Cardiac Enkephalins Interrupt Vagal Bradycardia Via $\delta$-2 Opioid Receptors in the Sinoatrial Node.

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Abstract

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 δ-2 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) and assorted δ-1 opioid (TAN-67 and DPDPE) and δ-2 (deltorphin II) agonists. The vagolytic effects of intranodal MEAP and deltorphin were then challenged with the δ-1 and δ-2 opioid receptor antagonists 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 percent. 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 δ-2 opioid receptors located in the sinoatrial node. These data also support the existence of vagotonic δ-1 opioid receptors also in the sinoatrial node.
Introduction

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 have observed that enkephalins suppressed vagal bradycardia in vivo suggesting that enkephalins function as governors of vagal control (3,4,10,13,22,24).

Several enkephalin sequences are concentrated in the heart (32) including the heptapeptide, methionine-enkephalin-arginine-phenylalanine (MEAP). MEAP attenuated vagally mediated bradycardia by more than seventy percent 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. Intra-nodal 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 sinoatrial node which were most likely located prejunctionally on vagal nerve terminals.

In order 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 δ-opioid receptors (13). The nodal delivery of MEAP and the δ-2 agonist, deltorphin II produced equipotent vagolytic responses and both effects were reversed by the δ-antagonist, naltrindole. Mu 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.

Though distinct transcripts corresponding to δ-receptor subtypes have not been isolated have (1,9,17) there are considerable functional and pharmacological evidence for the existence of distinct δ-1- and δ-2-receptor mediated responses (1,15,25,28,29,30,33). The nature of subtype specific actions on cardiac function is not well defined but Schultz et al (27) demonstrated
that pretreatment with the selective δ-1-agonist, TAN-67 significantly reduced infarct size in the ischemic rat heart. The cardioprotection conferred by TAN-67 was subsequently reversed by the selective δ-1 antagonist, BNTX. Chien et al. (5) also reported that δ-1-agonists helped to preserve the viability of multi-organ preparations. Since 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-opiate 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 (δ-1 and δ-2) may mediate respectively the opposing
vagotonic and vagolytic effects of opioids. Consistent with the suggestion that
the vagotonic effect is mediated by δ-1-receptors, Shultz et al (27) reported that
TAN-67 reduced resting heart rate in the rat. In contrast δ-activation by
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 δ-2 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 δ-2 opioid
receptors in the sinoatrial node were responsible for the vagolytic effect of the
cardiac opioid MEAP and to rule out the participation of δ-1 opioid receptors.
This was accomplished with two strategies. In one, the vagolytic effects of
MEAP and the δ-2 agonist, deltorphin II were first demonstrated and then the
endogenous opioid, MEAP was challenged with δ-1 and δ-2 selective
antagonists. In the second, the vagolytic effects of MEAP and deltorphin II were
compared with those of the selective δ-1 agonists, 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 δ-2 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 sodium pentobarbital, intubated and mechanically ventilated with room air. Fluid filled catheters were inserted into the femoral artery and vein then advanced into the descending aorta and inferior vena cava, respectively. The arterial line was attached to a Statham PD23XL pressure transducer 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 (Instrumentation Laboratories blood gas analyzer) and the pO$_2$ (90-120 mmHg), pH (7.34 - 7.45), and pCO$_2$ (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 minutes 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 sinoatrial node. To confirm the probe placement in the SA node, norepinephrine (1 x 10^{-9} moles/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 have determined that deliberate repositioning of the probe as little as 2 mm lateral to the node eliminates the norepinephrine mediated tachycardia (14). The microdialysis probe was constructed from a single one centimeter length of dialysis fiber (220 um OD, 200 um ID) and hollow silica inflow and outflow tubes (120 um ID, 170um 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 δ-2 opioid receptor subtype was responsible for the vagolytic effect of nodal enkephalins. Two strategies were employed. In the first the influence of δ-subtype specific agonists [D-pen²⁵] enkephalin (DPDPE), TAN-67 and deltorphin II were 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 (7-benzylidenenaltrexone [BNTX] and naltriben) 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 revealed that the δ-2 selective agonist, deltorphin II (1.5 x 10^{-9} moles/min) blocked vagally mediated bradycardia (13). The vagolytic effect of deltorphin II was successfully reversed by the δ-selective antagonist, naltrindole. These findings suggested participation of a δ-2 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 intra-nodal administration of δ-1-selective agonists were capable of interrupting vagal bradycardia. After microdialysis probe insertion, the sinoatrial node was perfused (5 µl/min) with saline for 60 minutes. After this period of equilibration, control vagal responses were obtained by stimulating the right vagus nerve at 1, 2, and 3 hertz. The nerve was stimulated at a supramaximal voltage for 15 seconds followed by one min 45 sec for recovery. Deltorphin II was then infused (5 µl/min) into the sinoatrial node for five minutes to establish a functional vagolytic effect. The effective dose used for deltorphin II (1.5 x 10^{-9} moles/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. Following this procedure, dose responses were constructed for the selective δ-1 agonist DPDPE or TAN-67. Doses were selected to provide molar equivalent ranges
(0.05 – 5 x 10^{-9} moles/min) to those previously determined to be vagolytic for MEAP and deltorphin II (13). Each dose of each agent was infused for five minutes before evaluating the vagus nerve. After each dose evaluation, the agent was washed out for 15 minutes 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 δ-1 antagonist, BNTX, to determine if the unexpected improvement in vagal function was mediated by a δ-1 opioid receptor.

