Bimodal δ-opioid receptors regulate vagal bradycardia in canine sinoatral node

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Farias, M., K. Jackson, D. Yoshishige, and J. L. Caffrey. Bimodal δ-opioid receptors regulate vagal bradycardia in canine sinoatral node. Am J Physiol Heart Circ Physiol 285: H1332–H1339, 2003; 10.1152/ajpheart.00353.2003.—Methionine-enkephalin-arginine-phenylalanine (MEAP) introduced into the interstitium of the canine sinoatral (SA) node by microdialysis interrupts vagal bradycardia. In contrast, raising endogenous MEAP by occluding the SA node artery improves vagal bradycardia. Both are blocked by the same δ-selective antagonist, naltrindole. We tested the hypothesis that vagal responses to intranodal enkephalin are bimodal and that the polarity of the response is both dose- and opioid receptor subtype dependent. Ultralow doses of MEAP were introduced into the canine SA node by microdialysis. Heart rate frequency responses were constructed by stimulating the right vagus nerve at 1, 2, and 3 Hz. Ultralow MEAP infusions produced a 50–100% increase in bradycardia during vagal stimulation. Maximal improvement was observed at a dose rate of 500 fmol/min with an ED50 near 50 fmol/min. Vagal improvement was returned to control when MEAP was combined with the δ-antagonist naltrindole. The dose of naltrindole (500 fmol/min) was previously determined as ineffective vs. the vagolytic effect of higher dose MEAP. When MEAP was later reintroduced into the same animals at nanomoles per minute, a clear vagolytic response was observed. The δ1-selective antagonist 7-benzylidenenaltrexone (BNTX) reversed the vagal improvement with an ED50 near 1 × 10−21 mol/min, whereas the δ2-antagonist naltrexin had no effect through 10−8 mol/min. Finally, the improved vagal bradycardia previously associated with nodal artery occlusion and endogenous MEAP was blocked by the selective δ1-antagonist BNTX. These data support the hypothesis that opioid effects within the SA node are bimodal in character, that low doses are vagotonic, acting on δ1-receptors, and that higher doses are vagolytic, acting on δ2-receptors.

heart rate; enkephalins; opiate receptors; microdialysis; parasympathetic nervous system

THE NORMAL CARDIAC RHYTHM arises in the sinoatral (SA) node. The resulting heart rate is largely determined by the intrinsic activity of the resident pacemaker cells and the tonic restraint applied to them by the efferent postganglionic vagal nerves that innervate the node. The intrinsic rate of the pacemaker cells is significantly higher than the resting heart rate, and rapid increases in heart rate are generally accomplished by reducing vagal nerve traffic. Concentrated cholinesterase activity within the SA node provides for nearly instantaneous hydrolysis of interstitial neurotransmitter and a rapid increase in heart rate as the rate of transmitter release is reduced. An effective heartbeat is highly dependent on the coordinated distribution of electrical signals from the SA node and on the subsequent electromechanical coupling of conducting and contractile elements. Disturbances in this delicate electromechanical network lead to serious arrhythmias, and an active healthy vagal function appears to exert a trophic influence over the rhythmic stability of the heart. Patients who reestablish vagal function quickly after myocardial infarction are far more likely to survive than those who do not (17). Physiologically improving vagal function through exercise training also dramatically reduces the susceptibility to infarction-induced sudden cardiac death (3). Although a large number of factors moderate heart rate, including significant adrenergic input, recent studies suggested that the paracrine environment of the SA node may be an important determinant of the vagal pacemaker interaction (11). This report concentrates on ultrapotent excitatory interactions between cardiac opioids and vagal control of heart rate.

