Glycyl-L-glutamine [β-endorphin-(30—31)] attenuates hemorrhagic hypotension in conscious rats

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Owen, Medge D., Sibel Gürün, Gary P. Zaloga, and William R. Millington. Glycyl-L-glutamine [β-endorphin-(30—31)] attenuates hemorrhagic hypotension in conscious rats. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R1598–R1606, 1997.—The profound hypotension caused by acute hemorrhage is thought to involve opioid peptide neurons. In this study, we tested whether glycyl-L-glutamine [Gly-Gln; β-endorphin-(30—31)], a nonopioid peptide derived from β-endorphin processing, prevents the cardiovascular depression induced by hemorrhage in conscious and anesthetized rats. Previously, we found that Gly-Gln inhibits the hypotension and respiratory depression produced by β-endorphin and morphine but does not affect opioid antinociception. Hemorrhage (2.5 ml/100 g body wt over 20 min) lowered arterial pressure in conscious rats (from 120.1 ± 2.9 to 56.2 ± 4.7 mmHg) but did not change heart rate significantly. Intracerebroventricular Gly-Gln (3, 10, or 30 nmol) pretreatment inhibited the fall in arterial pressure and increased heart rate significantly. The response was dose related and was sustained during the 35-min posthemorrhage interval. Pentobarbital sodium anesthesia potentiated the hemodynamic response to hemorrhage and attenuated the effect of Gly-Gln. Gly-Gln (10 or 100 nmol icv) did not influence arterial pressure or heart rate in normotensive rats. These data indicate that Gly-Gln is an effective antagonist of hemorrhagic hypotension.

opioid; dipeptide; posttranslational processing; proopiomelanocortin; cardiovascular

SINCE THE LATE 1970s, opioid peptides have been implicated in the development of cardiovascular depression during hemorrhagic shock (18, 38, 48). This concept originated from the finding that naloxone, an opioid receptor antagonist, reverses the precipitous fall in arterial pressure induced by hemorrhage (8) or endotoxemia (19). Opioid receptor antagonists attenuate hemorrhagic hypotension whether injected intravenously (8, 19) or intracerebroventricularly, indicating that their site of action is, at least in part, within the central nervous system (7, 20). These findings support the hypothesis that severe hemorrhage activates opioid peptide neurons, although the specific neuronal pathways involved in hemorrhage have yet to be fully elucidated.

Several lines of evidence suggest that β-endorphin-releasing neurons contribute to the reduction in sympathetic nerve activity and resulting fall in arterial pressure that accompanies severe blood loss. Intracerebroventricular injection of a β-endorphin antiserum attenuates hemorrhagic hypotension whereas, conversely, intracerebroventricular β-endorphin injection exacerbates it (36, 46). β-Endorphin also lowers blood pressure and heart rate in normotensive animals when injected either intracerebroventricularly (40, 44) or directly into the nucleus of the solitary tract (NTS) (28, 33). Hypotension and bradycardia are also produced by electrical stimulation of the arcuate nucleus (27), one of two brain sites where neurons that synthesize proopiomelanocortin (POMC), β-endorphin's precursor, originate (22). This response can be inhibited by injecting naloxone intravenously or by infusing a β-endorphin antiserum into the NTS (27). These findings support the hypothesis that POMC neurons projecting from the arcuate nucleus to the NTS participate in the central regulation of cardiovascular function.

The brain stem is also innervated by a second group of POMC neurons located in the commissural nucleus of the NTS (21, 22, 31). The function of these NTS POMC neurons is not well understood, although it may be quite different from those in the arcuate nucleus. Chromatographic analysis of regional β-endorphin processing indicates that the two POMC cell groups posttranslationally process β-endorphin quite differently. POMC neurons in the arcuate nucleus primarily synthesize β-endorphin-(1—31), the opioid form of the peptide, whereas NTS neurons convert β-endorphin-(1—31) almost entirely to nonopioid derivatives (6, 24, 51). Indeed, the predominant forms of β-endorphin in the brain stem, α-*N*-acetyl-β-endorphin-(1—27), α-*N*-acetyl-β-endorphin-(1—26), and β-endorphin-(1—26) (51), are devoid of both antinociceptive (5, 24) and cardioregulatory (17, 47) activity. This means that POMC neurons in the NTS primarily, if not exclusively, synthesize nonopioid β-endorphin peptides.

The posttranslational conversion of β-endorphin-(1—31) to β-endorphin-(1—27) and β-endorphin-(1—26) also generates a dipeptide, glycyl-L-glutamine [Gly-Gln; β-endorphin-(30—31)], from the carboxy terminal of β-endorphin-(1—31) (24, 32). Gly-Gln is a biologically active peptide, as first demonstrated by Parish et al. (32), who reported that Gly-Gln inhibits the firing frequencies of brain stem reticular neurons when applied iontophoretically. Their studies also confirmed that Gly-Gln is present in the brain stem in amounts equivalent to the sum of β-endorphin-(1—27) and β-endorphin-(1—26) concentrations, as one might predict (31). Evidence that Gly-Gln is a major end-product of β-endorphin processing in the brain stem prompted us to test whether central Gly-Gln administration influences cardiovascular or respiratory function. We found that Gly-Gln was inactive when administered intracerebroventricularly to normotensive rats but potently

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inhibited the hypotension and respiratory depression produced by central β-endorphin or morphine administration (44, 45). The response was dose dependent and stereospecific but was not attributable to Gly-Gln hydrolysis because equimolar amounts of glycine and glutamine were ineffective. Gly-Gln did not inhibit morphine (30) or β-endorphin (unpublished data) antinociception, however, consistent with evidence that it lacks affinity for opioid receptors (44). Gly-Gln thus inhibits the hypotension and respiratory depression, but not the antinociception, produced by opioids.

