Autonomic control of heart rate in dogs treated chronically with morphine

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Napier, Leslie D., Amber Stanfill, Darice A. Yoshishige, Keith E. Jackson, Barbara A. Barron, and James L. Caffrey. Autonomic control of heart rate in dogs treated chronically with morphine. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H2199–H2210, 1998.—The vagotonic effect of chronic morphine on the parasympathetic control of the heart was examined in dogs treated with morphine for 2 wk. Because normal vagal function is critical to myocardial stability, the study was conducted to evaluate for potential impairments following chronic vagal stimulation. The hypothesis that persistent vagal outflow would result in a loss of vagal reserve and reduced vagal control of heart rate was tested. Heart rate and the high-frequency variation in heart rate (power spectral analysis) declined shortly after initiation of subcutaneous morphine infusion. A progressive bradycardia correlated well with the rising plasma morphine. The resting bradycardia (57 beats/min) was maintained through day 2 and was accompanied by a significant parallel increase in vagal effect and a decline in the intrinsic heart rate (160 vs. 182 beats/min). A compensatory increase in the ambient sympathetic control of heart rate was evident on day 2 and was supported by an increase in circulating catecholamines.

The lowered intrinsic heart rate and elevated sympathetic activity were maintained through day 10 despite a return of the resting heart rate and plasma catecholamines to pretreatment values. These observations suggested that chronic morphine alters either the intrinsic function of the sinoatrial node or reduces the postvagal tachycardia normally attributed to nonadrenergic, noncholinergic agents. Both acute and chronic morphine depressed the rate of development of bradycardia during direct vagal nerve stimulation without altering the rate of recovery afterward. This last observation suggests that acute morphine reduces the rate of acetylcholine release. Results provide insight into the mechanisms that maintain vagal responsiveness. The results are also relevant clinically because opiates are increasingly prescribed for chronic pain and opiate abuse is currently in resurgence.

parasympathetic nervous system; opiates; power spectral analysis; intrinsic heart rate; catecholamines

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Intracath (14 gauge, 5.1 cm) was inserted subcutaneously with a local anesthetic (bupivacaine HCl, 5 mg), and a flexible catheter was prepared in the midscapular region. The area was infiltrated with 1.5 mg/kg acepromazine, 0.15 mg/kg), and a sterile field was placed under mild sedation (ketamine, 2.5 mg/kg; xylazine, below). Three days before initiation of the protocol, dogs were selected for their tolerance of the protocol. That they proceeded through the protocol in mixed groups assigned to control or morphine treatment groups to ensure that dogs were suitable for the study and ambulatory vest used to support the infusion pump. After it was established that dogs were suitable for the study and acclimatized to the laboratory setting, they were alternately assigned to control or morphine treatment groups to ensure that they proceeded through the protocol in mixed groups (controls and morphine treated) of two or three. Thus parasympathetic control of cardiovascular function appears to be altered in opiate addicts and related animal models. This study employed a canine model to test the hypothesis that persistent increases in efferent vagal traffic associated with chronic morphine treatment would reduce vagal reserve and attenuate or downregulate the parasympathetic control of heart rate. Parasympathetic and sympathetic contributions to heart rate variability were examined before, during, and after chronic morphine treatment with the aid of power spectral analysis. Autonomic blockade was utilized to determine whether the heart itself adapts to chronic morphine. Finally, direct vagal nerve stimulations were conducted to determine whether chronic morphine alters efferent vagal responses. The findings provide insight into basic autonomic adaptations to chronic vagal stimulation. The results are pertinent to tolerance and dependence models and are particularly important because a resurgence in opiate use is occurring in recent years (14). The study also provides relevant clinical information because opiates are increasingly prescribed for the relief of chronic pain (6).

**METHODS**

**Dog Selection and Training**

Mongrel dogs (15–20 kg) that tested heartworm free were accommodated to the laboratory and trained to stand quietly in a restraining sling on at least three occasions before the protocol was begun. In addition to size and demeanor, the animals were further selected for their tolerance of the ambulatory vest used to support the infusion pump. After it was established that dogs were suitable for the study and acclimatized to the laboratory setting, they were alternately assigned to control or morphine treatment groups to ensure that they proceeded through the protocol in mixed groups (controls and morphine treated) of two or three.

