Altered Pituitary Hormone Response to Naloxone in Hypertension Development

James A. McCubbin, Richard S. Surwit, Redford B. Williams, Charles B. Nemeroff, and Maya McNeilly

Endogenous opioid regulation of blood pressure is altered during stress in young adults at risk for hypertension. We studied the effects of the opioid antagonist naloxone on the secretion of corticotropin and β-endorphin during psychological stress in young adults with mildly elevated casual arterial pressures. Naloxone-induced secretion of both corticotropin and β-endorphin was significantly diminished in persons at enhanced risk for hypertension compared with the low blood pressure control group. Results suggest that opioidergic inhibition of anterior pituitary function is altered in hypertension development. (Hypertension 1989;14:636–644)

Endogenous opioids interact with circulatory control nuclei at several distinct anatomic sites, including adrenal medullae and peripheral sympathetic ganglia,1 nucleus tractus solitarius,3 and hypothalamic paraventricular nuclei.4,5 This multiplicity of pathways results in pressor or depressor actions of opioid antagonists, depending on the dose, site of action, behavioral state, and the species under investigation.6–8 Recent work with the stereospecific opioid antagonist naloxone suggests diminished opioid inhibition of circulatory responses during stress in young adults with blood pressure dysregulation and enhanced risk for essential hypertension.9 Pretreatment with naloxone potentiates blood pressure responses during mild psychological stress in persons with low casual blood pressure, but has no pressor effect in persons with high casual blood pressure. This diminished pressor effect of naloxone is not observed during orthostatic challenges, suggesting that the altered opioid-inhibitory mechanism of blood pressure control in hypertension development occurs in circuitry that is parallel with or rostral to baroreceptor reflex pathways.10

The hypothalamo-pituitary-adrenocortical and sympathoadrenomedullary axes are both apparently regulated by opioidergic input, and this may be important in the development of essential hypertension. Corticotropin releasing factor (CRF) cell bodies in hypothalamic paraventricular nuclei are believed to stimulate central autonomic efferents as well as secretion of corticotropin (ACTH),4,5 but the functional relation between these two effector systems in essential hypertension remains obscure. Naloxone-induced stimulation of plasma ACTH and epinephrine concentrations in humans11,12 suggests hypothalamic opioid integration of stress-related pressor mechanisms. We now present data that suggest that opioidergic inhibition of anterior pituitary function is altered in young adults at risk for hypertension.

Subjects and Methods

The experimental protocol was divided into two parts: 1) an on-campus blood pressure screening and 2) the placebo-controlled, in-laboratory stress tests. Two hundred Duke University undergraduate men between 18 and 24 years of age participated in a casual blood pressure screening at the student activity center on campus. Subjects with a history of major medical problems or who were receiving prescription medication were excluded from the study. Volunteers completed a brief family medical history questionnaire and were then accompanied to a quiet, semidarkened room where they rested for blood pressure measurement. After a 5-minute rest period, four automatic blood pressure determinations were made at 1-minute intervals via oscillometric techniques using a Dinamap Vital Signs Monitor (Critikon, Inc., Tampa, Florida). The distribution of mean arterial pressures was examined and subjects were rank-ordered by the mean of their
third and fourth pressure reading. Twenty-six individuals with pressures in the upper (High BP) or lower (Low BP) quintiles were recruited for in-laboratory drug studies. Although all recruits had casual pressure within the normotensive range, the level of pressure in college-aged males has been shown to predict both the level of pressure and incidence of essential hypertension in later life. Thus, relative to the Low BP group, the High BP group can be considered at enhanced risk for development of essential hypertension.

Volunteers reported to the clinical research unit, where the study was explained in detail and informed consent was obtained. Participants were told of two major risks for naloxone (Narcan, Du Pont Pharmaceuticals, Inc., Manati, Puerto Rico) use in narcotic abusers: 1) the immediate precipitation of withdrawal syndrome in addicts and 2) the possibility of an overdose in a nonaddicted abuser attempting to overcome receptor blockade after the experiment. A physical examination by a physician's assistant included careful screening for history or signs of opiate usage, pheochromocytoma, or other contraindications for opiate antagonism.

