bpm)	111.4 ± 4.4	113 ± 5.4	109.2 ± 4.9	107.4 ± 5.9	108.4 ± 6.1	107.4 ± 6.3	107.4 ± 4.9
MAP(mm Hg)	96.2 ± 2.4	96.4 ± 1.9	98.8 ± 1.7	97.1 ± 2.8	98.6 ± 2.2	98.2 ± 2.3	98.8 ± 2.4
	U-50488H (pmol/min)						
	Control	0.05	0.5	5	50	500	1500
Study 2 ($N = 7$)							
HR(bpm)	109 ± 1.4	108.8 ± 1.5	109.5 ± 1.5	108.5 ± 2.4	105.3 ± 2.7	102.3 ± 1.7	100.2 ± 2.2
MAP(mm Hg)	93.5 ± 3.2	93.8 ± 3.8	94.7 ± 4.3	95.3 ± 3.9	96.3 ± 3.7	96.0 ± 3.2	96.2 ± 3.2
	U-50488H (1.5 nmol/min)						
	Control	5 nmol/min	90 mins	15 mins	30 mins	45 mins	60 mins
Study 3 ($N = 3$)							
HR(bpm)	108.7 ± 8.3	107 ± 6.8	101.7 ± 4.4	99.0 ± 6.7	94.3 ± 10.4	95.0 ± 9.2	94.7 ± 9.8
MAP(mm Hg)	98.0 ± 4.4	98.7 ± 3.1	96.7 ± 1.9	97.7 ± 1.9	98.3 ± 1.2	98 ± 2.1	100.7 ± 0.9
norBNI	−	+	+	−	−	−	−
U-50488H	−	−	+	+	+	+	+

rate of 20.4 ± 2.6 and 42 ± 8.1 bpm (Fig. 1). MEAP produced a significant improvement in vagal transmission at 0.5 pmol/min. The bradycardia during low-frequency stimulation reduced the heart rate an additional 31% (29.6 ± 5.4 bpm). At the higher frequency, the increase in enkephalin-mediated bradycardia was a less-dramatic 10% (46.0 ± 7.4 bpm) and did not reach significance. At the higher end of the dose-response (1500 pmol/min), the bimodal character of the response was evident and MEAP reduced the low-frequency bradycardia by 62% (7.6 ± 2.1 bpm) compared to control. The high-frequency response (18.5 ± 5.7 bpm) was similarly reduced by 55%. The vagolytic effects were statistically different from control and from the peak vagotonic response. When the treatments were discontinued and the system was perfused again with vehicle, vagal stimulation produced bradycardia similar to that observed in the control. The restoration of control responses on washout also indicated that the changes in response to MEAP infusion were reversible and nontoxic. These observations were expected and confirmed findings from earlier studies (34, 35).

After a 2-min recovery from each vagal stimulation sequence, the sympathetic nerves were stimulated for 45 secs at 1 Hz. Sympathetic stimulation produced a mean increase of 38.8 ± 5.3 bpm (Fig. 2). MEAP did not alter the rate of change in heart rate (not shown) or the maximal tachycardia at any of the six dose rates. Despite an upward trend between control and 5 pmol/min, the increase was not significantly different. The inability of MEAP to modify heart rate during sympathetic stimulation indicated that both the vagotonic and vagolytic effects of MEAP on vagal transmission are independent of sympathetic input and that unlike LE, MEAP had no intrinsic κ-receptor activity.

Study 2. The κ-Agonist U-50488H Is Sympatholytic. Study 1 indicated that MEAP at ultra-low doses enhanced parasympathetically mediated bradycardia within the SA Node without altering the sympathetically induced tachycardia. In Study 2, the κ-receptor opioid agonist U-50488H was infused into the nodal interstitium to determine