Low concentrations of enkephalins were identified in myocardial extracts soon after they were first isolated from brain homogenates (14). The mRNA for preproenkephalin was subsequently determined to be highly abundant in rat heart and comparable to that found in parts of the brain rich in opioids (13). The preproenkephalin mRNA was localized in isolated cardiomyocytes, which were also found to be able to translate the message into peptide and secrete the products into the surrounding culture medium (18, 30). The evaluation of cardiac opioid content suggested that there was substantially more opioid activity present in heart than accounted for by the primary preproenkephalin product, methionine-enkephalin (2, 4, 7, 30). This discrepancy was explained in part when chromatographic analysis identified significantly higher concentrations of preproenkephalin and its partially processed intermediates in heart extracts (2, 19, 21, 34). In particular, unexpectedly high concentrations of the heptapeptide...
methionine-enkephalin-arginine-phenylalanine (MEAP) were identified.

The systemic infusion of MEAP interrupted the vagal control of heart rate, coronary blood flow, and contractile activity (6, 8). Opiate receptor participation was confirmed when each effect was prevented by pretreatment with the high-affinity nonselective opiate antagonist diprenorphine. The responsible opiate receptor appeared to be located prejunctionally because MEAP was ineffective when bradycardia was produced by the direct-acting muscarinic agonist methacholine. This observation was consistent with earlier reports that endogenous and exogenous opioids opposed the vagal control of heart rate, presumably through the inhibition of neurotransmitter release (23, 32). When MEAP was delivered directly into the interstitium of the SA node by microdialysis, the vagolytic effect was identical to that observed after systemic infusion (11), indicating that the opiate receptors were located within the SA node.

Careful analysis of the vagolytic effect indicated that the interruption of vagal function within the SA node was mediated by δ-opiate receptors (15). The response was duplicated by the δ-agonist deltorphin and abrogated by the general δ-antagonist naltrindole. The intranodal delivery of μ- and κ-agonists and antagonists all failed to alter vagal function. Surprisingly, when the endogenous nodal concentration of MEAP was elevated after a "preconditioning-like" protocol and subsequent occlusion of the SA node artery, vagal function was consistently improved and the improvement was reversed by the same δ-antagonist, naltrindole. These opposing observations suggested several potential explanations. First, vagotonic responses at low doses may indicate that vagotonic responses are more sensitive than companion vagolytic responses. Second, distinct δ-receptor subtypes may mediate the two responses, with one of the receptors (the vagotonic receptor) inaccessible by microdialysis. The third explanation would include a combination of both, in which one of the two responsible receptor subtypes was significantly more sensitive to the peptide.

Recent studies identified the vagolytic response as δ2 in character. These studies unexpectedly provided support for distinct receptor subtypes mediating the opposing vagolytic and vagotonic responses. The vagolytic effect of MEAP was blocked by a δ2-antagonist and was unaltered by a δ1-antagonist (12). Although ineffectiv
gly vagolytically in that study, the δ1-agonist 2-methyl-4a-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a-octahydroquinolino[2,3,3-g]isoquinoline (TAN-67) produced a vagotonic response very similar to that observed previously during nodal artery occlusion. The δ1 character of the vagotonic response to TAN-67 was further verified by its blockade with the δ1-antagonist 7-benzylidenenaltrexone (BNTX). The assignment of the δ1-receptors as vagotonic might explain in part their attributed role in preconditioning (9, 22, 26–28). Improved vagal function during ischemia would presumably be cardioprotective by locally reducing myocardial work and oxygen demand in the area at risk (1, 33). These observations led to the proposal that ultra-
low doses of MEAP released during coronary arterial occlusion improve vagal function by interacting with δ1-opioid receptors.

