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 vasoconstrictor 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: peptides • cardiovascular hemodynamics • baroreceptor • regional blood flow

The pro-opiocortin-derived peptides and analogues, including adrenocorticotropic hormone (ACTH4-10), 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.3-4 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 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 γ2-MSH was being mediated by central nervous system activation of sympathetic outflow; (2) to determine if peripheral cholinergic blockade would unmask a γ2-MSH-mediated increased sympathetic drive to the heart to produce cardioacceleration; and (3) to characterize the effects of systemically administered γ2-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 x 21 x 29 cm). After an acclimation period of at least 15 minutes, all animals were given infusions of phenylephrine (PHE, 3–5 μg/300 μL) or γ2-MSH (10–20 μg/300 μL) 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 γ2-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 cardiodynamometer.

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 γ2-MSH (2.5, 5.0, and 10.0 μg/300 μL) and PHE (1.0 and 2.5 μg/300 μL). Blockade of the γ2-MSH-induced or PHE-induced increases in vascular resistance were accomplished by prazosin (0.5 μg/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 μL/min followed by 10 μL/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 euvelo-

The stability of the preparation was enhanced by this technique.

Half the 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, Milis, MA) through which 0.5 N Normosol-R solution (Abbott Laboratories, North Chicago, IL) was pumped at 97 μL/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-μL 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).
All drug and peptide solutions were made fresh daily in distilled water. The \( \gamma_2 \)-MSH was purchased from Bachem, Inc. (Torrance, CA). Prazosin and chlorisondamine were generously donated by Pfizer, Inc. (Grotton, CT) and CIBA Pharmaceutical Co. (Summit, NJ) respectively. Norepinephrine, PHE, and scorpion venom were obtained from Sigma Chemicals (St. Louis, MO).

Statistical analysis of responses to PHE or \( \gamma_2 \)-MSH before and after pharmacological blockade was by paired \( t \) tests. The MAP, heart rate, and vascular resistance responses to graded doses of \( \gamma_2 \)-MSH and PHE were by analysis of variance followed by paired \( t \) tests comparing predose and postdose values where appropriate.

### Results

Bolus injections of \( \gamma_2 \)-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 \( \gamma_2 \)-MSH produced little change in MAP (±10 mm Hg) and no change in heart rate following ganglionic blockade. The pressor response to PHE was unaffected (+81 mm Hg) by ganglionic blockade, while the baroreceptor-induced decrease in heart rate was attenuated (—17 beats/min versus —130 beats/min decrease before blockade).

In Experiment 2 (see Table 1), administration of \( \gamma_2 \)-MSH produced a significant (45 mm Hg) increase in MAP that was accompanied by a significant increase in heart rate (44 beats/min; \( p < 0.05 \)). Following blockade of cholinergic receptors with methylatropine, \( \gamma_2 \)-MSH still produced an increase in MAP and heart rate. Administration of PHE produced a significant elevation of MAP both before (45 mm Hg) and after (47 mm Hg; \( p < 0.05 \)) cholinergic receptor blockade; however, the cardiodeceleration (—111 beats/min) produced by baroreceptor activation was not observed following peripheral cholinergic blockade (—16 beats/min).

A typical cardiovascular response to i.v. infusions of 5.0 \( \mu \)g/300 \( \mu \)L \( \gamma_2 \)-MSH for 6 seconds can be seen in Figure 1. In this animal, MAP rose approximately 60 mm Hg while heart rate increased 60 beats/minute during the peak pressure response. At the peak pressure response, mesenteric resistance was 202% of baseline while abdominal aorta resistance was 259% of baseline. In contrast, renal resistance was only 138% of baseline.