**Protocol 2.** This protocol was designed to test whether vagolytic effects of MEAP and deltorphin II were blocked by a selective δ-2 opioid receptor antagonist and not by a selective δ-1 opioid receptor antagonist. MEAP and deltorphin II (1.5 x 10^{-9} moles/min) were introduced into the interstitium of the sinoatrial node and vagal stimulations were performed as previously described in order to establish the vagolytic effect of each. After washout of these initial tests, MEAP was combined with increasing doses of the selective δ-1 antagonist, BNTX, or the selective δ-2 antagonist, naltriben. 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 δ-2 antagonist, naltriben should overcome the vagolytic effect of MEAP and deltorphin and verify participation of the δ-2 opioid receptor. BNTX should not reverse the vagolytic effect of MEAP or deltorphin indicating the absence of participation by δ-1 opioid receptors.
**Materials.** Methionine enkephalin-arginine-phenylalanine and deltorphin II were synthesized by American Peptide Co, Sunnyvale, CA. TAN-67, DPDPE and BNTX were obtained from Tocris Cookson Inc, Ellisville MO. Naltriben was obtained from Sigma Chemical Co, St. Louis, MO.

**Statistical methods.** All data were expressed as means and standard errors. 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 δ-1- and δ-2-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 prior to treatment. Resting heart rate and blood pressure were also unaltered by any of the opioid agonists and antagonists, regardless of dose.

Deltorphin vagolysis. Deltorphin II was used as a positive control to demonstrate the functional integrity of the system in each animal prior to testing other agents. 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 x 10^{-9} moles/min) reduced vagally mediated bradycardia by 75-85 percent at all vagal frequencies employed and was significantly different from control.

