Cardiac enkephalins interrupt vagal bradycardia via δ₂-opioid receptors in sinoatrial node

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Farias, III, Martin, Keith E. Jackson, Darice Yoshishige, and James L. Caffrey. Cardiac enkephalins interrupt vagal bradycardia via δ₂-opioid receptors in sinoatrial node. Am J Physiol Heart Circ Physiol 284: H1693–H1701, 2003. First published January 9, 2003; 10.1152/ajpheart.00730.2002.—Local cardiac opioids appear to be important in determining the quality of vagal control of heart rate. Introduction of the endogenous opioid methionine-enkephalin-arginine-phenylalanine (MEAP) into the interstitium of the canine sinoatrial node by microdialysis attenuates vagally mediated bradycardia through a δ-opioid receptor mechanism. The following studies were conducted to test the hypothesis that a δ₂-opiate receptor subtype mediates the interruption of vagal transmission. Twenty mongrel dogs were anesthetized and instrumented with microdialysis probes inserted into the sinoatrial node. Vagal frequency responses were performed at 1, 2, and 3 Hz during vehicle infusion and during treatment with the native agonist MEAP, the δ₁-opioids 2-methyl-4aa-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a-octahydroquinolino[2,3-3-g]isoquinoline (TAN-67) and [D-pen₂,5]-enkephalin (DPDPE), and the δ₂ opioid deltorphin II. The vagolytic effects of intranodal MEAP and deltorphin were then challenged with the δ₁- and δ₂-opioid receptor antagonists 7-benzylidenenaltrexone (BNTX) and naltriben, respectively. Although the positive control deltorphin II was clearly vagolytic in each experimental group, TAN-67 and DPDPE were vagolytically ineffective in the same animals. In contrast, TAN-67 improved vagal bradycardia by 30–35%. Naltriben completely reversed the vagolytic effects of MEAP and deltorphin. BNTX was ineffective in this regard but did reverse the vagal improvement observed with TAN-67. These data support the hypothesis that the vagolytic effect of the endogenous opioid MEAP was mediated by δ₂-opioid receptors located in the sinoatrial node. These data also support the existence of vagotonic δ₁-opioid receptors also in the sinoatrial node.

TAN-67; heart rate

The role of endogenous opioid peptides in the local control of heart rate is not yet well understood. When administered exogenously, these peptides are effective modulators of cardiac vagal function. Weitzell et al. (31) first reported that enkephalin inhibited vagal transmission in isolated rabbit hearts. The inhibition was reversed by the nonselective opiate antagonist naloxone. Other investigators (3, 4, 10, 13, 22, 24) have observed that enkephalins suppressed vagal bradycardia in vivo, suggesting that enkephalins function as “governors” of vagal control.

Several enkephalin sequences are concentrated in the heart (32), including the heptapeptide methionine-enkephalin-arginine-phenylalanine (MEAP). MEAP attenuated vagally mediated bradycardia by >70% when infused intra-arterially in anesthetized dogs and did not appear to involve a direct interaction with the pacemaker cells (3, 4). The high affinity but nonselective opioid antagonist diprenorphine completely reversed the effect of MEAP, restored vagal control of heart rate, and indicated that opiate receptors were involved (3, 4).

Prejunctional vagal nerve terminals in the sinoatrial (SA) node and the nearby intracardiac parasympathetic ganglia were the most likely targets for MEAP. MEAP was delivered directly into the SA node by microdialysis to resolve these two potential targets. Intranodal MEAP attenuated vagally mediated bradycardia to the same extent as that observed during systemic infusion of the peptide and both nodal and systemic effects were reversed by the nodal delivery of diprenorphine (10). Collectively, these findings indicated that MEAP modulated vagal control of heart rate by acting on opioid receptors in the SA node, which were most likely located prejunctionally on vagal nerve terminals.

