Sympathetic Nervous System Mediation of Acute Cardiovascular Actions of γ2-Melanocyte-Stimulating Hormone

MICHAEL F. CALLAHAN, ROBERT F. KIRBY, DENNIS W. WOLFF, JACK W. STRANDHOY, JOHN R. LYMANGROVER, ALAN KIM JOHNSON, AND KENNETH A. GRUBER

SUMMARY Peptides of the pro-opiocortin class produce pronounced cardiovascular and natriuretic actions. We have investigated the acute cardiovascular effects of one of the most potent members of this class, γ2-melanocyte stimulating hormone (γ2-MSH), in rats. Pressor actions of γ2-MSH administered systemically were eliminated by ganglionic blockade with chlorisondamine. Peripheral cholinergic blockade failed to affect either the pressor or cardioaccelerator responses to γ2-MSH. Administration of γ2-MSH (2.0–10.0 μg) produced vasoconstriction primarily in the mesenteric and hindlimb vascular beds, while the renal bed showed little response. Infusions of phenylephrine produced pressor responses similar to those found with γ2-MSH, which were accompanied by a decrease in heart rate and vasoconstriction in the mesenteric and renal vascular beds. Hemodynamic changes produced by γ2-MSH and phenylephrine were blocked or attenuated by α1-adrenergic receptor blockade with prazosin. Direct injection of γ2-MSH into the renal artery produced an acute renal vasoconstriction that was not attenuated by α1-adrenergic or ganglionic blockade. These findings and the results of previous publications are consistent with the hypothesis that γ2-MSH may produce a centrally mediated activation of the sympathetic nervous system, have direct vasoconstriction actions on the renal vasculature, and inhibit baroreceptor function to produce an increase in blood pressure without an accompanying bradycardia. (Hypertension 7 [Suppl I]: 1-145–1-150, 1985)

KEY WORDS blood flow • peptides • cardiovascular hemodynamics • baroreceptor • regional

The pro-opiocortin-derived peptides and analogues, including adrenocorticotropic hormone (ACTH)4–10, D-phenylalanine (D-Phe) ACTH4–10 and γ-melanocyte stimulating hormone (γ-MSH), have profound influences on cardiovascular hemodynamics1 and on body fluid and mineral balance.2–4 Systemic infusions of ACTH4–10 or the sterically hindered analogue (D-Phe) ACTH4–10 produce pressor, cardioaccelerator, and natriuretic actions.4 The cardiovascular actions of the peptides appear to result from direct activation of the sympathetic nervous system, in that the pressor and cardioaccelerator actions are blocked by α1-adrenergic and β1-adrenergic receptor blockade respectively.

γ-Melanocyte stimulating hormone peptides, fragments of the 16K N-terminus of pro-opiocortin6–7 that contain an amino acid sequence analogous to ACTH4–10, have been observed to be 10 to 100 times more potent than ACTH4–10 in cardiovascular and natriuresis studies.7–8 We recently have demonstrated that γ2-MSH has significant pressor actions.8 This peptide did not produce dose-dependent cardioacceleration, and the rise in mean arterial pressure (MAP) was not accompanied by a bradycardia, which suggests an inhibition of baroreceptor function. Blockade of α1-adrenergic receptors abolished the pressor response, while β1-adrenergic blockade failed to reveal a bradycardia to the γ2-MSH pressor response. This finding suggested that γ2-MSH produces an increase in MAP by selective activation of efferent sympathetic mechanisms without equivalent activation of sympathetic drive to the heart (i.e., competition between sympathetic and parasympathetic drive to the heart was not unmasked as a cardiodeceleration following the β-adrenergic receptor blockade).
The purpose of the present studies were: (1) to determine if the pressor response produced by \( \gamma_2 \)-MSH was being mediated by central nervous system activation of sympathetic outflow; (2) to determine if peripheral cholinergic blockade would unmask a \( \gamma_2 \)-MSH-mediated increased sympathetic drive to the heart to produce cardioacceleration; and (3) to characterize the effects of systemically administered \( \gamma_2 \)-MSH on resistance in the mesenteric, renal, and hindlimb vascular beds.