In this study, we tested whether Gly-Gln would restore arterial pressure following acute hemorrhage, as predicted by the hypothesis that endogenous opioid peptides contribute to the cardiovascular depression produced by severe blood loss (38, 48). We found that Gly-Gln significantly increased arterial pressure and heart rate following hemorrhage in both conscious and anesthetized rats without affecting peripheral hemodynamics when given alone to normotensive animals.

MATERIALS AND METHODS

Animals and surgery. Male Sprague-Dawley rats (250–350 g; Zivic-Miller, Pittsburgh, PA) were housed with free access to food and water in a temperature-controlled room with a 12:12-h light-dark cycle. Rats were anesthetized with 1.5–4.0% halothane, and the left carotid artery was cannulated with PE-50 tubing filled with heparinized saline (150 U/ml). The catheter was exteriorized at the nape of the neck and sealed until use. For intracerebroventricular drug administration, a 20-gauge stainless steel guide cannula was implanted in the right lateral ventricle 1.5 mm lateral to midline, 1.0 mm posterior to bregma, and 4.0 mm ventral to the skull surface and fixed with polycarboxylate cement. After surgery, rats were housed individually and body temperature was maintained at 37°C during recovery from anesthesia. Experiments were conducted 4 h after rats regained consciousness.

The experimental protocols were approved by the Animal Care and Use Committee of the Bowman Gray School of Medicine and were conducted in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*.

Hemorrhage. At the beginning of each experiment, the arterial cannula was flushed with 0.2 ml of heparinized saline and connected to a volumetric pressure transducer. Baseline blood pressure and heart rate measurements were recorded at 1-min intervals for 8 min, and rats were administered Gly-Gln (3, 10, or 30 nmol), naloxone (30 or 100 nmol), glycyl-D-glutamine (10 nmol), glycine and glutamine (10 nmol each amino acid) or saline (10 μl) by intracerebroventricular injection. Two minutes later, controlled hemorrhage was initiated by withdrawing 2.5 ml/100 g body wt of blood through the carotid cannula over a 20-min period. Subsequently, systolic, diastolic, and mean arterial pressure (MAP) and heart rate were measured at 5-min intervals for 35 min using a Micro-Med BPA-200 blood pressure analyzer (Micro-Med, Louisville, KY).

For intracerebroventricular injections, Gly-Gln-related peptides and naloxone were dissolved in 10 μl sterile isotonic saline and injected through a 25-gauge cannula inserted through and extending 0.5 mm beyond the guide cannula. The injection cannula was attached to a 50-μl Hamilton syringe with PE-20 tubing, and intracerebroventricular injections were made over a 30-s time interval. At the conclusion of each experiment, the ventricular cannula placement was verified by injecting 10 μl of a methylene blue dye solution intracerebroventricularly.

Catecholamine analysis. Blood samples (1.0 ml) were collected at the beginning and end of the 20-min hemorrhagic period for catecholamine determination. Samples were centrifuged at 3,000 revolutions/min for 15 min, and serum was stored at −70°C until analysis. Norepinephrine, epinephrine, and dopamine concentrations were determined after alumina extraction by high-pressure liquid chromatography with electrochemical detection as previously reported (2). The method has an interassay coefficient of variation of <9% and a detection limit of 12 fmol.

Drugs and peptides. Gly-Gln and glycyl-D-glutamine were purchased from Bachem California (Torrance, CA). Glycine, L-glutamine, and naloxone were obtained from Sigma Chemical (St. Louis, MO), and pentobarbital sodium and halothane were purchased from Barber Veterinary Supply (Richmond, VA).

Statistical analyses. Data were analyzed using repeated-measures analysis of variance with the Fisher's protected least-significant difference approach and Bonferroni corrections as necessary. Analyses were performed using "Proc Mixed" of the SAS software package version 6.12 (SAS Institute, Cary, NC).

RESULTS

Gly-Gln increases MAP and heart rate following hemorrhage in anesthetized rats. Initially, we tested whether Gly-Gln administration attenuates hemorrhagic hypotension and bradycardia in rats anesthetized with pentobarbital sodium (50 mg/kg ip). Blood withdrawal (2.5 ml/100 g body wt; ~40% of blood volume) produced a severe fall in MAP in saline-treated controls; MAP fell from a baseline of 122.3 ± 3.9 mmHg, recorded at the zero time point immediately before hemorrhage was initiated, to 29.1 ± 3.3 mmHg at the 20-min time point, when blood withdrawal was completed (Fig. 1). Subsequently, arterial pressure rose toward baseline MAP values, although it did not fully recover by the end of the 40-min posthemorrhage period. Hemorrhage also lowered heart rate. In controls, heart rate fell from 331 ± 17 beats/min to 244 ± 17 beats/min at the end of blood withdrawal and remained at approximately the same low rates during the entire 40-min posthemorrhage period.