In-laboratory stress testing required insertion of an indwelling intravenous cannula for drug infusion and blood sampling. Subjects were tested in a private room in the outpatient ward of the Clinical Research Unit, where they were allowed to rest for 1 hour after cannulation while neuroendocrine parameters stabilized. Blood pressure determinations were made at 1-minute intervals throughout the experiment. After a rest period, 0.1 mg/kg naloxone HCl (Narcan, Du Pont Pharmaceuticals, Inc.) or saline placebo was then slowly infused over 10 minutes. Subjects rested for 10 minutes before the stressor. The psychological stressor was a 10-minute performance on a self-paced mental arithmetic task that entailed serial additions of three-digit numbers for speed and accuracy. A 10-minute recovery period followed task performance. All experimental procedures were completed within 30 minutes postinfusion (average half-life for naloxone [Narcan, Du Pont Pharmaceuticals, Inc.] is 64 minutes in adults). Participants returned for a similar stress test 1 week later. All subjects received naloxone on one visit and saline on the other visit, with the order counterbalanced within blood pressure groups. Subjects were blind to the infusion and reported being unaware of the order of administration. Because the dose of naloxone was high, the experimenter was aware of the order of administration to maximize protection of human subjects. However, all samples were encoded before assay to minimize bias. All of the procedures were under the supervision of a physician (R.B.W.). No unexpected side effects were observed. One subject's study was discontinued secondary to fainting. All procedures were approved by the Institutional Review Board for Clinical Investigations of Duke University Medical Center.

Blood samples were obtained at the end of each rest period, and twice during the arithmetic stressor. Stress samples were obtained at minutes 4–5 and minutes 9–10 during the continuous arithmetic task. Whole blood was drawn into EDTA-containing sample tubes for ACTH and β-endorphin assay. All samples were immediately centrifuged and the plasma supernate frozen at -90°C until assay. ACTH-like immunoreactivity was determined by using antisera that is produced in rabbits with thyroglobulin conjugated ACTH 11–24. This fragment maximizes immunoreactivity with the bioactive fraction of ACTH 1–39 while minimizing cross-reactivity with other proopiomelanocortin-derived peptides. The interassay coefficient of variation is 12% and the intra-assay coefficient of variation is 7%. All samples were extracted from plasma with a C-18 Sep-Pak cartridge (Waters Associates, Millford, Massachusetts). This allows sensitivity, given availability of sample, into the 0.2 to 0.5 pg/ml range. The extracted plasmas are run in the homologous double antibody radioimmunoassay. Poly-L-lysine is added to the assay buffer to minimize nonspecific interactions. Plasma total corticoids were measured by a modification of the microscale competitive protein binding technique of Murphy. Magnesium silicate (Florisil, Fisher, Fair Lawn, New Jersey) was used as adsorbent. Representative coefficients of variation are 10.8% for interassay and 3.9% for intra-assay comparisons. Some subjects (n = 10) had samples analyzed for β-endorphin–like immunoreactivity. The Allegro β-endorphin assay from the Nichols Institute (San Juan Capistrano, California) was used without significant modification. This method uses a solid phase, two-site immunoradiometric assay. The minimal detectable quantity of β-endorphin in this assay is 2.88 pmol/l. Interassay coefficients of variation are 9% at 55 pmol/l and 7.7% at 250 pmol/l. Intra-assay coefficients of variation are 4% at 61 and 275 pmol/l. Only human β lipotropin shows a significant cross-reactivity (16%). Additional blood was drawn into sample tubes containing reduced glutathione for catecholamine determination via high-performance liquid chromatography using electrochemical detection. Off-line alumina extraction of the perchloric acid eluate before injection into the enrichment column enhances the baseline stability and signal-to-noise ratio. The lower limit of detection sensitivity for this assay is 10 pg/ml for both epinephrine and norepinephrine using 1 ml assay volume.

