The monosialosyl ganglioside GM-1 reduces the vagolytic efficacy of δ2-opioid receptor stimulation

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The cardiac enkephalin, methionine-enkephalin-arginine-phenylalanine (MEAP), alters vagally induced bradycardia when introduced by microdialysis into the sinoatrial (SA) node. The responses to MEAP are bimodal; lower doses enhance bradycardia and higher doses suppress bradycardia. The opposing vagotonic and vagolytic effects are mediated, respectively, by δ1 and δ2 phenotypes of the same receptor. Stimulation of the δ1 receptor reduced the subsequent δ2 responses. Experiments were conducted to test the hypothesis that the δ-receptor interactions were mediated by the monosialosyl ganglioside GM-1. When the mixed agonist MEAP was evaluated after nodal GM-1 treatment, δ1-mediated vagotonic responses were enhanced, and δ2-mediated vagolytic responses were reduced. Prior treatment with the δ1-selective antagonist 7-benzylidenaltrexone (BNTX) failed to prevent attirion of the δ2-vagolytic response or restore it when added afterward. Thus the GM-1-mediated attrition was not mediated by δ1 receptors or increased competition from δ1-mediated vagotonic responses. When GM-1 was omitted, deltorphin produced a similar but less robust loss in the vagolytic response. In contrast, however, to GM-1, the deltorphin-mediated attrition was prevented by pretreatment with BNTX, indicating that the decline in response after deltorphin alone was mediated by δ1 receptors and that GM-1 effectively bypassed the receptor. Whether deltorphin has intrinsic δ1 activity or causes the release of an endogenous δ1-agonist is unclear. When both GM-1 and deltorphin were omitted, the subsequent vagolytic response was more intense. Thus GM-1, deltorphin, and time all interact to modify subsequent δ2-mediated vagolytic responses. The data support the hypothesis that δ1-receptor stimulation may reduce δ2-vagolytic responses by stimulating the GM-1 synthesis.

bradycardia; deltorphin; heart rate

the sinoatrial (SA) node or cardiac pacemaker is densely innervated by sympathetic and parasympathetic nerve fibers that moderate automaticity through the local nodal release of norepinephrine and acetylcholine. In addition to direct innervation, multiple factors within the node modify both spontaneous activity and neurotransmitter release. Among these modulators, cardiac opioids appear to be potentially important neuromodulators (3, 9, 10).

Proenkephalin mRNA is more abundant in the heart than most other tissues, including the brain (12). Enkephalins are found in the heart, and despite the stoichiometric advantage afforded met-enkephalin (ME), ME-arginine-phenylalanine (MEAP) concentrations are higher in the heart than any of the other three enkephalins (2, 19, 29). ME, leucine-enkephalin (LE), and MEAP have all been demonstrated to alter vagally induced bradycardia when introduced into the SA node by microdialysis (9–11, 13). The opioids identified in the heart alter heart rate by binding to specific opiate receptors, which are probably located prejunctionally on sympathetic and parasympathetic nerve terminals (10, 27).

Opioid receptors moderate a wide variety of physiological systems, primarily through the regulation of neurotransmitter release (20). The enkephalins are potent δ agonists and are generally viewed as the native ligands for the δ receptor (13, 20, 21, 25, 31). Although behavioral and pharmacological studies have provided support for distinct receptor subtypes of the δ receptor (1, 11, 13, 20, 22, 25), biochemical studies have identified a single protein transcript (1, 8, 18). In the heart, δ-receptor stimulation produced a bimodal response during vagal stimulation (11, 13). Lower doses (10–15 mol/min) of enkephalin enhanced vagal transmission (vagotonic), while higher doses (10–12 mol/min) suppressed vagal transmission (vagolytic) (11). The two opposing effects appeared to have been mediated by phenotypes of the δ-opioid receptor because each effect was blocked by subtype-specific antagonists.

