Activation of central opioid receptors determines the timing of hypotension during acute hemorrhage-induced hypovolemia in conscious sheep

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Frithiof, R., and M. Rundgren. Activation of central opioid receptors determines the timing of hypotension during acute hemorrhage-induced hypovolemia in conscious sheep. Am J Physiol Regul Integr Comp Physiol 291: R987–R996, 2006. First published April 20, 2006; doi:10.1152/ajpregu.00070.2006.—After an initial compensatory phase, hemorrhage reduces blood pressure due to a widespread reduction of sympathetic nerve activity (decompensatory phase). Here, we investigate the influence of intracerebroventricular naloxone (opioid-receptor antagonist) and morphine (opioid-receptor agonist) on the two phases of hemorrhage, central and peripheral hemodynamics, and release of vasopressin and renin in chronically instrumented conscious sheep. Adult ewes were bled (0.7 ml·kg⁻¹·min⁻¹) from a jugular vein until mean arterial blood pressure (MAP) reached 50 mmHg. Starting 30 min before and continuing until 60 min after hemorrhage, either artificial cerebrospinal fluid (aCSF), naloxone, or morphine was infused intracerebroventricularly. Naloxone (200 µg/min but not 20 or 2.0 µg/min) significantly increased the hemorrhage volume compared with aCSF (19.5 ± 3.2 vs. 13.9 ± 1.1 ml/kg). Naloxone also increased heart rate and cardiac index. Morphine (2.0 µg/min) increased femoral blood flow and decreased hemorrhage volume needed to reduce MAP to 50 mmHg (8.9 ± 1.5 vs. 13.9 ± 1.1 ml/kg). The effects of morphine were abolished by naloxone at 20 µg/min. It is concluded that the commencement of the decompensatory phase of hemorrhage in conscious sheep involves endogenous activation of central opioid receptors. The effective dose of morphine most likely activated µ-opioid receptors, but they appear not to have been responsible for initiating decompensation as 1) naloxone only inhibited an endogenous mechanism at a dose much higher than the effective dose of morphine, and 2) the effects of morphine were blocked by a dose of naloxone, which, by itself, did not delay the decompensatory phase.

naloxone; morphine; intracerebroventricular; vasopressin; renin

IN CONSCIOUS MAMMALS THE HEMODYNAMIC response pattern to acute hemorrhage consists of, at least, two phases (40). Initially, the arterial blood pressure is well maintained because of an increased baroreceptor-mediated vasoconstrictor drive and tachycardia (compensatory phase). If hemorrhage continues beyond a critical point, a widespread decrease in vascular resistance in combination with a decrease in heart rate causes the arterial blood pressure to fall (decompensatory phase).

Since Faden and Holaday (16) demonstrated that intravenous administration of the nonspecific opioid receptor antagonist naloxone increases arterial blood pressure in hemorrhagic shock, a number of studies have been conducted using naloxone as a treatment in sepsis and hemorrhage (see Ref. 6). The usefulness of naloxone in the clinical setting is still under evaluation (3), but the studies have revealed important information regarding the physiology of hemorrhage. Among other things, it was found that naloxone prevented the decompensatory phase in conscious rabbits (4, 26) and that the site of action is in the brain (47). This, together with the observation that similar doses of naloxone do not have a hypertensive effect in normovolemic animals (41), suggests a central opioid mechanism for the initiation of the decompensatory phase. Moreover, contradictory results have been obtained regarding species, dose, and anesthesia (40). One major problem is that general anesthesia distorts and sometimes abolishes the normal response pattern to hemorrhage (17, 28, 36). In addition, anesthesia also severely alters cardiovascular responses to exogenous administered opioids (38). This makes it preferable to study the central mechanisms of the decompensatory phase in unanesthetized animals. Studies on central opioidergic mechanisms in hemorrhage, using conscious animals, have been made in rats, rabbits, and sheep. In hypovolemic rabbits, the increase in arterial blood pressure by central naloxone was found to be mediated by increased sympathoadrenal activity and peripheral vasoconstriction (45). Naloxone postponed the fall in arterial blood pressure, by an unknown mechanism, in conscious rats subjected to hemorrhage (31). In the only study in conscious sheep, centrally administered naloxone did not affect the cardiovascular responses to hemorrhage (5), but the dose was lower than that used in other species.

Paradoxically, morphine, primarily a µ-opioid receptor agonist, given intracerebroventricularly, has also been shown to delay or abolish the decompensatory phase (34). This indirectly suggests that naloxone exerts its effect in preventing the decompensatory phase by acting on δ- or κ-opioid receptors. However, this study was done in anesthetized rats and the question remains whether centrally administered morphine affects the cardiovascular response to hemorrhage in conscious animals.