**Catheter Implantation and Treatment Protocol**

A baseline cardiovascular evaluation was performed 4–5 days before the beginning of the treatment protocol (see below). Three days before initiation of the protocol, dogs were placed under mild sedation (ketamine, 2.5 mg/kg; xylazine, 1.5 mg/kg; acepromazine, 0.15 mg/kg), and a sterile field was prepared in the midscapular region. The area was infiltrated with a local anesthetic (bupivacaine HCl, 5 mg), and a flexible Intracath (14 gauge, 5.1 cm) was inserted subcutaneously and sutured in place. A CORMED ambulatory infusion pump (model ML-6–6) and accompanying tubing were attached to the catheter, and the dog was fitted with a vest designed to support the pump (Alice King Chatham Medical Arts). Saline was infused for 3 days in all animals to allow accommodation to the vest and pump. On day 0, morphine was infused at an initial rate of 5.75 mg·kg⁻¹·day⁻¹ and adjusted as required during the subsequent 24 h to achieve a target plasma concentration of 80–120 ng/ml (0.40–0.75 ml/h). This concentration of morphine is sufficient to induce physical dependence in dogs (49) and, in our experience, is well tolerated. Morphine or vehicle (saline) was infused for 14 days, during which time the dogs were monitored daily. The catheter was flushed daily to ensure patency, and the site was cleaned thoroughly and sprayed with gentamicin sulfate to prevent infection. The treatment protocol is summarized in Table 1.

**Table 1. Treatment protocol for dogs treated for 14 days with saline or morphine.**

<table>
<thead>
<tr>
<th>Day</th>
<th>-4</th>
<th>-3</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVA</td>
<td>Catheter insertion</td>
<td>PM</td>
<td>PM</td>
<td>PM</td>
<td>PM</td>
<td>PM</td>
<td>CVA</td>
<td>PM</td>
</tr>
<tr>
<td>Saline infusion</td>
<td>PC</td>
<td>PC</td>
<td>PC</td>
<td>PC</td>
<td>PC</td>
<td>CVA</td>
<td>Terminal</td>
<td></td>
</tr>
</tbody>
</table>

CVA, cardiovascular analyses; PM, plasma morphine; PC, plasma catecholamines.

**Power Spectral Analysis Evaluation of Conscious Animals**

**General description.** The sympathetic and parasympathetic nervous systems are the primary regulators of short-term (seconds to minutes) cardiovascular control. Fluctuations in heart rate reflect the compensatory responses of these systems to a variety of physiological perturbations. Power spectral analysis (PSA) allows one to assess sympathetic and parasympathetic contributions to heart rate by partitioning the variation into specific frequencies that are characteristics of each (1, 36, 42). Because the parasympathetic nervous system reacts quickly to changes in the physiological environment, high-frequency fluctuations in heart rate (>0.15 Hz) predominantly reflect the activity of this system. The sympathetic nervous system responds more slowly, and its activity is associated with variations in heart rate at frequencies below 0.15 Hz. PSA of heart rate in dogs and humans (1, 36, 42) has revealed the presence of three primary frequency peaks: 1) a peak centered at 0.25 Hz corresponding to the respiratory frequency and mediated primarily by the parasympathetic nervous system, 2) a peak near 0.10 Hz important in arterial blood pressure control, and 3) a peak at 0.04 Hz related to peripheral vasomotor control. The two lower-frequency peaks are mediated by both sympathetic and parasympathetic influences.

**Recording and analysis of heart rate signals.** The heart rate signal was captured from a surface electrocardiogram (ECG) and continuously displayed on a monitor (Hewlett-Packard 78354A). The signal was relayed to a 12-bit analog-to-digital converter (ADC, CIO-DAS16 Jr) connected to an IBM-compatible personal computer equipped with a 486-MHz microprocessor.

The system digitized heart rate signals at a rate of 512 Hz. Signals were then analyzed by an algorithm that allows for continuous, online PSA in real time (21). The digitized
signals were truncated into 32-s time segments (windows). For each segment, the algorithm used fast Fourier transforms to estimate the power density of the spectral components. The components were quantified by integration of the area of power spectral density between specific upper and lower frequency limits. The selected frequency ranges were 1) high frequency (HF), 0.15–0.40 Hz; 2) low frequency (LF), 0.08–0.15 Hz; and 3) very low frequency (VLF), 0.01–0.08 Hz. The graphical results were continuously displayed on the monitor and printed. Numerical output was listed and printed at the end of each recording session. Data were recorded for later retrieval on an external Zip drive. All data were edited manually for artifacts caused by ectopic or premature beats, ECG signal interference, or gross subject movement. The raw power spectral density data were routinely normalized within subjects using the standard formulas, HF/(HF + LF) and LF/(HF + LF). The formulas allow a determination of the relative influence of high- and low-frequency fluctuations on heart rate variability (minus the VLF).