To explore the physiology of opioids in the SA node, an extended series of dose-response relationships with specific opioid agonists and antagonists were conducted to identify the responsible opioid receptor. Those studies have established a clear δ-receptor profile, indicating that the vagolytic effect of MEAP was mediated by δ₂-opioid receptors (13). The nodal delivery of MEAP and the δ₂-agonist deltorphin II produced equipotent vagolytic responses and both effects were reversed by the δ-antagonist naltrindole. The μ- and κ-agonists had no effect on vagally mediated bradycardia, and μ- and κ-antagonists were ineffective versus MEAP (13). These data strongly indicated that δ-opioid receptors within the SA node were responsible for the vagolytic effect of MEAP.

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Although the distinct transcripts corresponding to δ-receptor subtypes have not been isolated (1, 9, 17), there is considerable functional and pharmacological evidence for the existence of distinct δ₁ and δ₂-receptor-mediated responses (1, 15, 25, 28–30, 33). The nature of subtype-specific actions on cardiac function is not well defined but Schultz et al. (27) demonstrated that pretreatment with the selective δ₁ agonist 2-methyl-4-α-(3-hydroxyphenyl)-1,2,3,4,4α,5,12,12α-octahydroquinolinol(2,3,3-g)isoquinoline (TAN-67) significantly reduced infarct size in the ischemic rat heart. The cardioprotection conferred by TAN-67 was subsequently reversed with the use of the selective δ₁ antagonist 7-benzylidenenaltrexone (BNTX). Chien et al. (5) also reported that δ₁-agonists helped to preserve the viability of multiorgan preparations. Because the activation of cholinergic receptors has also been implicated in cardioprotection (34), a potential link between opioids and vagal function might be physiologically important. However, the vagolytic action of added MEAP cited above would be difficult to reconcile with reported cardioprotective effects of cholinergic stimulation.

The application of a preconditioning-like protocol to the SA node artery stimulated a reproducible increase in the endogenous MEAP recovered by dialysis from the nodal interstitium (14). In contrast to the vagolytic effect of exogenously administered MEAP, the rise in endogenous MEAP was accompanied by a consistent enhancement of vagally mediated bradycardia. The δ-antagonist naltrindole reversed the vagotonic effect and suggested participation by δ-opioid receptors (14). An opioid-mediated increase in vagal function during arterial occlusion makes a role in cardioprotection mechanistically easier to explain. An increase in cholinergic stimulation during oxidative stress could reduce tissue loss by lowering metabolic demand locally.

These collected observations suggest the hypothesis that different subtypes of the δ-receptor (δ₁ and δ₂) may mediate respectively the opposing vagotonic and vagolytic effects of opioids. Consistent with the suggestion that the vagotonic effect is mediated by δ₁-receptors, Shultz et al. (27) reported that TAN-67 reduced resting heart rate in the rat. In contrast, δ-activation with the use of administered enkephalin in the dog produced a clear attenuation of vagal bradycardia. These opposing observations would be compatible if the vagolytic activity in the dog is mediated by δ₂-receptors. The two subtypes of δ-receptors may serve distinctly different roles in the regulation of heart rate.

The purpose of these studies was to test the hypothesis that δ₂-opioid receptors in the SA node were responsible for the vagolytic effect of the cardiac opioid MEAP and to rule out the participation of δ₁-opioid receptors. This was accomplished with two strategies. In one strategy, the vagolytic effects of MEAP and the δ₂-agonist deltorphin II were first demonstrated, and the endogenous opioid MEAP was then challenged with δ₁- and δ₂-selective antagonists. In the second, the vagolytic effects of MEAP and deltorphin II were compared with those of the selective δ₁-agonists [d-pen2,5]-enkephalin (DPDPE) and TAN-67. This endeavor arose as a result of previous studies, which established a role for δ-receptors in the vagolytic actions of MEAP. The efficacy of deltorphin II in those studies suggested the vagolytic effect might involve a δ₂ response, but the definitive comparisons were not available.

METHODS

Experiments conformed to the Guide for the Care and the Use of Laboratory Animals published by the National Institutes of Health.

Surgical preparation. Twenty Mongrel dogs were anesthetized with pentobarbital sodium, intubated, and mechanically ventilated with room air. Fluid-filled catheters were inserted into the femoral artery and vein and then advanced into the descending aorta and inferior vena cava, respectively. The arterial line was attached to a pressure transducer (model PD23XL; Statham) to monitor heart rate and blood pressure continuously online (PowerLab). The venous line was used to administer additional anesthetic as needed. Arterial blood gases were monitored with a blood gas analyzer (Instrumentation Laboratories) and the PO₂ (90–120 mmHg), pH (7.34–7.45), and PCO₂ (35–45 mmHg) were adjusted to normal with supplemental oxygen, bicarbonate, or by altering the minute volume.