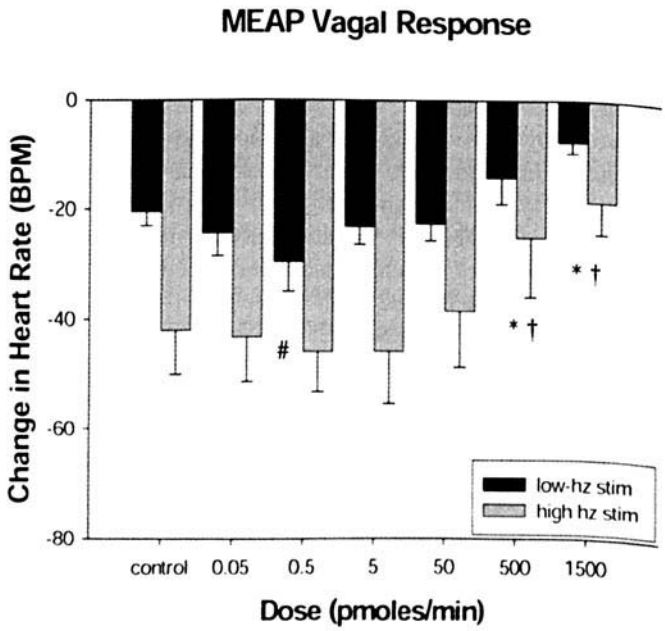

Figure 1. Changes in heart rate mediated by low (1–2 Hz)– and high (3–4 Hz)–frequency stimulation of the right vagus nerve are illustrated during exposure to increasing doses of MEAP introduced into the interstitium of the SA node by microdialysis. Values are means and standard error of the mean from six subjects. The three symbols indicate that the change in heart rate was greater (#) than control, less (*) than control, or less (†) than the peak vagotonic response ($P < 0.05$).

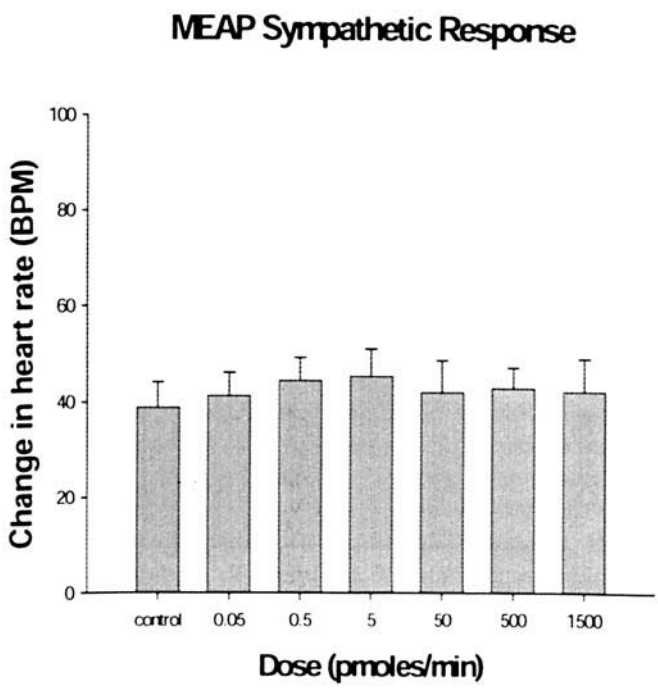

Figure 2. Changes in heart rate mediated by stimulation (1 Hz) of the right thoracic sympathetic nerves are illustrated during exposure to increasing doses of MEAP introduced into the interstitium of the SA node by microdialysis. Values are means and standard error of the mean from six subjects. No treatment effects were observed.