Morphine initiation protocol. The heart rate power spectrum was observed and recorded during the first 3 days of morphine treatment on day 0 of the protocol. Heart rate was monitored continuously while the dog rested quietly in the sling. Every attempt was made to reduce extraneous noise and other disturbances in the laboratory. The room temperature was controlled at 24–26°C. When a steady state was achieved, a 10- to 20-min baseline recording was obtained during saline infusion. The morphine infusion was then initiated, and the power spectrum was recorded and printed continuously for 3 h. Blood samples for plasma morphine determination were obtained via an intravenous catheter at 15, 30, 60, 90, 120, 150, and 180 min. The PSA data were divided into time periods for analysis as follows: 0–15 min, 15–30 min, 30–60 min, 60–90 min, 90–120 min, 120–150 min, and 150–180 min. PSA data were also obtained from control animals for the same number of minutes during saline infusion.

Autonomic blockade protocol. Autonomic blockade with simultaneous PSA was conducted before treatment (day –4), and both early (day 2) and late (day 10) in the treatment protocol. A venous catheter was inserted into the forelimb to obtain blood samples and to administer drugs. Heart rate was monitored continuously while the dog rested quietly in the sling as described above. When a steady state was achieved, the heart rate power spectrum was recorded for 10–20 min. The muscarinic antagonist atropine methyl bromide (AMB, 75 µg/kg iv) was then administered to induce parasympathetic blockade. Because AMB does not readily cross the blood-brain barrier, the secondary central effects of atropine were avoided (12). The selected dose of AMB results in complete muscarinic blockade without interrupting ganglionic transmission (47). The adequacy of the blockade was verified in each animal by demonstrating no further change in heart rate after doubling of the dose. Sympathetic blockade with atenolol (3.0 mg/kg iv) was then induced to determine the heart rate in the absence of neural input (intrinsic heart rate). Blockade with atenolol was previously verified in animals similarly pretreated with atropine and challenged with isoproterenol (1 µg). The heart rate power spectrum was recorded continuously during the experiment. Data were edited as described above, and 5–10 min of steady-state data following each injection were used for analysis.

Plasma Catecholamines

Given the complex interplay between parasympathetic and sympathetic control mechanisms and the effects of opiates on autonomic function, plasma catecholamines were measured at pretreatment and on days 2 and 10 of the treatment protocol. Venous blood samples were collected into iced tubes containing EGTA and glutathione. The plasma was separated by centrifugation, spiked with metabisulfite, stored at –90°C, and assayed within 30 days. Catecholamines were adsorbed onto alumina and eluted with 0.1 M perchloric acid. Catecholamines were then separated by HPLC and quantitated amperometrically by integration of the signal from the electrochemical detector (BAS) as previously described (2).

Surgical Procedure

Following the chronic infusion (day 14), dogs were anesthetized with pentobarbital sodium (32.5 mg/kg), intubated, and mechanically ventilated with room air (225 ml·kg⁻¹·min⁻¹). Catheters filled with heparinized saline were inserted into the right femoral artery and vein and advanced into the descending aorta and inferior vena cava, respectively. The arterial catheter was attached to a Statham PD 23 XL transducer to monitor arterial pressure. Heart rate was monitored continuously by a tachometer tracking the arterial pulse pressure. The venous catheter was used to obtain blood samples and for the administration of additional anesthetic as required. Arterial blood gases and pH were monitored throughout the experiment using a Corning 178 Blood Gas Analyzer and were adjusted to within normal limits (Po₂, 90–120 mmHg; PCO₂, 30–40 mmHg; pH, 7.35–7.40) by supplementing oxygen, adjusting the minute volume, or administering bicarbonate. The right and left vagus nerves were isolated through a midline cervical incision and ligated to eliminate afferently mediated sympathetic responses during different vagal stimulation. Heparin (1,500 units/kg) was administered to provide for sustained anticoagulation.

Vagal Stimulation

After surgical preparation, the animals were allowed to stabilize for 30 min, during which time blood gases and anesthesia were evaluated and adjusted as required. Test stimulations of the right vagus nerve were conducted to determine the supramaximal voltage for vagal stimulations (usually 15 V). The right vagus was stimulated at frequencies between 0.5 and 4.0 Hz according to the protocol in Table 2.

Heart rate was sampled during each 15-s stimulation. A 105-s interval followed each stimulation to allow heart rate to return to normal. The times to reach the minimum heart rate during vagal stimulation and to return to the prestimulation heart rate were recorded for each frequency. Data were sampled at appropriate times, digitized, and recorded online (MacLab).

