

# Effect of Morphine on the Hemodynamic and Neuroendocrine Responses to Hemorrhage in Conscious Rats

Giora Feuerstein, Anna-Leena Sirén, David S. Goldstein, Alan K. Johnson, and Robert L. Zerbe

Laboratory Support Division, Department of Neurology, Uniformed Services University of the Health Sciences (G.F., A.-L.S.); Endocrine Metabolic Branch, NHLBI, National Institutes of Health, Bethesda, Maryland (D.S.G.); Department of Psychology, University of Iowa, Iowa City (A.K.J.); and Eli-Lilly & Co., Lilly Research Laboratory, Indianapolis, Indiana (R.L.Z.)

We have previously reported that analgesic doses of morphine accelerate mortality of rats exposed to hemorrhage (Feuerstein and Sirén: *Circ Shock* 19:293-300, 1986). To study the potential mechanisms involved in this phenomenon, rats were chronically implanted with catheters in the femoral vessels and morphine (1.5 or 5 mg/kg) was administered 30 min or 24 hr after bleeding (8.5 ml/300 g over 5 min) while arterial blood pressure and heart rate were continuously monitored. Furthermore, the effect of morphine (5 mg/kg) on cardiac output (CO) response to hemorrhage was studied in rats chronically equipped with a minithermistor for CO monitoring by a thermodilution technique. In addition, plasma catecholamines (HPLC), plasma renin activity (PRA, RIA), vasopressin (RIA), pH, and blood gases were also determined. Morphine administration 30 min after hemorrhage produced a pressor response and tachycardia which were in marked contrast to its depressor effect in intact rats. Morphine elevated PRA and epinephrine but not vasopressin, while blood pH and gases showed no consistent change as compared to saline-treated hemorrhaged rats. Morphine given after the bleeding resulted in enhanced cardiac depression in response to a second bleed of 2 ml/300 g. Our data suggest that activation of pressor mechanisms by morphine during hypovolemic hypotension might enhance vasoconstriction in essential organs, depress cardiac function, and further reduce effective tissue perfusion.

**Key words:** hemorrhagic shock, opiates, catecholamines, renin, vasopressin

Submitted for publication March 4, 1988; revised November 29, 1988.

Address reprint requests to Dr. Giora Feuerstein, Department of Neurology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799.

## INTRODUCTION

Cardiovascular stress situations have been shown to activate the endogenous opioid system [for review see 1,2] as indicated by increased levels of opioid peptides in the circulation [3] and brain [3,4] and changes in opioid receptors in peripheral and brain tissue [5,6]. Furthermore, a role for the opioid peptides in cardiovascular responses to several shock and trauma paradigms has also been suggested based on the cardiostimulatory and improved hemodynamic responses to treatments with naloxone, a potent opiate receptor antagonist [7-9]. Although several studies failed to demonstrate improved cardiovascular status in hemorrhaged animals treated with naloxone [10-14], including failure to demonstrate improved survival [11,13,14], it is still commonly believed that activation of the endogenous opioid system plays a depressor and hence a detrimental role in cardiorespiratory recovery after bleeding.

This concept is not only of primary importance for understanding the mechanisms leading to the decompensatory phase of hemorrhagic shock but also for making practical therapeutic decisions involving administration of opiate analgesic drugs in states of shock and trauma. Thus, if opioid-mediated cardiovascular depression is indeed an important detrimental mechanism, one would assume that administration of opiates in a state of cardiovascular shock would further compromise vital functions, aggravate the shock, and lead to increased mortality. This hypothesis has recently been supported by studies conducted in our laboratories [14] showing that minimal or full analgesic doses of morphine administered to rats exposed to bleeding enhanced mortality in a dose-dependent manner; however, in this latter study, no attempt has been made to explore the hemodynamic consequences of morphine in hemorrhage, nor were the compensatory pressor systems (renin-angiotensin, vasopressin, catecholamines) examined.

The present studies are a continuation of our effort to further evaluate the effect of opiates on hemodynamic and neuroendocrine responses to hypovolemic hypotension by evaluation of systemic hemodynamic indices, cardiac output, respiratory and metabolic variables, as well as plasma renin, vasopressin, and catecholamines.

## MATERIALS AND METHODS

### Measurement of Blood Pressure and Heart Rate

Male Sprague Dawley rats (250-300 g) were purchased from Taconic Farms (Germantown, NY) and kept at 22°C and 12 hr/12 hr light/dark cycle. Rats were anesthetized with pentobarbitone (40 mg/kg, i.p.) and polyethylene catheters (PE-50) were inserted into the femoral artery and vein through a small incision in the groin. The catheters were then threaded under the skin of the back and exteriorized at the nape of the neck. Thereafter, the catheters were secured by a spring wire and an adhesive collar loosely attached around the neck. Rats were allowed to recover from surgery for 24-36 hr with food and tap water ad libitum. This procedure is slightly modified from a previously described technique [15] and has been successfully used in several hemorrhagic shock studies in our laboratory [16-18].

### Measurement of Cardiac Output

Rats were anesthetized with an intramuscular injection of ketamine-acepromazine (0.13 ml/100 g of a solution of ketamine, 100 mg/ml, and acepromazine, 1 mg/ml), and PE-50 tubing was inserted into the femoral arteries. The catheters were

tunneled beneath the back skin and exteriorized at the back of the neck. Then, an incision was made at the midline of the neck from the cricoid to the clavicle, and a PE-50 tubing was inserted into the right atrium through the right external jugular vein. Then, the left common carotid artery was exposed and ligated, and a thermistor (MX2-780-33 model THMP f#1.5, Teflon reusable, Columbus Instruments, OH) was advanced through the carotid into the ascending aorta. (Placement above the aortic valve was confirmed in each animal at the end of the experiment and by the shape of the dilution curve before the probe was finally sutured to the neck muscles.) The jugular vein catheter and the thermistor were tunneled under the skin to the back of the neck. All lines were secured by a soft spring wire (attached by an adhesive collar) from the back of the neck and outside of the cage. The animals were allowed to recover from surgery for 24–48 hr. On the day of the experiment, the arterial line was connected to a blood pressure transducer (Narco RP 1500i) and continuous recordings of arterial blood pressure (mean, pulse pressure) and heart rate were carried out by the Narcotrace 80 computerized dynograph. The cardiac output was measured by thermodilution technique, as the thermistor was attached to the computerized Cardiomax II (CMX2-780-k with the microprobe option R, Columbus Instruments, OH). The dead-space of the venous line was first flushed with 0.05 ml of 0.9% (w/v) NaCl (saline) at room temperature, 22°C; after a brief stabilization period (10 sec to assure normal core temperature) an additional injection of 0.2 ml normal saline (22°C) was rapidly injected by using a 1 ml syringe. Cardiac output was recorded in the following manner: A control period of 15 min included two or three cardiac output measurements made to test for consistency and placement of the probe and also to get control values for mean arterial pressure and heart rate. The timer on the automatic data collection system was then started and data points were taken at  $t_0$ ,  $t_5$ ,  $t_{30}$ ,  $t_{35}$ ,  $t_{60}$ ,  $t_{90}$ ,  $t_{95}$ ,  $t_{130}$ , and  $t_{160}$  min. Total peripheral resistance (TPR) was calculated by dividing the mean arterial pressure by the cardiac output; values of cardiac output and TPR were further indexed per unit of weight (kg).