Gly-Gln (10 nmol icv) pretreatment significantly increased MAP and heart rate during the posthemorrhage period (Fig. 1). MAP rose by 53.9 ± 5.4 mmHg in Gly-Gln-treated animals compared with 18.9 ± 4.3 mmHg in saline-treated controls 25 min after hemorrhage was completed. Analysis of variance revealed a significant effect of Gly-Gln treatment [$F(1,12) = 14.00$, $P < 0.01$] and time and a significant treatment × time interaction. Heart rate also increased markedly in Gly-Gln-treated animals (Fig. 1). Heart rate began to rise immediately after blood withdrawal and was fully restored to prehemorrhage values within 30 min (Fig. 1). Analysis of variance revealed a significant treatment × time interaction [$F(7,73) = 7.63$, $P = 0.0001$], although the effect of Gly-Gln treatment was marginally significant [$F(1,12) = 4.33$, $P = 0.0595$]. Gly-Gln pretreatment did not significantly influence the reduction in MAP and heart rate recorded immediately after

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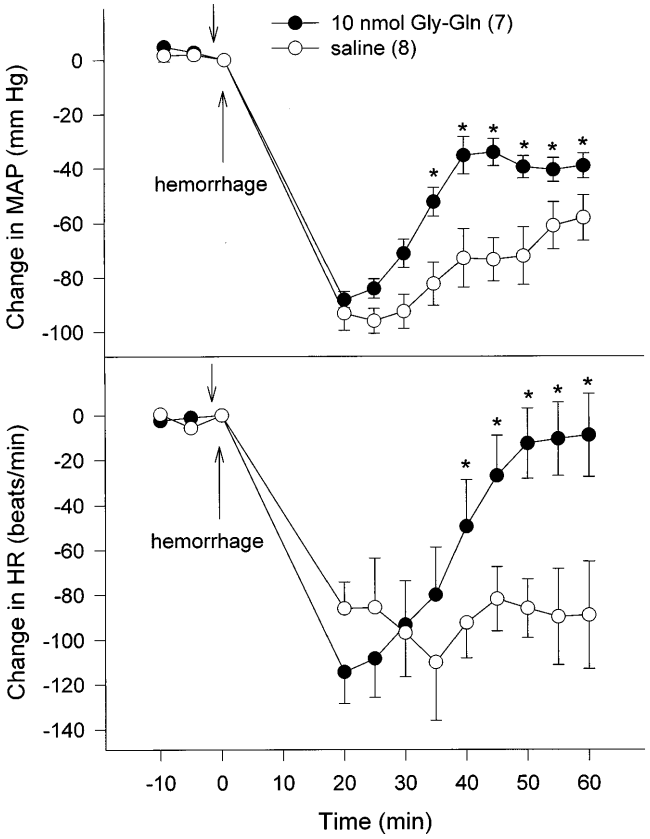

Fig. 1. Glycyl-L-glutamine (Gly-Gln) facilitates recovery of mean arterial pressure (MAP) and heart rate (HR) following acute hemorrhage in pentobarbital sodium-anesthetized rats. Rats were treated intracerebroventricularly with Gly-Gln (10 nmol) or saline, and, after a 2-min delay, blood was withdrawn (2.5 ml/100 g body wt) over a 20-min interval. Data represent mean ± SE change in MAP (*top*) and heart rate (*bottom*) from baseline values. Numbers in parentheses indicates no. of animals in each group. Baseline MAP and heart rate at the zero time point were 122.3 ± 3.9 mmHg and 331 ± 17 beats/min for controls and 111.7 ± 3.2 mmHg and 334 ± 13 beats/min for the Gly-Gln-treated group, respectively. $*P < 0.05$ vs. saline-treated controls.

blood withdrawal was completed, however (Fig. 1). Three of eight control animals died between 15 and 30 min after hemorrhage, whereas all animals in the Gly-Gln treated group survived. Baseline MAP (111.7 ± 3.2 mmHg) and heart rate (334 ± 13 beats/min) did not differ significantly from saline-treated controls. Thus Gly-Gln administration to pentobarbital sodium-anesthetized rats facilitated recovery of arterial pressure and heart rate following hemorrhage, although it did not prevent the hypotension and bradycardia that occurred during blood withdrawal.

Gly-Gln increases MAP and heart rate during hemorrhage in conscious animals. Anesthesia can influence the hemodynamic response to blood loss significantly (38), and pentobarbital sodium, in particular, has been reported to inhibit reflex sympathetic (52) and vagal (29) activation. To test whether the response to Gly-Gln was influenced by pentobarbital sodium, rats were cannulated under halothane anesthesia and allowed to recover from surgery for at least 4 h before hemorrhage was initiated. Hemorrhage (2.5 ml/100 g body wt over a 20-min period) did not affect MAP and heart rate as severely in conscious rats as it did in pentobarbital sodium-anesthetized animals. In conscious, saline-treated control rats, blood withdrawal lowered MAP from baseline values of 120.1 ± 2.9 mmHg to a low of 56.2 ± 4.7 mmHg 15 min after the end of the hemorrhage period (Fig. 2). Unlike the response in anesthetized animals, MAP continued to fall for 10 min after blood removal was discontinued and failed to recover spontaneously during the 35-min posthemorrhage period in saline-treated rats (Fig. 2). Heart rate did not change significantly during hemorrhage in conscious rats, in marked contrast to pentobarbital sodium-anesthetized animals (Fig. 3). A brief period of tachycardia followed by a slight bradycardia occurred during the posthemorrhage period, although these effects were not significant statistically.

Gly-Gln pretreatment (3, 10, or 30 nmol icv) increased MAP (Fig. 2) and heart rate (Fig. 3) following hemorrhage in conscious animals. Both the 10 and 30 nmol Gly-Gln doses increased MAP significantly above control values during the recovery period following hemorrhage. Statistical analysis indicated a significant effect of Gly-Gln treatment [$F(3,24) = 10.60$, $P = 0.0001$], although the time and treatment × time interaction effects were not significant. The increase in arterial pressure was sustained above control values during the entire 35-min posthemorrhage period. Baseline MAP (Fig. 2) and heart rate (Fig. 3) did not differ significantly between saline- and Gly-Gln (3, 10 or 30 nmol)-treated animals. Gly-Gln treatment also improved survival in hemorrhaged rats. Four of nine control rats died during the posthemorrhage period, whereas one of five rats treated with 3 nmol Gly-Gln died, and all animals treated with 10 or 30 nmol