**Methods**

Male Sprague-Dawley rats (300–450 g) were used in the first three studies. For Experiments 1 and 2, rats were anesthetized with sodium pentobarbital (50 mg/kg) and received long-term indwelling catheters in the right common carotid artery for the determination of arterial pressure and heart rate and in the jugular vein for intravenous drug infusions. Following a recovery period of at least 24 hours, rats were removed from their home cage, catheters were connected, and the animals were placed in a clear Plexiglas testing chamber (24 × 21 × 29 cm). After an acclimation period of at least 15 minutes, all animals were given infusions of phenylephrine (PHE, 3–5 \( \mu \)g/300 \( \mu \)L) or \( \gamma_2 \)-MSH (10–20 \( \mu \)g/300 \( \mu \)L) designed to produce a 30 to 70 mm Hg increase in MAP. Animals then received an infusion of either chlorisondamine (0.5 mg/kg) or methylatropine (0.5 mg/kg). Following return to a stable baseline, PHE and \( \gamma_2 \)-MSH were readministered. Direct blood pressures were measured by a Statham pressure transducer (Statham Inc., Hato Rey, Puerto Rico) and plotted on a stripchart recorder (Fisher Scientific, Pittsburgh, PA). Heart rate was determined by a cardiotachometer.

In Experiment 3, rats were anesthetized with pentobarbital and instrumented with miniaturized pulsed Doppler flow probes (Valpey Fisher, Hopkinton, MA) on the superior mesenteric and left renal arteries and on the lower abdominal aorta to determine blood flow in these individual vascular beds. The flow in the lower abdominal aorta is taken as a measure of flow to skeletal muscle in the hindquarters, as this is the major bed served by the vessel. The pulsed Doppler flowmeter (Biomedical Engineering, University of Iowa, Iowa City, IA) measures blood cell velocity as a change in Doppler shift (e.g., voltage shift) that is directly proportional to the blood flow in the vascular bed.

Animals were not tested after operation until they attained at least 90% of preoperative body weight and were consuming more than 8.0 ml of water/100 g body weight per 24 hours. At this time animals were anesthetized and received long-term catheters as described for Experiments 1 and 2. Following a recovery period of at least 24 hours, animals in Experiment 3 were removed from their home cage, connected to the pressure transducer and Doppler flowmeter, and placed in the testing chamber. The animals then received i.v. boluses of \( \gamma_2 \)-MSH (2.5, 5.0, and 10.0 \( \mu \)g/300 \( \mu \)L) and PHE (1.0 and 2.5 \( \mu \)g/300 \( \mu \)L). Blockade of the \( \gamma_2 \)-MSH-induced or PHE-induced increases in vascular resistance were accomplished by prazosin (0.5 \( \mu \)g/kg).

Resistance in each vascular bed was calculated according to the following formula: resistance in arbitrary units = MAP/kHz Doppler shift. Following drug infusion, resistance was calculated simultaneously in each vascular bed when MAP reached a peak. Values for resistance changes are expressed as a percentage of the preceding baseline.

In Experiment 4 male Wistar rats weighing 450 to 640 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The change in species was dictated by the presence of a suprarenal branch of the renal artery in the majority of Wistar rats, which provides a means for direct injection of drugs into the kidney. Cannulas were placed in a femoral artery and vein, and the trachea was intubated for spontaneous respiration supplemented with 100% oxygen. Plasma oncotic pressure was maintained by infusing 8% ficoll 70 (1 ml/300 g at 51 \( \mu \)L/min followed by 10 \( \mu \)L/min for duration)

The ficoll infusion acted as a plasma expander to maintain euvolemia. A consequence of laparotomy has been found to be an increase in hematocrit and a decrease in plasma protein concentration. This effect is unrelated to blood loss. The use of a plasma expander restores renal and cardiovascular function to a level approaching that of the unanesthetized state. To further reduce evaporative loss, the surgical site was covered with plastic kitchen wrap. While the renal vascular responses were not different without ficoll euvelemia, the stability of the preparation was enhanced by this technique.