### Experimental Protocols for Hemorrhage and Morphine Treatment

**Single hemorrhagic model.** The effects of morphine on blood pressure and heart rate were studied in both intact and hemorrhaged conscious rats. On the day of the experiment, one arterial line was connected to a pressure transducer (Narco RP1500i) and the mean arterial pressure (pulse pressure) and heart rate were continuously recorded on the computerized Narco-trace 80 dynograph (with automatic sampling every min). Three experimental protocols were studied as follows: 1) basal hemodynamic monitored in intact conscious rats for 30 min followed by administration of morphine, 1.5 or 5 mg/kg or 0.9% NaCl; hemodynamic variables were monitored for 60 additional min; 2) in intact conscious rats bleeding (8.5 ml/300 g body weight) was done over a 5 min period; the rats were allowed to recover for 24 hr and then blood pressure and heart rate were monitored for 30 min. After this basal recording, morphine (1.5 or 5 mg/kg) or 0.9% NaCl was administered i.a., and hemodynamic variables were followed up to 90 min after the treatment; 3) after 30 min of basal hemodynamic monitoring, bleeding was conducted as described above; 0.9% NaCl or morphine (1.5 or 5 mg/kg) was given for 30 min after the bleeding. Hemodynamic variables were continuously monitored up to 60 min after drug administration; 4) a separate group of rats was prepared in the same way described for the other groups and the rats were exposed to bleeding as described; the first and last ml of blood withdrawn from the arterial line were saved for control and

end of bleeding levels of catecholamines, vasopressin, and PRA. In addition, 0.5 ml of blood was again taken 45 and 90 min after the bleeding (to match the 15 and 60 min time points after morphine injection). This protocol was pursued in two groups of rats which were given either 0.9% NaCl or morphine 1.5 mg/kg, 30 min after the bleeding. Only the blood sample taken 45 min after the bleeding was replenished by fresh rat blood, so that this protocol will match protocol 3 described for the same dose of morphine.

**Double hemorrhage model.** The effect of morphine on cardiac output and TPR was studied in conscious rats exposed to an initial bleeding of 8.5 ml/300 g over a 5 min period followed by 3 ml/300 g 90 min later. Morphine (5 mg/kg) or saline was administered i.a. 30 min after the first bleeding.

### **Rationale for Doses and Experimental Procedures**

The doses of morphine selected for this study were chosen according to our previous studies on survival of morphine (0.5–5 mg/kg)-treated rats which were exposed to the same hemorrhagic shock paradigms [14]. We have chosen doses of morphine which already provide analgesia in the rat to reflect the potential use of analgesic doses of morphine in human.

### **Preparation of Plasma Samples**

Each blood sample withdrawn was rapidly centrifuged (Microfuge B, Beckman) for 30 sec and plasma was rapidly aliquoted in ice-cooled Eppendorf tubes. Plasma was further aliquoted for catecholamine assay (100–150  $\mu$ l), PRA (50–100  $\mu$ l), and vasopressin assay (100–150  $\mu$ l). The plasma was stored at  $-70^{\circ}\text{C}$  until assayed (within few weeks).

### **Assay of Plasma Catecholamines**

Catecholamines (epinephrine, norepinephrine, dopamine) were isolated by alumina extraction and assayed by high pressure liquid chromatography with electrochemical detection as previously described [19,20].

### **Assay of PRA**

Plasma renin activity (PRA) was determined by methods described in detail previously [21]. In brief, the assay procedure included 1 hr of incubation of plasma ( $37^{\circ}\text{C}$ ) to generate angiotensin I. Angiotensin I is then assayed by radioimmunoassay by using the angiotensin I RIA kit (NEN, North Billerica, MA). Assay sensitivity is 10 pg angiotensin I/tube, and assay range is 10–500 pg/tube. This method has been successfully utilized previously in our hemorrhagic shock studies in the conscious rat [17].

### **Assay of Plasma Vasopressin**

Plasma samples remaining after removal of a fraction for catecholamine and PRA determination were stored at  $-20^{\circ}\text{C}$  for subsequent measurement of plasma vasopressin by radioimmunoassay [22]. Prior to assay, 100  $\mu$ l of each sample was diluted in 150  $\mu$ l of 0.1 M phosphate buffer containing 0.3% sodium chloride and 0.1% bovine serum albumin at pH 7.6 and then extracted by using acetone and petroleum ether. The remaining acetone-water solution was completely dried at room temperature by a spin evaporator (Savant, Hicksville, NY). The extracted material was

resuspended in 500  $\mu\text{l}$  of 0.1% bovine serum albumin solution just prior to assay; cold recovery using this method was 77%.

The radioimmunoassay utilizes a highly sensitive and specific rabbit antibody diluted 1:1,000,000 and used in a volume of 200  $\mu\text{l}$ /assay tube (final antibody dilution in assay: 1:2,500,000). A total of 1,500 counts/min of iodinated arginine vasopressin with a specific activity of 1,330  $\mu\text{Ci}$  (New England Nuclear, Boston, MA) was added to each tube in a volume of 100  $\mu\text{l}$ . Two 100  $\mu\text{l}$  aliquots of standards and unknowns were assayed. Standard, tracer, and antibody were diluted in a 0.1 M phosphate buffer containing sodium chloride and albumin (see above). The assay was incubated for 7 days at 4°C and separated with bovine  $\gamma$ -globulins and polyethylene glycol. When done in this way, the assay will consistently detect 0.1 pg arginine vasopressin/tube and cross-reacts less than 1% with oxytocin. The intraassay coefficient of variation is 24% at 1 pg, 4% at 5 pg, and 6% at 20 pg.

All plasma samples in this study were extracted at the same time and measured in the same assay. The plasma concentration of vasopressin in picograms per milliliter was determined by multiplying the amount in each tube by 25 to correct for dilution; no correction was made for recovery. This method was previously used in several of our studies on the role of vasopressin in hemorrhage [17,22].

### Assay of Blood pH, $\text{pO}_2$ , and $\text{pCO}_2$

In order to evaluate the effect of morphine on the respiratory and metabolic responses to hemorrhage the experimental protocol described for the double-bleeding paradigm was utilized. The following blood samples were taken: the first and last 0.2 ml of the first bleed; 0.2 ml of blood was taken 30 min after the end of first bleeding and 15 and 30 min after morphine or saline administration, and 30 min after the second bleed. All blood samples were collected in ice-cooled syringes, and blood pH,  $\text{pO}_2$ , and  $\text{pCO}_2$  165/2 was assayed by a blood gas analyzer model IL 1303 (Instrument Laboratories).

### Statistical Analysis of the Data

All data in text and figures are means  $\pm$  S.E.M. for the indicated number of rats. The hemodynamic changes were analyzed by analysis of variance and covariance for repeated measures (BMD - P2V [23]). The biochemical data were evaluated by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keul test and the nonparametric ANOVA followed by the Mann-Whitney test or by a Student's t-test, when appropriate. Differences were considered significant at  $P < 0.05$ .

## RESULTS

### Effects of Morphine on Blood Pressure and Heart Rate

Mean arterial pressure, pulse pressure, and heart rate of the various groups of rats studied were not significantly different in the baseline period or postbleeding and prior to morphine administration (Figs. 1-3, left panel).

The effects of morphine in nonbled conscious rats are presented in Figure 1. The low dose of morphine (1.5 mg/kg) decreased blood pressure (5-7 mmHg) and produced bradycardia ( $>50$  bpm). The high dose (5 mg/kg) showed a lesser depressor response and no bradycardia. All these effects were short lasting, and baseline levels were reached 15 min after morphine administration.

