Evidence That Melanocortin 4 Receptor Mediates Hemorrhagic Shock Reversal Caused by Melanocortin Peptides

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ABSTRACT
Melanocortin peptides are known to be extremely potent in causing the sustained reversal of different shock conditions, both in experimental animals and humans; the mechanism of action includes an essential brain loop. Three melanocortin receptor subtypes are expressed in brain tissue: MC3, MC4, and MC5 receptors. In a volume-controlled model of hemorrhagic shock in anesthetized rats, three melanocortin peptides with a longer C-terminal extension, including ACTH-(4–10), ACTH-(1–17), ACTH-(1–24), etc., have dramatic cardiovascular effects in severe hypotensive conditions (for review see Gruber and Callahan, 1989; Versteeg et al., 1998). However, melanocortin peptides with a longer C-terminal extension, including γ1-MSH, ACTH-(4–10), ACTH-(1–17), ACTH-(1–24), etc., are devoid of these cardiovascular effects in the normotensive, normovolemic animal (Klein et al., 1985; Bertolini et al., 1986c, 1989). However, melanocortin peptides lacking the C-terminal Arg-Phe sequence [ACTH-(4–10), α-MSH, ACTH-(1–17), ACTH-(1–24), etc.] have dramatic cardiovascular effects in severe hypotensive conditions (for review see Bertolini, 1995).

In a model of volume-controlled hemorrhagic shock in rats and dogs that caused the death of all saline-treated animals within 20 to 30 min, the i.v. bolus injection of any of these peptides induces, within a few minutes, an adrenal-indepen-dent, dose-dependent (minimum and maximum ED 20 and 160 μg/kg, respectively) restoration of cardiovascular and respiratory functions. An equimolar dose of γ1-melanocyte stimulating hormone (selective agonist at MC3 receptors) was completely ineffective. The selective antagonist at MC4 receptors, HS014, although having no influence on cardiovascular and respiratory functions per se, dose-dependently prevented the antishock activity of adrenocorticotropin fragment 1–24, with the effect being complete either at the i.v. dose of 200 μg/kg or at the i.c.v. dose of 5 μg/rat (17–20 μg/kg). We concluded that the effect of melanocortin peptides in hemorrhagic shock is mediated by the MC4 receptors in the brain.

Melanocortin peptides have important cardiovascular effects. In conscious rats, as well as in rats under light urethane anesthesia (where reflexes and sufficient sympathetic tone are maintained), the adrenocorticotropin fragment 4–10 (ACTH-(4–10)) and γ1- and γ2-melanocyte-stimulating hormones [MSH; 10 times more potent than ACTH-(4–10)] induce a dose-dependent, short-lasting increase in blood pressure, heart rate (HR), and pulse amplitude following their i.v. administration (for review see Gruber and Callahan, 1989; Versteeg et al., 1998). However, melanocortin peptides with a longer C-terminal extension, including γ1-MSH, α-MSH, ACTH-(1–17), ACTH-(1–24), etc., are devoid of these cardiovascular effects in the normotensive, normovolemic animal (Klein et al., 1985; Bertolini et al., 1986c, 1989). However, melanocortin peptides lacking the C-terminal Arg-Phe sequence [ACTH-(4–10), α-MSH, ACTH-(1–17), ACTH-(1–24), etc.] have dramatic cardiovascular effects in severe hypotensive conditions (for review see Bertolini, 1995).

A model of volume-controlled hemorrhagic shock in rats and dogs that caused the death of all saline-treated animals

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ABBREVIATIONS: ACTH, adrenocorticotropin; MSH, melanocyte-stimulating hormone; HR, heart rate; PP, pulse pressure; RR, respiratory rate; MAP, mean arterial pressure.
blood flow in vital organs greatly extends the time limit for an effective and curing blood reinfusion. Although all rats reinfused with their own shed blood at 15 min after hemorrhage die within 6.6 ± 4.4 h, a substantial number of rats treated with a melanocortin peptide shortly after bleeding (within 5 min) survive, even if blood reinfusion is performed 30, 60, or 120 min later (Bertolini et al., 1989). This resuscitating effect of melanocortins also has been confirmed in a model of hypovolemic shock produced in rabbits by the graded occlusion of the inferior vena cava (Ludbrook and Ventura, 1995) and in the splanchic artery occlusion shock in rats (Squadrito et al., 1999), as well as in human subjects with hemorrhagic or cardiogenic shock (Bertolini et al., 1987; Pinelli et al., 1989; Noera et al., 1989, 1991).