Considering the conflicting data concerning the significance and effects of central endogenous and exogenous opioids in hemodynamic control during bleeding and the difficulties associated with studies of the central nervous system (CNS) mechanisms responsible for hemorrhagic hypotension in anesthetized animals, we decided to investigate the effects of different doses of centrally administered naloxone and morphine on the decompensatory phase of hemorrhage in conscious, unrestrained sheep. We hypothesized that central opioidergic mechanisms are involved in causing the decompensatory phase of hemorrhage in conscious sheep.

METHODS

The experimental protocol was approved in advance by the regional ethics committee in Stockholm and adheres to “European
Convention for Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Council of Europe No 123, Strasbourg 1985) as well as the “APS Guiding Principles in the Care and Use of Animals.”

**Animals and Surgical Preparation**

Nine Adult Texel cross-bred ewes weighing 49 to 79 kg (mean 64 ± 10 kg) were used. The animals were housed in individual cages, where they were fed twice a day and had free access to water and a salt block. Within a minimum of 7 wk before commencement of experimentation, the sheep were anesthetized, and their carotid arteries were exteriorized and placed in cervical skin loops bilaterally. Standard surgical anesthesia included premedication with acepromazin (0.3 mg/kg iv), anesthesia induction with sodium thiopental injection (10 mg/kg iv), followed by succinylcholine (1 mg/kg iv) and maintenance with isoflurane (2.1–2.3% end tidal concentration) in an O₂/air mixture (40/60) via a respirator. After 2 wk of recovery, the animals were anesthetized in the same manner and supplied with two permanent stainless-steel guide cannulas placed with their tip 2–3 mm above the lateral ventricles. In the final surgical preparation (after another 2 wk recovery period) ultrasonic flow probes (Transonic System, Ithaca, New York) were placed unilaterally around the left renal and right femoral artery, respectively. Four animals were also, at the same time, equipped with a third flow probe around the superior mesenteric artery (cranial mesenteric artery in quadrupeds). Connecting tubes were tunneled subcutaneously, and their connectors placed at a paralumbar position. Postoperative treatment for two days with buprenorphine (0.002 mg/kg im) and benzylpenicillin (20,000 IU/kg)/dihydrostreptomycin (0.0025 g/kg) were made routinely.

**Experimental Preparation**

The experiments were performed with the animals standing in a cage in their habitual environment. Intravascular catheterizations were made at least 60 min before starting the experiments and under local anesthesia (lidocaine hydrochloride 5 mg/ml). A cannula (OD 1.0 mm) was inserted in one of the exteriorized carotid arteries for measurement of arterial blood pressure and blood sampling. A central venous catheter was introduced through the right jugular vein and used for blood removal and retransfusion. Finally, a flow-directed thermodilution catheter (Swan-Ganz, Edward Labs, Santa Ana, CA) was inserted in one of the exteriorized carotid arteries for measurement of arterial blood pressure via an introducer in the right jugular vein and used for measuring mean pulmonary artery pressure (MPAP), central venous pressure (CVP), cardiac output (CO), and mixed venous oxygen saturation (SVO₂).

**Hemodynamic Recordings**

The different blood pressures were continuously measured via pressure transducers (DPT-6003, PVB Medizin Technik, BMBH, Kirchseen, Germany). Signals from the thermodilution catheter were fed into a Vigilance Edwards Critical Care Monitor (Baxter Healthcare, Irvine, CA), where the CO was calculated every 30 s and SVO₂ continuously. The flow probes were connected to two Transonic T208 two-channel flowmeters, and renal blood flow (RBF), femoral blood flow (FBF), and superior mesenteric blood flow (SMBF) were recorded continuously. Continuous online data acquisition was achieved by using the MP150/Acknowledge 3.7.2 system (BIOPAC Systems, Goleta, CA) with a sampling rate of 100 Hz. Heart rate (HR) and mean arterial pressure (MAP) were computed from the arterial blood pressure signal and displayed online.

**Intracerebroventricular Infusions**

Intracerebroventricular infusions were made by lowering a probe of suitable length into one of the permanently placed guide tubes. Free communication with the cerebrospinal fluid was assured by gentle siphoning of an artificial cerebrospinal fluid (aCSF)-filled tube. For infusion, another silicone tube connected the probe to a 5-ml syringe placed in an infusion pump (802 Syringe pump, Univentor, Żejtun, Malta). The intracerebroventricular infusion rate (20 μl/min) corresponds to 15–25% of the total CSF production rate in sheep (37).

Morphine chloride (AstraZeneca, London, UK) or naloxone hydrochloride (Tocris, Bristol, UK) were dissolved and diluted to the required concentrations in sterile aCSF. The aCSF solution had the following composition (in mM): Na⁺, 150; K⁺, 2.9; Ca²⁺, 1.1; Mg²⁺, 0.9; Cl⁻, 155; HCO₃⁻, 24; HPO₄²⁻/H₂PO₄⁻, 0.5 with a pH of 7.39.