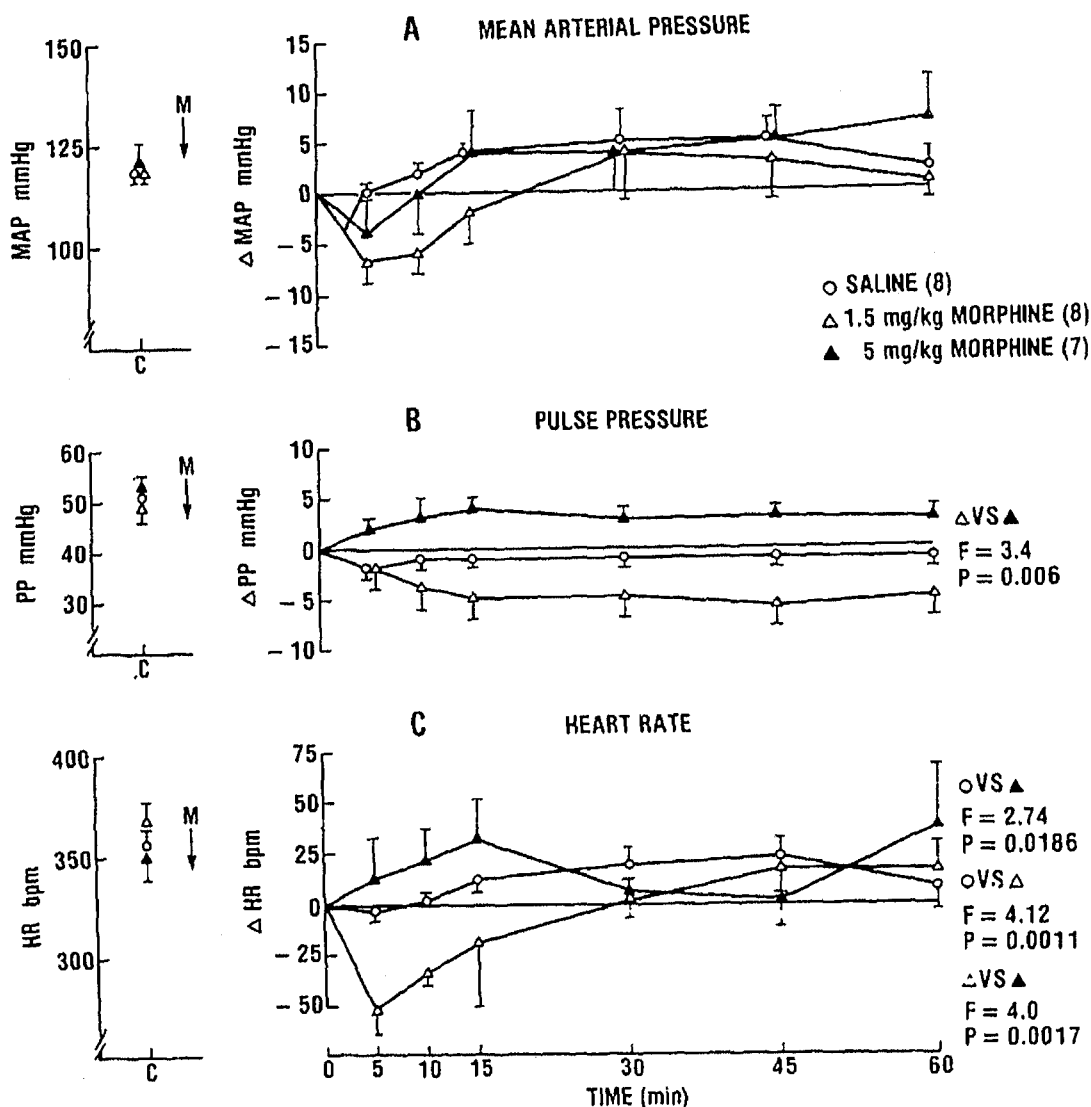


Fig. 1. Cardiovascular effects of morphine in the intact conscious rat. The baseline levels of mean arterial pressure (A), pulse pressure (B), and heart rate (C) are indicated on the left of the figure. Time of intraarterial morphine injection is given as !M. Vertical bars indicate S.E.M. Number of rats in each group is given in parentheses. Analysis of variance with repeated measures was used to evaluate statistical difference between the groups, and F and P values for the variables are given in the figure.

Hemorrhage induced sharp decreases in mean arterial pressure and pulse pressure with a variable effect on heart rate (Fig. 2, left panel). In the control (saline-treated) rats MAP fell to <50 mmHg but recovered to almost 85 mmHg 30 min after the bleeding. Thereafter, MAP as well as pulse pressure showed only a slight tendency for further decline (<5 mmHg).

Administration of morphine 30 min after the end of the bleeding produced an additional pressor response (Fig. 2, panel A, right) which was equally pronounced after 1.5 or 5 mg/kg morphine. The pressor response (up to 20 mmHg above that of the control group) was sustained over the duration of the experiment. Furthermore, in both morphine-treated groups tachycardia was noted, which exceeded 100 bpm at maximal effects (Fig. 2C) and was sustained throughout the experimental period.

Twenty-four hours after the bleeding the mean arterial pressure was slightly below prehemorrhage level (Fig. 3, left panel) while heart rate and pulse pressure were