**MATERIALS AND METHODS**

**Surgical preparation.** Mongrel dogs of either gender weighing 15–25 kg were assigned at random to experimental protocols. All protocols were approved by the Institutional Animal Care and Use Committee and were in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). The animals were anesthetized with pentobarbital sodium (32.5 mg/kg), intubated, and mechanically ventilated with room air, initially at 225 ml·min⁻¹·kg⁻¹. Fluid-filled catheters were then 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 Statham PD23XL pressure transducer, and heart rates and arterial pressures were measured continuously on-line (PowerLab). The venous line was used to administer supplemental anesthetic, as required. The arterial acid-base balance and blood gases were determined (Instrumental Laboratories Blood Gas Analyzer), and pH (7.35–7.45), PO₂ (90–120 mmHg), and PCO₂ (30–40 mmHg) were adjusted to normal by administering supplemental oxygen or bicarbonate or modifying the minute volume.

The right and left cervical vagus nerves were isolated through a ventral midline incision. The nerves were doubly ligated with umbilical tape to abort afferent nerve traffic and were returned to the prevertebral compartment for later retrieval. Surgical anesthesia was carefully monitored, and a single dose of succinylcholine (1 mg/kg) was administered intravenously to temporarily reduce involuntary muscle movements during the 10–15 min required for electrosurgical incision of the chest and removal of ribs 2–5. The heart was exposed from the right aspect. The pericardium was opened, and the dorsal pericardial margins were sutured to the body wall to support and stabilize the heart.

A 25-gauge stainless steel spinal needle containing the microdialysis line was inserted into the long axis of the SA node at the junction of the superior vena cava and the right atrium. The needle was removed, and the probe was positioned with the dialysis window completely within the SA node (11). The microdialysis probes were fabricated from 1-cm lengths of dialysis fiber from a Clirants TAF 08 (Asahi Medical) artificial kidney. The dialysis tubing restricted the passage of solutes with molecular weights >36,000 and had a 200-μm inner diameter and a 220-μm outer diameter (OD). The inflow and outflow lines were constructed of hollow 170-μm-OD silica tubing (SGE, Austin, TX) sealed into the dialysis fiber with cyanoacrylate glue.

The dialysis line was perfused with saline (5 μl/min) for 1 h to permit the tissue to equilibrate before the experimental protocol began. Previous reports indicated that metabolites recovered from the interstitium had returned to a low basal steady state during the first hour after insertion of the probe (31). At the end of each experiment, norepinephrine (1 nmol/μl in saline and 0.1% sodium ascorbate) was introduced into the microdialysis line to confirm the position of the probe. When the probe was properly positioned in the SA node, the heart rate increased by 30–40 beats/min within 30 s. This brisk tachycardia was not observed when the probe was positioned in the atrial wall as little as 1–2 mm lateral to the node.

**Protocol I: Is ultralow-dose MEAP vagotonic?** After the 1-h equilibration, the right vagus nerve was stimulated at a
supramaximal voltage (e.g., 15 V) at 1, 2, and 3 Hz for 15 s each. The new lower heart rate was recorded when it reached a new steady state during the 15-s stimulus. Heart rate/ frequency-response curves were constructed. The heart rate was allowed 105 s for complete recovery after each stimulation frequency was completed. Increasing doses of MEAP were then added to the perfusate in 10-fold increments beginning with 5 fmol/min until a maximal vagotonic response was observed. Each new dose was infused for 5 min at 5 µl/min, and the vagal response was reevaluated. The dose was then washed out and perfused with saline until the baseline vagal response was reconfirmed. Once the dose at which a maximal vagal improvement was determined, that dose of MEAP was combined with the nonselective δ-antagonist naltrindole (500 fmol/min) for 5 min. The dose of naltrindole was previously determined as ineffective versus the vagolytic effect of MEAP (15). After 5-min exposure to the combined agonist and antagonist, the vagus was again evaluated. The combination was washed out, and the baseline vagal function was reestablished. Next, a higher vagolytic dose of MEAP (50 nmol/min) was introduced into the nodal perfusate in 10-fold increments beginning with 5 fmol/min until a maximal vagotonic response was observed. After washout and reequilibration, increasing doses of the δ1-specific antagonist BNTX were combined with MEAP in the nodal perfusate for 5 min each. The initial dose of BNTX was 5 × 10⁻⁹ mol/min, and the subsequent doses were increased in 1,000-fold increments. After blockade was obtained, the combination was washed out and the effect of BNTX alone on vagal function was evaluated.