**Experimental Protocol**

The protocol for the experiments is illustrated in Fig. 1.

**Dose titration experiments.** The first experiments were made to establish the doses to be used in later experiments. The aim was to determine the lowest intracerebroventricular dose of morphine and naloxone able to affect the occurrence of the decompensatory phase during a venous hemorrhage. Two sheep were each subjected to four hypotensive hemorrhages, concomitant with different intracerebroventricular infusions, performed at 7-day intervals. The procedure was similar to that used in the main protocol (see Fig. 1 and below). The intracerebroventricular infusions (20 μl/min) were aCSF (control) and morphine at doses of 0.2, 2.0, and 20 μg/min, respectively (Sheep 1) and aCSF (control) and naloxone at a dose of 2.0, 20, and 200 μg/min, respectively (Sheep 2).

**Main experiments.** The animals were subjected to an intracerebroventricular infusion (20 μl/min) of either aCSF (control; n = 6), morphine 2.0 μg/min (MOR; n = 6), naloxone 2.0 μg/min (NLX 2; n = 4), or naloxone 200 μg/min (NLX 200; n = 6) before, during, and after a hypotensive hemorrhage (Fig. 1). The doses of morphine and naloxone were based on previous experiments in sheep (naloxone 200 μg/min) (5) and dose-titration experiments (morphine 2.0 μg/min and naloxone 200 μg/min).

![Fig. 1. Experimental protocol for the dose titration- and main experiments. An intracerebroventricular infusion of either artificial cerebrospinal fluid (aCSF), morphine, or naloxone was started after baseline cardiovascular recordings. The doses of morphine and naloxone, and the number of experiments, are presented for the four different treatments in the main experiment. After 30 min of infusion a venous hemorrhage commenced and continued until the mean arterial blood pressure (MAP) had dropped to <50 mmHg. Thereafter, the animals were allowed to spontaneously recover for 60 min before the intravenous infusion was discontinued and the blood retransfused.](http://ajpregu.physiology.org/)

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All intracerebroventricular infusions were preceded by a 10-min hemodynamic sampling period. Circulatory effects of the infusion per se were followed for 30 min before hemorrhage commenced. Thereafter, blood was aspirated from the left jugular cannula at 0.7 ml·kg⁻¹·min⁻¹ until the decompensatory phase occurred, defined as a sudden drop in MAP to 50 mmHg. Recovery was studied for 60 min followed by retransfusion. The intracerebroventricular infusions were stopped at the beginning of retransfusion (total infusion time: 90 min + hemorrhage time). Blood samples (17 ml of venous blood and 2 ml of arterial blood) were drawn at five separate occasions: before the infusion started, at start of hemorrhage, at end of hemorrhage, 30 min after hemorrhage was stopped, and finally 60 min after hemorrhage stopped. The minimum interval between experiments was 7 days.

Additional experiments. A separate experiment was performed in one sheep to 1) rule out any hypotensive effects of the current dose of morphine and 2) further confirm the hemodynamic effects of morphine per se. The animal was prepared in the same manner as in the main experiments, and a 60-min recovery period after connecting the catheters and probes was allowed. An intracerebroventricular infusion (20 µl/min) of morphine (2.0 µg/min) was started after 10 min of baseline registration and was discontinued after 150 min. Hemodynamic recordings were performed, and the same parameters as in the main experiments were measured.

To ensure that the hemodynamic effects of morphine were elicited by activation of opioid receptors, an additional experiment was performed. The protocol was equivalent to the main experiments apart from the type of intracerebroventricular infusion. The animal was given an intracerebroventricular infusion of naloxone only (1.0 mg/ml; 0.3 ml in 10 min) before commencement of an intracerebroventricular infusion (20 µl/min) of morphine (0.1 mg/ml) and naloxone (1.0 mg/ml).

Blood and Plasma Analyses

The venous blood was portioned into prechilled tubes with heparin, respectively, EDTA as anticoagulants and centrifuged at +4°C (3,000 rpm). Plasma aliquots were stored at −20°C until assayed for vasopressin concentration and renin activity. Other portions were taken for determination of hematocrit, plasma osmolality (Auto & Stat Om 6010 osmometer; Kagaku, Japan), and protein concentration by refractometry (Atago, Japan). The carotid blood samples were used for immediate arterial blood gas analyses, and determinations of sodium and potassium concentrations (ion optodes using ion-selective ionophores) were performed on an Opti Critical Care analyzer (AVL Scientific, Roswell, GA).

Commercial radioimmunoassay kits were used for determinations of plasma vasopressin concentration (Euria-Vasopressin; Euro-Diagnostica, Malmö, Sweden) and plasma renin activity (PRA; Rianen ANG II[125I] RIA kit, New England Nuclear, DuPont Scandinavia, Västervik, Sweden).