Data were analyzed by univariate (ANOVA) and multivariate (MANOVA) analyses of variance with SYSTAT. Two levels of the between-subjects factor (High versus Low casual blood pressure), five levels of the within-subjects measurement periods (preinfusion, postinfusion, early stress, late stress, and recovery), and the within-subject drug condition (naloxone vs. saline) were used in analysis. Order effects were analyzed separately. Drug effect and...
Table 1. Effects of Saline Infusion on Blood Pressure and Neuroendocrine Responses During an Arithmetic Stressor in Young Adults With High and Low Casual Arterial Pressure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Low BP group</th>
<th>Saline</th>
<th>High BP group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Infusion</td>
<td>Math1</td>
</tr>
<tr>
<td>Mean arterial</td>
<td>mean</td>
<td>78.6</td>
<td>79.8</td>
</tr>
<tr>
<td>pressure (mm Hg)</td>
<td>SEM</td>
<td>1.41</td>
<td>2.43</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>mean</td>
<td>29.0</td>
<td>31.2</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>6.2</td>
<td>4.3</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>mean</td>
<td>224.2</td>
<td>220.8</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td>SEM</td>
<td>35.5</td>
<td>39.1</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>mean</td>
<td>13.1</td>
<td>16.6</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>1.57</td>
<td>2.12</td>
</tr>
<tr>
<td>ACTH (fmol/ml)</td>
<td>mean</td>
<td>1.66</td>
<td>1.40</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.56</td>
<td>0.41</td>
</tr>
<tr>
<td>ß-endorphin (pmol/l)</td>
<td>mean</td>
<td>10.12</td>
<td>11.00</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.70</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Math1, first 5-minute block of arithmetic stress; Math2, second 5-minute block of arithmetic stress; BP, blood pressure; ACTH, corticotropin. n=5-13 samples/cell.

Reactivity transformations were employed before some analyses. Estimates of the drug effect were derived by subtraction of values obtained after naloxone administration from comparable saline control periods. Reactivity scores were derived by subtraction of postinfusion baseline periods from stress periods.

### Results

The mean±SEM of mean arterial blood pressures and neuroendocrine variables are summarized by casual blood pressure subgroups in Table 1 for saline and Table 2 for naloxone experiments. Analysis of order effects revealed no significant differences when saline was administered during the first versus the second laboratory visit and results are collapsed across order. The mean arterial pressures obtained on campus from recruited subjects were 94.2±0.97 and 70.8±2.3 mm Hg in the High BP and Low BP groups, respectively. Although the group differences in mean pressures obtained on campus were more pronounced than those obtained after an hour of rest in the laboratory, the difference between groups remained significant.

Figure 1 shows the effect of naloxone on plasma ACTH levels during stress in young adults with high versus low casual blood pressure. Although naloxone had no apparent effect on basal plasma ACTH concentrations in the High BP group (t<1), it marginally increased plasma ACTH concentrations in the Low BP group (t(9)=2.167, p<0.058). MANOVA on the drug effect scores revealed a significant groups×periods interaction for the ACTH-stimulating effect of naloxone (F(4,15)=3.243, p<0.05). Relative to saline control experiments, naloxone significantly increased plasma ACTH concentrations during both stress periods as well as during recovery in the Low BP group. In contrast, naloxone was a relatively poor stimulus for ACTH release in the High BP group, enhancing plasma ACTH concentrations only during the late stress period.

The effect of naloxone on plasma cortisol concentration is shown in Figure 2. There were no significant effects of opioid blockade on basal cortisol levels; however, naloxone potentiated cortisol
TABLE 2. Effects of Naloxone Infusion on Blood Pressure and Neuroendocrine Responses During an Arithmetic Stressor in Young Adults With High and Low Casual Arterial Pressure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Low BP group</th>
<th>Naloxone</th>
<th>High BP group</th>
<th>Naloxone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Infusion</td>
<td>Math1</td>
<td>Math2</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>mean</td>
<td>76.7</td>
<td>74.9</td>
<td>83.5</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>mean</td>
<td>44.1</td>
<td>84.6</td>
<td>87.3</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>mean</td>
<td>22.5</td>
<td>43.1</td>
<td>18.8</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>mean</td>
<td>11.5</td>
<td>17.3</td>
<td>21.8</td>
</tr>
<tr>
<td>ACTH (fmol/ml)</td>
<td>mean</td>
<td>1.13</td>
<td>3.09</td>
<td>3.18</td>
</tr>
<tr>
<td>β-endorphin (pmol/l)</td>
<td>mean</td>
<td>9.66</td>
<td>14.64</td>
<td>16.46</td>
</tr>
</tbody>
</table>