Protocol II: Are δ1-receptors responsible for the vagotonic effect of low-dose MEAP? In this protocol, a low vagotonic dose of MEAP (500 fmol/min) was introduced into the nodal perfusate. After 5 min, the vagal function was evaluated and the improvement was recorded. After washout and reequilibration, increasing doses of the δ2-specific antagonist naltriben were combined with MEAP in the nodal perfusate for 5 min each. The initial dose of naltriben was 5 × 10⁻¹⁰ mol/min, and the subsequent doses were increased in 1,000-fold increments through 5 × 10⁻⁹ mol/min in the same animals.

Protocol III: Do δ2-receptors participate in the vagotonic effect of low-dose MEAP? In this protocol, a low vagotonic dose of MEAP (500 fmol/min) was introduced into the nodal perfusate. After 5 min, the vagal function was evaluated and the improvement was recorded. After washout and reequilibration, increasing doses of the δ2-specific antagonist naltriben were combined with MEAP in the nodal perfusate for 5 min each. The initial dose of naltriben was 5 × 10⁻²⁴ mol/min, and the subsequent doses were increased in 1,000-fold increments through 5 × 10⁻⁹ mol/min. After the dose response was completed, the combination was washed out and the effect of naltriben alone on vagal function was evaluated.

Protocol IV: Do δ2-receptors mediate the vagotonic effects of nodal artery occlusion? A preconditioning-like protocol similar to that used in earlier studies (16) was used to raise endogenous MEAP and to produce a vagotonic effect. Because this effect was previously blocked with the nonselective δ-antagonist naltrindole, this protocol was conducted to determine whether this vagotonic effect was more specifically dependent on the δ2-receptor subtype. A suture was placed around the SA node artery to allow for a reversible arterial occlusion. A 10-min initial dialysate collection was made. Leucine-arginine (30 nmol/µl) was then added to the microdialysis perfusate and continued thereafter as a competitive inhibitor to reduce the enzymatic breakdown of MEAP (24) and to conform to the prior experimental design (16). After 5-min preinfusion, a 10-min control collection was made and then the SA node artery was temporarily occluded to produce four preconditioning-like 10-min periods of reduced blood flow. Each occlusion was followed by a 10-min reperfusion. Two 30-min occlusions separated by one 30-min reperfusion period followed the preconditioning-like sequences. After the preconditioning-like protocol was completed, the right vagus nerve was stimulated for 15 s at 3 Hz. The vagus nerve was then reevaluated at 15 and 20 min of each of the three subsequent 30-min periods. During the third period (second 30-min occlusion) BNTX was added to the perfusate between the 15 and 20 min stimulations.

Materials. MEAP was synthesized by American Peptide (Sunnyvale, CA). Naltrindole and naltriben were obtained from Sigma RBI (St. Louis, MO). BNTX was obtained from Tocris (Ellisville, MO). 8

Data Analyses. The data are expressed as means ± SE. Differences were evaluated by analysis of variance, and multiple post hoc comparisons were made with Tukey’s test (GB-STAT; Dynamic Microsystems, Silver Spring, MD). Repeated-measures designs were used where appropriate, and P < 0.05 was accepted as a statistically significant difference.

RESULTS

Twenty-three animals were studied, and three to five animals were evaluated for each treatment. The resting heart rate and blood pressure for each treatment are listed in Table 1. As illustrated, there was some variability of initial cardiovascular indexes between groups but the indexes were very stable within groups.