Calculations and Statistical Analysis

Cardiovascular parameters were averaged off-line using a moving window of 2,500 data points (25 s). Each value in the window was replaced with the mean value of the whole window. Renal vascular resistance (RVR), femoral vascular resistance (FVR), and superior mesenteric arterial resistances (SMVR) were calculated by dividing the difference between MAP and CVP by the RBF, FBF, and SMBF, respectively. Cardiac output was divided by body weight to obtain cardiac index. Statistical analyses were performed with Statistica 7.1 (Statsoft). Data are expressed as means ± SD or as confidence intervals (CI). Differences in hemorrage volumes between groups were evaluated with one-way ANOVA. Changes in parameters over time were analyzed according to a two-way repeated-measures ANOVA, the repeating variable being time. If the overall F ratio was significant, Tukey’s post hoc test was used for comparisons of means. In case of significant interaction between time and intracerebroventricular infusion, simple main effects were examined. As this procedure consists of multiple testing, the P values were adjusted according to Bonferroni (1, 24). Differences were considered significant at P ≤ 0.05.

RESULTS

The animals showed no signs of obvious discomfort during the experiments. When the decompensatory phase occurred, the sheep usually calmly lay down. The respiratory rate was not monitored, but it was noted to increase in association with the occurrence of hypotension.

The baseline plasma concentrations of Na⁺, K⁺, and protein, as well as plasma osmolality and hematocrit, were all within physiological limits in all groups. Blood gases, pH, and base excess did not change significantly during the course of the experiment in any of the groups. However, there was a tendency toward a respiratory alkalosis (increase in pH and decrease in pCO₂).

Dose Titration Experiments

Typical MAP responses to the different doses of morphine and naloxone are illustrated in Fig. 2. Morphine, at the rate of 2.0 µg/min and 20 µg /min, decreased the hemorrhage volumes needed to induce decompensation compared with control (Fig. 2A). Naloxone (200 µg/min) increased the hemorrhage volumes needed to induce decompensation compared with control (Fig. 2B).

Effects of intracerebroventricular naloxone and morphine on the appearance of decompensation. Hemorrhage volumes needed to reduce MAP to 50 mmHg, that is, induce decompensation are shown in Fig. 3. Statistical evaluation revealed a highly significant main effect for intracerebroventricular infusion treatment, F(3,18) = 25.7, P < 0.001. Compared with control (icv aCSF), 37% less blood loss was required for decompensation to occur in sheep treated with morphine intracerebroventricularly (2.0 µg/min) (8.9 ± 1.5 ml/kg, n = 6 vs. 13.9 ± 1.1 ml/kg, n = 6, P < 0.001). This effect of morphine at 2.0 µg/min was abolished by concomitant infusion of naloxone at a dose of 20 µg/min. (Fig. 2A).

Intracerebroventricular naloxone had the opposite effect compared with that of morphine, but only in the animals treated with the highest dose (200 µg/min). With this dose of naloxone, the hemorrhage volumes needed to induce hypotension was on average 40% larger than in control animals (19.5 ± 3.1 ml/kg, n = 6 vs. 13.9 ± 1.1 ml/kg, n = 6, P < 0.001). The low dose of naloxone (2.0 µg/min) did not affect the onset of decompensation compared with control animals (13.1 ± 2.0 ml/kg, n = 4 vs. 13.9 ± 1.1 ml/kg, n = 6, P = 0.96).

The average hemorrhage time for the different treatments was as follows: 13 min (MOR), 19 min (aCSF), 20 min (NLX 2.0), and 28 min (NLX 200).

Cardiovascular effects of intracerebroventricular naloxone and morphine per se. At baseline, there were no significant cardiovascular differences between groups. In control animals (icv aCSF) and the NLX 2.0 groups, all cardiovascular parameters measured remained unchanged during the prehemorrhage infusion period. In contrast, the higher dose of naloxone (200 µg/min) significantly affected MAP, cardiac index, and HR (Table 1 and Fig. 4). On average, MAP increased by 10%
caused a marked vasodilation of the femoral artery; FVR was reduced by 33% (mean 0.35 mmHg/ml, 95% CI 0.06 to 0.63, n = 6, P = 0.02) (Fig. 5), increasing the blood flow from an average of 90 ± 37 ml/min to 145 ± 49 ml/min (P = 0.02). Naloxone at 20 μg/min abolished the effect of morphine on FVR (n = 1).

The decrease in FVR and increase in FBF was also evident in the single experiment where morphine 2.0 g/min was infused ICV without concomitant hemorrhage. As illustrated in Fig. 6, there was a progressive vasodilation during the first 50 min of infusion until the blood flow stabilized just below 200 ml/min. MAP remained at control levels during the entire infusion period.