The studies on the mechanisms underlying the antishock effect of melanocortins suggest that in conditions of failure of the circulatory homeostasis, these peptides inhibit the overproduction of tumor necrosis factor-α (Altavilla et al., 1998) and nitric oxide (Guarini et al., 1997) (these effects are probably related), and activate or restore a complex vasomotor reflex that eventually leads to the mobilization of the peripherally pooled residual blood (for review see Bertolini, 1995), and which is seemingly obtunded by the massive release of endogenous opioids that occurs in such conditions (Bernton et al., 1985; Schadt, 1989). Opioids inhibit sympathetic outflow and noradrenaline release from sympathetic terminals (Schadt, 1989), whereas melanocortins have an opposite effect (Szabo et al., 1987).

Together, these effects of melanocortins would remove the main causes of hemorrhage-induced circulatory decompensation, namely, the blunted release of noradrenaline from sympathetic terminals and the reduced responsivity of resistance vessels to noradrenaline. The vasomotor reflex that melanocortins activate/restore in shock conditions (Bertolini, 1985) includes an essential brain loop. Indeed, the shock reversal induced by the i.v. injection of melanocortins is prevented or greatly impaired by 1) bilateral vagotomy at the cervical level (Guarini et al., 1986); 2) the i.v. injection of capsaicin (Guarini et al., 1992) (which induces a defunctionalization of primary afferent Substance P containing nerve fibers) or of a Substance P antagonist (Guarini et al., 1992); 3) the i.c.v. injection of hemicholinium-3 (Guarini et al., 1990a); 4) the blockade of brain M₃-muscarinic receptors (Guarini et al., 1990b); and 5) the i.c.v. injection of the N-calcium channel blocker ω-conotoxin (Guarini et al., 1993). Finally, shock reversal also can be obtained with the i.c.v. injection of melanocortins (Guarini et al., 1987b).

Molecular cloning of five melanocortin receptor subtypes (MC₁–MC₅) (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992; Gantzt et al., 1993a,b; Chhajlani et al., 1993; Schiöth et al., 1996; Adan and Gispen, 1997) has provided tools for the study of the mechanisms of the effect of melanocortins. MC₁ is the specific α-MSH receptor expressed in melanocytes, melanoma cells, and macrophages; MC₂ is the ACTH receptor expressed in the adrenal gland; and MC₃, MC₄, and MC₅ receptors are also (MC₂), mainly (MC₃) or exclusively (MC₄) expressed in brain tissue. The present study was aimed at defining whether (and, in the affirmative, which) brain melanocortin receptors are involved in the antishock effect of melanocortin peptides.
Results

The baseline values of the recorded parameters (MAP, PP, HR, RR) were not significantly different in any of the experimental groups. As repeatedly described (for review see Bertolini, 1995), the acute and severe hypovolemia induced in our model of volume-controlled hemorrhagic shock in anesthetized rats was incompatible with survival and, hence, all saline-treated animals died within 30 min after saline injection (Figs. 1, 2, and 4). The i.v. bolus injection of ACTH-(1–24) 5 min after the termination of bleeding, at the maximum ED (Bertolini et al., 1989) of 160 μg/kg, produced, within a few minutes, an almost complete restoration of cardiovascular and respiratory functions that 10 to 15 min after treatment were not significantly different from baseline (Fig. 1). This effect of ACTH-(1–24) remained unchanged throughout the observation period (60 min). An i.v. equimolar dose (54 nmol/kg) of γ1-MSH, also injected 5 min after the termination of bleeding, was completely ineffective (Fig. 2).

HS014, although having no influence per se on cardiovascular and respiratory functions, both in normal, nonbled rats (Fig. 3) and in hemorrhage-shocked rats (Fig. 2), dose-dependently prevented the antishock effect of ACTH-(1–24) that was completely antagonized by HS014 either at the i.v. dose of 200 μg/kg (Fig. 1) or at the i.c.v. dose of 5 μg/rat (Fig. 4).