Table 1. Cardiovascular indexes during treatments

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Control</th>
<th>Treatment</th>
<th>Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR, beats/min</td>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
</tr>
<tr>
<td>High-dose MEAP (8)</td>
<td>125 ± 3</td>
<td>107 ± 3</td>
<td>125 ± 4</td>
</tr>
<tr>
<td>BNTX (5)</td>
<td>136 ± 6</td>
<td>101 ± 4</td>
<td>136 ± 4</td>
</tr>
<tr>
<td>NTB (3)</td>
<td>129 ± 2</td>
<td>116 ± 8</td>
<td>131 ± 3</td>
</tr>
<tr>
<td>Low-dose MEAP (8)</td>
<td>133 ± 1</td>
<td>100 ± 1</td>
<td>132 ± 2</td>
</tr>
<tr>
<td>BNTX + MEAP (5)</td>
<td>145 ± 2</td>
<td>124 ± 3</td>
<td>146 ± 8</td>
</tr>
<tr>
<td>NTB + MEAP (3)</td>
<td>130 ± 4</td>
<td>102 ± 3</td>
<td>131 ± 5</td>
</tr>
<tr>
<td>MEAP + NT (3)</td>
<td>120 ± 4</td>
<td>102 ± 3</td>
<td>129 ± 5</td>
</tr>
<tr>
<td>Ischemia (control) (5)</td>
<td>132 ± 2</td>
<td>101 ± 3</td>
<td>132 ± 2</td>
</tr>
<tr>
<td>Ischemia + BNTX (5)</td>
<td>120 ± 4</td>
<td>112 ± 9</td>
<td>119 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE of resting heart rate (HR) and mean arterial pressure (MAP) for numbers of animals indicated in parentheses before (Control) and during (Treatment) each experimental treatment. Washout indicates the HR and MAP after the nodal perfusate was returned to vehicle at the end of selected experimental treatments. MEAP; methionine-enkephalin-arginine-phenylalanine; NT, naltrindole; NTB, naltriben; BNTX, 7-benzylidenenaltrexone.

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There were no systematic differences in heart rate and mean arterial pressure as a result of any treatment compared with control, including nodal artery occlusion.

Protocol I: Is ultralow-dose MEAP vagotonic? The results of these experiments are illustrated in Figs. 1 and 2. In the first group of experiments, the dose of MEAP was gradually increased until a consistent improvement was observed. An improvement was observed at 50 fmol/min, and the effect was always distinguishable at 500 fmol/min (Fig. 1, bottom curve). At that juncture, an equimolar dose of the nonselective δ-antagonist naltrindole was combined with the MEAP in the nodal perfusate. The antagonist reversed the vagotonic effect and restored the vagal bradycardia to control. The agonist and antagonist were washed out, and a dose of MEAP previously determined to be vagolytic (5 nmol/min) was introduced into the node. As observed in the top curve of Fig. 1, this higher dose of MEAP nearly eliminated vagal control of heart rate. Of note, the dose of naltindole used was previously demonstrated to be completely ineffective versus the vagolytic effect of MEAP (15). A full low-dose dose response was conducted in the second group of animals and is illustrated in Fig. 2. In this group of animals, the effect of 5 fmol/min was inconsistent and not significantly different from control. However, a statistically significant improvement in vagal function was observed at 50 fmol/min, which reached an apparent maximum at 500 fmol/min. Higher doses of MEAP gradually reduced vagal function back toward the control response, and a vagolytic response was again clearly evident when the dose was raised to 5 nmol/min. The higher doses of MEAP were washed out, and the maximal vagotonic dose (500 fmol/min) was reintroduced to demonstrate that the vagotonic response was reproducible and could

![Figure 1](http://example.com/figure1.png)

**Fig. 1.** Vagotonic effect of low doses of methionine-enkephalin-arginine-phenylalanine (MEAP; bottom curve) on heart rate/frequency-response relationships during right vagal stimulation and their reversal when combined with low doses of the δ-antagonist naltrindole (NT). In this example, the dose rates for MEAP and naltrindole were 500 fmol/min. The vagolytic effect of higher dose rates (5 nmol/min) is also shown in the same animals. Individual values represent means ± SE; n = 5. The top and bottom curves are significantly different from control. bpm, Beats per minute.