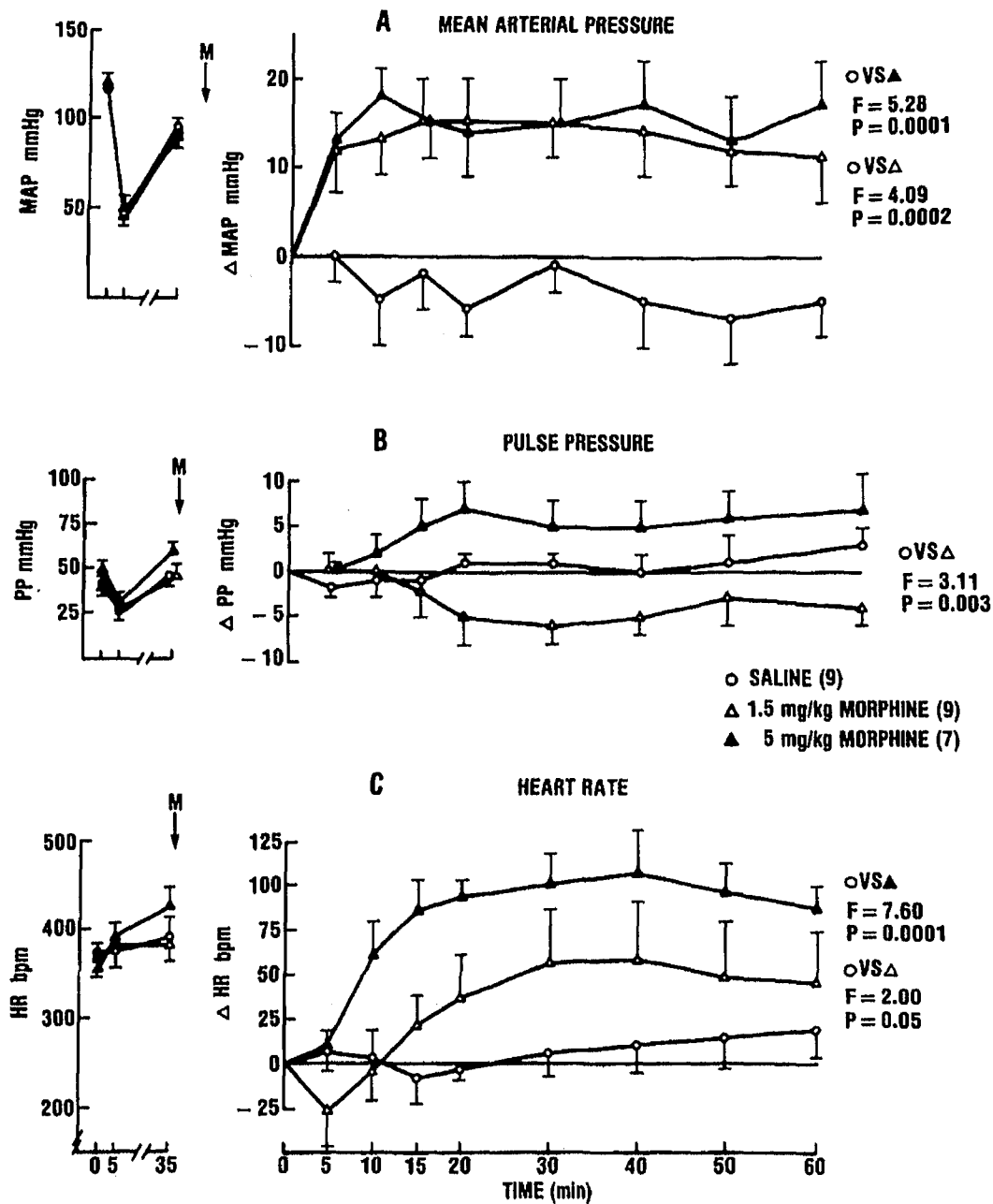


Fig. 2. Cardiovascular effects of morphine injected 30 min after hemorrhage (8.5 ml/300 g/5 min) in the conscious rat: mean arterial pressure (A), pulse pressure (B), and heart rate (C). The effect of bleeding is indicated on the left-hand panels. Bleeding was started at  $T_0$  and completed at  $T_5$ . Time of intraarterial morphine injection is given as  $M$ . Vertical bars indicate S.E.M. Number of rats in each group is given in parentheses. The statistical difference between morphine-treated and control groups was evaluated by analysis of variance with repeated measures, and  $F$  and  $P$  values for the variables are given in the figure.

within the normal range for conscious rats (Fig. 3). When injected 24 h after the bleeding, the lower dose of morphine significantly decreased MAP (5–10 mmHg) and heart rate ( $>50$  bpm); the morphine-induced bradycardia reached its maximum ( $-73 \pm 10$  beats/min,  $P < .01$ ) 5 min after the injection and subsided in 45 min. In contrast to the low dose, the high dose of morphine (5 mg/kg) produced a sustained pressor response, which was not preceded by a depressor phase, and MAP was still clearly elevated 90 min after morphine administration. The pulse pressure also increased while the heart rate briefly decreased initially and then slightly increased.

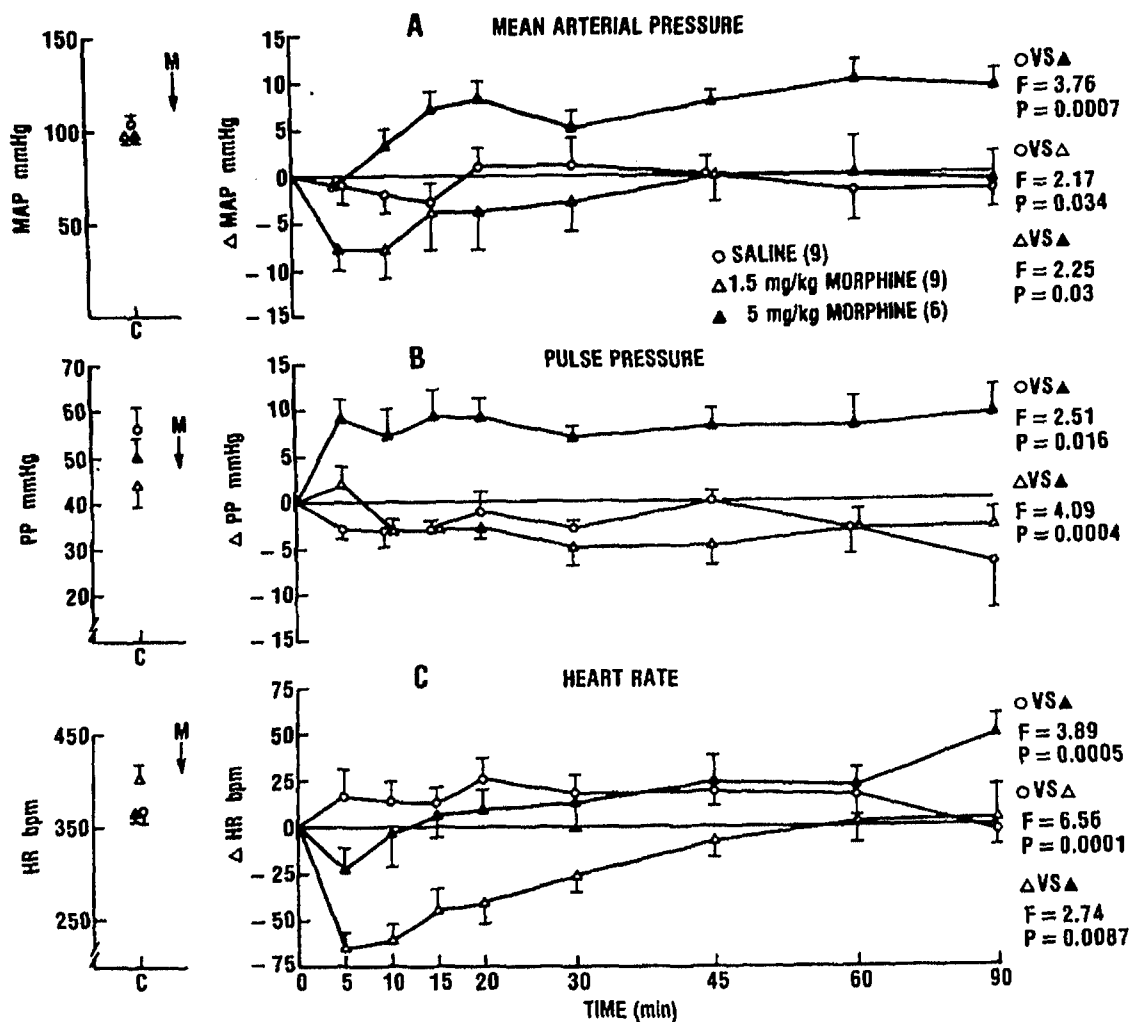


Fig. 3. Cardiovascular effects of morphine injected 24 hr after hemorrhage (8.5 ml/300 g/5 min) in the conscious rat. The baseline levels of mean arterial pressure (A), pulse pressure (B), and heart rate (C) are indicated on the left of the figure. Time of intraarterial morphine injection is given as IM. Vertical bars indicate S.E.M. Number of rats in each group is given in parentheses. Analysis of variance with repeated measures was used to evaluate statistical difference between the groups, and F and P values for the variables are given in the figure.

### Effect of Morphine on Plasma Catecholamines After Hemorrhage (Table I)

The baseline levels of norepinephrine, epinephrine, and dopamine of saline-treated and morphine-treated rats did not significantly differ from each other (Table I). Hemorrhage markedly increased plasma epinephrine (50-fold), while plasma norepinephrine levels were raised only about twofold. The plasma dopamine levels also modestly increased after hemorrhage. There was no difference in plasma catecholamine response to bleeding between the two groups of rats prior to saline or morphine administration. Injection of morphine (1.5 mg/kg) 30 min after the hemorrhage further raised plasma epinephrine 15 and 60 min after morphine injection. Plasma norepinephrine also tended to increase after morphine, but this effect did not reach statistical significance when compared to the control group. Morphine had no effect on plasma dopamine level. It is noteworthy that while the levels of all the catecholamines in the control group returned toward baseline at the end of the experiment, an opposite trend was noticed in the morphine-treated group (Table I).