The right and left vagus nerves were isolated in the cervical region through a midline surgical incision and tied off tightly with umbilical tape and were returned to their position in the neck for later retrieval. A single dose of succinylcholine (1 mg/kg) was administered intravenously to temporarily reduce involuntary muscle movements during the 10–15 min required for the electrosurgical incision of the right thorax and removal of right ribs 2–5. The pericardium was opened and the upper margins were sutured to the body wall to provide a pericardial cradle. A 27-gauge stainless steel cannula was used to introduce the microdialysis probe into the SA node. To confirm the probe placement in the SA node, norepinephrine (1 x 10⁻⁹ mol/μl) was introduced into the microdialysis probe. The observation of a brisk 30–40-beat increase in heart rate provided a functional confirmation of the probe location within the SA node. Prior studies (14) have determined that deliberate repositioning of the probe as little as 2 mm lateral to the node eliminates the norepinephrine-mediated tachycardia. The microdialysis probe was constructed from a single 1-cm length of dialysis fiber (220 μm OD, 200 μm ID) and hollow silica inflow and outflow tubes (120 μm ID, 170 μm OD). The dialysis tubing permits molecules with a molecular weight of 36,000 or less to freely cross from the lumen into the nodal interstitium. This technique allows one to both alter and sample the local nodal interstitial environment while minimizing alterations in systemic hemodynamics and reflex compensations.

Protocols. These experiments were conducted to demonstrate that the δ₂-opioid receptor subtype was responsible for the vagolytic effect of nodal enkephalins. Two strategies were employed. In the first strategy, the influence of the δ subtype-specific agonists DPDPE, TAN-67, and deltorphin II was compared for their vagolytic action. In the second strategy, a vagolytic effect of the endogenous agonist MEAP was established and then the ability of subtype-selective antagonists (BNTX and naltrindone) to reverse this effect were evaluated.

All treatments were introduced locally into the interstitium of the SA node by microdialysis at a flow rate of 5 μl/min.

Previous studies (13) revealed that deltorphin II (1.5 x 10⁻⁹ mol/min) blocked vagally mediated bradycardia. The vagolytic effect of deltorphin II was successfully reversed by
the δ-selective antagonist naltrindole. These findings suggested participation of a δ₂-opioid receptor in this effect. This study will determine the subtype of δ-opioid receptor responsible for the inhibition of vagally mediated bradycardia by MEAP.

Protocol 1. This protocol tested whether the intranodal administration of δ₁-selective agonists was capable of interrupting vagal bradycardia. After microdialysis probe insertion, the SA node was perfused (5 μl/min) with saline for 60 min. After this period of equilibration, control vagal responses were obtained by stimulating the right vagus nerve at 1, 2, and 3 Hz. The nerve was stimulated at a supramaximal voltage for 15 s, followed by 1 min 45 s for recovery. Deltorphin II was then infused (5 μl/min) into the SA node for 5 min to establish a functional vagolytic effect. The effective dose used for deltorphin II (1.5 × 10⁻⁹ mol/min) was determined previously (13). Once established, the effect of deltorphin II served as a positive control in cases where the subsequent agonists under evaluation were without effect. After this procedure, dose responses were constructed for the selective δ₁-agonist DPDPE or TAN-67. Doses were selected to provide molar equivalent ranges (0.05–5 × 10⁻⁹ mol/min) to those previously determined to be vagolytic for MEAP and deltorphin II (13). Each dose of each agent was infused for 5 min before evaluating the vagus nerve. After each dose evaluation, the agent was washed out for 15 min and vagal function was retested to ensure that it had returned to normal. The length of washout was based on previous experiments (13). At the end of the TAN-67 protocol, this agent was combined with the δ₁-antagonist BNTX to determine whether the unexpected improvement in vagal function was mediated by a δ₁-opioid receptor.