To separate chronic adaptive responses from effects due to morphine circulating at the time of the experiment, the vagal stimulation protocol was also conducted in animals treated acutely with morphine (1 mg/kg sc). This dose produced plasma morphine concentrations of approximately double those observed in chronically treated animals (230 ± 14 vs. 108 ± 5 ng/ml). The dose was selected to produce central nervous system (CNS) morphine concentrations equal to those in animals treated with chronic morphine. After acute

Table 2. Vagal nerve stimulation protocol

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, s</td>
<td>Off</td>
<td>Off</td>
<td>On</td>
<td>Off</td>
<td>On</td>
</tr>
<tr>
<td>15</td>
<td>Off</td>
<td>105</td>
<td>15</td>
<td>105</td>
<td>15</td>
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<tr>
<td>105</td>
<td>15</td>
<td>Off</td>
<td>15</td>
<td>105</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>105</td>
<td>Off</td>
<td>15</td>
<td>105</td>
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<td>15</td>
<td>105</td>
<td>15</td>
<td>105</td>
<td>Off</td>
</tr>
</tbody>
</table>
morphine administration, CNS morphine concentrations in dogs are approximately one-half those observed in plasma (29, 30). Surgical preparation was as described above for chronically treated animals.

Statistics

Values are expressed as means ± SE. Experiments were analyzed using within-subjects (repeated measures) ANOVA and/or between-group randomized two- or three-factor ANOVA. Multiple post hoc comparisons were made using Tukey’s protected t-test. The significance of relationship between plasma morphine and heart rate was tested by simple correlation and linear regression (GB-STAT; Dynamic Microsystems, Silver Spring, MD). P < 0.05 was accepted as statistically different.

RESULTS

Body Mass

Figure 1 illustrates the weights of saline control and morphine-treated dogs over the 14-day treatment period. The weight of control animals did not change significantly. During the initial 24–48 h, the morphine-treated animals were sedated and ate little. As a result, the weight of morphine-treated dogs declined through day 4 of the protocol and, although their food intake returned to normal, their weight remained significantly below baseline for the remainder of the treatment period. The maximum weight loss was 1.7 kg, observed on days 4 and 7. By day 10, dogs receiving morphine were behaviorally indistinguishable from control animals and had begun to regain their lost body mass.

Plasma Morphine

Plasma morphine concentrations during the first 3 h of morphine infusion and during the 14-day treatment period are presented in Fig. 2. By the end of the initial 3-h observation period, plasma morphine concentration had reached an average of 42 ng/ml, or ~50% of the target concentration, 80–120 ng/ml. In most animals, the target concentration was achieved within 24 h of the beginning of the infusion. Throughout the remainder of the protocol, infusion rates were adjusted as required to maintain plasma concentrations within the target range.
Initial Exposure to Morphine

Heart rate. The decreases in heart rate during the first 3 h of morphine and saline infusion are presented in Fig. 3. The average heart rate of dogs treated with saline did not differ significantly from baseline (70 ± 4 beats/min) at any time during the 3-h period. The average heart rate of dogs receiving morphine decreased during the 3 h and was significantly different from baseline (78 ± 2 beats/min) and from control after 60 min. This decline at 60 min corresponded to a plasma morphine concentration of 27 ng/ml and, as indicated in Fig. 4, the decrease in heart rate was significantly correlated with plasma morphine concentration during the 3-h initiation period (r = 0.74, P < 0.01).

PSA. PSA data from the initial 3-h saline or morphine period are presented in Fig. 5. Variation in the raw power spectral data necessitated that the data be normalized within subjects. Normalization of HF and LF power using the formulas HF/(HF + LF) and LF/(HF + LF), respectively, provides an estimate of parasympathetic-sympathetic balance. As illustrated (Fig. 5), HF power represented 70–90% of combined power (excluding VLF) while LF power represented the remaining 10–30%. This confirms the predominance of vagal control of heart rate variability in the conscious dog (36). Normalized HF and LF powers were similar in both groups, indicating that morphine infusion did not change relative parasympathetic or sympathetic contributions to heart rate variability. The stability of autonomic balance is also demonstrated by the LF-to-HF ratio, which did not change significantly during the initial 3 h in either group (not shown). The VLF power represents a small portion of the total power and did not change significantly in either group during the 3 h. The raw HF power declined during the 3-h period in
morphine-treated animals and was significantly lower than baseline by 60 min (P < 0.05, data not shown).