DPDPE dose responses: In this protocol DPDPE was introduced directly into the sinoatrial node to rule out the participation of δ-1 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 (Figure 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 (lower two curves). The
The vagolytic effect of deltorphin II is illustrated in the upper curve. The complete
dose responses for all three frequencies are illustrated in Figure 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 second selective δ-1 opioid
receptor agonist, TAN-67 was used in a second group of animals to provide
further evidence that δ-1 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 (Figure 3, middle curve).
Deltorphin II produced a vagolytic response similar to that observed (80%
inhibition) in the prior group (Figure 3, upper curve). 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 (Figure 3, lower curve). The maximum effect was
observed at the 1.5 x 10^{-9} moles/min (Figure 4) with an apparent ED_{50} of 1.0 x
10^{-10} moles/min. The maximal improvement at 1.5 x 10^{-9} moles/min was 28-37
percent 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 δ-1 receptor, TAN-67 (1.5 x 10^{-9} moles/min) was then combined
with the δ-1 antagonist BNTX (1.5 x 10^{-9} moles/min) and infused directly into the
sinoatrial node via microdialysis. BNTX effectively prevented the vagotonic
effect of TAN-67 since the vagally mediated bradycardia during the combined infusion was similar to control values (Fig 3, middle curve). 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 vs 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 δ-1 and δ-2 antagonists to verify which δ-receptor subtype was responsible for the interruption of vagal bradycardia. The control frequency response is illustrated among the lower curves in Figure 5. The vagolytic effect of deltorphin II and MEAP are illustrated in the two upper curves. Increasing doses of the selective δ-2, 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 (Figure 6). The reversal was obtained with an ID$_{50}$ approximating 1.5 x 10$^{-10}$ moles/min and a maximal effect near molar parity with the agonist (1.5 x 10$^{-9}$ moles/min). The similar blockade of the deltorphin and MEAP effects are illustrated among the lower curves in Figure 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 δ-2 receptors.
**MEAP vs BNTX dose responses:** The selective δ-1-opioid receptor antagonist BNTX was used to confirm that the vagolytic effect of MEAP was mediated by δ-2- and not by δ-1-opioid receptors. This was achieved by combining increasing doses of BNTX with an effective vagolytic dose of MEAP (1.5 x 10^{-9} moles/min). The rationale presumed that if naltriben identified a functional δ-2-response, then combining MEAP with increasing doses of BNTX would find BNTX ineffective or much less effective than naltriben. The lower two curves in Figure 7 illustrate the control bradycardia response in this group and the absence of an effect of BNTX alone. The 50-70 percent inhibition by both MEAP and deltorphin II are indicated among the upper curves in Figure 7. When BNTX was combined with MEAP or deltorphin II, the resulting curves were very similar to those for MEAP and deltorphin alone (Figure 7, upper curves). 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 Figure 8. Though 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 δ-2-agonist, deltorphin II further supports the exclusive δ-2-character of the vagolytic effect.
Discussion

The data reported above support the primary hypothesis that the vagolytic effect of the endogenous opioid, MEAP on heart rate is mediated by $\delta$-2-opioid receptors in the sinoatrial node. This conclusion is based on the observation that vagolytic response to MEAP was duplicated by the $\delta$-2 agonist deltorphin II when the $\delta$-1-agonists, DPDPE and Tan-67 were both vagolytically 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 $\delta$-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 δ-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 TAN-67 had reached the nodal interstitium. The δ-1-opioid receptor antagonist, BNTX subsequently reversed the TAN-67 mediated vagal improvement. Thus δ-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 δ-receptor.

Selectivity issues: TAN-67 and DPDPE. The existence of δ receptor subtypes has been based entirely on biological responses which can be distinguished by agonists and antagonists reported as selective for the respective subtypes (1,13,25,28,29,30,33). 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 δ-1- and δ-2-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 $\delta$-1-, $\delta$-2-, or mixed responses are evident (7).

Subtype specific responses have been used to quantify the relative $\delta$-selectivity of various agents. DPDPE and deltorphin II have been widely employed respectively as preferential $\delta$-1- and $\delta$-2-agonists. Each has approximately 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 $\delta$-1- and $\delta$-2-antagonists (15,25).