![Figure 2](http://example.com/figure2.png)

**Fig. 2.** Change in heart rate at each frequency of right vagal stimulation (Stim) (1 (A), 2 (B), and 3 (C) Hz) as the dose rate for MEAP is gradually raised from very low initial rates. After the dose response was completed, the MEAP was washed out and the maximally vagotonic effect (500 fmol/min) was reestablished and then combined with the δ-antagonist naltrindole (500 fmol/min). Individual values represent means ± SE; n = 5. Values at $5 \times 10^{-14}$, $5 \times 10^{-13}$, and $5 \times 10^{-9}$ mol/min are significantly different from controls.
be restored. When combined with an equimolar dose of naltrindole, the vagotonic effect at each frequency was returned to the original control response.

**Protocol II: Are δ₁-receptors responsible for the vagotonic effect of low-dose MEAP?** The results of this experiment are presented in Fig. 3. The control vagal responses are illustrated in the top group of curves. As in protocol I, the nodal introduction of MEAP at 500 fmol/min produced a significant increase in vagal bradycardia (Fig. 3, bottom curve). Once the response was established, the peptide was washed out and the return to the control response was confirmed. Increasing doses of the selective δ₁-antagonist BNTX were then combined with MEAP in the nodal perfusate. As indicated, 5 × 10^{-24} mol/min (5 ymol/min) did not alter the vagotonic effect. However, 5 × 10^{-21} mol/min (5 zmol/min) completely restored vagal function to control (Fig. 3, top curves). Raising the concentration of BNTX another 1,000-fold had no additional effect on the vagal response. In addition, the later introduction of BNTX alone at 500 nmol/min had no effect on vagal function independent of the ability of BNTX to oppose MEAP, indicating that BNTX is not itself vagolytic.

**Protocol III: Do δ₂-receptors participate in the vagotonic effect of low-dose MEAP?** The summary of this experiment is presented in Fig. 4. As in protocol II, the introduction of MEAP at 500 fmol/min improved vagal function by >50% (Fig. 4, bottom curve). Subsequent combinations with increasing doses of the δ₂-receptor antagonist naltriben (5 × 10^{-24} through 5 × 10^{-9} mol/min) had no effect on the existing vagotonic response. After the agonist-antagonist combinations were washed out, the addition of naltriben alone (5 nmol/min) also had no effect on the control vagal function.

**Protocol IV: Do δ₁-receptors mediate the vagotonic effects of nodal artery occlusion?** In these experiments, a series of nodal artery occlusions and reperusions were conducted (Fig. 5). This protocol was previously determined to increase nodal MEAP and improve vagal function in a δ₁-opioid receptor-dependent manner. When vagal function was evaluated during the first 30-min occlusion, the vagal bradycardia at 3 Hz was improved by about one-third. This vagal evaluation was repeated 5 min later to demonstrate that repeated stimulation does not alter the response. During the second stimulation, a very similar improvement was evident. The two stimulations applied during the subsequent 30-min reperfusion were both reduced and very similar to the postconditioning, preocclusion control and to one another. When the artery was reocluded, the vagal function once again improved to a value comparable to those recorded during the first 30-min occlusion. BNTX (5 × 10^{-13} mol/min) was then

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**Fig. 3.** Vagotonic effect of low doses of MEAP (bottom curve) on heart rate/frequency-response relationship during right vagal stimulation and their reversal when combined with the δ₁-antagonist 7-benzylidenenaltrexone (BNTX). In this example, the dose rate for MEAP was 500 fmol/min and the dose rate tested for BNTX extended from 5 × 10^{-24} to 5 × 10^{-15} mol/min. A significant reversal was observed at a BNTX dose rate of 5 × 10^{-21} mol/min and above. BNTX alone (5 nmol/min) had no effect. Individual values represent means ± SE; n = 5.