Early and Late Effects of Chronic Morphine

Heart rate. Heart rates at rest and during sequential autonomic blockade are presented in Fig. 6. Resting heart rates were significantly lower on day 2 in morphine-treated animals (76 ± 4 vs. 57 ± 3 beats/min, P < 0.05) but had recovered to normal by day 10 (71 ± 4 beats/min). In control animals, atropine increased heart rate (vagal effect) by 170 beats/min. This postatropine heart rate, which represents the intrinsic heart rate and the ambient sympathetic tone combined, did not differ across days in control animals. However, morphine treatment increased the postatropine heart rate and, by day 10, the increase was more than 20 beats/min over the pretreatment, postatropine rate (246 ± 8 vs. 222 ± 12, P < 0.05). The difference between the resting heart rate and the postatropine heart rate reflects the amount of vagal activity required to maintain resting heart rate at the observed value. This “vagal effect” was significantly greater on days 2 and 10 in morphine-treated animals compared with pretreatment (P < 0.05). The subsequent administration of atenolol provides an estimate of the intrinsic heart rate, and the difference between the postatropine and intrinsic heart rate indicates the ambient sympathetic tone. In control animals, atenolol reduced heart rate by 52 ± 4 beats/min from the postatropine rate to reveal an intrinsic heart rate of 187 ± 11 beats/min. The ambient sympathetic tone and the resultant intrinsic heart rates were not different on subsequent days in control animals. The intrinsic heart rates obtained after 2 (160 ± 8 beats/min) and 10 (164 ± 9 beats/min) days of morphine were significantly lower than those obtained before treatment (182 ± 12 beats/min) in the same animals (P < 0.05) and those obtained in controls on days 2 and 10 (P < 0.01). This difference suggests that chronic morphine alters intrinsic properties of the heart or its regulation by nonadrenergic, noncholinergic factors. Chronic morphine also increased ambient sympathetic tone on days 2 and 10 when compared with measurements made before treatment (P < 0.01) or in comparable controls on the same days (P < 0.05). In an additional set of dogs (n = 4), intrinsic heart rates were determined on separate occasions under control conditions and after the acute administration of morphine (1 mg/kg). The intrinsic heart rates were determined 45 min after morphine administration, when plasma morphine concentrations approximated those observed in dogs receiving chronic morphine (135 ± 13 ng/ml). Acute morphine had no effect on intrinsic heart rate in these animals, suggesting that a direct effect of circulating morphine was not responsible for the changes in intrinsic heart rates observed on days 2 and 10 of chronic morphine treatment.

PSA. PSA data obtained before treatment and on days 2 and 10 of the saline or morphine infusion are presented in Table 3. Although the control and morphine-treated dogs differed initially, within-group analyses indicated no change from baseline in any PSA variable on day 2 or 10 of saline or morphine infusion. This is again best illustrated in the normalized units. A representative tracing is presented in Fig. 7. The administration of AMB nearly abolished the HF power, verifying that HF fluctuations in heart rate primarily reflect vagal influences. The LF power was substantially reduced by atropine, indicating that parasympathetic influences also contribute to heart rate variation in this range. As reflected by the normalized units, AMB shifted the autonomic balance toward greater sympathetic influence. Atenolol further reduced HF power and almost completely abolished the LF power. Relative parasympathetic and sympathetic control of remaining heart rate variability was approximately equal following atenolol, as reflected by the HF (52%) and LF (48%) normalized units. The HF and LF power after autonomic blockade were unaffected by morphine on days 2 and 10. The VLF power was reduced by the administration of AMB and almost eradicated with
subsequent administration of atenolol. There were no differences in the VLF within treatment groups over time or between treatment groups before autonomic blockade. A small increase in VLF was observed in morphine-treated animals after blockade; however, any conclusion in this regard is suspect because VLF power was very near the baseline noise in all blocked animals.

### Plasma Catecholamines

Plasma norepinephrine and epinephrine concentrations remained unchanged in control animals throughout the treatment period (Fig. 8). Plasma concentrations of both catecholamines were increased on day 2 in the morphine-treated dogs compared with pretreatment ($P < 0.05$). On day 10, norepinephrine and epinephrine concentrations were still slightly elevated compared with pretreatment but were no longer significantly different.