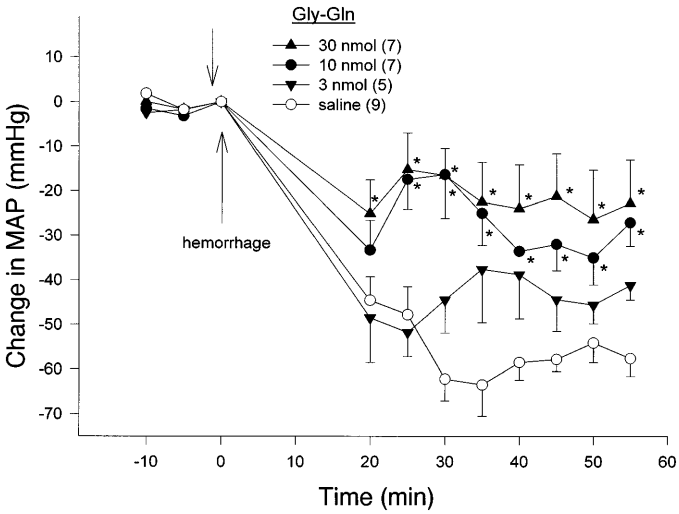

Fig. 2. Gly-Gln inhibits hemorrhagic hypotension in conscious rats. Rats were treated intracerebroventricularly with Gly-Gln (3, 10, or 30 nmol) or saline, and, beginning 2 min later, blood (2.5 ml/100 g body wt) was withdrawn over a 20-min interval. Numbers in parentheses indicate no. of animals in each group. Baseline MAP at the zero time point was 120.1 ± 2.9 mmHg for controls and 119.4 ± 1.5 mmHg, 114.5 ± 4.5 mmHg, and 122.2 ± 5.4 mmHg for 3, 10, and 30 nmol Gly-Gln-treated groups, respectively. $*P < 0.05$ vs. saline-treated controls.

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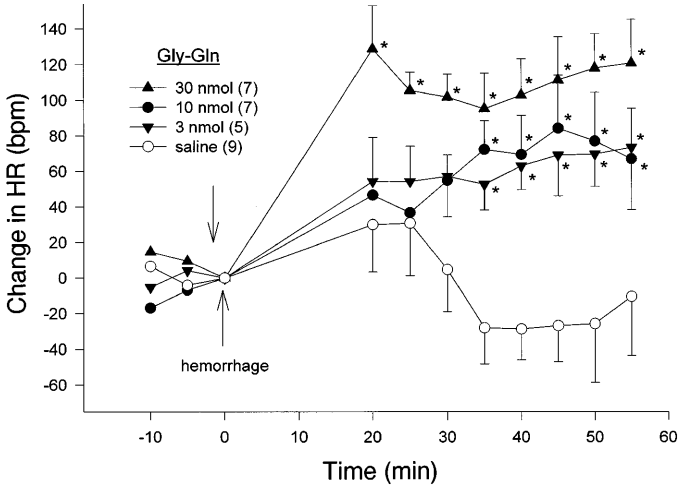

Fig. 3. Gly-Gln administration increases HR following acute hemorrhage. Conscious rats were injected intracerebroventricularly with the indicated dose of Gly-Gln or saline, and hemorrhage (2.5 ml/100 g body wt in 20 min) was initiated after a 2-min delay. Numbers in parentheses indicate no. of animals in each treatment group. Data are presented as mean ± SE change in HR from baseline values. Baseline HR at the zero time point was 346 ± 9 beats/min for controls and 359 ± 8, 361 ± 9, and 367 ± 14 beats/min for the 3, 10, and 30 nmol Gly-Gln-treated groups, respectively. bpm, Beats/min. *$P <$ 0.05 vs. saline-treated animals.

Gly-Gln survived until the experiment was terminated 1 h after the end of the posthemorrhage period. Thus Gly-Gln produced a sustained increase in arterial pressure both during and after blood withdrawal and enhanced survival in conscious rats.

The Gly-Gln-induced rise in MAP during hemorrhage was accompanied by marked tachycardia (Fig. 3). Following the highest Gly-Gln dose (30 nmol icv), heart rate rose from a baseline of 367 ± 14 beats/min to 496 ± 24 beats/min at the end of the 20-min hemorrhage period. Analysis of variance indicated that the effect of Gly-Gln treatment on heart rate was significant [$F(3,24) = 6.45$, $P = 0.002$], and post hoc analysis confirmed that all three Gly-Gln doses increased heart rate significantly above saline-treated controls during the recovery period following hemorrhage.

Gly-Gln (10 nmol icv) elevated pulse pressure significantly in pentobarbital sodium-anesthetized rats, but not in conscious rats (Fig. 4). Interestingly, hemorrhage itself produced quite different effects on pulse pressure in anesthetized and conscious animals. In anesthetized rats, pulse pressure declined significantly during blood withdrawal but returned toward baseline values during the posthemorrhage interval [time effect: $F(7,73) = 7.20$, $P = 0.0001$]. By contrast, pulse pressure did not change significantly either during or after hemorrhage in conscious rats. The effect of Gly-Gln on pulse pressure was also different in anesthetized compared with conscious rats. Whereas 10 nmol Gly-Gln increased pulse pressure significantly during the posthemorrhage period in anesthetized rats [treatment effect: $F(1,12) = 10.66$, $P < 0.01$], it produced no effect whatsoever in conscious animals (Fig. 4); 3 and 30 nmol Gly-Gln also failed to change pulse pressure significantly in conscious animals (data not shown). Hence, the effects of both hemorrhage and Gly-Gln were altered by pentobarbital sodium anesthesia.