The observations made in the heart are consistent with those made in sensory neurons that indicated that opioids were excitatory in some circumstances and inhibitory in others (4, 22, 24). Crain and Shen (4) proposed that the quality and sensitivity of the response were governed by the ganglioside content of the cell membrane surrounding the opiate receptor. Membranes rich in the monosialosyl ganglioside GM-1 favored excitatory opioid responses at very low doses. The excitatory response was further proposed to activate a positive-feedback loop that increased excitatory activity by stimulating the synthesis of more GM-1. Ultralow opioid concentrations stimulate δ1-opioid receptors coupled through Gs to activate adenyl cyclase. The hypothesis suggested that the resulting increase in the cAMP-dependent protein kinase phosphorylated glycosyltransferase and increased the synthesis of GM-1. This increase in GM-1 theoretically improved the efficiency of excitatory opioid receptor coupling and counteracted the inhibitory opioid receptor effects. In the absence of GM-1, these same opioids reduced cyclase activity through Gs/Gt-coupling (4, 5, 24). Thus they suggested that the excitatory stimulation and the resulting changes in the environment around the receptor modified the response in isolated systems.

Because only one transcript has been isolated for the δ receptor, we have suggested that the δ-receptor coupling in the SA node is fluid, and the receptor subtype-dependent responses

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might be interconvertible. In support of this thesis, a decrement in the intensity of δ2-mediated vagolytic responses was noted after a lengthy exposure of the SA node to δ1-receptor stimulation (7). These observations prompted the suggestion that similar ganglioside-mediated plasticity might be operative in parasympathetic nerves regulating heart rate. The following studies were designed to test that hypothesis that adding GM-1 into the interstitium of the SA node will reduce the intensity of δ2-mediated vagolytic responses secondary to an increase in competing δ1-mediated vagotonic responses.

MATERIALS AND METHODS

Surgical Preparation

Thirty-two mongrel dogs of either gender weighing 15–25 kg were assigned at random to various experimental protocols. All protocols were approved by the Institutional Animal Care and Use Committee and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were anesthetized with pentobarbital sodium (32.5 mg/kg), intubated, and mechanically ventilated initially at 225 ml·min<sup>−1</sup>·kg<sup>−1</sup> with room air. 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 to monitor heart rate and arterial pressure. The venous line was used to administer supplemental anesthetic, as required. The acid-base balance and the blood gases were determined periodically with an Instrumental Laboratories Blood Gas Analyzer. The P<sub>O2</sub> (90–120 mmHg), the pH (7.35–7.45), and the P<sub>CO2</sub> (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 surgical incision. The nerves were double ligated with umbilical tape to prevent afferent nerve traffic during electrical stimulation. The isolated nerves were then returned to the prevertebral compartment for later retrieval. Surgical anesthesia was carefully monitored, and a single dose of succinylcholine (50 µg/kg) was administered intravenously to temporarily reduce involuntary muscle movements during the 10–15 min required for electrosurgical incision of the chest. The costosternal cartilage for ribs 2–5 was severed to permit access to the thoracic cavity, and 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 the heart. The left femoral artery was isolated, and a high-fidelity catheter pressure transducer (Millar) was inserted and advanced into the abdominal aorta to measure heart rate and blood pressure continuously online thereafter (PowerLab).

Nodal Microdialysis

A 25-g stainless steel needle containing the microdialysis line was inserted into the center of the SA node along its long axis (9, 14). The needle was removed, and the probe was then positioned so that the dialysis window was completely within the substance of the SA node. The microdialysis probes were constructed of a single 1-cm length of dialysis fiber from a Clarian TAF08 (Asahi Medical) artificial kidney (200 µm ID, 220 µm OD) and hollow silica (SGE; Austin, Texas) inflow and outflow tubes (120 µm ID, 170 µm OD). The dialysis tubing permits molecules with a molecular mass of 35,000 kDa or less to cross from the lumen into the nodal interstitium. This technique allows the precise introduction of agents directly into the nodal interstitium for extended periods without provoking complicating systemic reflexes. After placement of the probe in the SA node, the preparation was allowed to equilibrate for 1 h while being perfused with saline at 5 µL/min.

Materials

MEAP and deltorphin II were synthesized by American Peptide. GM-1 was obtained from Sigma Chemical, and the δ<sub>1</sub>-antagonist 7-benzylidenaltrexone (BNTX; Ref. 20) was obtained from Toecris.