Cardiovascular responses to hemorrhage. Representative, original tracings from one animal illustrating the MAP and HR responses to hemorrhage when subjected to the different intracerebroventricular infusions are shown in Fig. 7. In all three experiments, the two different phases of hemorrhage are easily detectable in both MAP and HR recordings, that is, MAP was maintained together with an increase in HR until the decompensatory phase was elicited when both MAP and HR fell. Also, the effects of morphine (reduction) and naloxone (200 μg/min) (increase) on hemorrhage volume necessary to induce decompensation are evident.

In Figs. 4 and 5, the cardiovascular responses to hemorrhage are not plotted on a time scale. Because there is a significant individual variation in hemorrhage, tolerance expressing the cardiovascular parameters as a function of time or volume would make the transition from the compensatory and decompensatory phase difficult to distinguish. Instead, the individual values of each animal at different stages of hemorrhage are grouped according to intracerebroventricular infusion and expressed as means and 95% CIs. The animals are, however, at largely different degrees of hypovolemia at corresponding stages of hemorrhage (compare Fig. 7).

In the NLX 200 group, MAP was elevated at the beginning of hemorrhage but during the decompensatory phase, it fell to the same level as controls (Fig. 4). The characteristic mainte-
Intracerebroventricular infusion of artificial cerebrospinal fluid, morphine, or naloxone

Table 1. Heart rate and systemic vascular resistance in conscious sheep subjected to hemorrhage and an intracerebroventricular infusion of artificial cerebrospinal fluid, morphine, or naloxone

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline</th>
<th>Start Hemorrhage</th>
<th>Maximum Value During Comp.</th>
<th>Minimum Value During Decomp.</th>
<th>End Hemorrhage +30</th>
<th>End Hemorrhage +60</th>
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<tr>
<td>aCSF</td>
<td>6</td>
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<td>116±21*</td>
<td>65±4</td>
<td>68±9</td>
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<td>103±16*</td>
<td>58±12</td>
<td>68±5</td>
<td>89±17</td>
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<td>4</td>
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<td>70±4</td>
<td>124±23*</td>
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<td>64±7</td>
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<td>NLX 200</td>
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<td>89±9*†</td>
<td>169±28*†</td>
<td>96±32*†</td>
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<td>17±6</td>
<td>17±4</td>
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Values are expressed as means ± SD. Effects of intracerebroventricular infusions (20 µl/min) of artificial cerebrospinal fluid (aCSF), morphine 2.0 µg/min (MOR), naloxone 2.0 µg/min (NLX 2.0) and naloxone 200 µg/min (NLX 200) on heart rate (HR) and systemic vascular resistance (SVR) before, during, and after a slow (0.7 ml/min kg⁻¹) hypotensive hemorrhage in conscious sheep. HR and SVR during hemorrhage are expressed as the maximum value during the compensatory phase (Comp.) of hemorrhage and the minimum value during the compensatory phase (Decomp.) of hemorrhage. The infusion started 30 min before hemorrhage and lasted during the whole experiment. The hemorrhage was stopped when the mean arterial blood pressure was reduced to 50 mmHg. See METHODS for calculation of SVR. *P < 0.05 for difference from baseline. †P < 0.05 for difference from control (aCSF).

HR increased in parallel with the level of hypovolemia in all groups. Naloxone (200 µg/min) postponed the compensatory phase and augmented the tachycardia seen in the compensatory phase (Table 1). In addition, naloxone (200 µg/min) attenuated, but did not prevent, the fall in HR typical for the compensatory phase (Table 1). SVR did not increase during the compensatory phase and did not differ between control animals and any of the treatment groups. Irrespective of intracerebroventricular infusion, SVR fell at the time of decompen-
sation (Table 1).

Cardiac index was largely unaffected during hemorrhage, although in the NLX 200 group, a statistically significant reduction was obtained (mean 17.0 ml·min⁻¹·kg⁻¹ between 20 and 100% of total hemorrhage, 95% CI 5.0 to 28.9, n = 6, P = 0.008) (Fig. 4). However, in the minutes after onset of hypotension, the cardiac index dropped significantly in all groups and remained reduced. Considering the rather slow response time of the equipment used to measure CO, it is likely that the substantial reduction in cardiac index coincided with the compensatory phase.

In Fig. 5, the resistance in three different vascular beds is illustrated. RVR remained unchanged during the compensatory phase. In the NLX 200 group, RVR rose significantly (P = 0.006, n = 6) during the compensatory phase (80–100% of hemorrhage, Fig. 5). The apparent increase in RVR during decompen-
sation in the other groups was not statistically significant.