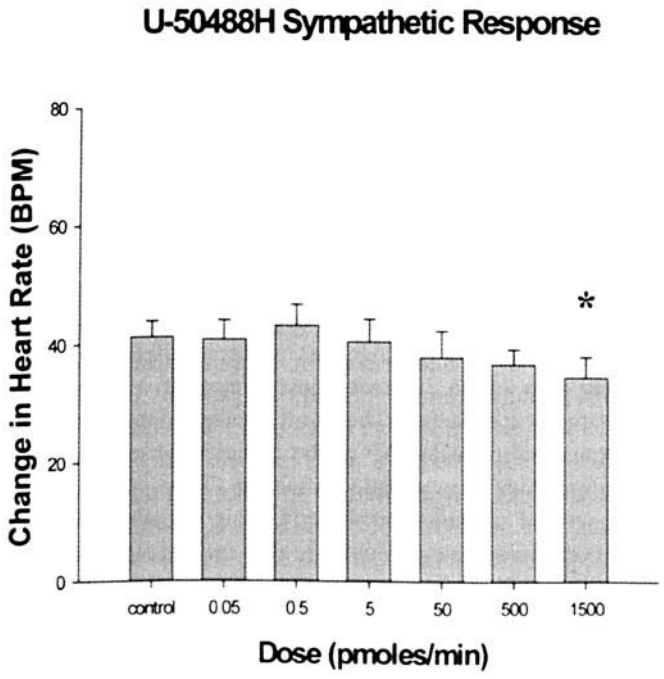

Figure 3. Changes in heart rate mediated by stimulation (1 Hz) of the thoracic sympathetic nerves are illustrated during exposure to increasing doses of the κ-agonist U-50488H introduced into the interstitium of the SA node by microdialysis. Values are means and standard error of the mean from seven subjects. The symbol (*) indicates that the change in heart rate was significantly different from that of the control ($P < 0.05$).

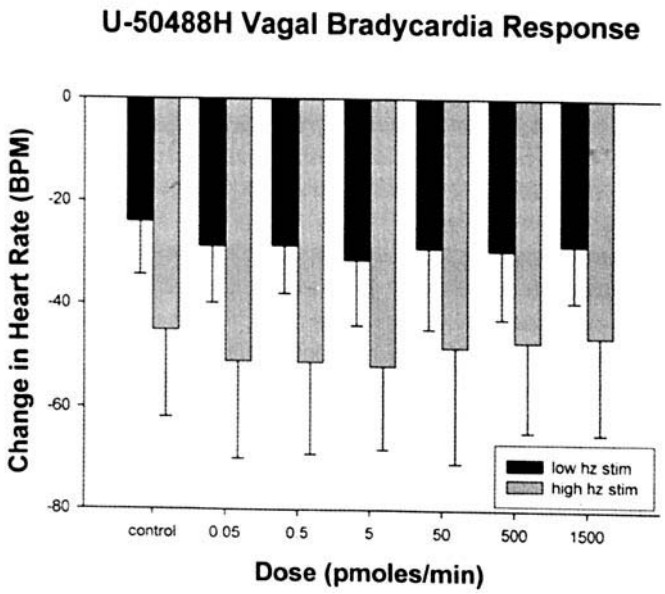

Figure 4. Changes in heart rate mediated by low (1–2 Hz)- and high (3–4 Hz)-frequency stimulation of the right vagus nerve are illustrated during exposure to increasing doses of the κ-agonist U-50488H, introduced into the interstitium of the SA node by microdialysis. Values are means and standard error of the mean from seven subjects. No treatment effects were observed.

the role of nodal κ-receptors in sympathetic and parasympathetic mechanisms. Sympathetic stimulation increased heart rate an average of 41.2 ± 3.2 bpm (Fig. 3). U-50488H produced a gradual decline in sympathetically mediated tachycardia starting at 5 pmol/min and became significantly different from control at 1500 pmol/min. The resulting 34.4 ± 4.7 bpm represented a 17% reduction from control. Low- and high-frequency vagal stimulations reduced heart rate by 24.0 ± 3.9 and 45.0 ± 6.4 bpm, respectively (Fig. 4). Vagal bradycardia was unaltered by U-50488H. During infusion of the maximal dose of U-50488H, the vagal stimulation reduced heart rate by 28.4 ± 4.2 and 46.3 ± 7.1 bpm, which was not significantly different from control. The significant reduction in tachycardia during the administration of U-50488H indicated that nodal κ-receptors influence sympathetic transmission independent of any vagal interaction.

Inconsistencies arose during the postinfusion evaluations after the U-50488H was discontinued. The sympathetic effect observed before U-50488H was not restored on washout, and the tachycardia remained depressed even after 30-min washout with vehicle (35.8 ± 5.6). Furthermore, addition of the κ-antagonist (norBNI) for another 30 mins also failed to reverse the sympatholytic effect (27.6 ± 2.9) and to restore the sympathetically induced tachycardia observed before U-50488H. The sympathetic stimulation after norBNI was not significantly different from the maximally sympatholytic dose of U-50488H. Vagal evaluation at these same intervals indicated again that neither U-50488H nor norBNI had any effect on the vagal response. In summary, the selective κ-agonist produced a clear sym-