Protocol 2. This protocol was designed to test whether vagolytic effects of MEAP and deltorphin II were blocked by a selective δ₂-opioid receptor antagonist and not by a selective δ₁-opioid receptor antagonist. MEAP and deltorphin II (1.5 × 10⁻⁹ mol/min) were introduced into the interstitium of the SA node, and vagal stimulations were performed as previously described to establish the vagolytic effect of each. After washout of these initial tests, MEAP was combined with increasing doses of the selective δ₁-antagonist BNTX or the selective δ₂-antagonist naltrindole. At the end of the protocol, the specific subtype was further confirmed by combining deltorphin II with the maximum effective dose of one or the other antagonist. As predicted by the hypothesis, the δ₂-antagonist naltrindole should reverse the vagolytic effect of MEAP and deltorphin and verify participation of the δ₂-opioid receptor. BNTX should not reverse the vagolytic effect of MEAP or deltorphin indicating the absence of participation by δ₁-opioid receptors.

Materials. MEAP and deltorphin II were synthesized by American Peptide (Sunnyvale, CA). TAN-67, DPDPE, and BNTX were obtained from Tocris Cookson (Ellisville, MO). Naltrindole was obtained from Sigma (St. Louis, MO).

Statistical methods. All data were expressed as means ± SE. Differences were evaluated with ANOVA for repeated measures. Individual treatment differences were determined by post hoc analysis with Tukey’s test for multiple comparisons. Differences determined to occur by chance with a probability of P < 0.05 were accepted as statistically significant.

RESULTS

Twenty dogs were randomly assigned to various protocols employing δ₁- and δ₂-agonists and antagonists. Table 1 represents the resting cardiovascular parameters for all animals across all treatments. There were no significant differences in heart rate or blood pressure among groups before treatment. Resting heart rate and blood pressure were also unaltered by any of the opioid agonists and antagonists, regardless of the dose.

Deltorphin vagolyysis. Deltorphin II was used as a positive control to demonstrate the functional integrity of the system in each animal before other agents were tested. This pretest also served to verify the appropriate placement of the dialysis probe in the proximity of the nodal opiate receptors responsible for the interruption of vagal bradycardia. The nodal administration of deltorphin II (1.5 × 10⁻⁹ mol/min) reduced vagally mediated bradycardia by 75–85% at all vagal frequencies employed and was significantly different from control.

DPDPE dose responses. In this protocol, DPDPE was introduced directly into the SA node to rule out the participation of δ₁-opioid receptors in the opioid-mediated interruption of vagal bradycardia. Control vagal stimulations during vehicle infusion produced a normal graded decline in heart rate at all vagal frequencies used (Fig. 1). The nodal delivery of DPDPE had no effect on heart rate during the vagal frequency response as indicated by the superimposition of the DPDPE and vehicle responses (Fig. 1, bottom two traces). The vagolytic effect of deltorphin II is illustrated in the top trace. The complete dose responses for all three frequencies are illustrated in Fig. 2.

TAN-67 dose responses. In the absence of an effect as observed with DPDPE, it is difficult to say with confidence that the agent successfully crossed the dialysis membrane into the interstitium. In this regard, a sec-