### Vagal Stimulation Data

After the 14-day treatment period, animals were anesthetized and prepared for the vagal stimulation

**Table 3. Effects of autonomic blockade on normalized heart rate power spectral analysis data before treatment and on days 2 and 10 of morphine or saline infusion**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-AMB</th>
<th>Post-Atenolol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF</td>
<td>LF</td>
<td>HF</td>
</tr>
<tr>
<td>Pre Saline</td>
<td>76.23 ± 3.33*</td>
<td>23.77 ± 3.33*</td>
<td>31.99 ± 5.02</td>
</tr>
<tr>
<td>Saline</td>
<td>90.03 ± 1.76</td>
<td>8.30 ± 0.94</td>
<td>30.83 ± 2.52</td>
</tr>
<tr>
<td>Morphine</td>
<td>87.78 ± 1.60</td>
<td>12.22 ± 1.60</td>
<td>36.57 ± 2.49</td>
</tr>
<tr>
<td>Saline</td>
<td>83.32 ± 2.60</td>
<td>16.68 ± 2.60</td>
<td>27.49 ± 2.35</td>
</tr>
<tr>
<td>Morphine</td>
<td>78.43 ± 3.21</td>
<td>21.58 ± 3.21</td>
<td>38.21 ± 4.66</td>
</tr>
<tr>
<td>Saline</td>
<td>83.32 ± 3.59</td>
<td>18.68 ± 3.59</td>
<td>28.99 ± 2.36</td>
</tr>
<tr>
<td>Morphine</td>
<td>78.43 ± 3.59</td>
<td>21.58 ± 3.21</td>
<td>38.21 ± 4.66</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8$ for saline controls and 9 for morphine-treated animals. Pre, pretreatment; HF, high frequency; LF, low frequency; AMB, atropine methyl bromide. *$P < 0.05$, saline vs. morphine.

![Fig. 7. Representative tracing of continuous, online, real-time power spectral analysis of heart rate (HR) variability during sequential autonomic blockade. ECG, electrocardiogram; HPSD, heart rate power spectral density; HHF, heart rate high frequency; HLF, heart rate low frequency; HVLF, heart rate very low frequency.](http://ajpheart.physiology.org/)

Downloaded from http://ajpheart.physiology.org by 10.220.33.6 on October 23, 2017
protocol. A separate group of nonaddicted animals was similarly prepared and given 1 mg/kg morphine subcutaneously 30 min before the vagal stimulation protocol. The average plasma morphine concentration was 230 ng/ml in these animals. Initial cardiovascular parameters and blood gases are presented in Table 4. Heart rates in dogs treated with morphine acutely were significantly lower than those of controls (P < 0.05) or chronically treated animals (P < 0.01), consistent with the bradycardic effect of acute morphine. Mean arterial pressure was lowered by both chronic and acute morphine treatment (P < 0.01).

The right vagus nerve was stimulated at 0.5, 1.0, 2.0, and 4.0 Hz. Both vagi were ligated before stimulation to eliminate afferent vagal traffic while efferent bradycardic response was obtained. In all groups, heart rate declined further with each increase in the frequency of stimulation, reaching a decrease of 55–60 beats/min at 4 Hz. The frequency-response relationship was not significantly different between the three groups at any frequency.

The kinetics of heart rate responses during vagal stimulation were monitored, and the times required for heart rate to reach 50% of the maximum decrease during stimulation and to return to 50% of the prestimulation rate are presented in Figs. 9 and 10, respectively. In all groups, the heart rate declined more quickly as the frequency of stimulation increased (Fig. 9). The 50% down-time intervals were significantly different among the three groups across frequencies (P < 0.01), with the control group reaching 50% of maximum bradycardia more quickly, followed by the chronic and acute morphine groups. In all groups, heart rate returned to the prestimulation rate more quickly as the frequency of stimulation increased (Fig. 9). Although chronic morphine appeared to slow the recovery, these time intervals were not significantly different among treatment groups at any frequency.

### Table 4. Postanesthesia cardiovascular parameters and blood gases

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>PO2, mmHg</th>
<th>PCO2, mmHg</th>
<th>pH</th>
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<tr>
<td>Saline control</td>
<td>149 ± 5.21</td>
<td>128 ± 3.94</td>
<td>105 ± 3.08</td>
<td>39 ± 1.57</td>
<td>7.36 ± 0.01</td>
</tr>
<tr>
<td>Chronic morphine</td>
<td>155 ± 5.25</td>
<td>106 ± 4.65</td>
<td>113 ± 2.89</td>
<td>39 ± 1.17</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td>Acute morphine</td>
<td>123 ± 7.37*</td>
<td>97 ± 5.36</td>
<td>116 ± 2.96</td>
<td>40 ± 1.65</td>
<td>7.37 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 for controls, 11 for chronic morphine, and 8 for acute morphine. HR, heart rate; MAP, mean arterial pressure.