Figure 2A presents a summary of the vascular responses to increasing doses of \( \gamma_2 \)-MSH. All doses of \( \gamma_2 \)-MSH produced an increase in MAP with no change in heart rate. Analysis of variance indicated that increasing doses of \( \gamma_2 \)-MSH produced an increase in vascular resistance (\( F = 16.2, p < 0.01 \)). Comparisons of preressistance and postresistance values indicate that the vasoconstriction produced by \( \gamma_2 \)-MSH was primarily in the mesenteric (at the 5.0 and 10.0 \( \mu \)g doses) and hindlimb (all doses) beds (\( p < 0.05 \)).

Cardiovascular responses to PHE are presented in Figure 2B. Although blood pressure responses to 1.0 and 2.5 \( \mu \)g of PHE were comparable to those produced with low doses of \( \gamma_2 \)-MSH, the pattern of vascular resistance changes appeared to differ. In the PHE response, the mesenteric and renal beds showed the greatest changes while the hindlimb showed little change in vascular resistance. There was relatively little change in renal resistance in response to \( \gamma_2 \)-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 \( \gamma_2 \)-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 1

<table>
<thead>
<tr>
<th>Blockade</th>
<th>Before blockade</th>
<th>After blockade</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>HR</td>
</tr>
<tr>
<td>Ganglionic (chlorisondamine, ( n = 5 ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>111 ± 7</td>
<td>444 ± 19</td>
</tr>
<tr>
<td>Response to ( \gamma_2 )-MSH (10–20 ( \mu )g)</td>
<td>171 ± 9*</td>
<td>458 ± 15</td>
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<tr>
<td>Baseline</td>
<td>114 ± 5</td>
<td>446 ± 10</td>
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<tr>
<td>Response to PHE (3–5 ( \mu )g)</td>
<td>172 ± 6*</td>
<td>316 ± 9*</td>
</tr>
<tr>
<td>Cholinergic receptor (methylatropine, ( n = 6 ))</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>117 ± 5</td>
<td>411 ± 18</td>
</tr>
<tr>
<td>Response to ( \gamma_2 )-MSH (10–20 ( \mu )g)</td>
<td>162 ± 12</td>
<td>456 ± 8*</td>
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<tr>
<td>Baseline</td>
<td>120 ± 7</td>
<td>436 ± 26</td>
</tr>
<tr>
<td>Response to PHE (3–5 ( \mu )g)</td>
<td>165 ± 12*</td>
<td>335 ± 21*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. MAP = mean arterial pressure, HR = heart rate, \( \gamma_2 \)-MSH = \( \gamma_2 \)-melanocyte-stimulating hormone, PHE = phenylephrine.

*\( p < 0.05 \), correlated \( t \) test.
FIGURE 1  The effect of a bolus infusion of γ2-MSH (5 µg/300 µL 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.

![Graph showing blood pressure and blood flow changes](image)

FIGURE 2  A Effects of three doses of γ2-MSH on MAP, heart rate (HR), and vascular resistance in mesenteric, renal, and hindlimb vascular beds. All doses of γ2-MSH caused an increase in MAP with no significant change in HR. The increased vascular resistance was mediated primarily by the mesenteric (50 and 100 µg of γ2-MSH) and the hindlimb (all doses of γ2-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 γ2-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.

![Bar chart showing vascular resistance changes](image)

of α₁-adrenergic receptors significantly attenuated the pressor response to 10 µg of γ2-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 γ2-MSH and PHE were either absent or significantly attenuated (p < 0.05). After prazosin administration, the mesenteric vascular resistance response to 10 µg of γ2-MSH was reduced from 227% of baseline to 148%.
These findings do not agree with the report by Mues et al. of action to increase sympathetic efferent activity. Elimination of the baroreceptor reflex by these peptides may have a central nervous system site of action. Reduction of the pressor response to y2-MSH was blocked by hexamethonium. This finding suggests that the peptide may be the result of activation of renal sympathetic nervous system. In Experiment 1, the pressor responses of these rats to y2-MSH were compared with those of norepinephrine. Norepinephrine (0.03–0.3 μg/kg) increased renal blood flow from 50 to 100% in these rats. Comparing these responses to a regression line through the responses to norepinephrine and y2-MSH, the peptide may be the result of activation of renal sympathetic nervous system. In Experiment 1, the pressor response to y2-MSH was blocked by ganglionic blockade with mecamylamine. We now have evidence that there was incomplete blockade to the effects of ACTH 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 y2-MSH.