Table 1. Cardiovascular indexes

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Control HR, beats/min</th>
<th>Control MAP, mmHg</th>
<th>Treatment HR, beats/min</th>
<th>Treatment MAP, mmHg</th>
<th>Washout HR, beats/min</th>
<th>Washout MAP, mmHg</th>
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<td>128 ± 5</td>
<td>114 ± 7</td>
<td>132 ± 7</td>
<td>113 ± 7</td>
<td>125 ± 5</td>
<td>114 ± 8</td>
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<td>13</td>
<td>127 ± 4</td>
<td>118 ± 4</td>
<td>128 ± 6</td>
<td>114 ± 6</td>
<td>126 ± 4</td>
<td>112 ± 9</td>
</tr>
<tr>
<td>DPDPE</td>
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<td>129 ± 5</td>
<td>112 ± 2</td>
<td>127 ± 5</td>
<td>109 ± 7</td>
<td>128 ± 4</td>
<td>112 ± 6</td>
</tr>
<tr>
<td>TAN-67</td>
<td>5</td>
<td>122 ± 6</td>
<td>117 ± 7</td>
<td>110 ± 4</td>
<td>119 ± 9</td>
<td>117 ± 5</td>
<td>114 ± 8</td>
</tr>
<tr>
<td>BNTX</td>
<td>5</td>
<td>125 ± 5</td>
<td>117 ± 5</td>
<td>111 ± 2</td>
<td>108 ± 9</td>
<td>121 ± 4</td>
<td>109 ± 7</td>
</tr>
<tr>
<td>Naltrindole</td>
<td>5</td>
<td>136 ± 7</td>
<td>112 ± 7</td>
<td>123 ± 6</td>
<td>118 ± 5</td>
<td>120 ± 2</td>
<td>114 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects in each treatment group. HR, heart rate; MAP, mean arterial pressure; MEAP, methionine-enkephalin-arginine-phenylalanine; DPDPE, d-[Pen⁻⁵]-enkephalin; TAN-67, 2-methyl-4aa-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a-octahydroquinolino[2,3,3-g]isoquinoline; BNTX, 7-benzylidenenaltrexone. Resting HR and MAP are shown before (control) and during (treatment) each experimental period. Washout indicates HR and blood pressure after nodal perfusate was returned to vehicle at the end of each experiment.
ond selective δ₁-opioid receptor agonist, TAN-67, was used in a second group of animals to provide further evidence that δ₁-opioid receptors were not vagolytic. During vehicle infusions, control vagal stimulations produced a normal graded decline in heart rate as the frequency of stimulation was increased (Fig. 3, middle trace). Deltorphin II produced a vagolytic response similar to that observed (80% inhibition) in the prior group (Fig. 3, top trace). The administration of TAN-67 into the SA node had no vagolytic effect during the vagal frequency response at any dose employed. Rather, TAN-67 produced a greater vagal bradycardia as the dose was increased (Fig. 3, bottom trace). The maximum effect was observed at the $1.5 \times 10^{-9}$ mol/min (Fig. 4) with an apparent ED$_{50}$ of $1.0 \times 10^{-10}$ mol/min. The maximal improvement at $1.5 \times 10^{-9}$ mol/min was 28–37% and was significantly different from control at all vagal frequencies.

Acting on the presumption that the vagotonic effect of TAN-67 was perhaps mediated by a δ₁-receptor, TAN-67 ($1.5 \times 10^{-9}$ mol/min) was then combined with the δ₁-antagonist BNTX ($1.5 \times 10^{-9}$ mol/min) and infused directly into the SA node via microdialysis. BNTX effectively prevented the vagotonic effect of TAN-67 because the vagally mediated bradycardia during the combined infusion was similar to control values (Fig. 3, middle trace). The administration of BNTX alone had no effect on vagal bradycardia and once again produced values that were similar to control. Vagal stimulations were performed after washout of each treatment and were again similar to control values.

**MEAP versus naltriben dose responses.** In the second strategy, deltorphin II and the endogenous cardiac opioid MEAP were introduced into the SA node at vagolytically effective doses. Then each agonist was subsequently combined with selective δ₁- and δ₂-antagonists to verify which δ-receptor subtype was responsible for the interruption of vagal bradycardia. The control frequency response is illustrated in Fig. 5, bottom traces. The vagolytic effects of deltorphin II and MEAP are illustrated in the two top traces. Increasing doses of the selective δ₂-opioid receptor antagonist naltriben were combined with MEAP in the dialysis perfusate. Naltriben progressively reversed the effect of MEAP and restored vagal regulation of heart rate to control (Fig. 6). The reversal was obtained with an ID$_{50}$ of $1.5 \times 10^{-10}$ mol/min and a maximal effect near molar parity with the agonist ($1.5 \times 10^{-9}$ mol/min).

**Fig. 1.** The heart rate/frequency response [in beats/min (bpm)] is mediated by right vagal nerve stimulation during the nodal delivery of deltorphin II ($1.5 \times 10^{-9}$ mol/min) and [D-Pen²,⁵]-enkephalin (DPDPE) ($5 \times 10^{-9}$ mol/min) by microdialysis. The data illustrated are for the maximal dose of DPDPE employed in its dose-response curve. *P < 0.05, significantly different from control.