Statistical Methods

All data were expressed as means ± SE. Differences were evaluated with an ANOVA; a repeated-measures approach was employed where appropriate. Individual treatment differences were determined by post hoc analysis with Dunnett’s or Tukey’s test, respectively, when multiple comparisons to control or multiple comparisons among treatments were necessary. Differences determined to occur by chance with a probability of P < 0.05 were accepted as statistically significant.

Experimental Protocols

Protocol 1: Interactions between GM-1 and the native agonist MEAP (n = 7). After equilibration for 1 h, the right cervical vagus nerve was stimulated at a supramaximal voltage (15 V) for 15 s at low (1–2 Hz) and high (3–4 Hz) frequencies selected to produce, respectively, 10–20 and 30–40 beats/min decreases in heart rate. Subthreshold vagotonic (5 fmol/min, Lo-MEAP) and submaximal vagolytic (1.5 nmol/min, Hi-MEAP) doses of MEAP were administered by dialysis for 5 min each (11). After 5-min exposure to the first dose, the two-step heart rate/vagal frequency evaluation was repeated. The dose was then increased, and the vagal transmission was reevaluated 5 min later. The MEAP infusion was then discontinued, and the line was washed with vehicle until control vagal function was restored. GM-1 was then added to the dialysis inflow (5 nmol/min) and continued for 30 min. The dose of GM-1 was extrapolated from the effective dose employed by others in vitro (4, 5). After 30 min, the GM-1 was discontinued, and the vagal responses to the two doses of MEAP used earlier were tested again.

Protocol 2: Interactions between GM-1 and the δ<sub>2</sub>-agonist deltorphin II (n = 5). After the initial equilibration, control vagal responses were obtained by sequentially stimulating the right vagus nerve in two steps as described above. The δ<sub>2</sub>-agonist deltorphin was then introduced into the SA node by microdialysis at a submaximal dose of 0.7 nmol/min for 5 min (13). The two-step vagal evaluation was repeated to quantify the initial δ<sub>2</sub> (vagolytic) response before exposure to GM-1. This test of efficacy was designated δ<sub>2</sub>-250 to indicate the time in the protocol. After determining the δ<sub>2</sub> response, deltorphin was discontinued, and the system was washed out with saline (45–60 min) until the control vagal responses were restored. GM-1 (5 nmol/min) was perfused for 1 h, and the vagus was stimulated every 15 min to evaluate the effects of GM-1 alone. Excess GM-1 was washed out for 30 min, and post-GM-1 control responses were retested. Deltorphin was reintroduced, and after 5 min, the vagolytic δ responses were also retested and designated δ<sub>2</sub>-155. Deltorphin was discontinued but was introduced again 25 min later for another vagal test designated δ<sub>2</sub>-180 to evaluate the progression of changes in the δ<sub>2</sub> response. The deltorphin was discontinued and washed out (45–60 min). The δ<sub>1</sub>-selective antagonist (BNTX) was then introduced at a maximal dose of 5 nmol/min (11) for 5 min, and the right vagus nerve was stimulated to evaluate the effects of δ<sub>1</sub>-receptor blockade with BNTX alone. BNTX and deltorphin were then reintroduced together (1:1) for 5 min, and a two-step vagal stimulation designated δ<sub>2</sub>-250 was conducted to determine (by subtraction) the contribution of δ<sub>1</sub>-mediated (vagotonic) response to any change in the δ<sub>2</sub> response. The treatments were then discontinued, the area was washed, and the vagal responses were tested periodically until the reappearance of the original control response.

Protocol 3: GM-1 and δ<sub>1</sub>-receptor blockade (n = 4). This protocol was conducted to test whether the effect of GM-1 was dependent on
Δ1-receptor activity. The protocol was identical to that in protocol 2 except the Δ1-antagonist BNTX was infused throughout the protocol.

Protocol 4 (time controls): Vehicle, duration, and repeated deltorphin II (n = 4). The purpose of this study was to determine the potential influence of the duration of the protocol, the repeated vagal stimulation, and/or the prior exposure to deltorphin on the subsequent deltorphin-mediated, Δ2-vagolytic responses. This protocol was identical to the first 150 min in protocol 2 except vehicle (saline) was substituted for GM-1 during the treatment period.