There was no significant change in FVR during the compensatory phase. However, decomposition was associated with a reduction in FVR (P = 0.04, n = 22) that was independent of intracerebroventricular treatment. Hemorrhage caused SMVR to increase progressively (P = 0.02, n = 13) (Fig. 4) until decomposition. Like FVR, SMVR fell significantly between 80% and 100% of hemorrhage (P = 0.02, n = 13). Apart from influencing the timing of the decompen-
sation phase, none of the treatments had an effect on FVR or SMVR during hemorrhage.

Hemorrhage progressively reduced CVP in all groups (Fig. 4). However, at a blood loss of 8.9 ml/kg (i.e., the volume necessary to cause decomposition in the MOR group), the fall in CVP was more pronounced in sheep treated with morphine compared with the NLX 200 group (mean 2.8 mmHg, 95% CI 0.6 to 5.1, n = 6, P = 0.02). This might reflect a tendency for a more pronounced central hypovolemia in the morphine-
treated animals.

The SvO₂ and MPAP showed no intergroup differences. During hemorrhage, both variables were well maintained (data not shown) until the decompen-
satory phase occurred, which caused a reduction from 74.1 ± 0.9% to 57.8 ± 1.4% (P < 0.001, n = 22) and from 15.3 ± 0.7 mmHg to 11.9 ± 0.8 mmHg (P = 0.004, n = 22), respectively.

Posthemorrhage recovery

_Hemodynamics._ Despite a larger degree of hypovolemia, animals treated with naloxone (200 µg/min) recovered faster with respect to MAP, and 30 min after the end of hemorrhage, MAP had increased significantly more than in the control group (mean increase 13.6 mmHg, 95% CI 0.5 to 26.8 mmHg, P = 0.04, n = 6) (Fig. 4).

Controls and animals treated with morphine or the lower dose of naloxone (2.0 µg/min) did not have tachycardia during the course of recovery (Table 1). In the NLX 200 group, however, heart rate remained elevated for the remainder of the posthemorrhage recording period (Table 1). As noted above, cardiac index fell and remained below baseline values after end of hemorrhage. There was a slight recovery during the last 30 min of the posthemorrhage recovery period (Fig. 4). These changes did not differ between groups.

The vascular resistance tended to increase during 30 min after hemorrhage, except in the renal artery in the NLX 200 group and the femoral artery in the morphine-treated animals (Fig. 5 and Table 1). In the latter group (MOR), no change in FVR was seen during the posthemorrhage recovery period (Fig. 5). Apart from this exception, all vascular resistances were at baseline levels in all groups at the end of the experiment.

CVP increased gradually, but only animals in the MOR-group reached prehemorrhage levels. The recovery of MPAP...
and SvO₂ were faster, and at 60 min, all groups were at baseline levels.

**AVP and PRA.** None of the infusions caused changes in PRA or plasma arginine vasopressin (P-AVP) levels per se (Fig. 8). The P-AVP concentration increased in response to hypotensive hemorrhage in all groups (*P* < 0.001, *n* = 6 in groups aCSF, NLX 200, MOR, *n* = 4 in NLX 2.0, Fig. 8). In animals treated with naloxone (200 μg/min), the plasma level of AVP was significantly higher than in controls (+645 pmol/l, 95% CI 380 to 910, *P* = 0.03, *n* = 6) at end of hemorrhage (Fig. 8). Changes in PRA followed the same pattern; it increased during hemorrhage (*P* < 0.05, *n* = 6 in all groups) (with the exception of NLX 2.0; *P* = 0.06, *n* = 4), and the high dose of naloxone (200 μg/min) augmented the response (+1.8 ng ANG I/ml/h, 95% CI 0.7 to 2.9, *P* = 0.04, *n* = 6) (Fig. 8). During the posthemorrhage recovery period, the AVP and PRA levels in the treatment groups did not significantly differ from controls (Fig. 8).

**DISCUSSION**

In this study, we demonstrate that intracerebroventricular naloxone at 200 μg/min, but not 0.2 or 2.0 μg/min, are able to postpone the onset of the decompensatory phase of a continu-
ous hemorrhage in conscious sheep and thus prolong the maintenance of blood pressure and blood flow to vital organs. Furthermore, it significantly attenuates the associated bradycardia and facilitates a more rapid recovery of arterial blood pressure. Intracerebroventricular morphine, on the other hand, causes the decompensatory phase to occur at an earlier stage of blood loss. That central opioid receptor blockade with naloxone is able to postpone the decompensatory phase in rats and rabbits is well known (2, 14) but has previously not been reported in sheep. The opposite effect obtained here by intracerebroventricular morphine is also a novel observation in sheep and is in contrast to that observed in other species.

Hypotension and bradycardia are reported to occur at a blood loss corresponding to 20–30% of the estimated blood volume in all mammalian species studied (40). The present results show that the sheep is no exception in this regard. As a ruminant, the estimated blood volume in sheep is somewhat lower in relation to body weight (60 ml/kg) than in other mammals (43).