Hemorrhagic hypotension is not reversed by Gly-Gln's constituent amino acids or by glycyl-D-glutamine. Control experiments investigated whether the effect of Gly-Gln on peripheral hemodynamics during hemorrhage might result from the enzymatic hydrolysis of Gly-Gln to glycine and glutamine. To test this possibility, conscious rats were treated intracerebroventricularly with equimolar amounts of glycine and glutamine (10 nmol) immediately before blood withdrawal was initiated. Amino acid pretreatment had no significant effect on MAP (Fig. 5) or heart rate (data not shown) following hemorrhage. The Gly-Gln stereoisomer, glycyl-D-glutamine, was similarly ineffective (Fig. 5), which shows that Gly-Gln's hemodynamic activity is not readily attributable to a nonspecific effect of peptide administration. These data indicate that Gly-Gln's hemodynamic effect during hemorrhage is stereospecific and evidently does not result from Gly-Gln hydrolysis.

Gly-Gln lacks hemodynamic activity in normotensive animals. In subsequent experiments, we investigated

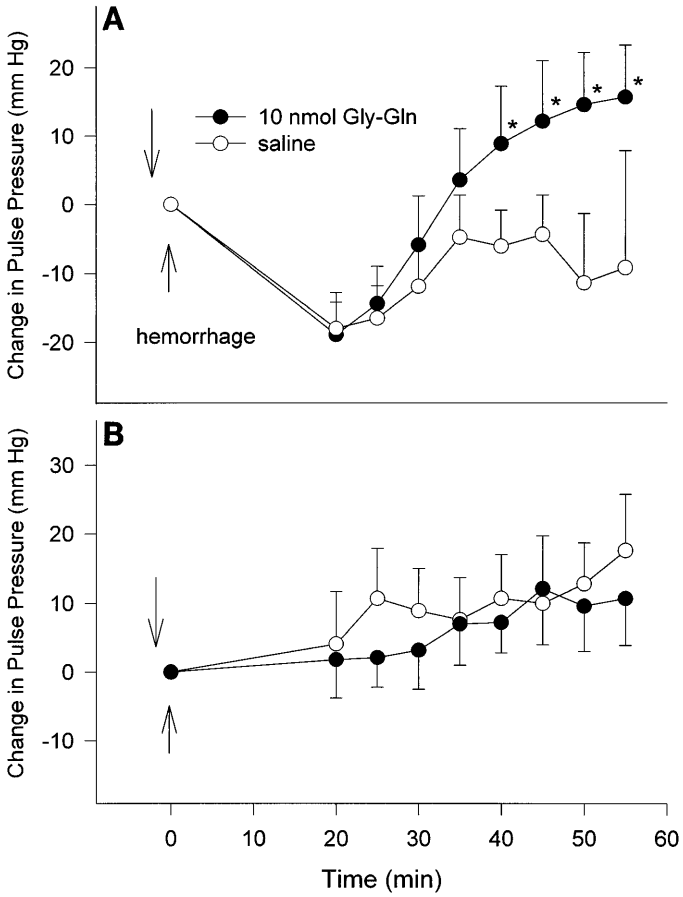

Fig. 4. Effect of Gly-Gln on pulse pressure following acute hemorrhage in anesthetized (*A*) and conscious (*B*) rats. Groups of 7–9 rats received Gly-Gln (10 nmol) or saline 2 min before blood withdrawal (2.5 ml/100 g body wt over 20 min). Baseline pulse pressure values at the zero time point were control = 37.5 ± 5.6 mmHg, treated = 36.5 ± 4.8 mmHg (*A*); control = 27.4 ± 3.5 mmHg, treated = 30.7 ± 4.9 mmHg (*B*). *$P <$ 0.05 vs. saline-treated controls.

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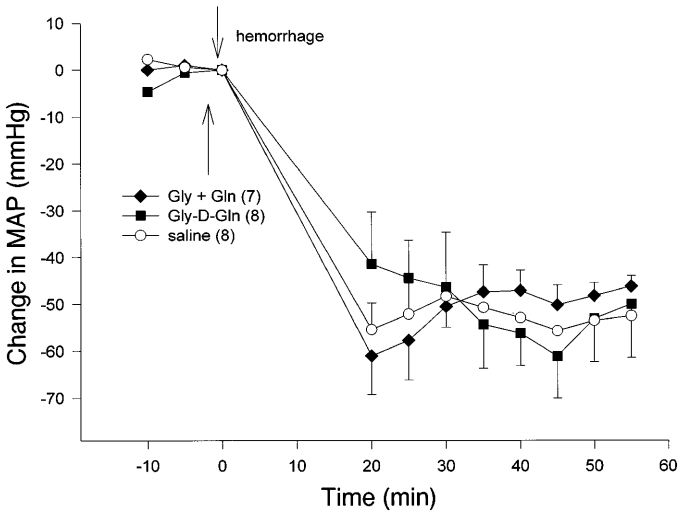

Fig. 5. Intracerebroventricular injection of glycyl-D-glutamine (Gly-D-Gln) or glycine plus glutamine (Gly + Gln) does not affect MAP during acute hemorrhage. Glycyl-D-glutamine (10 nmol), glycine plus glutamine (10 nmol each), or saline was injected intracerebroventricularly 2 min before blood withdrawal (2.5 ml/100 g body wt in 20 min). Numbers in parentheses indicate no. of rats in each group. Baseline MAP values at the zero time point were saline = 126.1 ± 2.2 mmHg, glycyl-D-glutamine = 125.5 ± 4.5 mmHg, glycine + glutamine = 118.7 ± 2.5 mmHg.

whether Gly-Gln affects arterial pressure or heart rate in normotensive, nonhemorrhaged rats. In earlier studies, we found that Gly-Gln did not affect peripheral hemodynamics when administered intracerebroventricularly to pentobarbital sodium-anesthetized rats (44), but it is possible that anesthesia may be an important variable. As illustrated in Fig. 6, Gly-Gln injection (10 or 100 nmol) was ineffective when administered intracerebroventricularly to conscious animals and had no effect on MAP or heart rate for up to 40 min after intracerebroventricular administration.