Protocol 5 (time controls): Influence of Δ1 blockade on Δ2 responses (n = 4). The purpose of this study was to test whether apparent contributions of deltorphin to the erosion of the Δ2 response observed in protocol 4 depended on Δ1-receptor activity. This protocol was identical to protocol 4 except that the Δ1-antagonist BNTX was added to the dialysis inflow after equilibration and before Δ2-5 deltorphin. BNTX was then continued throughout the following 2 h and the Δ2 challenges at Δ2-155 and Δ2-180.

Protocol 6 (time controls): Vehicle, duration, and naive deltorphin II (n = 5). The purpose of this study is to remove the influence of prior deltorphin exposure on the Δ2 response. This protocol is similar to protocol 2 except initial exposure to deltorphin (Δ2-5) and the treatment with GM-1 were omitted. Vehicle was perfused for 2.5 h with vagal stimulations every 15 min during the 2nd hour as in protocol 2. The subsequent deltorphin challenges with and without BNTX were then applied in a sequence equivalent to Δ2-155, Δ2-180, and Δ2-250 in protocol 2.

Protocol 7A: GM-1 and naive deltorphin (no wash, n = 5). The purpose of this study was to test whether GM-1 was effective alone without prior deltorphin. The protocol was designed to test, by omission of Δ2-5, whether the decline in the Δ2-vagolytic response depended on the initial exposure to deltorphin (Δ2-5), the treatment with GM-1, or a combination of both. The initial two-step vagal stimulation was conducted followed by 1 h of perfusion with vehicle to simulate the Δ2-5 exposure to deltorphin and its washout. GM-1 was infused at a dose of 5 nmol/min for the 2nd hour, and the right vagus nerve was tested at 15-min intervals as described in the other protocols to evaluate progressive effects of GM-1. GM-1 was discontinued, and immediately afterward, the deltorphin challenges with and without BNTX were then applied in a sequence equivalent to Δ2-155, Δ2-180, and Δ2-250, as described in protocol 2.

Protocol 7B: GM-1 and naive deltorphin (with wash, n = 4). The purpose of this study was to test whether the influence of GM-1 was sustained after discontinuing its perfusion or whether the Δ2-response began to recover. The sequence of the protocol was identical to that in protocol 7A except that a 30-min wash was inserted between stopping the GM-1 at 150 min and the first two Δ2-evaluations now designated Δ2-180 and Δ2-205. BNTX was not tested in this protocol.

RESULTS

Basal cardiovascular parameters for all subjects across all treatments are presented in Tables 1 and 2. Animal subjects were assigned randomly to various protocols, and there were no significant differences in blood pressure or heart rate among groups before treatment. Resting heart rate and blood pressure were also unaltered by any of the treatments applied.

Table 1. Resting cardiovascular indexes for protocol 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lo MEAP</th>
<th>High MEAP</th>
<th>GM-1</th>
<th>GM-1+Lo MEAP</th>
<th>GM-1+High MEAP</th>
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</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>132±7</td>
<td>128±5</td>
<td>128±6</td>
<td>131±6</td>
<td>130±5</td>
<td>126±5</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>102±6</td>
<td>97±7</td>
<td>99±8</td>
<td>94±9</td>
<td>96±10</td>
<td>90±8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Lo MEAP and High MEAP refer to subthreshold vagotonic (5 fmol/min) and submaximal vagotonic (1.5 nmol/min) doses of methionine-enkephalin-arginine-phenylalanine (MEAP), respectively. For further description of protocol 1, see Experimental Protocols.

Protocol 1: Interactions Between GM-1 and the Native Agonist MEAP

The filled squares in Fig. 1 illustrates the two step decline in heart rate after right vagal stimulation at 2 and 4 Hz (control). Pretreatment with a subthreshold dose of MEAP (Lo MEAP) had no effect on vagal transmission. The higher dose (Hi MEAP) reduced the decline in heart rate by approximately two-thirds. GM-1 had no demonstrable effect on the control response (GM-1). However, after pretreatment with GM-1, the vagolytic effect of Hi MEAP was reduced, and a clear vagotonic effect of Lo MEAP emerged. These data led to the hypothesis that GM-1 improved the Δ1-mediated vagotonic effect of MEAP at the expense of a decline in its Δ2-mediated vagolytic effect. The subsequent protocols in this report were designed to evaluate the Δ2 portion of this thesis with the aid of the selective Δ2-agonist deltorphin.

