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Role of carbon monoxide in heme-induced vasodilation

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Abstract

We investigated the effects of carbon monoxide and heme-L-lysinate on the diameter of isolated, pressurized gracilis muscle arterioles. Both agents increased arteriolar diameter. The vasodilatory effect of heme-L-lysinate, but not of carbon monoxide was prevented by an inhibitor of heme oxygenase. Hence, heme-L-lysinate-induced vasodilation appears to be mediated by a product of vascular heme metabolism, presumably carbon monoxide. This implies that vascular formation of carbon monoxide may subserve a vasodilatory function.

Keywords: Vasomotor control; Heme-L-lysinate; Chromium mesoporphyrin

Heme oxygenase catalyzes the metabolism of heme to biliverdin, free iron and carbon monoxide (CO). Heme oxygenase is expressed both in arterial smooth muscle and endothelial cells (Ewing et al., 1994; Christodoulides et al., 1995; Morita and Kourembanas, 1995; Zakhary et al., 1996), and heme oxygenase-catalyzed formation of CO was documented in vascular tissue (Morita and Kourembanas, 1995). Since CO dilates arterial vessels (Furchgott and Jothianandan, 1991) and heme-derived CO lowers blood pressure (Johnson et al., 1996), the possibility arises that vascular CO contributes to vasomotor control. We tested this hypothesis in isolated gracilis muscle arterioles by studying the effects of CO and a heme oxygenase substrate, heme-L-lysinate, on arteriolar diameter in the presence and absence of a photostable inhibitor of heme oxygenase, chromium mesoporphyrin (Vreman et al., 1993).

Chromium mesoporphyrin and biliverdin (Porphyrin Products, Logan, UT, USA) stock solutions (15 mM) were prepared in 50 mM Na_2CO_3. CO was purchased from Tech Air (White Plains, NY, USA). Heme-L-lysinate was prepared as previously described (Johnson et al., 1996).

Male Sprague-Dawley rats (150–250 g; Charles River, Wilmington, DE, USA) were anesthetized (sodium pentobarbital, 60 mg/kg, i.p.), injected with heparin (1000 U/kg, i.v.) and the gracilis anticus muscles removed. First-order gracilis arteriolar segments were isolated as previously described (Sun et al., 1994), and cannulated in an 18 ml vessel chamber, containing two Krebs'-filled micropipettes and Krebs' buffer. The proximal micropipette was connected to a pressure servo controller (model CH/200/Q, Living Systems Instrumentation; Burlington, VT, USA); the distal micropipette was connected to a stopcock. Continuous non-recirculating superfusion with Krebs' buffer (5 ml/min, 37°C), bubbled with 95% O_2/5% CO_2, was started and the distal stopcock was closed. Intraluminal pressure was slowly increased to 80 mmHg and maintained at this level throughout the experiment. Vessel length was adjusted to remove buckling. For internal diameter measurements the vessel chamber was mounted on a stage of a microscope fitted with a video camera leading to a video caliper (Texas A&M, College Station, TX, USA), monitor and recorder.

After a 60 min equilibration period the nitric oxide synthesis inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 1 mM), was included into the superfusion to minimize the possible interactions between the nitric oxide and CO systems. Experiments were initiated 20 min later by administrating heme-L-lysinate (2 μM), Krebs' buffer saturated with CO (1:10 dilution), biliverdin (15 μM), or chromium mesoporphyrin (15 μM). Concurrently, these agents were infused into the superfusion media to achieve the final concentrations noted above. Arteriolar responses to heme-L-lysinate also were studied in preparations superfused with media containing chromium mesoporphyrin or

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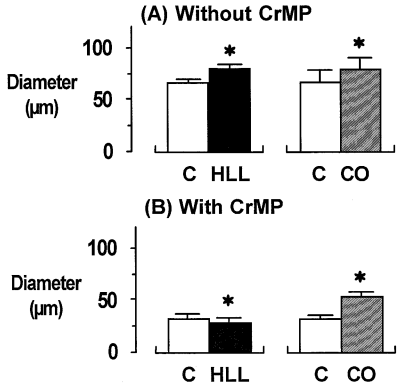

Fig. 1. Diameter of pressurized (80 mmHg) gracilis muscle arterioles before (C) and during exposure to heme-L-lysinate (HLL, 2 μM) or carbon monoxide (CO) in preparations superfused with media without (panel A) and with (panel B) chromium mesoporphyrin (CrMP, 15 μM). Data are means ± S.E.M.; asterisks indicate $P < 0.05$ relative to control (C).

deferoxamine (15.2 μM). Responses to CO also were examined in preparations superfused with media containing chromium mesoporphyrin.

The internal diameter of arterioles decreased from 124.8 ± 2.9 to 89.9 ± 4.8 μm ($n = 24$, $P < 0.05$) during the equilibration period; the addition of L-NAME caused a further decrease to 68.7 ± 3.8 μm ($n = 24$, $P < 0.05$). As shown in Fig. 1, heme-L-lysinate increased the diameter in the absence of chromium mesoporphyrin ($\Delta + 12.4 \pm 2$ μm, $n = 6$, $P < 0.05$), but reduced it in the presence of the heme oxygenase inhibitor ($\Delta - 5.3 \pm 1.8$ μm, $n = 8$, $P < 0.05$). Heme-L-lysinate also increased the diameter of arterioles in the presence of the iron chelator deferoxamine (from 75.0 ± 8.6 to 86.1 ± 8.5 μm, $n = 5$, $P < 0.05$). The heme oxygenase product biliverdin did not affect arteriolar diameter (from 80.4 ± 3.9 to 79.3 ± 4.4 μm, $n = 5$, $P > 0.05$). However, as shown in Fig. 1, CO increased ($P < 0.05$) the diameter in the absence ($\Delta + 11.8 \pm 2.8$ μm, $n = 6$), and as well as in the presence ($\Delta + 18.2 \pm 4.4$ μm, $n = 6$) of chromium mesoporphyrin. Chromium mesoporphyrin alone caused a sustained reduction of arteriolar diameter (from 63.5 ± 12.6 to 32.6 ± 9.6 μm, $n = 6$, $P < 0.05$). Matched vehicles did not affect arteriolar diameter.

The major finding of this study is that heme-L-lysinate elicits dilation of gracilis muscle arterioles. That chromium mesoporphyrin prevented the response is evidence that the vasodilatory effect of heme-L-lysinate is mediated by generation of a heme oxygenase product. Neither biliverdin nor free iron are likely to contribute to the dilatory effect of heme-L-lysinate, since biliverdin did not cause vasodilation and chelation of free iron did not interfere with the dilatory effect of heme-L-lysinate. However, that CO can dilate gracilis muscle arterioles in a manner which is not attenuated by chromium mesoporphyrin suggests that the vasoactive heme oxygenase product is CO. Collectively, these observations support the notion that vascular formation of CO may subserve a vasodilatory function.

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