**Fig. 2.** Change in heart rate produced during right vagal stimulation during exposure (5 min) to increasing doses of the δ₁-selective opioid agonist DPDPE. A: 1 Hz; B: 2 Hz; C: 3 Hz. The units for the doses listed within the bars are 0.05, 0.15, 0.5, 1.5, and $5.0 \times 10^{-9}$ mol/min. Deltorphin ($1.5 \times 10^{-9}$ mol/min) was included as a positive control. All treatments were infused into the sinoatrial node of the dog via microdialysis. *P < 0.05, significantly different from control.
The data reported above support the primary hypothesis that the vagolytic effect of the endogenous opioid MEAP on heart rate is mediated by δ₂-opioid receptors in the SA node. This conclusion is based on the observation that vagolytic response to MEAP was duplicated by the δ₂-agonist deltorphin II when the δ₁-agonists DPDPE and TAN-67 were both vagolytically

**DISCUSSION**

The similar blockade of the deltorphin and MEAP effects is illustrated among the bottom traces in Fig. 5 for the last dose in the naltriben dose-response curve. Perfusion with the highest dose of naltriben alone was similar to control indicating that naltriben had no effect on vagal function independent of it ability to obstruct the access of MEAP and deltorphin II to nodal δ₂-receptors.

**MEAP versus BNTX dose responses.** The selective δ₁-opioid receptor antagonist BNTX was used to confirm that the vagolytic effect of MEAP was mediated by δ₂- and not by δ₁-opioid receptors. This was achieved by combining increasing doses of BNTX with an effective vagolytic dose of MEAP (1.5 × 10⁻⁹ mol/min). The rationale presumed that if naltriben identified a functional δ₂ response, then combining MEAP with increasing doses of BNTX would find BNTX ineffective or much less effective than naltriben. The bottom two traces in Fig. 7 illustrate the control bradycardia response in this group and the absence of an effect of BNTX alone. The 50–70% inhibition by both MEAP and deltorphin II is indicated among the top traces in Fig. 7. When BNTX was combined with MEAP or deltorphin II, the resulting curves were very similar to those for MEAP and deltorphin alone (Fig. 7, top traces). BNTX had no effect on the vagolytic properties of either MEAP or deltorphin. The complete dose-response curves for BNTX versus MEAP are described in Fig. 8. Although a subtle reversal of the effect of MEAP might be suggested from these data, the observed bradycardia was never different from MEAP alone. The absence of an effect of BNTX versus both MEAP and the δ₂ agonist, deltorphin II further supports the exclusive δ₂ character of the vagolytic effect.
ineffective in the same animals. Participation by $\delta_2$-receptors was verified further by demonstrating the vagolytic effect of MEAP was reversed by the $\delta_2$-antagonist naltriben and unaltered by equimolar doses of the $\delta_1$-antagonist BNTX. The $\delta_1$-character of the vagolytic effect of MEAP was rigorously determined earlier (13), and the current findings suggest that the vagolytic effect was mediated by $\delta_2$-receptors without a measurable $\delta_1$-receptor contribution.

Deltorphin II served as positive control in these experiments to confirm the location of the dialysis probe within functional reach of the nodal opioid receptors responsible for the vagolytic response. The absence of a response when introducing agents by microdialysis can be ambiguous because it is often difficult to verify that every agent has successfully crossed the dialysis membrane into the interstitium in biologically effective concentrations. In this instance, functionally similar but molecularly distinct $\delta_1$-agonists were used to reduce the probability of interference with diffusion due to molecular charge, adsorption, or solubility. In this case, both DPDPE and TAN-67 are $\delta_1$-agonists but DPDPE is a modified peptide and TAN-67 is a heterocyclic isoquinoline. This dramatically reduces the probability that the absence of a $\delta_1$-effect resulted from a failure to reach the target due to adsorption or failure to diffuse freely.