**TABLE I. Effect of Morphine (1.5 mg/kg) on Plasma Catecholamines of Conscious Rats Exposed to Hemorrhage†**

Catecholamine	Saline	Morphine
<b>Norepinephrine</b>		
Control	169 ± 27	119 ± 10
EOB	303 ± 62	259 ± 47
15 min	210 ± 40	393 ± 110
60 min	130 ± 121	1,066 ± 432
<b>Epinephrine</b>		
Control	124 ± 42	117 ± 21
EOB	5,091 ± 1,772	6,138 ± 1,696
15 min	1,024 ± 182	3,570 ± 991*
60 min	1,065 ± 138	5,172 ± 1,247**
<b>Dopamine</b>		
Control	34 ± 8	49 ± 7
EOB	117 ± 38	62 ± 29
15 min	215 ± 98	173 ± 24
60 min	125 ± 21	327 ± 141

†Eight rats were studied in each group. Asterisks denote statistical significance from saline-treated group (Student's t-test) at \* $P < .05$ , \*\* $P < .02$ . All data are pg/ml. EOB = end of bleeding. The time points 15 and 60 min represent the respective sampling points after morphine administration.

### Effect of Morphine on Plasma Renin Activity in Hemorrhaged Rats

Plasma renin activity (PRA) in the control group gradually increased after the bleeding and throughout the experimental protocol up to tenfold at the end of the experiment (Fig. 4).

Injection of morphine (1.5 mg/kg, i.a.) 30 min after the bleeding caused further increase in PRA. The PRA 60 min after morphine administration was  $138 \pm 23$  ng/ml/hr as compared to  $56 \pm 6$  ng/ml/hr in saline-treated animals ( $P < .02$ ,  $n = 7$ ).

### Effect of Morphine on Plasma Vasopressin in Hemorrhaged Rats

Plasma vasopressin levels did not differ at the control and end of bleeding time points (Table II). Morphine (1.5 mg/kg) injected 30 min after the hemorrhage tended to further increase plasma vasopressin but this difference was not significant.

### Effect of Morphine on Cardiac Output

Figures 5 and 6 summarize the hemodynamic changes induced by the double hemorrhage paradigm in morphine- and vehicle-treated rats. The baseline values of cardiovascular variables were not statistically different in morphine- and saline-treated animals (Figs. 5, 6; panel A). Also, the first bleeding (8.5 ml/300 g/5 min) induced changes of similar magnitude in both of these groups (Figs. 5, 6; panel A). Morphine (5 mg/kg) injected 30 min after the first hemorrhage tended first to increase cardiac index (Fig. 5, panel B), but after the second bleeding (2.0 ml/300 g/5 min) it resulted in severe cardiac depression (Fig. 5, panel C). The mean arterial pressure was also significantly lower after the second bleed in the morphine-treated group (Fig. 6, panel C) while there was no significant difference in TPR between these groups (Fig. 5, panel C).

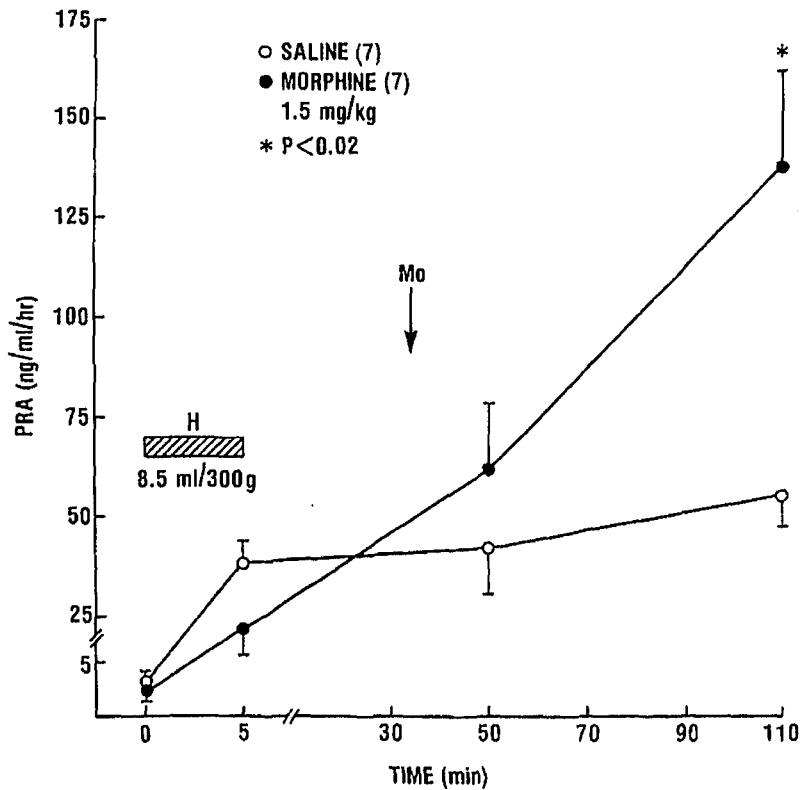


Fig. 4. Effect of morphine on plasma renin activity (PRA) after hemorrhage (8.5 ml/300 g) in the conscious rat. Bleeding is denoted as H and time of intraarterial injection of morphine administration as Mo. Number of rats in each group is given in parentheses. Vertical bars indicate S.E.M. Asterisk indicates statistical significance relative to the saline-treated group (Student's t-test) at  $P < 0.02$ .

TABLE II. Effect of Morphine (1.5 mg/kg) on Plasma Vasopressin of Conscious Rats Exposed to Hemorrhage\*

Time	Saline treated	Morphine treated
Control	2.0 ± 0.3	2.6 ± 0.9
EOB	200 ± 40	486 ± 164
15 min	87 ± 22	80 ± 12
60 min	69 ± 9	177 ± 69

\*Five rats were studied in the saline-treated group and three in the morphine group. All data are pg/ml. EOB = end of bleeding.

### Effect of Morphine on Blood pH, pO<sub>2</sub>, and pCO<sub>2</sub>

In all the rats exposed to bleeding a rapid and significant increase in pO<sub>2</sub> and pH and a profound decrease in pCO<sub>2</sub> were seen at the end of the bleeding (Table III). However, all these indices approached the basal level at 30 min after the end of the bleeding. Administration of morphine did not produce a consistent effect on any of these indices. In response to the second bleed, all the rats again exhibited a tendency to increase pO<sub>2</sub> and decrease pCO<sub>2</sub>, but since the only measurement was done 30 min after the latter bleed, these changes were not significant (as also observed 30 min after the first bleed). In the three morphine-treated rats which survived until the end of the experiment (30 min after the second bleed) blood pCO<sub>2</sub> was significantly lower and