Discussion

Our present results confirm that in a condition of severe hemorrhagic shock invariably causing the death of all salinetreated animals within 30 min, the i.v. bolus injection of ACTH-(1–24) induces within a few minutes an almost complete and steady normalization of cardiovascular and respiratory parameters. Furthermore, these results show that a selective antagonist at MC4 receptors (HS014) (Schioth et al., 1998), either i.v.- or i.c.v.-injected, dose-dependently prevents the shock reversal induced by ACTH-(1–24); the antagonism being complete after an i.c.v. dose of 5 μg/rat or an i.v. dose of 200 μg/kg. Moreover, γ1-MSH, a selective agonist at MC4 receptors, injected in an i.v. bolus at a dose equimolar to the maximum ED of ACTH-(1–24) was completely ineffective in our experimental condition of hemorrhagic shock.

The MC3, MC4, and MC5 receptors are expressed in the brain (for review, see Hol et al., 1995; Adan and Gispen, 1997). ACTH-(1–24) has maximum agonist potency and efficacy at MC4 (equal to α- and β-MSH) (Gantz et al., 1993b) and MC5 receptors (equal to α-MSH and [Nle4, d-Phe7]α-MSH) (Griffon et al., 1994). The MC3 receptors have the highest affinity for γ1-MSH and desacetyl-α-MSH (Schioth et al., 1995), and γ-MSHs are selective agonists at MC5 receptors (for review, see Versteeg et al., 1998). HS014 is a cyclic...
Fig. 2. Effect of i.v. treatment with γ-MSH or HS014, at doses (81 and 91 μg/kg, respectively) equimolar to 160 μg/kg ACTH-(1–24), in rats subjected to hemorrhagic shock. Only data concerning MAP are shown; the effect on PP, HR, and RR was similar. Values are means ± S.E., n = 6 to 8 rats per group. *P < .05, at least, versus the corresponding value in saline-treated rats (Student-Newmann-Keuls test). In some cases, for the sake of clarity, S.E.M. was omitted. Percentage of animals surviving 60 min after treatment (value at the end of lines): **P < .005 versus saline group (Fisher’s test).

Fig. 3. Influence of i.v. (100 and 200 μg/kg) or i.c.v. (5 μg/rat) treatment with HS014 on cardiovascular and respiratory functions in normal, nonbled rats. Saline, 1 ml/kg i.v. or 5 μl/rat i.c.v. Only data concerning MAP are shown; the effect on PP, HR, and RR was similar. Values are means ± S.E., n = 6 to 8 rats per group. In some cases, for the sake of clarity, S.E.M. was omitted. Statistical assessment between groups was not significantly different (ANOVA).

Fig. 4. Influence of i.c.v. pretreatment with the MC4 antagonist HS014 (0.5–5 μg/rat) on the resuscitating effect of ACTH-(1–24) (160 μg/kg i.v.) in rats subjected to hemorrhagic shock. Only data concerning MAP are shown; the effect on PP, HR, and RR was similar. Values are means ± S.E., n = 6 to 8 rats per group. *P < .05, at least, versus the corresponding value in saline-pretreated and ACTH-treated rats (Student-Newmann-Keuls test). In some cases, for the sake of clarity, S.E.M. was omitted. Percentage of animals surviving 60 min after treatment (value at the end of lines): **P < .005 and ***P < .0005 versus the saline-ACTH group (Fisher’s test).

Our present data show that reversal of hemorrhagic shock is antagonized by morphine. Thus, the present results further confirm that it is the MC4 receptor that mediates the hemorrhagic shock reversal caused by melanocortin peptides. This may suggest that selective MC4 receptor agonists would be specifically effective in shock conditions.

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References


MSH analog that is a potent and selective antagonist at MC4 receptors and a partial agonist at MC1 and MC5 receptors; its selectivity for the MC4 receptors is 34-, 17-, and 220-fold higher than that for the MC1, MC5, and MC6 receptors, respectively (Schiotth et al., 1998).

Our present data show that reversal of hemorrhagic shock is produced by the selective agonist at MC4 and MC5 receptors [ACTH-(1–24)], but not by a selective agonist at MC3 receptors (γ-MSH), and that the antishock effect of ACTH-(1–24) can be completely prevented by a selective antagonist at MC4 receptors (HS014), injected either i.v. or i.c.v.; the i.c.v. ED being 10 to 15 times lower than the i.v. ED.

Thus, the present results further confirm that the mechanism of action of melanocortins in hemorrhagic shock rever-


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