Although TAN-67 had no vagolytic effect, it produced a consistent improvement in vagal bradycardia and thus provided additional direct evidence that $\delta_1$-receptor antagonists BNTX subsequently reversed the TAN-67-mediated vagal improvement. Thus $\delta_1$-receptors were present in the SA node and were vagotonic rather than vagolytic. These observations suggested that the opioid modulation of vagal function is bimodal with opposite poles of the response mediated by different subtypes of the $\delta$-receptor.

Selectivity issues: TAN-67 and DPDPE. The existence of $\delta$-receptor subtypes has been based entirely on biological responses that can be distinguished by agonists and antagonists reported as selective for the respective subtypes (1, 13, 25, 28–30, 33). Each receptor subtype stimulated responses that were reversed by agonists preferential to that subtype. Mixed results were obtained when cross-tolerance or cross-desensitization experiments were conducted (1, 21, 29). A single receptor transcript has been isolated, and attempts to identify distinct receptor proteins associated with $\delta_1$-

Fig. 5. The heart rate/frequency response is mediated by right vagal nerve stimulation during the nodal delivery of vehicle, deltorphin II ($1.5 \times 10^{-9}$ mol/min), methionine-enkephalin-arginine-phenylalanine (MEAP) ($1.5 \times 10^{-9}$ mol/min), and MEAP or deltorphin II ($1.5 \times 10^{-9}$ mol/min) combined with an equimolar dose of naltriben (NTB), and naltriben ($5 \times 10^{-9}$ mol/min) alone. *$P < 0.05$, significantly different from control.

Fig. 6. A–C: change in heart rate produced during right vagal stimulation during exposure (5 min) to increasing doses of the $\delta_2$-antagonist, naltriben combined with MEAP ($1.5 \times 10^{-9}$ mol/min). The units for the doses listed in the bars are 0.05, 0.15, 0.5, 1.5, and $5.0 \times 10^{-9}$ mol/min. Deltorphin ($1.5 \times 10^{-9}$ mol/min) was included as confirmation of the $\delta_2$-character of the naltriben blockade. All treatments were infused into the sinoatrial node of the dog via microdialysis. *$P < 0.05$, significantly different from control.
and δ2-mediated responses have been as yet unsuccessful (1, 9, 17). Contradictory findings in some isolated systems in vitro support the suggestion that differences in coupling, agonist concentration or local membrane conditions may determine whether δ1-, δ2-, or mixed responses are evident (7).

Subtype-specific responses have been used to quantify the relative δ-selectivity of various agents. DPDPE and deltorphin II have been widely employed respectively as preferential δ1- and δ2-agonists. Each has ~80- to 100-fold selectivity for its respective receptor subtype in antinociceptive and binding studies (6, 8, 30). Antagonists for each receptor subtype have been characterized as well. BNTX and naltriben currently serve respectively as prototypical δ1- and δ2-antagonists (15, 25).

DPDPE reportedly has some mixed δ2-agonist activity in some biological systems (33). This aspect might complicate the interpretation of the absent response with DPDPE during vagal stimulations and may help to explain the difference observed between DPDPE and TAN-67. Because δ2-opioid receptors were clearly vagolytic, the absence of a response to DPDPE would suggest either the absence of δ1-receptors or the absence of a δ1-effect on vagal function. If DPDPE has measurable δ2-activity, one might expect to see a vagolytic response at the high end of the dose-response curve. TAN-67, which is significantly more selective for δ1-opioid systems (6, 16), improved vagal bradycardia by 35% and was reversed by BNTX. This suggests that δ1-receptors were present and they did alter vagal function through an apparent δ1-mechanism. If DPDPE acted on both δ1- and δ2-receptors simultaneously, the opposing vagotonic and vagolytic actions may have cancelled out one another. In summary, selective activation of δ1-receptors had no demonstrable vagolytic effect. In contrast, δ1-receptors appeared to facilitate vagal function.