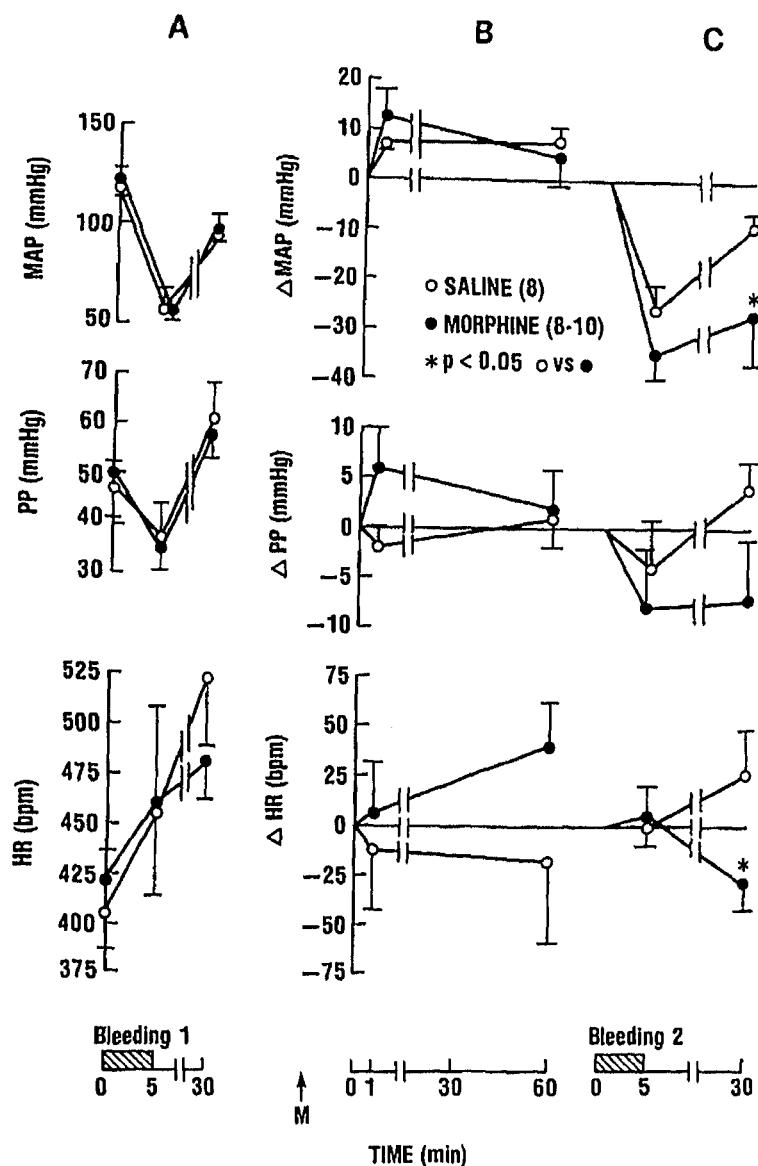


Fig. 5. Effect of morphine on mean arterial pressure (MAP), pulse pressure (PP), and heart rate (HR) in the conscious rat exposed to hemorrhage. The first bleeding (panel A) was instituted by withdrawal of blood (8.5 ml/300 g) over 5 min. Morphine (5 mg/kg) or saline was injected (†M) i.a. 30 min after the end of the bleeding 1 (panel B). A second bleeding (3 ml/300 g over 5 min introduced 60 min after the morphine/saline injection (panel C). †M denotes injection of morphine or saline. Vertical bars indicate S.E.M. Number of rats in each group is given in parentheses. Kruskal Wallis one-way ANOVA followed by Mann-Whitney test was used to evaluate statistical difference between morphine- and saline-treated groups.

$pO_2$  significantly higher than the levels monitored prior to the second bleed; the  $pO_2$  and  $pCO_2$  levels in these rats were also different from the saline-treated rats in the respective manner, but due to the small group of surviving rats the changes were not significantly different.

In this group of experiments, none of the saline-treated rats have died in comparison to 50% (three out of six rats) mortality of the morphine-treated rats.

### Effect of Morphine on Survival

Morphine significantly reduced the survival of rats exposed to the double-bleeding paradigm (Table IV). Twenty-four hours after the hemorrhage three out of

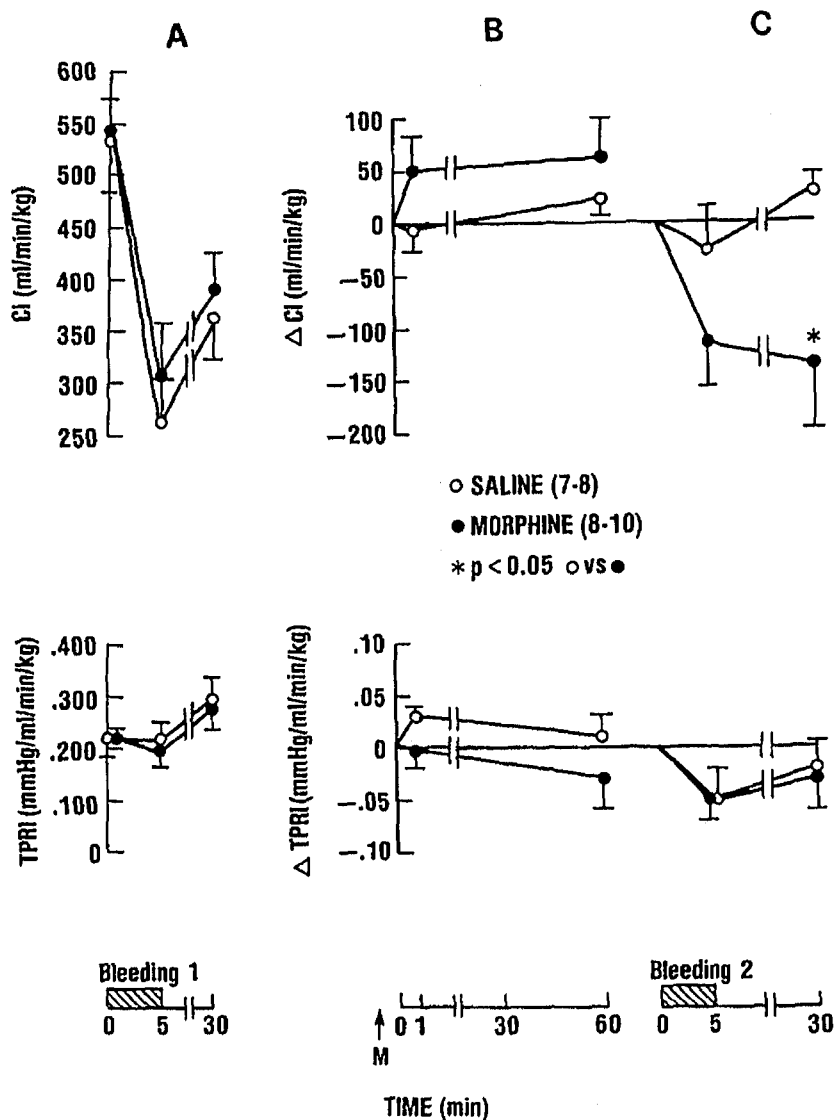


Fig. 6. Effect of morphine on cardiac index (CI) and total peripheral resistance index (TPRI) in the conscious rat exposed to hemorrhage. The first bleeding (panel A) was instituted by withdrawal of blood (8.5 ml/300 g) over 5 min. Morphine (5 mg/kg) or saline was injected i.a. 30 min after the end of the bleeding 1 (panel B). A second bleeding (3 ml/300g over 5 min) was introduced 60 min after the morphine/saline injection (panel C). †M denotes injection of morphine or saline. Vertical bars indicate S.E.M. Number of rats in each group is given in parentheses. Kruskal-Wallis one-way ANOVA followed by Mann-Whitney test was used to evaluate statistical difference between morphine- and saline-treated groups.

11 rats in the morphine group were alive as compared to six out of eight rats in the saline-treated group.

### DISCUSSION

The present report confirms and extends our previous studies which showed depressor and bradycardic responses to morphine in both anesthetized [24] and conscious rats [25]. However, the present study shows a still-undocumented effect of morphine, i.e., a strong pressor response produced by an otherwise depressor dose of morphine in rats exposed to hypovolemic hypotension. After the high dose of mor-

TABLE III. Effect of Morphine on Blood Gases and pH of Rats Exposed to Hemorrhage†

Variable	Basal	EOB	Minutes after				
			1st bleed 30	Drug injection		2nd bleed 30	
				15	60		
pH	Control	7.436	7.490	7.418	7.411	7.417	7.391
		±0.012	±0.021	±0.012	±0.017	±0.017	±0.022
	Morphine	7.447	7.481	7.360	7.325	7.345	7.313
		±0.004	±0.054	±0.018	±0.016	±0.021	±0.061
pO <sub>2</sub>	Control	98.1	128.8	118.8	124.5	123.5	137.9
		±3.0	±10.0	±2.0	±4.0	±8.0	±11.0
	Morphine	106.2	119.3	121.8	115.4	114.6	145.7*
		±2.0	±4.0	±5.0	±7.0	±6.0	±12.0
pCO <sub>2</sub>	Control	43.8	29.2	38.7	39.6	41.6	35.8
		±1.0	±1.0	±1.0	±2.0	±1.0	±3.0
	Morphine	42.0	27.6	35.7	42.1	41.7	23.9*
		±.5	±2.0	±2.0	±2.0	±2.0	±4.0

†Data in table are mean value ± S.E.M. for six rats in each group except for the morphine group, where three rats only were examined at the last time point. EOB = end of bleeding. \* $P < .01$  as compared to levels prior to the second bleed.