Through a midline incision, the right suprarenal artery was gently isolated and a tapered PE-10 catheter was inserted to the junction of the renal artery, as described by Smits et al. This cannula was connected to a Harvard syringe pump (Harvard Instruments, Millis, MA) through which 0.5 N Normosol-R solution (Abbott Laboratories, North Chicago, IL) was pumped at 97 \( \mu \)L/minute throughout the experiment. A Valco high-performance liquid chromatography valve (Valco, Inc., Houston, TX) was interspersed in the infusion line so that reproducible 6-\( \mu \)L volumes could be injected without stopcock dead space or the introduction of air bubbles. Renal blood flow was measured with a Doppler flow probe and flowmeter as described by Haywood et al. Because of the intrarenal injection the peak decrease in renal flow (maximum increase in renal resistance) did not occur at the same time as the peak increase in MAP. Thus, we did not think it would be valid to express the decrease in renal blood flow in resistance units with the MAP during the maximal renal response. Decreases in renal blood flow were therefore expressed as a percentage of control flow. The baseline Doppler signal was considered to be 100%, and zero flow was the polygraph recording with the flowmeter set to zero. Renal blood flow, blood pressure, and heart rate were continuously recorded with a Model 7 Grass polygraph (Grass Instruments, Quincy, MA).
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er, the cardiodeceleration (—111 beats/min) produced

Administration of PHE produced a significant eleva-

tion of MAP both before (45 mm Hg) and after (47 mm

Hg; p < 0.05) increase in blood pressure with no slowing of heart rate. In contrast, a

small and insignificant concurrent increase in heart rate also was noted (from 444 ± 32 beats/min to 498 ± 29 beats/min). Blockade

Results

Bolus injections of γ2-MSH (Table 1) produced a significant (60 mm Hg; p < 0.05) increase in blood pressure with no slowing of heart rate. In contrast, a similar rise in MAP produced by PHE was accompanied by a significant (—130 beats/min; p < 0.05) drop in heart rate. The γ2-MSH produced little change in vascular resistance. There was relatively greatest changes while the hindlimb showed little change in vascular resistance. There was relatively little change in renal resistance in response to γ2-MSH, while mesenteric and hindquarter resistance increased twofold (see Figure 2A).

Administration of prazosin significantly attenuated the systemic cardiovascular and regional blood flow responses to γ2-MSH and PHE. In these rats (n = 5), MAP was reduced from 121 ± 5 mm Hg to 67 ± 10 mm Hg (p < 0.05). A small and insignificant concomitant increase in heart rate also was noted (from 444 ± 32 beats/min to 498 ± 29 beats/min). Blockade

<table>
<thead>
<tr>
<th>Blockade</th>
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<th>After blockade</th>
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<tr>
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<td>HR</td>
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<td>Baseline</td>
<td>111 ± 7</td>
<td>444 ± 19</td>
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<td>Response to γ2-MSH (10–20 μg)</td>
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<td>114 ± 5</td>
<td>446 ± 10</td>
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<tr>
<td>Response to PHE (3–5 μg)</td>
<td>172 ± 6*</td>
<td>316 ± 9*</td>
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<td>Cholinergic receptor (methylatropine, n = 6)</td>
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<tr>
<td>Baseline</td>
<td>117 ± 5</td>
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<tr>
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<tr>
<td>Baseline</td>
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<td>436 ± 26</td>
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<tr>
<td>Response to PHE (3–5 μg)</td>
<td>165 ± 12*</td>
<td>335 ± 21*</td>
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Values are means ± SEM

MAP = mean arterial pressure, HR = heart rate, γ2-MSH = γ2-melanocyte-stimulating hormone, PHE = phenyl-


ephine.

*p < 0.05, correlated t test.
**FIGURE 1**  
The effect of a bolus infusion of \( \gamma_2 \)-MSH (5 \( \mu \)g/300 \( \mu \)L for 6 seconds) Tracings from top to bottom show arterial pressure and blood flow to mesenteric (MES), renal (REN), and hindlimb (HL) vascular beds. Note that at the peak pressure response (60 mm Hg) blood flow to MES and HL were decreased, while REN flow remained essentially unchanged.

**FIGURE 2**  
A Effects of three doses of \( \gamma_2 \)-MSH on MAP, heart rate (HR), and vascular resistance in mesenteric, renal, and hindlimb vascular beds. All doses of \( \gamma_2 \)-MSH caused an increase in MAP with no significant change in HR. The increased vascular resistance was mediated primarily by the mesenteric (5 \( \mu \)g and 10 \( \mu \)g of \( \gamma_2 \)-MSH) and the hindlimb (all doses of \( \gamma_2 \)-MSH) beds. The renal vascular bed showed relatively less responsiveness to increasing doses than did the other beds.  
B Effects of two doses of PHE on MAP, HR, and regional vascular resistance. These doses of PHE produced pressor responses equivalent to those of the lower doses of \( \gamma_2 \)-MSH. However, the pressor responses to PHE were accompanied by significant decreases in HR (p < 0.05). In addition, PHE produced changes in vascular resistance primarily in the mesenteric and renal beds (p < 0.05) with little change in the hindlimb beds.  