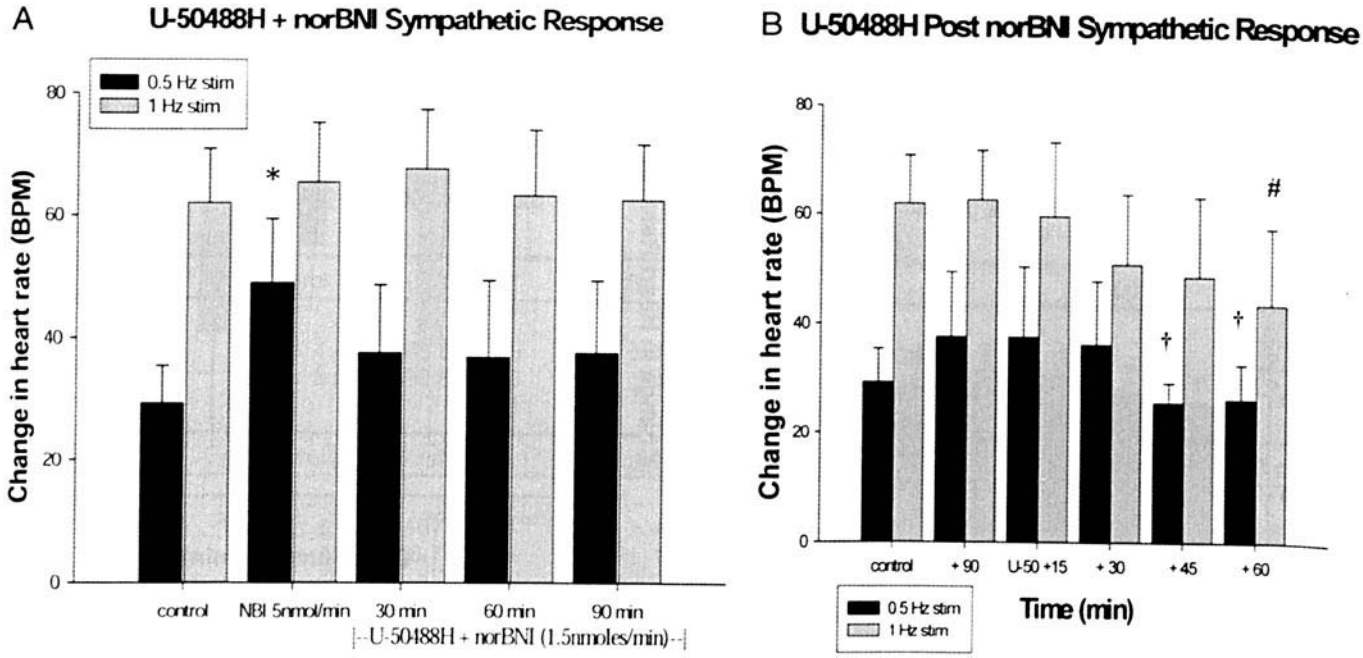

Figure 5. (A) Changes in heart rate mediated by lower (0.5 Hz) and higher (1 Hz) frequency stimulation of the right thoracic sympathetic nerves are illustrated during 5-min exposure to excess norBNI alone (5 nmol/min) followed by 90 mins of norBNI combined with U-50488H at equimolar dose rates (1.5 nmol/min) introduced into the interstitium of the SA node by microdialysis. (B) After 90 mins, the norBNI was discontinued and the U-50488H was continued alone for 60 mins. Values are means and standard error of the mean from three subjects. In Panel A, the symbol (*) indicates that the change in heart rate was greater than control ($P < 0.05$). In Panel B, the symbols indicate that the change in heart rate was less than control (†), less than at 90 mins, and control (#) ($P < 0.05$).

patholytic response, but the control response was not restored on washout or after administering a selective κ-antagonist. The failure to restore normal sympathetic transmission indicated that exposure to U-50488H precipitated a very slowly resolving effect downstream from the κ-receptor or that the sympatholytic response was not mediated by κ-receptors. Study 3 was designed to differentiate between those alternatives.