**Fig. 4.** Vagotonic effect of low doses of MEAP (bottom curve) on heart rate/frequency-response relationships during right vagal stimulation and the failure of the δ₂-antagonist naltriben (NTB) to alter the response. In this example, the dose rate for MEAP was 500 fmol/min and the dose rates illustrated for naltriben extended from 5 × 10^{-24} to 5 × 10^{-9} mol/min. Doses of naltriben through 5 nmol/min were tested and did not alter the vagotonic response to MEAP. Naltriben alone (5 nmol/min) had no effect. Individual values represent means ± SE; n = 3.
**DISCUSSION**

This study was prompted by contradictory reports about the influence of enkephalin on vagal function. Initial reports indicated that intravenous enkephalin reduced vagal control of heart rate (8, 23). The vagolytic effect was localized to opiate receptors in the SA node when MEAP introduced directly into the nodal interstitium by microdialysis was equally as effective as that administered systemically (11). The contradiction became apparent when endogenous MEAP was elevated during nodal artery occlusion (16). Vagal function during nodal artery occlusion was consistently improved and both vagolytic and vagotonic responses were reversed by the same \( \delta \)-receptor antagonist, naltrindole (15, 16). This led to the hypothesis that the two opposing responses were mediated by different \( \delta \)-receptor subtypes and that the subtype responsible for the vagotonic response was far more sensitive to ultralow doses of the native peptide.

The vagal response to MEAP was clearly bimodal in character. MEAP improved vagal function at doses well below those needed to interrupt vagal function. The dose responses may overlap one another, providing an explanation for a central dose range in which the opposing influences balance one another with no net effect. This would explain the failure to observe an enhanced vagal response at the beginning of earlier dose-response studies focused on vagolytic actions (15). In those prior studies vagolytic effects were largely absent between 5 and 50 pmol/min, which coincides well with the waning of the vagotonic effect observed here. The difference in the sensitivity of these two responses to reversal by naltrindole further confirmed their fundamental differences in sensitivity to enkephalin. Naltrindole produced a complete reversal of the vagotonic effect at 500 pmol/min, whereas the threshold for reversal of the vagolytic effect was 100 pmol/min (15).

In addition to the difference in sensitivity, the opposing responses also appear to be mediated by different receptor subtypes. The blockade of both responses by naltrindole strongly suggests that both were mediated by \( \delta \)-receptors. A recent report presented evidence that the vagolytic response was mediated by \( \delta_2 \)-receptors (12). Those authors found that the vagotonic response was blocked by the \( \delta_2 \)-antagonist naltriben and unaltered by the \( \delta_1 \)-antagonist BNTX. The data reported here complement those findings and illustrate that the vagotonic response is mediated by \( \delta_1 \)-receptors. In this case, the vagotonic response was blocked by BNTX and unaltered by naltriben. The sensitivity to the blockade by BNTX was remarkable. The \( \delta_1 \) character was suggested earlier when the \( \delta_1 \)-agonist TAN-67 produced an improved vagal response that was subsequently reversed by BNTX (12). Therefore, these data do collectively support the hypothesis that the vagotonic and vagolytic effects of MEAP are mediated by \( \delta_1 \) and \( \delta_2 \)-receptor subtypes, respectively, and that the \( \delta_1 \) response is far more sensitive.

The last question addressed was whether the changing vagotonic responses associated with the occlusion and reperfusion of the SA node artery were likewise mediated by \( \delta_1 \)-receptors. Prior studies indicated that the improvement associated with occlusion was accompanied by an increased recovery of MEAP and that the improved response was reversed by naltrindole (16). In this case, the extraordinary selectivity of BNTX was used to probe this response. This too appears to be mediated by \( \delta_1 \)-receptors, because the response was completely reversed by a very low concentration of intranodal BNTX.

