ENDOGENOUS opioids may participate in circulatory control. Administration of synthetic opioid pentapeptides to the cisterna magna of dogs produces a fall in blood pressure. The specific opiate receptor antagonist, naloxone, reverses the fall in blood pressure associated with both endotoxic and hypovolemic shock in rats, and preliminary data suggest similar findings in humans. A physiological role for opioids in blood pressure control is suggested by the observation that naloxone prevents the fall in blood pressure in humans that normally occurs during sleep. A possible site of opioid action is in the brain stem at primary baroreceptor synapses in the nucleus of the solitary tract. This area is known to have a high concentration of opioid containing fibers and opiate receptors. In dogs and rabbits, the circulatory reflex responses to both pressor and depressor stimuli are attenuated by morphine or opioid analogs and potentiated by naloxone. We have studied the influence on circulatory control in humans of a metenkephalin analog and of naloxone. Our findings suggest that the endogenous opioids modulate baroreflex function in humans.

SUMMARY We describe two studies designed to elucidate the role of endogenous opioids in blood pressure control in humans. In the first study, nine normal subjects received infusions of DAMME (a metenkephalin analog), naloxone, or saline, and blood pressure, heart rate, and plasma norepinephrine concentration were determined supine and following 5 minutes of 70° head-up tilt at intervals for 6 hours. Blood pressure following tilt was significantly decreased by DAMME but not influenced by naloxone, the effect being most marked at 3 hours (placebo = 110 ± 6/78 ± 7 mm Hg; naloxone = 106 ± 10/79 ± 5 mm Hg; DAMME = 96 ± 16/67 ± 8 mm Hg (p < 0.01). However, heart rate and plasma norepinephrine did not rise in response to this hypotension. Heart rates at 3 hours were: placebo = 87 ± 16 bpm; naloxone = 88 ± 19 bpm; DAMME = 89 ± 23 bpm. Plasma norepinephrine levels (nmol/liter) at 3 hours were: placebo = 6.0 ± 2.2; naloxone = 5.8 ± 1.9; DAMME = 6.0 ± 1.9. In the second study, seven normal subjects had blood pressure reduced by incremental infusions of sodium nitroprusside, and the effects of placebo, naloxone, and DAMME on the slope of the heart period/blood pressure relationship investigated. Naloxone significantly increased the slope by 90% and DAMME significantly reduced the slope by 30%. It is concluded that endogenous opioids modulate the baroreflex control of blood pressure in normal humans. (Hypertension 5: 535–538, 1983)

METHODS

The study was performed in two phases, during each of which the subjects were studied on three occasions at weekly intervals. They received in randomized and single-blind fashion intravenous infusions of D-Ala², MePhe³, Met(O)²-OH, enkephalin (DAMME, Sandoz, Basel, Switzerland) 0.5 mg, naloxone (Narcan, Sterling Winthrop, Surbiton, United Kingdom) 0.2 mg/kg or vehicle (0.9% saline), each treatment being made up to 50 ml with 0.9% saline and infused over 30 minutes. Blood pressure was recorded by the Bosomat 2D Semi-automated sphygmomanometer which has a coefficient of variation of 3%. Heart rate was recorded continuously using a Grass Polygraph. Blood for norepinephrine analysis was drawn from an indwelling venous cannula, centrifuged at 4°C, stored at −70°C, and analyzed by single isotope radioenzymatic assay using PNMT. This method in our laboratory has an intrasay coefficient of variation of 10%.