BP = blood pressure

of \( \alpha_1 \)-adrenergic receptors significantly attenuated the pressor response to 10 \( \mu \)g of \( \gamma_2 \)-MSH (64 mm Hg before versus 9 mm Hg after blockade; p < 0.05) and abolished the pressor response to 2.5 PHE (53 mm Hg before versus −1 mm Hg after blockade; p < 0.05). In addition, all changes in vascular resistance produced by \( \gamma_2 \)-MSH and PHE were either absent or significantly attenuated (p < 0.05). After prazosin administration, the mesenteric vascular resistance response to 10 \( \mu \)g of \( \gamma_2 \)-MSH was reduced from 227% of baseline to 148%...
while the hindlimb resistance response was reduced from 251% to 108% of baseline. Following the prazosin administration, the mesenteric vascular resistance response to 2.5 μg of PHE was reduced from 589% of baseline to 94% of baseline. No significant change in heart rate was noted in prazosin-treated rats following administration of PHE or γ2-MSH.

Intrarenal arterial boluses of γ2-MSH (5–20 μg/kg) caused 40 to 90% decreases in renal blood flow in 6 of 11 rats studied. These decreases in renal blood flow were followed by 5 to 70 mm Hg increases in MAP. The remaining five rats were much less responsive to intrarenal arterial injection of γ2-MSH; 20 to 60 μg/kg boluses decreased renal blood flow only 10 to 25%. A consistent correlation between decreases in renal blood flow and increases in MAP was not evident. In the responsive rats doses of γ2-MSH were compared with those of norepinephrine. Norepinephrine (0.03–0.3 μg/kg) decreased renal blood flow from 50 to 100% in these rats. Comparing these responses to a regression line through the responses to norepinephrine suggests that 20 μg/kg of γ2-MSH is equipotent with 0.27 μg/kg of norepinephrine (12.8 nmol/kg of γ2-MSH versus 0.84 nmol/kg of norepinephrine). The renal vasoconstriction produced by γ2-MSH was not blocked by mecamylamine (20 mg/kg, i.v.) Prazosin (50–1000 ng/kg/min i.a.) attenuated responses to norepinephrine (1 μg/kg); PHE (10 μg/kg); and scorpion venom (10 μg/kg), an indirect agonist13; but did not block the responses to γ2-MSH.

Discussion

Recent experimental evidence has suggested that γ2-MSH and related peptides increase sympathetic nervous system activity.8,14,15 In Experiment 3, this was reflected by the ability of γ2-MSH to produce an increase in vascular resistance in the mesenteric and abdominal aorta beds in the face of increased MAP. These responses appeared to be mediated by the sympathetic nervous system in that α1-adrenergic receptor blockade with prazosin attenuated the pressor renal vascular resistance responses to γ2-MSH infusion. The renal vascular bed appeared to be less responsive than the mesenteric and hindlimb beds to γ2-MSH. The relatively small increase in renal resistance appeared to be a response to the increase in blood pressure, as flow was not significantly diminished (see Figure 1).

The experiments on direct renal injection of γ2-MSH suggest that there are some direct (non-neurally mediated) effects of the peptide on vascular resistance. Although the doses of peptide we used were relatively high, a rather dramatic decrease in blood flow was observed. These effects may be the mammalian homologue of the direct smooth muscle actions that structurally related peptides have in invertebrates.14,17 The inability of some rats to show a renal vasoconstriction to the peptide may be the result of activation of renal vasodilator systems. As anesthesia may be a complicating factor in these experiments, further studies are planned in conscious, unrestrained rats; however, our pharmacological blockade studies do not yet support any direct vascular effects of γ2-MSH.

A natriuretic and hypertensive factor has been hypothesized to play a role in the renal response to isotonic volume expansion or high salt diets.19 The biological activities of γ2-MSH appear to fulfill some of the requirements for such a factor. We also have found
similar biological activities in a related peptide, \( \gamma_1 \)-MSH [(des Gly\(^{12}\)] \( \gamma_2 \)-MSH-amide (unpublished observations)]. The preservation of renal blood flow in the face of highly significant increases in MAP is what might be expected for a class of peptides that may function as physiological natriuretic hormones. Clearly, further work is needed to ascertain the physiological roles of \( \gamma \)-MSH peptides.

References

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