Effects of Intracerebroventricular Naloxone

There are three well-established different opioid receptor types in the CNS; μ, κ, and δ. They are all G-protein coupled and control cell activity through ion-gating and by regulating intracellular Ca^{2+} levels, adenylyl cyclase, and the mitogen-activated protein (MAP) kinase cascade (25). Naloxone and morphine both show a significantly higher binding affinity for μ-receptors than for κ-receptors (10- and 20-fold, respectively) and δ-receptors (20-fold) (18). Opioid receptors are widespread throughout the CNS (27), and endogenous activation occurs in many situations, including hemorrhagic shock (32). Because opioid signaling in the brain stem and the hypothalamus also have implications for the neural control of blood pressure (9, 19) an attractive possibility is that endogenous opioids acting in the CNS are somehow involved in inducing the decompensatory phase. This question has been addressed in a number of studies, primarily made in rats and rabbits (for a review, see Refs. 23 and 40). The extensive work of Evans et al. (14) on the subject has shown that the decompensatory phase can be prevented by naloxone in conscious rabbits undergoing a simulated hemorrhage and possibly via sites of action in the brain stem (45). Further investigations using selective opioid-receptor antagonists has lead to the hypothesis that circulatory decompensation depends on stimulation of δ-receptors in the brain stem (15) or the spinal cord (2). The results in the present study also suggest a role for the central opioid system in the decompensatory phase of hemorrhage in conscious sheep, as naloxone delayed the onset of bradycardia and hypotension and morphine had the opposite effect. Our data could be interpreted as reflecting modulation of μ-receptor-mediated mechanisms, as the antagonist naloxone, as well as the agonist morphine has the greatest affinity for this receptor. However, considering the binding affinity and selectivity of the drugs in combination with the high dose of naloxone needed to postpone circulatory decompensation, it appears less likely that naloxone exerted its effect by blocking the μ-receptor. Also, naloxone at a dose ineffective by itself

![Figure 6](http://ajpregu.physiology.org/)

Fig. 6. Original recordings in one conscious sheep showing the effects of intracerebroventricular morphine (2.0 μg/min) on MAP, FVR, and femoral arterial blood flow (FABF) without concomitant hemorrhage. The dashed line represents the start of intracerebroventricular infusion (20 μl/min).

![Figure 7](http://ajpregu.physiology.org/)

Fig. 7. Blood pressure and heart rate responses to hemorrhage in one sheep subjected to different intracerebroventricular infusions: 1) intracerebroventricular morphine (2.0 μg/min), 2) intracerebroventricular artificial cerebrospinal fluid (aCSF), and 3) ICV naloxone (200 μg/min). The infusion was started 30 min before the hemorrhage.
Naloxone has been shown to reverse the attenuation of renal sympathetic nerve activity during hemorrhage by a central mechanism (47), but there is no evidence that it actually interacts with the neuronal circuitries responsible for the decompensatory phase. It is possible that intracerebroventricular naloxone gives rise to an increased activity in presympathetic neurons by pathways anatomically and functionally separate from those responsible for sympathoinhibition during hemorrhage. The results in this study partly support such a mechanism, as HR and cardiac index were significantly elevated by a high dose of naloxone per se, indicating an increased sympathetic nerve activity and/or decreased vagal activity to the heart before hemorrhage. However, given that there is no rise in vascular resistances [neither systemically (SVR) nor in any of the monitored vascular beds; RVR, FVR, SMVR] or P-AVP levels before hemorrhage, the change in autonomic nerve activity by intracerebroventricular naloxone in sheep appears to be limited to the heart.

During hemorrhage, intracerebroventricular naloxone prolonged the compensatory phase. The augmented increase in heart rate probably reflects a more pronounced hypovolemia but also the direct effect of naloxone. Presumably, the tachycardia helped counteract the diminished stroke volume and prevent a substantial decrease in CO. However, it appears unlikely that the shifting of the decompensatory phase was solely dependent on naloxone’s cardio-stimulatory effects and not its ability to inhibit cerebral mechanisms normally initiating hypotension. First, it has been shown in man that the fall in blood pressure appears, even though the bradycardia is present, that hypotension is dependent on a decrease in peripheral resistance. Second, in the present study, the peripheral vasodilation appears synchronous with the hypotension. If the effect of naloxone was to postpone hypotension via an increase in heart rate, the decrease in total peripheral resistance would have occurred at similar hemorrhage volumes as in control animals.

Thus we conclude that naloxone in high concentrations antagonized endogenous opioid mechanisms, which unopposed are involved in causing the peripheral vasodilation typical for the decompensatory phase. It is notable that the typical biphasic response pattern to hemorrhage remained, meaning that naloxone was only able to influence the timing, not the characteristics of the decompensatory phase.