The second phase of the study was designed specifically to investigate the role of opioids in baroreflex control. This was achieved by incremental reductions in blood pressure produced by increasing infusion rates
of sodium nitroprusside (Nipride, Roche Wellyn Garden City, United Kingdom). Seven normal subjects aged 20–25 years were involved. The subjects rested quietly for 30 minutes following insertion of a venous cannula; supine blood pressure and heart rate were then recorded at 10-minute intervals for 60 minutes. Sodium nitroprusside was then infused in six incremental doses ranging from 1.5 to 7.5 μg/kg/min, with each infusion lasting for 10 minutes. Blood pressure and heart rate were recorded as the mean of three readings taken at 8, 9, and 10 minutes of each infusion. Subjects then received DAMME, naloxone, or 0.9% saline, as described above; they rested quietly until 120 minutes had elapsed when their baseline blood pressures and heart rates were again recorded over a 60-minute period. The sodium nitroprusside infusions were then repeated during the 180- to 240-minute interval, which corresponded to the period of maximum effect as observed in the first phase of the study. Each stepwise lowering of blood pressure produced by the nitroprusside was accompanied by an increase in heart rate. In the analysis, heart period (reciprocal of heart rate expressed in msec) and mean arterial pressure (diastolic plus one-third pulse pressure) for each subject at each rate of nitroprusside infusion were fitted to a linear equation, and the slope of the regression relationship was used as an index of baroreflex sensitivity. Slopes before DAMME, naloxone, or saline administration were compared with those following for each of the 3 study days. Data from the first study were analyzed by Friedman two-way analysis of variance. Slopes of pressure-heart period relationships before and after infusion were compared by Wilcoxon matched pairs test.

Results

DAMME significantly lowered both systolic and diastolic blood pressure following 5 minutes of 70° head-up tilt but the effect was not apparent until 180 minutes and had resolved by 300 minutes (fig. 1 upper). The effect of DAMME on blood pressure is also apparent when the data are expressed as change in diastolic pressure from before to following a 5-minute 70° head-up tilt. At the 180-minute time point on the placebo day, diastolic blood pressure rose by an average of 13 mm Hg, and on the naloxone day by 8 mm Hg (p > 0.01), while after DAMME administration diastolic pressure fell by 3 mm Hg (p < 0.05). This fall in blood pressure was not accompanied by an increase in either heart rate or plasma norepinephrine concentration (fig. 1 lower). Naloxone had no effect on blood pressure or plasma norepinephrine concentration. Neither DAMME nor naloxone influenced blood pressure or plasma norepinephrine concentration in supine subjects at any time during the study. Average values for blood pressure (systolic/diastolic) at the 180-minute reading in supine subjects were: saline 104/67 mm Hg; naloxone 110/69 mm Hg; and DAMME 106/66 mm Hg (p > 0.1). Plasma norepinephrine concentrations at this time point were (nM): saline 3.8; naloxone 3.1 and DAMME 4.4 (p < 0.1).

Baroreflex sensitivity assessed by the slope of the linear relationship between heart period and mean arterial pressure is shown in table 1. There was considerable variation between days in the slope of this relationship. However, there was little difference between readings obtained on the same day before and following placebo administration. Naloxone significantly increased and DAMME significantly decreased baroreflex sensitivity.

Discussion

These observations with DAMME indicate that this metenkephalin analog lowers blood pressure by a mechanism involving attenuation of baroreflex response. Exogenous and endogenous opioid mechanisms appear to be involved in central blood pressure regulation. Opiate receptors have been characterized into at least three subtypes, μ, δ, and κ. DAMME is a relatively selective agonist at μ type opiate receptors. These results are consistent with observations in the rabbit in which centrally adminis-

### Table 1. Slopes (Heart Period/mm Hg) of Linear Regression Relationships Between Heart Period and Mean Arterial Pressure Before and After Placebo, Naloxone, or DAMME

<table>
<thead>
<tr>
<th>Subject</th>
<th>Placebo Pre</th>
<th>Placebo Post</th>
<th>Naloxone Pre</th>
<th>Naloxone Post</th>
<th>DAMME Pre</th>
<th>DAMME Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.5</td>
<td>15.4</td>
<td>13.3</td>
<td>44.6</td>
<td>41.2</td>
<td>22.1</td>
</tr>
<tr>
<td>2</td>
<td>53.2</td>
<td>76.2</td>
<td>28.6</td>
<td>81.2</td>
<td>20.4</td>
<td>17.4</td>
</tr>
<tr>
<td>3</td>
<td>18.2</td>
<td>10.2</td>
<td>45.5</td>
<td>46.8</td>
<td>46.9</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>65.4</td>
<td>68</td>
<td>7.6</td>
<td>19.1</td>
<td>16.2</td>
<td>8.5</td>
</tr>
<tr>
<td>5</td>
<td>20.1</td>
<td>23</td>
<td>9.4</td>
<td>42.2</td>
<td>11.2</td>
<td>6.6</td>
</tr>
<tr>
<td>6</td>
<td>20.1</td>
<td>30</td>
<td>18.1</td>
<td>26.5</td>
<td>40.1</td>
<td>20.3</td>
</tr>
<tr>
<td>7</td>
<td>29.5</td>
<td>31</td>
<td>26.2</td>
<td>24.3</td>
<td>24.6</td>
<td>28.2</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>31.8±9.1</td>
<td>36.2±12</td>
<td>21.2±6.1</td>
<td>40.7±9.3</td>
<td>28.6±6.3</td>
<td>20.4±5.4</td>
</tr>
</tbody>
</table>