Naloxone elevates MAP and heart rate during hemorrhage. As a final control, we compared the cardiovascular effects of Gly-Gln in hemorrhaged rats to those of naloxone. Intracerebroventricular naloxone injection (100 nmol) 2 min before blood withdrawal was initiated reduced the degree of hemorrhagic hypotension significantly compared with saline-treated controls; 30 nmol naloxone was ineffective (Fig. 7). Analysis of variance confirmed that naloxone produced a significant treatment effect [$F(3,25) = 14.65$, $P = 0.0001$], although the time and treatment × time interaction effects were not significant. Naloxone was less potent than Gly-Gln to the extent that the effect of 30 nmol naloxone on MAP was significantly lower than that of 30 nmol Gly-Gln [$F(1,25) = 6.94$, $P < 0.05$]. Naloxone (30 nmol icv) also increased heart rate significantly [treatment effect; $F(3,25) = 7.21$, $P = 0.001$], although the higher dose (100 nmol) was less effective. Thus Gly-Gln and naloxone both elevated MAP and heart rate following hemorrhage, although their potencies differed and, unlike Gly-Gln, the effect of naloxone on heart rate was not strictly dose related.

Gly-Gln elevates plasma norepinephrine concentrations. Naloxone elevates plasma norepinephrine concentrations following severe blood loss in conscious rabbits (37), although it reportedly does not alter circulating catecholamines significantly in conscious rats (11). To determine whether Gly-Gln affects plasma catecholamine levels, we analyzed norepinephrine, dopamine, and epinephrine concentrations in plasma samples withdrawn at the beginning and the end of the 20-min hemorrhage period (Table 1). Hemorrhage did not change circulating norepinephrine or dopamine concentrations significantly in saline-treated control animals, although it elevated plasma epinephrine levels markedly ($P < 0.01$); indeed, epinephrine was increased 17-fold above baseline values by the end of the hemorrhage period, consistent with previous reports (4, 10, 11).

Gly-Gln (30 nmol icv) pretreatment significantly increased plasma norepinephrine concentrations during hemorrhage ($P < 0.05$; Table 1). Plasma norepinephrine levels were two- to threefold higher in Gly-Gln treated rats than corresponding values for saline-treated controls at the end of the hemorrhage period. Gly-Gln pretreatment also elevated plasma dopamine concentrations ($P < 0.05$), but it did not change plasma epinephrine levels significantly compared with saline-treated controls (Table 1). Pretreatment with the Gly-

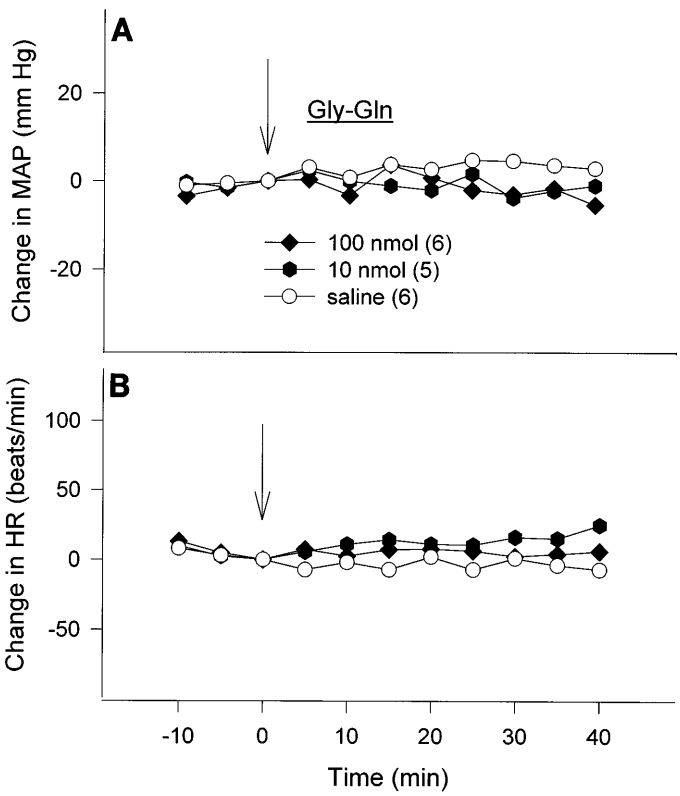

Fig. 6. Gly-Gln does not affect MAP or HR in normotensive rats. Gly-Gln (10 or 100 nmol) or saline was administered intracerebroventricularly to groups of 5 or 6 conscious, normotensive rats, and MAP (*A*) and HR (*B*) were recorded at 5-min intervals for 40 min. Baseline MAP values at the zero time point were saline = 119.1 ± 4.9 mmHg, 10 nmol Gly-Gln = 127.8 ± 9.1 mmHg, 100 nmol Gly-Gln = 137.2 ± 6.0 mmHg. Baseline HR values were saline = 326 ± 24 beats/min, 10 nmol Gly-Gln = 352 ± 2 beats/min, 100 nmol Gly-Gln = 356 ± 15 beats/min.