Protocol 2: Interactions Between GM-1 and the Δ2-Agonist Deltorphin II

Figure 2 shows that, like MEAP, deltorphin produced a significant vagolytic response when first introduced in to the nodal interstitium (Δ2-5). The GM-1 treatment had no measurable effect on the vagal responses during the 60-min treatment period, but the subsequent vagolytic responses at Δ2-155 and Δ2-180 were progressively reduced in magnitude. If the loss of the Δ2 response was due to rising opposition from a competing Δ1 response, the acute Δ1 blockade should immediately restore or unmask the original Δ2 response. If, however, the Δ2 response is gone, Δ1 blockade will have no effect. The deltorphin from Δ2-180 was washed out for 1 h to restore the original control vagal response. BNTX was first added alone and had no effect on the control vagal response (not shown). Deltorphin was then introduced, combined with BNTX (BNTX + Δ2-250). The vagolytic effect of deltorphin was not different from that observed at Δ2-180, suggesting that the loss of the response between Δ2-5 and Δ2-180 resulted from downregulation or desensitization of the Δ2 response. In fact, the vagolytic effect of deltorphin was reduced further. These data suggest that GM-1 suppresses the Δ2-receptor response without a coincident contribution from enhanced Δ1-mediated vagotonic activity.

Protocol 3: Effect of Δ1 blockade

In this protocol (Fig. 3), the Δ1-antagonist BNTX was combined with GM-1 throughout the protocol. BNTX alone had little effect on the baseline vagal response (not illustrated) or the initial vagolytic response to deltorphin at Δ2-5. Adding GM-1 produced a limited but significant increase in vagal...
function at 3 Hz only. BNTX was ineffective in altering the subsequent erosion of the δ2 response at δ2-155 and δ2-180. The progressive attrition of the response was very similar to that observed in protocol 2 without BNTX.

Protocol 4 (Time Controls): Vehicle, Duration, and Repeated Deltorphin II

The purpose of this study (Fig. 4) was to test whether the reduction in the δ2 response observed in earlier protocols occurs in the absence of added GM-1. Thus vehicle was substituted for GM-1 during the treatment period. The initial vagolytic effect of deltorphin was similar to the initial response in protocols 2 and 3. Surprisingly, after a vehicle-only infusion for a time interval matching the GM-1 treatment period in protocol 1, a smaller but qualitatively similar progressive loss in the δ2-mediated vagolytic effect of deltorphin was observed during the δ2-155 and δ2-180 evaluations. These data suggest that there is attrition of the δ2 response during the protocol, perhaps due to the length of the protocol or the repeated deltorphin administration. Once again, the lost vagolytic response was not restored by blocking the δ1 receptors afterward with BNTX (not shown).

Protocol 5: Time Control and BNTX

The purpose of this study (Fig. 5) was to test whether δ1 blockade prevents loss of the δ2 response observed after deltorphin only in protocol 4. BNTX was introduced into the SA node by microdialysis. After 5-min exposure to BNTX, the vagal stimulations were repeated, and there was no significant difference between this response and the control response. BNTX was then combined with deltorphin for 5 min, and the vagus was retested. A typical deltorphin-mediated vagolytic response was observed. Deltorphin was discontinued, and protocol 4 time control was then repeated with BNTX added throughout. In this case, the δ2-5, δ2-155, and δ2-180 evaluations were virtually identical, with no erosion in the subsequent vagolytic responses. These data led to the suggestion that the loss of the δ2 response in the deltorphin-only time controls was indeed mediated by activation of δ1 receptors. Thus either deltorphin has intrinsic δ1 activity or deltorphin provoked the release of an endogenous δ1-agonist or facilitated its activity. Furthermore, the data suggest that GM-1 bypasses the requirement for stimulating the δ1 receptor to erode the vagolytic response.