Naloxone, 0.1 mg/kg infused over 10 minutes; Math1, first 5-minute block of arithmetic stress; Math2, second 5-minute block of arithmetic stress; BP, blood pressure; ACTH, corticotropin. n=5-13 samples/cell.

responses during stress in both groups. Naloxone significantly stimulated cortisol release during all stress and recovery periods in the Low BP group, but only during recovery in the High BP group. The stimulating effect of naloxone on cortisol reactivity was significantly larger in the Low BP group than in the High BP group (F(1,23)=6.722, p<0.025).

Figure 3 shows the effect of naloxone on plasma β-endorphin concentration during stress in High and Low BP groups. Infusion of naloxone significantly increased plasma β-endorphin-like immunoreactivity in the Low BP group only (p<0.005) with no effect in the High BP group. The group difference in the endorphin-releasing effect of naloxone was maintained across postinfusion samples [F(1,8)=5.718, p<0.05]. There were no consistent effects of blood pressure grouping or stress on the ratio of ACTH to β-endorphin in plasma.

The effect of naloxone on blood pressure was similar to previous studies conducted in our laboratory.9,10 Figure 4 shows the changes in mean arterial pressure at the onset of the arithmetic stressor. Psychological stress produced significant elevations in mean arterial blood pressure in both groups after saline infusion; however, the stress response of the High BP group (8.4±1.14 mm Hg) was significantly larger than the response of the Low BP group [3.8±1.75 mm Hg, t(19)=2.361, p<0.05]. Naloxone significantly increased the stress response in the Low BP group [8.6±1.79 mm Hg, t(8)=2.36, p<0.05] with no effect on High BP group responses (9.3±1.86 mm Hg).

The group differences in blood pressure correspond with group differences in plasma epinephrine levels. Preinfusion resting epinephrine levels were comparable in High and Low BP groups, but after saline infusion, the High BP group had significantly higher levels than those obtained from Low BP subjects [F(1,15)=7.835, p<0.025]. In addition, naloxone significantly increased epinephrine levels during stress in the Low BP group only (p<0.05). The effect of naloxone on plasma epinephrine was significantly correlated with the effect of naloxone on mean arterial pressure in the Low BP group only. Additionally, the effect of naloxone on blood pressure levels during recovery was positively correlated with the plasma ACTH response during stress in the Low BP group.
A. Low Casual Blood Pressure Group

![Graph A. Low Casual Blood Pressure Group]

B. High Casual Blood Pressure Group

![Graph B. High Casual Blood Pressure Group]

**FIGURE 1.** Line graphs showing effect of opioid antagonism with 0.1 mg/kg naloxone on plasma corticotropin (ACTH)-like immunoreactivity (fmol/ml) in young adults with low (panel A) and high (panel B) levels of arterial pressure obtained casually during on-campus screenings. MATH1, MATH2 are the first and second 5-minute blocks of arithmetic stress, respectively. n=10 per group. *p<0.05, **p<0.01 compared with saline.