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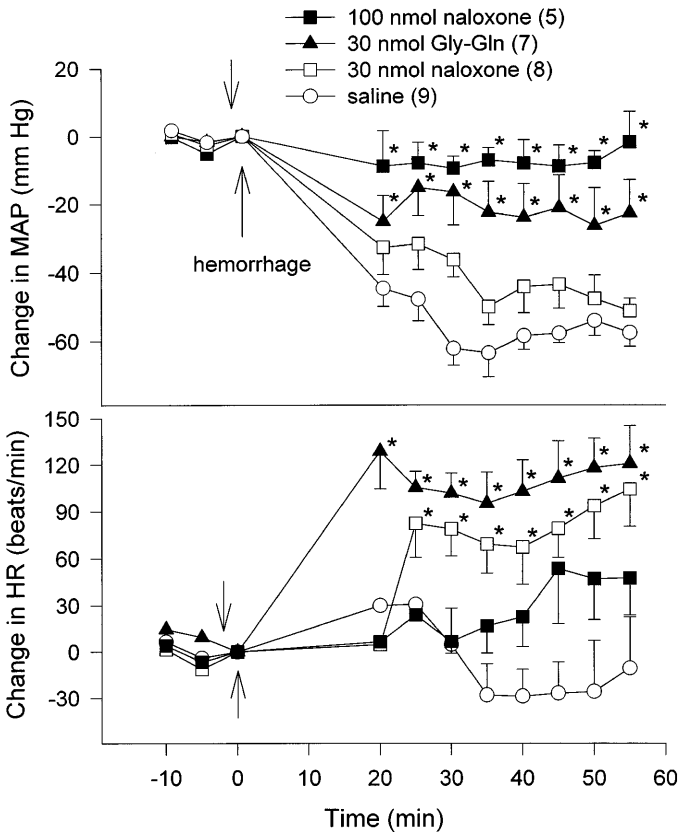

Fig. 7. Gly-Gln is more effective than equimolar amounts of naloxone in preventing hemorrhagic hypotension. Conscious rats received intracerebroventricular Gly-Gln (30 nmol), naloxone (30 or 100 nmol), or saline 2 min before initiation of controlled hemorrhage (2.5 ml/100 g in 20 min). The number of animals in each group is shown in parentheses. Baseline MAP values at the zero time point were saline = 120.1 ± 2.9 mmHg, 30 nmol naloxone = 121.5 ± 5.4 mmHg, 100 nmol naloxone = 117. ± 3.4 mmHg. Baseline HR values were saline = 346 ± 9 beats/min, 30 nmol naloxone = 382 ± 13 beats/min, 100 nmol naloxone = 379 ± 18 beats/min. *$P < 0.05$ vs. saline-treated controls.

Table 1. *Effect of Gly-Gln on plasma norepinephrine, dopamine, and epinephrine concentrations following hemorrhage*

	Plasma Catecholamines, pg/ml	
Treatment	Baseline	Hemorrhage
Norepinephrine		
Saline	92.0 ± 33.2	173.8 ± 56.9
Gly-Gln	70.9 ± 16.7	457.1 ± 132.3*†
Dopamine		
Saline	80.5 ± 26.9	163.5 ± 49.1
Gly-Gln	65.9 ± 15.3	397.4 ± 113.6*†
Epinephrine		
Saline	54.5 ± 24.8	887.8 ± 357.3*
Gly-Gln	66.7 ± 22.9	799.3 ± 226.2*

Values are means ± SE. Conscious rats were treated with glycyl-L-glutamine (Gly-Gln) (30 nmol; $n = 7$) or saline ($n = 6$) intracerebroventricular, and, beginning 2 min later, blood (2.5 ml/100 g body wt) was withdrawn over a 20-min period. Plasma norepinephrine, dopamine, and epinephrine concentrations were measured at the beginning (baseline) and end (hemorrhage) of the blood withdrawal period. Data were analyzed by 1-tailed *t*-test. *$P < 0.01$ vs. baseline values. †$P < 0.05$ vs. corresponding value for saline-treated animals.

Gln stereoisomer glycyl-D-glutamine (10 nmol) did not affect plasma norepinephrine, epinephrine, or dopamine (data not shown). These data are consistent with the hypothesis that Gly-Gln enhances norepinephrine release from sympathetic neurons but does not influence adrenal epinephrine secretion.

DISCUSSION

Gly-Gln is a major end-product of POMC processing in the pituitary and brain (32, 34), but relatively little is known about its physiological function. In this study, we showed that Gly-Gln significantly increases arterial pressure and heart rate following acute hemorrhage in both conscious and anesthetized rats. The response was dose related and stereospecific and was not secondary to Gly-Gln metabolism because it was not reproduced by Gly-Gln's constituent amino acids, glycine and glutamine. Gly-Gln also elevated plasma norepinephrine concentrations during hemorrhage, consistent with the hypothesis that it raises arterial pressure by increasing sympathetic activity. Peripheral hemodynamics were unaffected by Gly-Gln in normotensive animals, however, making it unlikely that Gly-Gln interferes with normal baroreflex mechanisms. Gly-Gln thus appears to be an effective antagonist of hemorrhagic hypotension.

These findings were predicated on evidence that endogenous opioid peptides are involved in the sympathoinhibitory phase of hemorrhage (38, 48). Progressive hemorrhage produces a biphasic response in humans and most laboratory animals. Initially, arterial pressure is maintained within normal limits by a compensatory increase in peripheral vascular resistance and heart rate, but after severe blood loss, a second phase develops in which sympathetic activity decreases and arterial pressure declines precipitously (38, 48). In rats, this sympathoinhibitory phase predominates, and arterial pressure begins to decline soon after blood withdrawal is initiated (4, 10, 13). The central mechanisms that generate the fall in arterial pressure and sympathetic nerve activity produced by hemorrhage are not completely understood, although the finding that naloxone inhibits hemorrhagic hypotension has been widely interpreted as evidence that opioid peptides are an important contributory factor (38, 48). The present finding that Gly-Gln elevates arterial pressure and heart rate during hemorrhage raises the possibility that, like naloxone, Gly-Gln prevents the sympathoinhibition triggered by endogenous opioid peptides.