How are these opposing responses mediated? Bimodal actions of opioids, although often unacknowledged, are not uncommon (25). The receptors in question may be located on different cellular targets. The vagolytic response was presumed to be mediated through the prejunctional inhibition of acetylcholine release. This assumption was based on the failure of

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Fig. 5. Change in heart rate during right vagal stimulation at 3 Hz at selected points during occlusion and reperfusion of the sinoatrial node artery. BNTX (5 \( \times \) 10\(^{-18} \) mol/min) was introduced into the nodal interstitium after 15 min of the second 30-min occlusion. Individual values represent means \( \pm \) SE; \( n = 5 \). The effect of occlusion was significantly different from control, and BNTX was significantly different from occlusion alone (\( P < 0.05 \)) but not different from control.
MEAP to alter the bradycardia following the addition of the direct-acting cholinergic methacholine (8). Although the δ2-receptor is probably located on the vagal nerve terminals, MEAP may act indirectly on an intermediate cell or interneuron. The case for the δ1-receptor is less clear because the pre- or postjunctional character of the response has not been addressed. However, no improved vagal function was noted when MEAP and methacholine were combined (8). The data thus far are, however, consistent with the hypothesis suggested by Crain and Shen (10) to explain the bimodal effects of opioids on the action potential duration in cultured dorsal root ganglion cells. They argued convincingly that subtypes of the opioid receptor in the same cell were coupled to adenylate cyclase through opposing stimulatory and inhibitory G proteins. They also reported that the excitatory receptors, like the δ1-receptors here, were far more sensitive to agonist. Because alterations in the cAMP within vagal nerve terminals can modify acetylcholine release, the bimodal effects of MEAP on vagal function may represent a functional expression of Crain and Shen’s observations in cultured neurons.

The normal function of these opposing receptors remains to be determined. The ubiquitous presence of enkephalin-degrading aminopeptidases in tissue and blood suggests that the cardiac enkephalins function primarily as paracrine hormones (5). The higher sensitivity associated with the vagotonic effect might suggest that the primary function of the cardiac enkephalins is one of facilitation. The release of enkephalin during hypoxia may serve to stabilize the myocardium during metabolic stress by increasing vagal function, reducing work, and decreasing local oxygen demand (1, 33). Consistent with this suggestion, opioid receptors have been implicated in the cardioprotection afforded by ischemic preconditioning (20, 22, 29) and preconditioning improves the efferent vagal component of the baroreflex (1). A number of investigators have demonstrated that opioids reduce the area of injury after coronary occlusion in a manner similar to ischemic preconditioning (9, 20, 22, 26–29). In addition, opioid antagonists will prevent this effect and, furthermore, opiate antagonists abort the beneficial effect of ischemic preconditioning (9, 22, 26, 27). Particularly consonant with the current findings, δ2-receptors were specifically implicated in opioid-mediated preconditioning based on positive preconditioning-like results with TAN-67 and their reversal with BNTX (26, 27).

On the other end of the spectrum, the vagolytic effect of MEAP is less easily rationalized. Impaired vagal function is often viewed as a liability (17), and strategies that improve vagal function have demonstrated therapeutic efficacy (3). However, too much of a good thing may also have undesirable consequences. The electrical stability of the intracardiac nervous system is the result of a delicate balance of multiple inputs, and an inappropriately intense vagal response under the wrong circumstances could be proarrhythmic. Similarly threatening to survival, intense unopposed vagal stimulation can trigger vasovagal syncope. Therefore, the vagolytic effect of MEAP may serve the organism as a governor of vagal activity at the other extreme. In summary, the data presented here support the hypothesis that ultralow-dose enkephalin improves vagal function by activation of δ2-receptors within the interstitium of the SA node. Furthermore, these same δ1-receptors appear to be involved in the vagotonic effect associated with nodal artery occlusion and reperfusion.

DISCLOSURES

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