TABLE IV. Effect of Morphine on Survival of Conscious Rats Exposed to Hemorrhage†

Treatment	Min after first hemorrhage				
	30	60	90	120	160
Morphine	11/0	11/0	11/0	9/2	6/5*
0.9% Nacl	8/0	8/0	8/0	8/0	8/0

†Values indicate survival (alive/dead). Morphine was injected into the arterial line 30 min after the first bleeding, at a dose of 5 mg/kg.

\* $P < .05$  vs. saline-treated group (Fisher's exact probability test).

phine, the pressor effect lasted over 1 hr; furthermore, the pressor effect of morphine was accompanied by marked and prolonged tachycardia. These findings differ from those reported in endotoxic shock wherein morphine produced a marked depressor response [26]. Therefore, the present findings may provide evidence to suggest that the endogenous opioid system, at the receptor level, is substantially different in these two shock models. The present data also negate previous suggestions (based primarily on the similar pressor effect of naloxone in hemorrhagic and endotoxic shock) that the opioid receptors mediate depressor effects in all forms of circulatory shock.

In agreement with the present findings, it was recently reported that microinjections of the highly selective  $\mu$ -opiate agonist, D-Ala<sup>2</sup>-MePhe<sup>4</sup>-Gly-ol<sup>5</sup>-enkephalin, into the hypothalamus enhanced cardiovascular recuperation in conscious rats exposed to a bleeding paradigm similar to the one used in the present study [18]. Thus, hemorrhage seems to increase sensitivity to the pressor and/or decrease sensitivity to the depressor effect of opiates.

Such a phenomenon may not be entirely surprising if one considers the suppression of the depressor effect of morphine and emergence of a pressor effect reported after repeated systemic injections of morphine [24,27]. Activation by hemorrhage of the endogenous opioid system in the brain [3,4] may in fact produce the same phenomenon as repeated pharmacological injections of morphine. Interestingly, chronic infusion of the opiate antagonist naloxone in rats resulted in the opposite effect, namely, enhanced depressor response to morphine which is associated with up-regulation of opiate receptors in the brain [25]. Although hemorrhage produces a different profile of opiate receptor changes in the rat brain than those produced by chronic naloxone, i.e., selective up-regulation of  $\kappa$ -opiate receptors, these receptor changes were measured only 24 hr after the bleeding [5]. Therefore, we cannot exclude the possibility that in hypovolemic hypotension, a rapid down-regulation of opioid receptors mediating depressor responses in discrete cardiovascular nuclei might follow the activation of the endogenous opioid system, thereby promoting pressor rather than depressor responses. This suggestion is supported by studies conducted in our laboratory showing a rapid down-regulation of opiate receptors in specific areas of the heart in response to the same bleeding paradigm [6]; however, a peripheral site for the cardiovascular actions of morphine is excluded by experiments conducted in the pithed rat (in which the brain and spinal cord are effectively eliminated) which failed to demonstrate any effect of a broad selection of highly specific  $\mu$ ,  $\delta$ ,  $\kappa$ , or  $\epsilon$  opioid agonists [28,29] or morphine [29] on blood pressure, on heart rate or catecholamine release. Thus, additional studies will be necessary to clarify the mode of opioid receptor changes in appropriate brain centers which might shed light on the conversion of the depressor action of morphine to a pressor one after bleeding.

The bleeding paradigm used in this study induced marked increases in plasma catecholamines, vasopressin, and plasma renin activity as previously reported in this model [17]. Morphine, injected 30 min after the bleeding, further increased plasma epinephrine and PRA, while norepinephrine, dopamine, and vasopressin showed only some tendency for elevated plasma levels.

Increased levels of plasma catecholamines have been reported after intravenous injection of morphine in normal conscious rats [30]. Sympathoadrenomedullary activation was previously shown to underlie the cardiostimulatory effects of centrally administered  $\mu$ -agonists (which include morphine) since elevated circulating levels of catecholamines are found during the pressor/tachycardic response to  $\mu$ -opiate agonists [31-33]; also, in conscious bilaterally adrenal-demedullated rats treated with the sympathetic blocker bretylium, the hypertensive and tachycardic effects of the highly selective  $\mu$ -opioid agonist D-Ala<sup>2</sup>-MePhe<sup>4</sup>-Gly-ol<sup>5</sup>-enkephalin were substantially attenuated [32]. In addition, a central site for the sympathoadrenomedullary activation by morphine is also supported by recent studies in the pithed rat showing lack of changes in resting or stimulated levels of plasma catecholamines even after high doses (10 mg/kg) of morphine [29].

The elevation of plasma catecholamines and renin are in accord with the additional pressor response observed after morphine administration. The primary elevation of plasma epinephrine most probably mediated the increase in heart rate and possibly cardiac contractility, which support the tendency for elevated cardiac output after morphine administration. It is important to note in this regard that no signs of respiratory or metabolic depression were seen after morphine administration. In fact, blood pH, pO<sub>2</sub>, and pCO<sub>2</sub> followed the same pattern of changes in both morphine- and

saline-treated rats, and all these changes are in agreement with previously reported data [34].

Following the second bleed, morphine-treated rats displayed signs of decompensation, a precipitous decrease in CO, and hypotension. In this group of rats, the survival rate was also reduced, as previously noted in similar hemorrhagic shock experiments [14].

A possible explanation for the higher rate of mortality in morphine-treated rats could be reduced organ blood flow due to the reduced cardiac output and impaired blood flow in some essential organs. Although morphine is known to produce respiratory depression, this might not have played a role in the enhanced mortality in the morphine-treated rats since no evidence for hypoxemia, acidosis, or hypercarbia was found. The sustained activation of the sympathoadrenomedullary and the renin-angiotensin systems might reduce blood flow to essential organs, possibly the heart and kidney, and contribute to the development of irreversible shock and death. While no direct measurements of organ blood flow were made in this study, this possibility is lent support from studies showing that stimulation of the  $\mu$ -opioid receptors in the hypothalamus produces mesenteric and renal vasoconstriction and reduction in blood flow in the intact rat [35]. If this phenomenon also exists in hemorrhaged rats, it might be possible that earlier failure of essential organ blood flow underlies the detrimental outcome of the morphine treatment; however, more detailed hemodynamic studies will be necessary to elucidate the mechanism of the detrimental effect of morphine in hemorrhagic shock.

## ACKNOWLEDGMENTS

This work was supported by USUHS protocol No. R09211. The opinions or assertions contained herein are the private ones of the author(s) and are not to be construed as official or as necessarily reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. There is no objection to its presentation and/or publication. The experiments reported herein were conducted according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Medicine, National Research Council, DHEW Publication No. (NIH) 80-23, 1980. The authors wish to thank Mrs. R. Press and Ms. E. Powell for excellent technical assistance and Mrs. L. Garza for preparing this manuscript.