Data were compared by Wilcoxon matched pairs test.
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BLOOD PRESSURE FOLLOWING A 5 MINUTE, 70° HEAD UP TILT

FIGURE 1. Upper two graphs: Systolic and diastolic blood pressure following 5 minutes of 70° head-up tilt in nine normal subjects following infusions of placebo (0.9% saline), naloxone 0.2 mg/kg, or the metenkephalin analog DAMME 0.5 mg. For ease of presentation, data are presented as mean together with standard errors at the time of maximum effect but analysis was by nonparametric testing (see text). Lower two graphs: Heart rate and plasma norepinephrine concentration following 5 minutes of 70° head-up tilt in the same subjects. There is no difference at any time point between the values, including the period when blood pressure was significantly reduced by DAMME.

Further experiments with additional compounds in humans are needed to evaluate the receptor specificity of opiate activity in the baroreceptor reflex.

The findings with naloxone are of greater interest since they probably result from the inhibition of endogenous opioid activity. This inference is based on the assumption that naloxone is here acting as a pure opiate receptor antagonist. It is usually assumed that inhibition of a physiologic process by naloxone is proof that endogenous opioids are mediating that process. While antagonism by naloxone is certainly a necessary criterion, there is accumulating evidence that the drug is not as specific for opiate receptors as initially believed. Many interactions with other systems, such as displacement of $^3$H-gamma amino butyric acid from synaptic binding sites, occur only at very high concentrations of naloxone. However, one interesting observation that could be relevant to our findings is that at quite modest concentrations naloxone potentiates the contractile response to norepinephrine in guinea pig vas deferens. This effect is calcium-dependent. At present, it is difficult to make anything but speculative comments concerning the mechanism by which naloxone exerted its observed effect in our study. However, it did produce the opposite effect to DAMME, suggesting that opiate receptor antagonism is perhaps the major basis for its action.

Naloxone did not influence the cardiovascular response to tilt but markedly attenuated the fall in blood pressure produced by sodium nitroprusside. The disparity between these observations is probably the result of the very different blood pressures attained during the two procedures. In response to tilt, the blood pressure ordinarily rises slightly, as we found on the placebo day. However, sodium nitroprusside lowered mean arterial pressure by over 20% at the highest doses. If naloxone is acting here as an opiate receptor antagonist, then it appears that endogenous opioids are involved in circulatory control only when substantial deviations from normal occur. Previous observations would be in keeping with this view.
There was clearly considerable day-to-day variations in the slopes of heart period/mean arterial pressure relationships (table 1). However, we feel justified in using this technique since the variations within each person on individual study days were very small, as shown in the placebo column of table 1.

The delayed time of onset was not unexpected since endocrine studies with DAMME in another laboratory showed a maximum effect of luteinizing hormone release at 180 minutes while naloxone has its greatest effect both on prolactin release and blood pressure during sleep at this same time. The reason for this delay is not clear. Naloxone reverses the sedative, analgesic, and respiratory depressant effects of exogenous opiates, such as morphine in a matter of seconds rather than hours which suggests that an alternative indirect explanation in the present study is involved. There may be differences in the relative affinity of naloxone and endogenous opioids compared to exogenous compounds. Alternatively, other central neurotransmitters or hormones may be involved. It has recently been proposed that naloxone could be acting by increasing the release of vasopressin. However, the evidence is circumstantial and this mechanism would not of itself explain the delayed action.

In conclusion, these findings suggest that endogenous opioids can indirectly modulate baroreflex function in normal man by an action on receptors of the \( \mu \) type.

Acknowledgments

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References

Endogenous opioids and baroreflex control in humans.
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