[r(6)=0.918, p<0.01], but the correlation in the High BP group was nonsignificant [r(10)=-0.480]. Using z-score transformations, there was a significant difference between groups (z=3.58, p<0.001) in the correlation of plasma ACTH concentrations and blood pressure. These correlations indicate that Low BP subjects demonstrating the largest pressor effect of naloxone also demonstrated the greatest epinephrine and ACTH-releasing effect of naloxone. The correlation of ACTH effects during stress with blood pressure effects during recovery may indicate that ACTH-induced cortisol stimulation in persons with low casual blood pressure suggests opioidergic inhibition of anterior pituitary function. Although the mechanism for opioid regulation of ACTH secretion is relatively unexplored in humans, animal investigations suggest that this may represent hypothalamic opioid effects on the corticotropin secretagogue CRF, because opioid compounds have no observable effect on pituitary tissue in vitro. The ACTH-stimulating effect of the naloxone/stress challenge is quantitatively diminished in young adults with high casual blood pressure, exaggerated circulatory stress responses, and enhanced risk for hypertension.

The effect of naloxone on ACTH secretion appears to have observable consequences on adrenocortical function. Naloxone potentiated cortisol responses to stress in the Low BP group, possibly via its effects on ACTH secretion. Although the High BP group showed some stimulation of the cortisol response to stress by naloxone, this effect was significantly smaller than that observed in the Low BP group. Given the correspondence between the stimulatory effects of naloxone on ACTH levels and cortisol responses, the latter is likely a result of circulating ACTH. Therefore, a defect in opioidergic inhibition of pituitary function has observable peripheral consequences at the level of the adrenal cortex.

Naloxone significantly increased circulating β-endorphin in the Low BP group, with no observable effect in High BP subjects. This effect was further exaggerated by psychological stress, with plasma β-endorphin-like immunoreactivity increased by 60% during stress and naloxone compared with stress.
In High BP subjects there was a trend (see Figure 3) for lower basal plasma concentrations of β-endorphin, and absence of an endorphin-stimulating effect by naloxone. Low concentrations of circulating β-endorphin have also been observed in young hypertensive patients, and the present findings suggest that this pattern may be part of multiple neuroendocrinologic abnormalities accompanying hypertension development, and not a pathophysiological consequence of clinically significant chronic pressure elevations.

Analysis of the ACTH/β-endorphin ratio showed no consistent effects of naloxone on plasma β-endorphin expressed by pituitary versus adrenal medullary secretory cells. The observed correspondence between naloxone effects on ACTH and β-endorphin suggests opioid inhibition of both proopiomelanocortin-derived peptides, possibly via adrenohypophyseal secretagogues, especially CRF. Diencephalic CRF-containing neurons are known to control pituitary secretion of both ACTH and β-endorphin, and these or similar circuits are believed to be of primary importance in central control of autonomic outflow. However, the relation between opioidergic inhibition of pituitary secretion and blood pressure reactivity remains to be determined. The correlation between the effect of naloxone on blood pressure and plasma ACTH concentrations suggests that there is some evidence for quantitative coupling of opioidergic regulation of pituitary and circulatory function. This is surprising given the multitude of factors that could obscure quantitative comparisons (e.g., time course of effects, nonopioid control mechanisms, and variations in sampling procedures). Nevertheless, these data suggest that opioid mechanisms of blood pressure control have several features in common with opioid mechanisms of pituitary regulation, including the effects on stress reactivity, the risk-group differences, and the correlation in effect sizes. The exact nature of this relation remains to be identified, and the interactions of hypothalamo-pituitary-adrenocortical and sympathoadrenomedullary hormones could contribute to the observed effects. For example, adrenocortical effects on phenylethanolamine N-methyltransferase could stimulate epinephrine synthesis, but this pathway may be more important for chronic versus acute stressors. Nevertheless, the possibility of a common central opioidergic regulatory mechanism for both the hypothalamo-pituitary-adrenocortical and sympathoadrenomedullary axes cannot be ruled out during psychological stress. Although there are multiple naloxone-sensitive sites on the sympathoadrenomedullary axis for interaction of opioids with blood pressure control mechanisms, these sites of interaction could not easily explain the correlation with pituitary mechanisms. Taken together, these considerations suggest that risk group differences in opioidergic regulation of blood pressure may be mediated via hypothalamic mechanisms linked to pituitary-adrenocortical regulation. The circulatory significance of altered opioidergic regulation of pitu-
A. Low Casual Blood Pressure Group

B. High Casual Blood Pressure Group

**Figure 3.** Line graphs showing effect of opioid antagonism with naloxone on plasma β-endorphin-like immunoreactivity (pmol/l) in young adults with low (panel A) and high (panel B) levels of casual arterial pressure. MATH1, MATH2 are the first and second 5-minute blocks of arithmetic stress, respectively. n=5 per group. *p<0.05, **p<0.01 compared with saline.