Intrarenal arterial boluses of y2-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 y2-MSH; 20 to 60 μg/kg boluses reduced 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 y2-MSH were compared with those of norepinephrine. Norepinephrine (0.03–0.3 μg/kg) increased renal blood flow from 50 to 100% in these rats. Comparing these responses to a regression line through the responses to norepinephrine and y2-MSH, we found that 20 μg/kg of y2-MSH is equipotent with 0.27 μg/kg of norepinephrine. The renal vasoconstriction produced by y2-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 agonist1, but did not block the responses to y2-MSH.

Discussion

Recent experimental evidence has suggested that y2-MSH and related molluscan and avian cardioexcitatory peptides (FMRFamide and LPLRFamide) can have pronounced physiological actions, including increased MAP and heart rate. We and others14, 15 have presented evidence that the pressor responses of these vasoactive peptides are due to activation of the sympathetic nervous system. In Experiment 1, the pressor response to y2-MSH was blocked by the ganglionic blocking agent chlorisondamine. These results suggest that y2-MSH has pressor actions by activating preganglionic sympathetic afferents. These findings are consistent with the work of Barnard and Dockray and associates, who found that the pressor response to intracerebrally administered LPLRFamide is blocked by phenolamine and guanethidine and reduced by hexamethonium. This finding suggests that these peptides may have a central nervous system site of action to increase sympathetic efferent activity. These findings do not agree with the report by Mues et al., who reported that the pressor actions of FMRFamide are independent of sympathetic activation and any known hormonal pressor system; nor do they agree with our initial report that these cardiovascular effects of ACTH were not totally blocked by ganglionic blockade with mecamylamine. We now have evidence that there was incomplete blockade to the effects of ACTH. At that time we were unaware of the total elimination of the baroreceptor reflex by these peptides and used the lack of bradycardia as evidence of complete ganglionic blockade.

In Experiment 2 blockade of peripheral cholinergic receptors with methylatropine had no effect on the pressor or cardioaccelerator response to y2-MSH. In contrast, methylatropine blocked the reflex cardioaccelerator response brought by PHE-induced increase in MAP. These findings, along with the results of a previous study, suggest that y2-MSH does not cause a direct increase in sympathetic drive to the heart that can compete with baroreceptor-mediated slowing of the heart. That is, we did not observe any further increase in heart rate following cholinergic or y2-adrenergic receptor blockade. Rather, it appears that y2-MSH may have a direct (though not dose-dependent) positive chronotropic action on the heart or that the cardioaccelerator effect may be due to circulating catecholamines. In addition, y2-MSH in some way interferes with baroreceptor function. All of these findings appear reasonable in that related peptides (FMRFamide and LPLRFamide) can have direct positive inotropic and chronotropic actions on molluscan hearts, have pressor effects in animals that are attenuated by sympathetic nervous system blockade, and have direct actions on medullary reticular and nucleus tractus solitarius neurons.

The results of previous experiments suggest that y2-MSH and related peptides increase sympathetic nervous system activity. We and others14, 15 in Experiment 3, this was reflected by the ability of y2-MSH to produce an increase in vascular resistance in the mesentery and abdominal aorta beds in the face of increased MAP. These responses appeared to be mediated by the sympathetic nervous system in that y2-MSH infusion. The renal vascular bed appeared to be less responsive than the mesenteric and hindlimb beds to y2-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 y2-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. 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 y2-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. The biological activities of y2-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.

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Sympathetic nervous system mediation of acute cardiovascular actions of gamma 2-melanocyte-stimulating hormone.
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Hypertension. 1985;7:1145
doi: 10.1161/01.HYP.7.3_Pt_2.1145

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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