Study 3. NorBNI Prevents the Sympatholytic Effect of U-50488H. In this study, the κ-antagonist norBNI was infused into the SA node by microdialysis before and concurrently with U-50488H to verify κ-receptor participation by preventing the subsequent sympatholytic effect of U-50488H. The sympathetic nerves were stimulated at lower (0.5 Hz) and higher (1 Hz) frequencies to produce average increases in heart rate of 29.3 ± 6.2 and 62.0 ± 8.9 bpm, respectively. During the initial 5-min infusion with excess norBNI alone (5 nmol/min), the heart rate response to sympathetic stimulation increased but was significant only at the lower frequency (Fig. 5). When equimolar doses of U-50488H and norBNI were combined at 1.5 nmol/min, no sympatholytic effect was observed throughout the 90-min period of infusion. When norBNI was discontinued and U-50488H was continued alone for an additional 60 mins (Fig. 5B), a sympatholytic effect gradually emerged. After 60 mins, both low- and high-frequency responses were reduced, respectively, to 25.6 ± 6.4 and 43.7 ± 13.9 bpm.

Discussion

The normal cardiac rhythms are primarily derived from interactions between the intrinsic rhythm of nodal pacemaker cells and incoming parasympathetic and sympathetic traffic. Changes in heart rate are quickly executed by increasing and decreasing efferent vagal transmission and less often by increasing sympathetic activity. As local neuromodulators, cardiac opioids are well positioned to modify these efferent autonomic influences as they innervate the pacemaker. The current study was prompted by the observation that enkephalins administered by dialysis into the SA node were both vagotonic and sympatholytic (34, 35, 42). The first study was designed to test whether the vagotonic effect of MEAP was a direct effect on vagal transmission or an indirect consequence of a coincident sympatholytic influence. The result obtained demonstrated a vagotonic effect of MEAP in the complete absence of any complimentary sympathetic influence. Thus, MEAP improves vagal transmission directly. This is consistent with the prior pharmacologic evidence that the vagotonic effects were mediated by δ_1-receptors (34, 35) and the sympatholytic effects were mediated by κ-receptors (42, 43). Since MEAP did not alter sympathetic transmission, the vagotonic effect cannot be attributed to a reduction in sympathetic opposition. The failure of MEAP to alter sympathetic transmission indicates that unlike LE, which appears to exert some κ-agonist activity in this system, MEAP has little or no effect, regardless of the dose applied.

LE is an established δ-agonist and thus an unlikely

candidate as the endogenous κ-agonist responsible for moderating sympathetic transmission. LE, like MEAP, is highly vagolytic, and, like MEAP, its vagolytic effect was eliminated by the δ-antagonist naltrindole (42). The sympatholytic effect of LE that was reported earlier may reflect the higher dose used in that earlier study (42), and some of the efficacy of LE at the κ-receptor may be attributed to the amino acid sequence it shares with the native κ-agonist dynorphin. The second study was designed to verify that the sympatholytic effect of LE could be duplicated by a more-selective κ-agonist and presumably with a much greater potency. Initial κ-agonist studies were conducted in several animals with the native κ-selective peptide dynorphin 1–13. Unfortunately, dynorphin is highly charged and routinely adsorbs to surfaces. Despite attempts to prevent adsorption, there was no demonstrable effect of dynorphin on sympathetically mediated tachycardia. However, in the absence of a response, it is difficult to determine whether dynorphin was ineffective or simply never reached the target. As a result, the subsequent dose-responses were conducted with the synthetic κ-agonist U-50488H, an alkaloid. As demonstrated in the second study, U-50488H was mildly sympatholytic. The reduction in sympathetic tachycardia appeared to emerge at lower doses, but only reached significance at the highest dose. The control sympathetic effect was not, however, restored when the U-50488H was washed out with vehicle, and the sympatholytic response was not reversed following the addition of the κ-antagonist norBNI. Though the data were not included, longer washouts and higher doses of the antagonist were evaluated in several animals without any change in the outcome. Thus, it appears that U-50488H exerted a downstream effect that was either irreversible or only slowly reversible. Whether this was mediated by κ-receptors or through a non–κ-opioid-receptor mechanism required further confirmation.