DPDPE reportedly has some mixed $\delta$-2-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. Since $\delta$-2-opioid receptors were clearly vagolytic, the absence of a response to DPDPE would suggest either the absence of $\delta$-1-receptors or the absence of a $\delta$-1-effect on vagal function. If DPDPE has measurable $\delta$-2-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 $\delta$-1-opioid systems (6,16) improved vagal bradycardia by 35 percent and was reversed by BNTX. This suggests that $\delta$-1-receptors were present and they did alter vagal function through an apparent $\delta$-1-mechanism. If DPDPE acted on both $\delta$-1- and $\delta$-2-receptors simultaneously,
opposing vagotonic and vagolytic actions may have cancelled out one another. In summary, selective activation of $\delta$-1-receptors had no demonstrable vagolytic effect. In contrast, $\delta$-1-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 heart’s prodigious capability 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 both in vivo and in isolated heart models demonstrated that opioids attenuated a variety of cardiac parasympathetic responses during vagal nerve stimulation (3,4,10,13,22,24,31). The $\delta$-2-mediated interruption of vagal bradycardia is consistent with the traditional view of opioids as inhibitory neuromodulators. The apparent bimodal character of $\delta$-receptor activation though not often acknowledged is also not that unusual (7,26). Since distinct $\delta$-1- and $\delta$-2-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. Since the later was blocked by BNTX, both responses may have been mediated by δ-1-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, Gsα 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 δ-1-receptors much like Tan-67. The activation of δ-1-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 δ-2-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 Glα might predominate with the opioids now serving as inhibitory governors of vagal activity. In accord with this proposed hypothesis, one might argue that the δ-1-activity provides a background environment of neurofacilitatory activity while the δ-2-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 δ-1-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 δ-1-receptors. Healthy vagal influences have been associated with better survival statistics after myocardial infarction (1, 18). The activation of δ-1-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 δ-2-opioid receptors are vagolytic suggests that their actions may be pathologic for instance during sustained excess. Circulating endogenous opioids rise significantly during congestive heart failure (11).
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 δ-1- and δ-2-receptor effects on heart rate during cardiovascular disease remains to be elucidated and may hold significant clinical potential.

Conclusions. In conclusion, the current results suggested that the endogenous cardiac enkephalin, MEAP, attenuated vagal bradycardia via δ-2-opioid receptors concentrated within the canine sinoatrial node. The data above also support the presence of δ-1-opioid receptors in the SA node that appear to facilitate vagal transmission. Whether δ-1- and δ-2-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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FIG. 1 This graph represents the heart rate/frequency response mediated by right vagal nerve stimulation during the nodal delivery of Deltorphin II (1.5 x 10^{-9} moles/min) and DPDPE (5 x 10^{-9} moles/min) by microdialysis. The data illustrated are for the maximal dose of DPDPE employed in its dose response curve. * = Significantly different from control, P< .05.

Fig 2(a,b,c). These graphs illustrate the change in heart rate produced during right vagal stimulation during exposure (5 min) to increasing doses of the δ-1-selective opioid agonist, DPDPE. The units for the doses listed within the bars are x 10^{-9} moles/min. Deltorphin (1.5 x 10^{-9} moles/min) was included as a positive control. All treatments were infused into the sinoatrial node of the dog via microdialysis. * = Significantly different from control, P< .05.

Fig 3. This graph represents the heart rate/frequency response mediated by right vagal nerve stimulation during the nodal delivery of vehicle, deltorphin II (1.5 x 10^{-9} moles/min), TAN-67 (5 x 10^{-9} moles/min) or BNTX alone (5 x 10^{-9} moles/min), and TAN-67 (1.5 x 10^{-9} moles/min) combined with an equimolar dose of BNTX. * = Significantly different from control, P< .05.

Fig 4 (a,b,c). These graphs illustrate the change in heart rate produced during right vagal stimulation during exposure (5 min) to increasing doses of the δ-1-selective opioid agonist, TAN-67. The units for the doses listed within the bars are x 10^{-9} moles/min. Deltorphin (1.5 x 10^{-9} moles/min) was included as a
positive control. All treatments were infused into the sinoatrial node of the dog via microdialysis. * = Significantly different from control, P< .05.

Fig 5. This graph represents the heart rate/frequency response mediated by right vagal nerve stimulation during the nodal delivery of vehicle, deltorphin II (1.5 x 10^{-9} moles/min), MEAP (1.5 x 10^{-9} moles/min), MEAP or deltorphin II (1.5 x 10^{-9} moles/min) combined with an equimolar dose of Naltriben, and Naltriben (5 x 10^{-9} moles/min) alone. * = Significantly different from control, P< .05.