*Significantly different from control (P < 0.05) and from chronic morphine (P < 0.01). †Significantly different from control (P < 0.01).
Because morphine stimulates vagal outflow, we predicted that HF fluctuations in heart rate would increase with morphine treatment. However, the power of the HF component represents fluctuations in heart rate mediated by efferent vagal activity and not vagal tone per se (28). If morphine produces an increased but invariant or less variant vagal outflow, fluctuations in heart rate could decrease. Lee et al. (36) measured the effects of the opioid anesthetic fentanyl on the arterial pressure power spectra in anesthetized rats. The power spectral components associated with fluctuations in arterial pressure reflect activity of the same control mechanisms as the heart rate power spectral peaks (36). Fentanyl substantially reduced HF power in these animals, which may also reflect an invariant or less variant increase in vagal activity. The decrease in HF power during the initial 3 h of morphine treatment in the present study is consistent with this theory. We have also observed this decline in HF power in ongoing studies in dogs given higher doses of acute morphine.

Sequential autonomic blockade provided several indexes of parasympathetic and sympathetic activity. The heart rate following parasympathetic blockade with AMB represents the combined influence of intrinsic heart rate and ambient sympathetic tone. The difference between resting heart rate and this post-AMB heart rate has been described as the vagal effect (35) because it reflects the amount of vagal activity that was required to maintain the lower resting heart rate before atropine. Subsequent administration of atenolol produced complete autonomic blockade, revealing the intrinsic heart rate. The difference between the post-AMB heart rate and intrinsic heart rate is a measure of ambient sympathetic tone.

The vagal influence on resting heart rate and the ambient sympathetic tone were increased on days 2 and 10 in morphine-treated animals. The increase in sympathetic tone suggests a compensatory sympathetic response to the increase in vagal activity induced by morphine treatment. This increase in sympathetic tone may have been responsible for returning the resting heart rate to normal on day 10. The intrinsic heart rate on day 2 was depressed to the same degree as the resting heart rate, suggesting that the decline in intrinsic heart rate was responsible for the change in resting heart rate. However, because sympathetic tone was also greater, an increase in parasympathetic activity must have accompanied this enhanced ambient sympathetic activity. The intrinsic heart rate remained depressed on day 10. The lower intrinsic heart rates on days 2 and 10 are not due to the acute influence of morphine because intrinsic rate was not lower in animals following acute morphine administration.

A decline in intrinsic heart rate may reflect a fundamental change in the function of sinoatrial nodal cells. Several possibilities have been suggested in this regard, including alterations in mechanical factors (26), cellular metabolism (17), and potassium kinetics (15). The intrinsic heart rate following double blockade may also involve contributions from other chronotropic mechanisms.
agents. The neuropeptide vasoactive intestinal peptide (VIP) is coreleased with acetylcholine from postganglionic parasympathetic neurons and produces an opposing increase in heart rate (40). This “excess tachycardia” is demonstrated by an additional reduction in heart rate after ganglionic blockade or vagotomy subsequent to the usual double receptor blockade (4, 41, 44). Thus the reduction in the intrinsic heart rate suggests that chronic morphine may reduce ganglionic transmission, the synthesis of VIP, or the release of VIP from the parasympathetic nerve terminals, thereby reducing the excess tachycardia and lowering the apparent intrinsic heart rate. Alternatively, the chronic parasympathetic activation associated with morphine treatment may deplete the neurons of their stores of VIP.

The intrinsic heart rates obtained in control animals (185 beats/min) in the present study were somewhat higher than those reported for dogs elsewhere in the literature (10) but virtually identical to those reported in the initial studies of excess tachycardia (41). Lower intrinsic heart rates in some studies may reflect a reduction in the excess tachycardia described above due to the gangliolytic properties of higher doses of atropine. The dose of AMB selected for our study was specifically titrated to provide for complete muscarinic blockade without blocking the ganglion.

An increase in plasma catecholamines paralleled the augmentation of ambient sympathetic activity after 2 days of morphine treatment. The sympathetic activity remained high through day 10 despite the return of plasma catecholamines toward the baseline concentrations. This suggests an increase in the responsiveness to catecholamines. This normalization of circulating catecholamines would be consistent with observations by Leung et al. (24), who found no change in arterial catecholamines in rats following 3 wk of morphine. Plasma concentrations were not determined during their treatment protocol, which precludes comparisons with our early observations of elevated catecholamines.

Contrary to expectations, efferent vagal control of the heart during direct vagal nerve stimulations was not altered by chronic morphine treatment. This contrasts with our earlier observation, when we found a significant attenuation of efferent vagal bradycardia in dogs treated with subcutaneous morphine pellets for 7 days (33). These conflicting results were not due to different plasma morphine concentrations because the concentrations were similar throughout the two protocols. The animals may have become tolerant to the effects of morphine between days 7 and 14. Leung et al. (25) similarly found no effect of chronic morphine on vagal bradycardia in rats that had received morphine in drinking water for 3 wk. The administered dose of morphine had been increased in their study but only during the first 8 days. Thereafter, the dose remained constant. Progressively increasing the dose of morphine might reduce the likelihood that animals would accommodate to the effects of the drug.