The normal role of cardiac opioids in the autonomic control of the heart remains unclear, but some of the details have begun to resolve. The presence of significant mRNA for proenkephalin in heart and the prodigious capability of the heart to degrade enkephalin suggest the cardiac enkephalins function primarily as a local paracrine hormones. The current studies reported here have concentrated on interactions with vagal control of heart rate. Earlier studies (3, 4, 10, 13, 22, 24, 31) both in vivo and in isolated heart models...
demonstrated that opioids attenuated a variety of cardiac parasympathetic responses during vagal nerve stimulation. The δ2-mediated interruption of vagal bradycardia is consistent with the traditional view of opioids as inhibitory neuromodulators. The apparent bimodal character of δ-receptor activation though not often acknowledged is also not that unusual (7, 26). Because distinct δ1- and δ2-receptor proteins have not been isolated, opposing responses in the same tissue presents some interesting mechanistic questions. One proposal suggested that the local membrane environment determined the functional expression of opposing opioid receptor responses by regulating how the receptors were coupled to their respective second messenger systems (7). How this local environment and the balance of these responses participate in normal heart rate control remains to be determined.

What purpose do these δ-subtypes serve in modulating heart rate during normal homeostasis? When endogenous nodal MEAP was elevated during occlusion of the nodal artery, vagal bradycardia was improved (14). The vagotonic effect was blocked by the general δ-antagonist naltrindole, and the vagal improvement was quantitatively very similar to that observed during administration of TAN-67 in this current report. Because the latter was blocked by BNTX, both responses may have been mediated by δ1-receptors. The coupling hypothesis cited above (7) also suggested that one side of the bimodal response was far more sensitive to agonist. The hypothesis argued that the positive coupling to adenylate cyclase through the G protein Gs α predominated at physiologically very low opioid concentrations. Thus the vagotonic effect associated with nodal artery occlusion would be consistent with the bimodal hypothesis if the modest increases in nodal MEAP also observed during occlusion (14) improved the efficiency of vagal transmission through δ1-receptors much like TAN-67. The activation of δ1-receptors during arterial insufficiency might serve to stabilize the heart by improving local vagal function and thereby reducing local oxygen demand and consequent irritability.

At the other end of the spectrum, vasovagal syncope poses a different threat to the organism during stressful circumstances. In this regard, higher rates of opioid release, combined with the activation of δ2-opioid receptors may suppress vagal function when that activity is inappropriately intense. Thus at higher concentrations the more widely recognized neuroinhibitory coupling to adenylate cyclase through the inhibitory G protein Giα might predominate with the opioids now serving as inhibitory governors of vagal activity. In accord with this proposed hypothesis, one might argue that the δ1 activity provides a background environment of neurofacilitatory activity, whereas the δ2-receptors provide a more episodic governor-like function.

The opioid receptor systems may also be of significance during cardiovascular pathologies such as myocardial infarction and congestive heart failure. Evidence that δ1-receptors mediate preconditioning suggested that these receptors might be therapeutically valuable during myocardial infarction (27). Nodal MEAP recovered in the dialysate was elevated during a series of brief nodal artery occlusions. As indicated above, this increase in nodal MEAP was accompanied by an improved vagal function (14) that in retrospect may have been mediated by δ1-receptors. Healthy vagal influences have been associated with better survival statistics after myocardial infarction (2, 18). The activation of δ1-receptors could enhance vagal function during myocardial infarction, and by slowing the heart, decrease work output and energy demand (23, 34). This would then reduce the damage caused by free radicals and help to maintain cellular integrity (23).

The observation that δ2-opioid receptors are vagolytic suggests that their actions may be pathological for instance during sustained excess. Circulating endogenous opioids rise significantly during congestive heart failure (11). The vagolytic action of these peptides may contribute to cardiac dysfunction and the rise in sympathetic activity. In support of this hypothesis, δ-opioid antagonists restored vagal function in atrial preparations from failing human hearts (19). However, the characterization of δ1- and δ2-receptor effects on heart rate during cardiovascular disease remains to be elucidated and may hold significant clinical potential.

In conclusion, the current results suggested that the endogenous cardiac enkephalin MEAP attenuated vagal bradycardia via δ2-opioid receptors concentrated within the canine SA node. The data above also support the presence of δ1-opioid receptors in the SA node that appear to facilitate vagal transmission. Whether δ1- and δ2-opioid receptors in the SA node are located prejunctionally on vagal nerve terminals and whether these receptors modify the release of acetylcholine both remain to be verified directly and as such constitute important future directions.

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