This hypothesis is supported by the finding that Gly-Gln pretreatment elevated plasma norepinephrine concentrations during acute blood loss (Table 1). Plasma epinephrine concentrations were not significantly affected by Gly-Gln, although they were increased markedly by hemorrhage in both control and treated rats. The finding that hemorrhage increases plasma epinephrine, but not norepinephrine, levels is consistent with earlier reports that blood loss increases impulse flow in adrenal nerves but reduces it in renal sympathetic nerves in anesthetized rats (41, 43, 49). Naloxone has

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consistently been shown to elevate plasma norepinephrine levels (37), renal sympathetic nerve activity (3, 15, 50), and peripheral vascular resistance (39) during acute hemorrhage in conscious rabbits, although its mechanism of action in rats is more controversial (9, 11, 38). Feuerstein et al. (11) reported that intra-arterial naloxone administration to conscious rats elevates arterial pressure following hemorrhage but fails to change plasma catecholamine concentrations significantly. In preliminary experiments, we found that intracerebroventricular naloxone administration also failed to elevate plasma norepinephrine concentrations (control = 173.8 ± 56.9 pg/ml; naloxone 100 nmol = 165.6 ± 26.6 pg/ml), further arguing against a primary role of sympathetic activation in the response to naloxone in rats. Naloxone does increase renal sympathetic nerve activity during hemorrhage in anesthetized rats, but the increased sympathetic activity occurs after the rise in arterial pressure, calling into question whether the two phenomena are causally related (26). Hence, it may be premature to conclude that either Gly-Gln or naloxone prevents hemorrhagic hypotension by increasing sympathetic nerve activity in rats.

Whether or not the same physiological mechanism is involved, Gly-Gln and naloxone almost certainly do not act through the same receptor mechanism. In earlier studies, we found that Gly-Gln fails to displace [^{3}H]naloxone binding to rat brain homogenates at concentrations ranging from 10 pM to as high as 10 mM, indicating that it does not act as an opioid receptor antagonist (44). Electrophysiological experiments support this conclusion. Gly-Gln's inhibitory effect on the firing frequencies of nucleus reticularis gigantocellularis neurons is not reversed by naloxone, again indicating that opioid receptors do not mediate the response (32). Strychnine was similarly ineffective, ruling out the involvement of glycine receptors (32). Gly-Gln produces a number of other pharmacological effects in brain and peripheral tissues unrelated to those of β-endorphin or other opioids (16, 23, 24, 35), which further supports the conclusion that it acts as an independent neurotransmitter rather than an opioid receptor antagonist. The receptor that does mediate Gly-Gln's pharmacological effects has not been identified; conceivably, a specific Gly-Gln receptor may be expressed in brain.

It remains to be determined whether endogenous Gly-Gln serves a comparable physiological role in the regulation of cardiovascular homeostasis. Measurement of Gly-Gln concentrations in the brain stem (32) as well as chromatographic analysis of regional β-endorphin processing (6, 51) indicate that most, if not all, the β-endorphin synthesized by POMC neurons in the NTS is converted to Gly-Gln and carboxy terminal-truncated β-endorphin peptides. POMC neurons in the NTS innervate a number of brain stem autonomic centers, including the vasomotor and vasodepressor areas of the ventrolateral medulla, nucleus ambiguus, and parabrachial nucleus (21, 22, 31), which supports the hypothesis that they participate in central cardiovascular regulation. Thus it seems paradoxical that NTS POMC neurons inactivate most, if not all, the β-endorphin they synthesize. It is important to emphasize, however, that POMC neurons do not solely release β-endorphin but are multitransmitter neurons that synthesize and release other bioactive peptides. Other POMC-derived peptides, γ-melanocyte-stimulating hormone (12, 42) and the joining peptide (14), produce hypertension and tachycardia when injected centrally, effects directly opposite those produced by β-endorphin. These pharmacological data indicate that Gly-Gln may be one of several peptides synthesized by NTS POMC neurons that are capable of elevating arterial pressure during hemorrhage.

Despite extensive evidence that naloxone reverses hemorrhagic hypotension in laboratory animals, its clinical utility remains controversial, in part, because its use is often contraindicated by the need for concurrent opioid therapy (38). Indeed, we recently found that the lowest naloxone dose required to elevate blood pressure during hemorrhage (30 nmol icv; Fig. 7) inhibits morphine antinociception completely (30). By contrast, intracerebroventricular Gly-Gln injection has no effect whatsoever on morphine antinociception at doses considerably higher than required to inhibit hemorrhagic hypotension (30). Thus we recently reported that Gly-Gln (1–300 nmol icv) did not change paw lift latencies when coinjected with morphine (30 nmol icv) or when administered alone to conscious rats (30). Gly-Gln was similarly ineffective in the tail flick reflex test when coinjected with β-endorphin (1.5 nmol icv) or when given alone at doses as high as 1 μmol (unpublished data). Gly-Gln's pharmacological selectivity is consistent with evidence that β-endorphin is processed to Gly-Gln and other nonopioid β-endorphin derivatives in the brain stem (51) and caudal medulla (6) but not, to a major extent, in the periaqueductal gray region (1) or other forebrain sites that influence pain perception (51). Together, these findings indicate that, unlike opioid receptor antagonists, Gly-Gln attenuates hemorrhagic hypotension without influencing opioid neuronal systems involved in pain perception.

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