Fig 6 (a,b,c). These graphs illustrate the change in heart rate produced during right vagal stimulation during exposure (5 min) to increasing doses of the δ-2-antagonist, naltriben combined with MEAP (1.5 x 10^{-9} moles/min). The units for the doses listed in the bars are x 10^{-9} moles/min. Deltorphin (1.5 x 10^{-9} moles/min) was included as confirmation of the δ-2-character of the naltriben blockade. All treatments were infused into the sinoatrial node of the dog via microdialysis. * = Significantly different from control, P< .05.

Fig 7. This graph represents the heart rate/frequency response mediated by right vagal nerve stimulation during the nodal delivery of vehicle, deltorphin II (1.5 x 10^{-9} moles/min), MEAP (1.5 x 10^{-9} moles/min), MEAP or deltorphin II (1.5 x 10^{-9} moles/min) combined with an equimolar dose of BNTX, and BNTX (5 x 10^{-9} moles/min) alone. * = Significantly different from control, P< .05.
Fig 8 (a,b,c). These graphs illustrate the change in heart rate produced during right vagal stimulation during exposure (5 min) to increasing doses of the δ-1-antagonist, BNTX combined with a fixed dose of MEAP (1.5 x 10⁻⁹ moles/min). The units for the doses listed in the bars are x 10⁻⁹ moles/min. Deltorphin (1.5 x 10⁻⁹ moles/min) was included as added confirmation of the absent δ-1 receptor participation in the response. All treatments were infused into the sinoatrial node of the dog via microdialysis. * = Significantly different from control, P< .05.
### Table 1. Cardiovascular Indices

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Treatment</th>
<th>Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (bpm)</td>
<td>MAP (mmHg)</td>
<td>HR (bpm)</td>
</tr>
<tr>
<td>MEAP (15)</td>
<td>128±5</td>
<td>114±7</td>
<td>132±7</td>
</tr>
<tr>
<td>Deltorphin II (13)</td>
<td>127±4</td>
<td>118±4</td>
<td>128±6</td>
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<tr>
<td>DPDPE (5)</td>
<td>129±5</td>
<td>112±7</td>
<td>127±5</td>
</tr>
<tr>
<td>TAN-67 (5)</td>
<td>122±6</td>
<td>117±7</td>
<td>110±4</td>
</tr>
<tr>
<td>BNTX (5)</td>
<td>125±4</td>
<td>117±5</td>
<td>111±2</td>
</tr>
<tr>
<td>Naltriben (5)</td>
<td>136±7</td>
<td>112±7</td>
<td>123±6</td>
</tr>
</tbody>
</table>
Figure 1

DPDPE vs Deltorphin II

Change in Heart Rate (bpm)

Right Vagal Stimulation Frequency (Hz)
DPDPE Dose Response Curve

1 Hz

Figure 2 (a)

2 Hz

Figure 2 (b)

3 Hz

Figure 2 (c)
Figure 3

TAN-67 vs BNTX

Change in Heart Rate (bpm)

Right Vagal Stimulation Frequency (Hz)

Deltorphin II
BNTX/TAN
BNTX
Control
TAN-67
TAN-67 Dose Response Curve

Change in Heart Rate (bpm)

Figure 4 (a) 1 Hz

Figure 4 (b) 2 Hz

Figure 4 (c) 3 Hz
Figures 5
Figure 6 (a) 1 Hz

Figure 6 (b) 2 Hz

Figure 6 (c) 3 Hz

MEAP vs Naltriben
BNTX vs. MEAP

Change in Heart Rate (bpm)

Right Vagal Stimulation Frequency (Hz)

Figure 7
Figure 8 (a)

1 Hz

Figure 8 (b)

2 Hz

Figure 8 (c)

3 Hz