Kosterlitz and Taylor (20) first reported the ability of morphine to inhibit vagal bradycardia in anesthetized rats and rabbits but were unable to show this effect in guinea pigs. Acute morphine has been shown to reduce the bradycardic response to vagal nerve stimulation (48) and to stimulation of cholinergic fibers in the sinoatrial node (18) in isolated rabbit heart preparations. The reduced vagal function was attributed to morphine-mediated inhibition of acetylcholine release from postganglionic nerve fibers (18). Musha et al. (32) found that acute morphine in doses similar to those used in the present study did not alter vagal bradycardia in anesthetized dogs. Similarly, acute morphine had no effect on stimulated vagal bradycardia in the present study. The 1 mg/kg dose of morphine administered in our acute experiments produced a plasma concentration of approximately double that of chronic animals. The acute dosage was selected to ensure that the resulting central interstitial concentrations would equal or exceed those obtained during chronic administration (29, 30). Although central morphine concentrations lag behind those in the plasma after acute administration, we made the assumption that they would eventually equilibrate with and approach those in the plasma during continuous delivery. These conflicting effects of acute morphine cited above may be due to the different experimental preparations (isolated versus intact) or to species differences in the distribution of m-receptors.

Neither acute nor chronic morphine treatment had any effect on the absolute decrease in heart rate observed at each vagal stimulation frequency. Perhaps not unexpectedly, the rate of decline and rate of recovery of heart rate during and after vagal stimulation was proportional to the frequency of stimulation. The time to reach 50% of each decline in heart rate was longer in dogs that had received morphine chronically. This time was extended even further in animals treated with morphine acutely. These results suggest that acute morphine altered the kinetics of vagal bradycardia potentially by decreasing the rate of acetylcholine release or by increasing its rate of degradation. Slower vagal responses might render the heart more vulnerable to sympathetically mediated arrhythmias. Acute morphine inhibits acetylcholine release in a number of systems (3, 11, 43, 45), but the effects of chronic morphine treatment are less consistent. Some studies have found no change in release (5, 31), whereas others have reported a decrease in release with chronic morphine (16, 22). However, none of these studies measured the rate of acetylcholine release. Because the rate of recovery after the stimulus was terminated was unaltered, an increase in acetylcholine degradation seems less likely. In fact, acute morphine inhibits serum and tissue cholinesterase in vitro, albeit at very high morphine concentrations (46). A chronic effect of morphine on myocardial cholinesterase has not been determined.

In summary, we predicted that chronic morphine accompanied by persistent efferent vagal traffic would attenuate or downregulate parasympathetic control of myocardial function on the basis of preliminary observations made after 1 wk of treatment with morphine. The initial decrease in heart rate and HF power spectrum are consistent with increased vagal activity...
with morphine. However, after 2 wk of treatment, an increasing vagal effect, a return of the resting heart rate to normal, and a progressive increase in ambient sympathetic activity all suggest that subsequent sympathetic and parasympathetic compensations may have overridden any early vagal impairment. Collectively, these results suggest an adaptive, compensatory response in sympathetic nervous activity to chronic morphine or to the resultant vagal outflow. The lower intrinsic heart rates with chronic morphine may reflect fundamental changes in sinoatrial nodal cell function or, alternatively, an attenuation in nonadrenergic, noncholinergic (VIP) mechanisms. The altered kinetics of vagal bradycardia suggests that morphine inhibits acetylcholine release. Thus, if morphine did decrease parasympathetic reserve through down-regulation early in the treatment, the effects were most likely subsequently masked by greater yet sympathetic adjustments. Muscarinic receptors and associated inhibitory G proteins were upregulated in these same animals (L. D. Napier, S. C. Roerig, D. A. Yoshishige, B. A. Barron, and J. L. Caffrey, unpublished observations), suggesting a secondary parasympathetic response to the augmented sympathetic activity indicated in the present study.

The consequences of these findings are not entirely clear. However, the progressive compensatory increase in sympathetic activity during exposure to morphine might have significant cardiovascular consequences if the pharmacologically augmented vagal opposition were suddenly withdrawn. The unopposed sympathetic activity during abrupt withdrawal might raise clinical concerns, especially for patients with existing arrhythmias and/or underlying ischemic heart disease. The resulting sympathetic imbalance may explain the common observation that opiate-related deaths are often associated with nontoxic opiate concentrations (7).

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REFERENCES