### Table 2. Resting cardiovascular indexes for protocols 2–6, 7A, and 7B

<table>
<thead>
<tr>
<th>Control</th>
<th>δ2-5</th>
<th>GM-1</th>
<th>δ2-155</th>
<th>δ2-180</th>
<th>BNTX</th>
<th>BNTX + δ2-250</th>
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</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
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<td>126±3</td>
<td>124±6</td>
<td>121±9</td>
<td>122±9</td>
<td>123±10</td>
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<td>MAP, mmHg</td>
<td>102±4</td>
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<td>100±6</td>
<td>98±6</td>
<td>87±9</td>
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<td>103±2</td>
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<td>δ2-155</td>
<td>δ2-180</td>
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<td></td>
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<td>124±8</td>
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<td>Heart rate, beats/min</td>
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<td>Heart rate, beats/min</td>
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<td>BNTX</td>
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Values are means ± SE. BNTX, 7-benzylidenaltrexone; δ2 refers to δ2-agonist deltorphin II; δ2-5, δ2-150, δ2-180, and δ2-250 refer to δ2 evaluations at 5, 150, 180, 205, and 250 min. See Experimental Protocols for further details.
responses observed in protocols 2 and 3 but clearly less intense than that observed without GM-1 at δ2-155 in protocol 6 (~85% vs. ~65%, P < 0.01). Deltorphin was discontinued and then reintroduced 25 min later. When the vagus was retested again at δ2-180, there was significant further attrition of the vagolytic response particularly compared with the same response in the absence of GM-1 in Fig. 6 (~61–69% vs. ~27–28%, P < 0.03). Thus GM-1 alone appeared to have diminished the δ2 response compared with vehicle. Furthermore, the greater subsequent attrition at δ2-180 suggests that GM-1 and deltorphin interact to accelerate the rate of loss in the δ2-mediated response. As in protocol 2, blockade of the δ1 receptors after the fact did not restore the vagolytic response (not shown). The vagolytic effect of deltorphin was in fact again reduced further. In a parallel study (n = 4, not shown), the GM-1 was washed out for 30 min to evaluate whether its influence on the vagolytic response was sustained. In this case, the two posttreatment evaluations were conducted at 180 and 205 min. The first evaluation at δ2-180 was very similar to that above without a prior wash [61–69% vs. 66–75%; not signif-
Second, the reduced vagolytic response was not restored by the subsequent blockade of opposing δ₁ receptors. The erosion of the response was not the result of arithmetic competition from an emerging δ₁-mediated opposition. By default then, the lost δ₂ response must include a reduction in available δ₂ receptors or a decrease in the efficacy of δ₂ coupling. The working hypothesis, however, suggested that positively coupled δ₁ receptors depend on GM-1 and that added ganglioside would recruit negatively coupled δ₂ receptors. While the loss of the δ₂ response was very consistent, the appearance of the vagotonic δ₁ response during GM-1 was not specifically tested after protocol 1. Thus the recruitment or interchange of receptor phenotypes was not rigorously investigated, and the fate of the “lost” δ₂ receptors remains to be demonstrated.

The third observation was that in the absence of treatment, the vagolytic response intensified as the protocol proceeded. This increase in the vagolytic response may represent an adaptation to the continued surgical stress and an increasing need to shift from parasympathetic to sympathetic control to sustain cardiovascular homeostasis. On the other hand, the increase in the magnitude of the vagolytic response with time might also reflect the gradual restoration of the normal status quo that was acutely disturbed during the initial surgical stress.

DISCUSSION

There are three primary conclusions from the current data. The initial data reported above for MEAP supported the hypothesis that GM-1 enhanced the δ₁-mediated vagotonic effect of MEAP and reduced the opposing δ₂-mediated vagolytic effect. When the more selective δ₁-agonist deltorphin was employed to verify the δ₂ component of the GM-1-effect, a similar reduction in the vagolytic response was observed. Unlike the prior findings with the δ₁-agonist TAN-67 (6), the effect of GM-1 was not altered by δ₁ blockade with BNTX. Like TAN-67, deltorphin alone produced a qualitatively similar loss in the vagolytic response that was prevented by BNTX. Thus GM-1 effectively bypassed the δ₁ receptor in a manner consistent with the hypothesis that the δ₁ receptor exerts its effect by stimulating GM-1 synthesis.
In any case, the data reinforce the fluid bipolar character of the vagolytic response over relatively short time intervals. GM-1 and \(\delta_1\)-receptor stimulation may reduce the vagolytic response through wholly independent mechanisms. The current data do not permit one to evaluate whether the effects of GM-1 and \(\delta_1\)-stimulation are additive because dose-effect relationships for GM-1 have not been determined. The effects of GM-1 alone are also potentially complicated by differences in endogenous GM-1. The vagotonic effects of enkephalin are far more variable than companion vagolytic responses. Some of this inherent variability may reflect differences in the endogenous GM-1 content.