...mitary function in hypertension development awaits further exploration.

Analysis of plasma catecholamine levels provides partial support for the contention that the pressor effect of naloxone in Low BP subjects is a result of opioid inhibition of sympathoadrenomedullary outflow. The epinephrine results suggest an opioidergic inhibition of adrenomedullary outflow in the Low BP group. The epinephrine-releasing effect of naloxone was significantly lower in the High BP group, suggesting diminished opioidergic inhibitory input to adrenomedullary control mechanisms. The noradrenaline levels are not consistently affected by naloxone in the present experiment. This could reflect at least two possibilities. First, the opioidergic inhibitory input in Low BP subjects is effective at the adrenal medulla but not at peripheral sympathetic fibers. Second, the norepinephrine levels in plasma may not be adequately sensitive to naloxone-induced changes in peripheral sympathetic nerve activity. This latter possibility could reflect the differences between the physiological characteristics of epinephrine and norepinephrine. Specifically, norepinephrine in plasma primarily reflects passive diffusion from wide-gap synaptic junctions, whereas epinephrine is released directly into the circulation and its concentration in blood closely reflects the amount available for receptor binding. Moreover, a major route of deactivation of norepinephrine is via reuptake, making the rate of spillover into the circulation a marginally reliable index of synaptic transmission. At present, the role of the peripheral sympathetic nerves in the present study is unclear, and more data is necessary to adequately evaluate opioidergic inhibition of sympathetic neurotransmission.
Paraventricular CRF-containing parykarya are believed to stimulate the hypothalamospinal upper motoneurons of the sympathetic nervous system. The effects of naloxone on pituitary function and blood pressure responses may be mediated via direct or indirect opioidergic input to hypothalamic CRF-containing neurons. Although paraventricular CRF control of central sympathetic outflow and ACTH/endorphin secretion may reflect two distinct populations of neurons, these two CRF systems may have a common opioidergic input, possibly via limbic, brainstem, or intrinsic afferents. This is plausible given the correlations between hypothalamic-pituitary-adrenocortical and sympathoadrenomedullary function and the present findings linking anterior pituitary function and the effects of naloxone on blood pressure responses to orthostatic baroreceptor reflex challenges, suggesting that impaired opioidergic inhibition of blood pressure responses during psychological stress is not a characteristic of reflex-stimulated sympathetic nerve discharge.

Recent findings of naloxone antagonism of the hypotensive effects of clonidine suggest altered brainstem opioids in hypertension, and the relation of these mechanisms to baroreceptor reflex and pituitary function has not been determined. Nevertheless, several facts are consistent with altered opioid input to hypothalamic mechanisms mediating risk-group differences, including the anatomic substrate, the effects of naloxone on orthostasis, and the behavioral concomitants of opioid antagonism, and the present findings linking anterior pituitary function and blood pressure reactivity. These data, taken together, suggest that naloxone-sensitive opioid pathways inhibit the hypothalamic-pituitary-sympathoadrenomedullary cascade, and reduced expression of these opioid mechanisms may have a role in the exaggerated stress reactivity observed in young adults at enhanced risk for essential hypertension.

Acknowledgment

We express grateful appreciation to Elizabeth M. Harlan for technical assistance.

References

23. Risch SC, Janowsky DS, Mott MA, Gillin JC, Kalir HH, Huey LY, Zeigler M, Kennedy B, Turken A: Central and peripheral cholinesterase inhibition: Effects on anterior pitu-


Altered pituitary hormone response to naloxone in hypertension development.
J A McCubbin, R S Surwit, R B Williams, C B Nemeroff and M McNeilly

Hypertension. 1989;14:636-644
doi: 10.1161/01.HYP.14.6.636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/14/6/636