Recent studies using microinjections, extracellular recordings, and immediate early gene expression have identified sites of importance for controlling efferent preganglionic sympathetic nerve activity, such as the paraventricular nuclei, the caudal midline medulla, ventrolateral periaqueductal gray, and the rostral ventrolateral medulla (8, 10, 11, 20, 42). All of these sites are situated close to the CSF compartment.

The increases in P-AVP concentrations and PRA by hemorrhage were augmented by the high dose of naloxone. It has been shown that peripherally administered naloxone augments AVP responses to several different stimuli (39). The inhibitory influence of opioids on the hormone release has been shown to
Effects of Intracerebroventricular Morphine

The premature development of the decompensatory phase caused by morphine intracerebroventricularly observed here is in contrast to what has been previously reported in rats and rabbits (13, 34, 35). In those species, circulatory decompensation is prevented by central stimulation of μ-opioid receptors. The disparity could be attributable to the major species differences in the distribution of opioid receptors in the brain (30) and also the relative proportion of receptor subtypes in various brain regions (29).

The nervous mechanisms that trigger the transition between compensated and decompensated hemorrhage remains unclear (12), but it is well established that the appearance of hypotension is strongly related to central hypovolemia (40). The inability of 2.0 and 20 μg/min naloxone intracerebroventricularly to extend the compensatory phase of hemorrhage suggests that μ-opioid receptors are not part of the CNS mechanism involved in initiating bradycardia and hypotension. Hence, the effect of morphine observed here is not likely due to a reinforcement of an endogenous opioid mechanism activated by hemorrhage. Instead, it is possible that the cardiovascular effects of morphine intracerebroventricularly changed the stimulus for the reflex by producing a peripheral vasodilation (Figs. 5 and 6), thus reducing central blood volume. Assessed in terms of CVP, the morphine group had a tendency toward a more rapid decrease in central blood volume during hemorrhage compared with the control group (significant compared with NLX 200 group). The decrease in peripheral vascular resistance by morphine is consistent with what has been reported in humans, where the effect is substantial (7). Furthermore, intravenous morphine has repeatedly been shown to inhibit the baroreceptor-heart rate reflex in man and animals (21), and although our results do not clearly indicate such a mechanism by intracerebroventricular morphine in sheep, it cannot be ruled out as a contributing mechanism to the fall in blood pressure.

Cardiac Index and Blood Flows

In contrast to other hemorrhage studies in conscious rabbits, dogs, and humans, we found that the cardiac index was well maintained in sheep during the compensatory phase. Stroke volume decreased owing to diminished venous return, but this was compensated by increased heart rate. A fall in cardiac index was not evident until the appearance of the decompensatory phase. This appears not to be the result of an erroneous measurement due to a slow response time of the CO monitor, as there were no major reductions in peripheral flows and SvO2 concomitant to hemorrhage, suggesting a more or less unaffected cardiac index. Furthermore, the gradual decrease in cardiac index, well correlated to the degree of hypovolemia, which is typically seen in response to hemorrhage in anesthetized sheep, has been recorded using the same equipment (17). Therefore, the time resolution of the CO computer to calculate the cardiac index during hemorrhage in these experiments seems sufficient.

Besides a modest increase in SMVR, no vasoconstrictor was seen during the compensatory phase. Consequently, the hypovolemic conscious sheep initially appears to defend the blood pressure more by preserving CO and less via peripheral vasoconstriction than that seen in other species.

Vascular resistance in the femoral and superior mesenteric artery dropped when decompensation started, coherent with the typical sympathoinhibition seen during hemorrhage. In the renal circulation, the opposite event takes place, an increase in vascular resistance, most prominent in the NLX 200 group. This is most likely not a neural mechanism since renal sympathetic nerve activity is known to decrease during decompensation. The levels of PRA in this study, on the other hand, increased during hemorrhage in correlation with the degree of vasoconstriction in the renal artery, and ANG II is known to increase vascular resistance in the kidney in conscious sheep (44). This indicates that a humoral factor counteracts the diminished sympathetic nerve activity to kidney vessels but not to the arteries in the gut or hindlimb.

In conclusion, endogenous activation of CNS opioid receptors initiates hypotension and bradycardia during hemorrhage in conscious sheep. In accordance with previous studies in other species, these opioid receptors appear to be of the κ- and/or δ-type. Opioid receptor blockade also results in increased cardiac performance.

The results in this study suggest species differences in response to exogenous activation of central μ-opioid receptors during hemorrhage, as results from studies in rats and rabbits show that this abolishes hemorrhage-induced hypotension and bradycardia. In sheep, these events are provoked by intracerebroventricular morphine, possibly through peripheral vasodilation.

In addition it is noted that the compensatory phase of hemorrhage in conscious sheep relies mainly on mechanisms aimed at maintaining CO.

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