Study 3 was thus designed to determine whether pretreatment with the κ-antagonist norBNI would prevent the sympatholytic effect of U-50488H and to confirm that the effect of U-50488H was mediated by interaction with κ-receptors. Surprisingly, norBNI alone at a high dose appeared to improve sympathetic transmission. Although this is indicative of a reversal of the effect of an endogenous κ-agonist, it may also represent a direct effect of norBNI. The sympatholytic dose of U-50488H was then combined with an equimolar dose of norBNI for 90 mins. The duration was selected to exceed the time required for the complete U-50488H dose-responses in the previous protocol. NorBNI completely prevented the sympatholytic effect of U-50488H, and the protection gradually eroded when the norBNI was discontinued. It is unclear whether the gradual onset reflects the temporal character of the U-50488H response or simply the time needed to wash out the residual interstitial norBNI. Thus, the sympatholytic effect of U-50488H was mediated by κ-opioid receptors. The resultant changes in function, however, may develop and resolve very slowly. In contrast, sympatholytic effect of LE and the vagolytic effects of ME, LE, and MEAP all develop and resolve quickly (33–42). Thus, the reason for the slow kinetics is unclear but may be related to either the long duration of exposure during the dose-response or may reflect the disposal characteristics of U-50488H in this specific model.

Opioids are generally believed to function as neuromodulators. The endogenous opioids at work in the heart are presumably local in origin, since the enzyme activities that degrade them are prodigious and widespread. The cardiac opioids then likely regulate the gain of the relationship between neural transmission and neurotransmitter release for the direct-acting transmitters norepinephrine and acetylcholine. The magnitude of the observed response depends on the ambient nerve traffic and the integrated influence of other prejunctional modifiers. In fact, some of the variability of response to added enkephalin may reflect differences in the concentrations of the endogenous moderators at the time of administration. In this regard, the vagotonic and vagolytic effects of the opioids have varied from as little as 10% to as much as 100%. Typically, however, the opioid alters the neural effect by one-third to one-half. The response to U-50488H was at the lower end of this scale and may reflect a lower potency compared with the peptides. Intracoronary dynorphin, by comparison, reduced norepinephrine spillover by 50% (43). In general, these opioid effects are similar in magnitude to effects mediated by other prejunctional moderators like catecholamines (44, 45), histamine (45), and adenosine (46). Although direct comparisons have not been made with delivery by microdialysis, the opioids appear to exert their effects at lower concentrations than these other neuromodulators.

The physiologic role of the responses observed above is currently speculative; however, the vagotonic effect of MEAP is probably primary, since the low concentrations required are more easily achieved *in vivo*. The release of endogenous enkephalins during coronary occlusion (37) improved vagal transmission and may thus serve to govern contractile function and moderate oxygen consumption when fuel supplies are limited. The same δ_1-receptor was implicated in opioid preconditioning (27, 28). The vagolytic influences of enkephalin (33–40) were observed at doses that are more likely to be associated with adrenergic stress (15, 17, 39). The vagolytic effect may serve to limit vagally mediated bradycardia when that bradycardia threatens cardiac output. Aside from its developmental aspects, less is known about cardiac dynorphin or the function of its presumed target, the κ-receptors. κ-Receptor stimulation has also been implicated in preconditioning (29), and a reduction in adrenergic transmission in the ischemic area could moderate an inappropriate increase in oxygen consumption in the local area and thus salvage more of the area at risk.

In summary, the vagotonic effects of MEAP are independent of any coincident sympatholytic influences.

The improved vagal transmission occurred at very low dose rates and may represent a salutary compensation. Enkephalins accumulate following ischemic preconditioning, and improved vagal transmission in the ischemic territory might reduce tissue injury by reducing work and oxygen consumption locally in the tissue served by the occluded artery (15, 34, 35, 37). An additional sympatholytic effect to reduce myocardial oxygen demand further would be complimentary to the vagotonic effect of enkephalin. Locally sympatholytic κ-receptors thus might explain the cardioprotective activity reported following the introduction of κ-opioids before ischemia (26, 29). Based on low myocardial LE content (1–5) and on the dose-response data presented above for MEAP, it seems unlikely that enkephalins would be responsible for a κ-mediated cardioprotective effect unless, for instance, LE was concentrated in close proximity to sympathetic neurons. The myocardial content of the prototypical endogenous κ-agonist dynorphin is also limited, but its selectivity and greater potency may make dynorphin a better candidate for regulating local sympathetic activity in heart. The sympatholytic effect of U-50488H was less potent than expected, although it was clearly mediated by κ-receptor activation. The magnitude of the response to U-50488H was limited and appeared to resolve very slowly if at all. Thus, the κ-receptor response seems more suited to long-term or background modulation.

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