## REFERENCES

1. Holaday JW: Cardiovascular effects of endogenous opiate systems. *Annu Rev Pharmacol Toxicol* 23:541-594, 1983.
2. Feuerstein G: The opioid system and central cardiovascular control: Analysis of controversies. *Peptides* 6(2):51-56, 1985.
3. Elam R, Bergmann F, Feuerstein G: Simultaneous changes of catecholamines and Leu-enkephaline-like immuno-reactivity in plasma and cerebrospinal fluid of cats undergoing acute hemorrhage. *Brain Res* 303:313-317, 1984.
4. Feuerstein G, Molineaux CI, Rosenberger JG, Zerbe RL, Cox BM, Faden AI: Hemorrhagic shock and the central vasopressin and opioid peptide system of rats. *Am J Physiol* 249:E244-E250, 1985.
5. Feuerstein G, Faden AI, Krumins SA: Alteration in opiate receptor binding after hemorrhagic shock. *Eur J Pharmacol* 100:245-246, 1984.

6. Krumins SA, Faden AI, Feuerstein G: Diprenophrine binding in rat heart: Modulation of binding sites in the brain. *Biochem Biophys Res Commun* 127:120-128, 1985.
7. Faden AI, Holaday JW: Opiate antagonists: A role in the treatment of hypovolemic shock. *Science* 205:317-318, 1979.
8. Gurll NJ, Reynolds DG, Vargish T, Lechner D: Naloxone without transfusion prolongs survival and enhances cardiovascular function in hypovolemic shock. *J Pharmacol Exp Ther* 220:621-624, 1982.
9. Isoyama T, Tarsa J, Sato T, Shatney C: Effect of naloxone and morphine in hemorrhagic shock. *Circ Shock* 9:375-382, 1982.
10. Brown DF, Burr RE: Naloxone fails to reverse hemorrhagic hypotension. *Am J Physiol* 251:H468-H470, 1986.
11. Van der Meer K, Valkenburg PW, Bastiaans AC, Vleeming W, Ufkes JCR, Ottenhof M: Effect of naloxone on blood pressure and survival in different shock models in rats. *Eur J Pharmacol* 124:299-308, 1986.
12. Rockhold RW, Crofton JT, Brooks DP, Share L: Naloxone does not improve cardiovascular or blunt vasopressin responses in spontaneously hypertensive rats following graded hemorrhage. *Neuroendocrinology* 43:657-663, 1986.
13. Traverro LW, Bellamy RF, Hollenbach SJ, O'Benar JD: Naloxone does not prevent death after rapid hemorrhage in swine. *Surg Gynecol Obstet* 161:229-239, 1985.
14. Feuerstein G, Sirén A-L: Effect of naloxone and morphine on survival of conscious rats after hemorrhage. *Circ Shock* 19:293-300, 1986.
15. Chiueh CC, Kopin IJ: Hypersensitivity of spontaneous hypertensive rats to indirect measurements of blood pressure. *Am J Physiol* 234:H690-H695, 1978.
16. Feuerstein G, Bayorh MA, Goldstein DS, Zerbe RL, Ramwell PW, Faden AI: Effect of Nafazotrom on the cardiovascular, sympathetic and endocrine responses to hemorrhage shock. *Circ Shock* 17:223-232, 1985.
17. Feuerstein G, Johnson AK, Zerbe RL, Davis-Kramer R, Faden AI: Anteroventral hypothalamus and hemorrhagic shock: Cardiovascular and neuroendocrine responses. *Am J Physiol* 246:R551-R557, 1984.
18. Feuerstein G, Powell E, Faden AI: Central effects of  $\mu$ ,  $\delta$  and  $\kappa$  receptor agonists in hemorrhagic shock. *Peptides* 6:11-13, 1985.
19. Goldstein DS, Feuerstein G, Kopin IJ, Keiser HR: Validity of liquid chromatography with electrochemical detection for measuring dopamine in human plasma. *Clin Chim Acta* 117:113-120, 1981.
20. Goldstein DS, Feuerstein G, Izzo JL, Kopin IJ, Keiser HR: Validity and reliability of high pressure liquid chromatography with electrochemical detection for plasma levels of norepinephrine and epinephrine in man. *Life Sci* 28:467-475, 1981.
21. Cohen EL, Grim CE, Conn JW, Brough WM, Jr, Guyer RB, Kem DC, Lucas CP: Accurate and rapid measurement of plasma renin activity by radioimmunoassay. *J Lab Clin Med* 77:1025-1028, 1971.
22. Zerbe RL, Feuerstein G, Meyer DK, Kopin IJ: Cardiovascular, sympathetic and renin angiotensin system responses to hemorrhage in vasopressin deficient rats. *Endocrinology* 111:608-613, 1982.
23. Dixon WJ, Brown MB: "Biochemical Computer Programs, P-series." Los Angeles, CA: University of California Press, 1981.
24. Evans AGJ, Nasmyth PA, Stewart HC: The fall of blood pressure caused by intravenous morphine in the rat and the cat. *Br J Pharmacol* 7:542-552, 1952.
25. Pfeiffer A, Pfeiffer DG, Feuerstein G, Faden AI, Kopin IJ: An increase in opiate receptor-sites is associated with enhanced cardiovascular depressant action of morphine. *Brain Res* 296:305-311, 1984.
26. Bernton EW, Black LE, Ruvio BA, Holaday JW: Morphine tolerance enhances endotoxic shock susceptibility and exaggerates the pressor response to naloxone in conscious rats. *Circ Shock* 3:45 (Abst), 1984.
27. Kiang JG, Wei ET: Peripheral opioid receptors influencing heart rate in rats: Evidence for endogenous tolerance. *Regul Pept* 8:297-303, 1984.
28. Eimerl J, Feuerstein G: The effect of  $\mu$ ,  $\delta$ ,  $\kappa$  and  $\epsilon$  opioid receptor agonists on heart rate and blood pressure of the pithed rat. *Neuropeptides* 8:351-358, 1986.



29. Feuerstein G, Zukowska-Grojec Z: Effect of dermorphin and morphine on the sympathetic and cardiovascular system of the pithed rat. *Neuropeptides* 9:139-150, 1987.
30. Conway EL, Brown MJ, Dollery CT: Plasma catecholamines and cardiovascular responses to morphine and D-ala<sup>2</sup>-d-leu<sup>5</sup>-enkephalin in conscious rats. *Anch Int Pharmacodyn* 265:244-258, 1983.
31. Feuerstein G, Zerbe RL, Faden AI: Opiate receptors and cardiovascular control in conscious SHR and WKY rats. *Hypertension* 5:663-671, 1983.
32. Pfeiffer A, Feuerstein G, Zerbe RL, Faden AI, Kopin IJ:  $\mu$ -Receptors mediate opioid cardiovascular effects at anterior hypothalamic sites through sympathoadrenomedullary and parasympathetic pathways. *Endocrinology* 113:929-938, 1983.
33. VanLoon GR, Appel NM, Ho D:  $\beta$ -Endorphin induced increases in plasma epinephrine, norepinephrine and dopamine in rats: Inhibition of adrenomedullary response by intracerebral somatostatin. *Brain Res* 212:207-214, 1981.
34. Sirén A-L, Powell E, Feuerstein G: Thyrotropin releasing hormone in hemorrhage shock: Effect on cardiac output and regional blood flow in the conscious rat. *Am J Physiol* 250:H1093-H1101, 1986.
35. Sirén A-L, Feuerstein G: Opioid  $\mu$ -receptors and cardiovascular control: Effects on cardiac output and regional blood flow. *Soc Neurosci Abst* 11:996, 1985.

The experiments described in this paper were performed in adherence to the NIH guidelines for the use of experimental animals.