Opioid-mediated downregulation of opiate receptors is a widely recognized phenomenon (30, 31). Prior studies with MEAP in the SA node provided little evidence for downregulation during continuous exposure (9). Deltorphin and MEAP do differ in that MEAP is a mixed \(\delta_1/\delta_2\)-agonist, and deltorphin is purportedly more \(\delta_2\) selective (11, 13, 31). The vagolytic effect of deltorphin was slow to wash out (45–60 min) compared with MEAP (10–20 min). The slower off-responses might reflect longer residence times within the \(\delta\)-receptor site and thus a greater likelihood of downregulation due to the longer exposure. The attrition in the response to deltorphin alone was initially attributed to either the duration of the protocol or the prior exposure to the \(\delta_2\)-agonist deltorphin. Simple homologous downregulation was ruled out when erosion of the response was prevented by the \(\delta_1\)-antagonist BNTX. The effect of the protocol duration was similarly dismissed when the omission of the initial exposure to deltorphin produced a significantly more intense response later in the protocol. Thus prior exposure to deltorphin must have contributed to the subsequent erosion of the vagolytic response. Therefore, either deltorphin has limited \(\delta_2\) activity or it facilitates an endogenous \(\delta_2\)-agonist. \(\delta_1\)-Receptor blockade prevented the loss of the \(\delta_2\)-mediated responses when applied in advance but was unable to reverse the responsible process once the downregulation was permitted time to proceed.

The increased intensity of the vagolytic response in the absence of intervening treatment suggested that the improved \(\delta_2\)-mediated vagolytic response may have resulted from the gradual metabolism of endogenous GM-1. This interpretation was supported by the observation that 30 min wash after GM-1 reduced the subsequent attenuation in the \(\delta_2\) response 60 min later (28% vs. 63%).

In the heart, the inhibitory (vagolytic) opioid effects dynamically respond to both \(\delta_1\)-receptor stimulation and GM-1. GM-1 shifts the mix of opioid receptors in vitro from predom-
The vagal regulation is central to the momentary adjustments in heart rate required for daily living (23). Furthermore, healthy vagal control is closely allied with resistance to sudden cardiac death and successful recovery from myocardial infarction (17). As such, opioid modulation of vagal transmission may represent an important therapeutic target. The development of pharmaceuticals specifically targeting the δ₁ receptor might be useful in improving vagal transmission in susceptible individuals after myocardial infarction. The fluid nature of this opioid influence reinforces the potential for intervention because one might be able to affect an improved mix of opioid receptors in heart within as little as 30 min. Heart disease is generally associated with impaired parasympathetic regulation of the heart (26). This could result in part from an unfavorable mix of δ receptors that might then be targeted for modification with an appropriate δ agonist or -antagonists. Little is known about the myocardial physiology of GM-1 and its synthesis. The GM-1 results above suggest that increased synthesis of GM-1 might be part of a therapeutic strategy to improve vagal transmission. GM-1 improves postischemic left ventricular performance and has thus been implicated in cardioprotection (15). A steady-state increase in GM-1 might contribute to improved vagal transmission and a beneficial reduction in resting heart rate. However, the effects of exercise or diet, for instance, on the content and distribution of GM-1 in heart or in parasympathetic neurons are largely unexplored. Thus what happens to GM-1 after exercise training, after ischemia and repertusion, and in chronic heart failure are all important questions regarding the physiological importance of the opioid, GM-1, and vagal interaction.

REFERENCES


10. Farias M, Jackson K, Johnson M, and Caffrey JL. Cardiac enkephalins attenuate vagal bradycardia: interactions with NOS-1-cGMP systems in


