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The role of the endothelium in ceramide-induced vasodilation

Douglas G. Johns [a,*], Jong-Shiaw Jin [b], R. Clinton Webb [a]

[a] *Department of Physiology, 7812 Medical Sciences Building II, University of Michigan Medical School, Ann Arbor, MI, 48109-0622, USA*
[b] *Department of Physiology, National Defense Medical Center, Taipei, China*

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Abstract

Ceramide, a novel sphingomyelin-derived second messenger mediates cellular signals of cytokines such as tumor necrosis factor-alpha (TNF-α). In the present study, we hypothesized that the endothelium contributes to ceramide-induced vasodilation. We report that relaxation to ceramide in endothelium-intact rat thoracic aortic rings is greater than in endothelium-denuded or endothelial nitric oxide synthase (endothelial NO synthase)-inactivated rings. We conclude that the endothelium contributes to ceramide-induced relaxation possibly through an interaction between sphingomyelin hydrolysis and endothelial NO synthase within caveolae.

Keywords: Ceramide; Vasodilation; Endothelium; vascular

Ceramide is a lipid second messenger generated by hydrolysis of membrane sphingomyelin by a neutral pH-operating, membrane-associated sphingomyelinase (Hannun, 1994). Cytokines such as tumor necrosis factor-alpha (TNF-α) have been shown to stimulate ceramide generation in a variety of cell types (Kim et al., 1991). TNF-α is a vasodilator which may be a causative factor in the severe hypotension seen in sepsis or endotoxemia (Damas et al., 1997). We have previously demonstrated that both ceramide and exogenously applied sphingomyelinase elicit concentration-dependent vasodilation in contracted rat thoracic aortic rings (no endothelium) (Johns et al., 1997). However, the role of the vascular endothelium in ceramide-induced vasodilation has not been investigated. This study tests the hypothesis that the endothelium contributes to ceramide-induced vasodilation.

Male Sprague–Dawley rats weighing 200–250 g were used for this study. The methods for isometric force recording in rat thoracic aortic rings were conducted as previously described (Johns et al., 1997). For the relaxation experiments an EC_{50} concentration of phenylephine was used to contract each vessel segment. During the plateau phase of the phenylephrine contraction, either C2-ceramide, ethanol vehicle, or acetylcholine was added to the muscle bath. Removal of endothelium was accomplished by gentle rubbing of the lumen of each ring with the tips of a pair of forceps. In some experiments, N^{ω}-nitro-L-arginine (L-NNA) (10^{-4} mol/l) or methylene blue (10^{-5} mol/l) was added prior to experimentation.

The following compounds were purchased from Sigma (St. Louis, MO): L-phenylephrine HCl, acetylcholine HCl, N^{ω}-nitro-L-arginine, methylene blue, and indomethacin. *N*-acetylsphingosine (C2-ceramide) was purchased from Calbiochem (La Jolla, CA). C2-ceramide was prepared as 2×10^{-2} mol/l stock solutions in 95% ethanol. Ethanol concentrations in the tissue baths did not exceed 0.1%.

Results are presented as the mean $\pm$ standard error of the mean (S.E.M.). For two-group comparisons, Student's *t*-test was used with the Bonferroni correction for multiple test procedures with a *P*-value of less than 0.05 being considered significant.

Fig. 1A illustrates ceramide-induced relaxation in the presence and absence of endothelium. In contracted aortic rings, 10^{-6} and 10^{-5} mol/l C2-ceramide caused a 13% and 30% relaxation in the endothelium-denuded rings, and a 27% and 55% relaxation in the endothelium-intact rings, respectively. Acetylcholine (10^{-8} mol/l and 10^{-7} mol/l) caused a 8% and 50% relaxation, respectively in endothelium-intact aortic rings, but did not elicit relaxation in endothelium-denuded rings (Fig. 1B).

Inhibition of endothelial NO synthase with L-NNA (10^{-4} mol/l) partially blocked the relaxation response to

* Corresponding author. Tel.: +1-734-763-5606; fax: +1-734-647-7950; e-mail: dgjohns@umich.edu

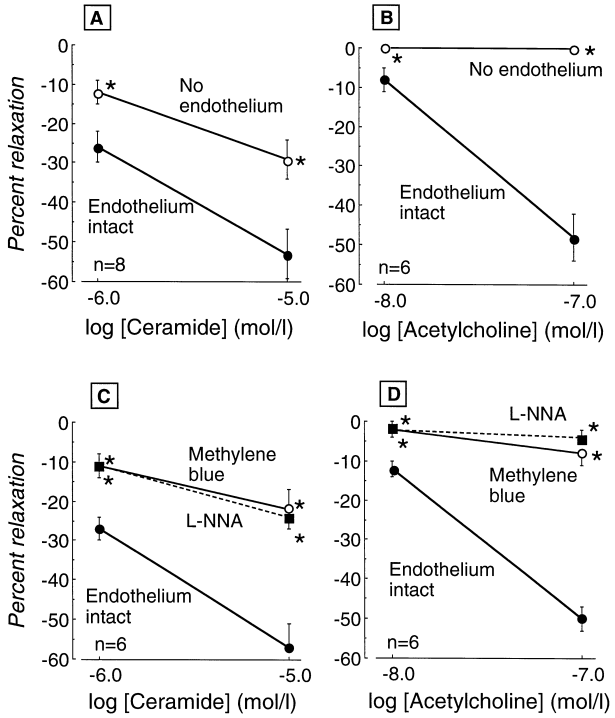

Fig. 1. Relaxation response to ceramide and acetylcholine in rat thoracic aortic rings. (A) Endothelium removal (open circles) partially inhibits the relaxation response to ceramide (10^{-6}, 10^{-5} mol/l) compared to endothelium-intact rat thoracic aortic rings (closed circles); (B) Endothelium removal (open circles) completely blocks the relaxation response to acetylcholine (10^{-8}, 10^{-7}) compared to endothelium-intact rat thoracic aortic rings (closed circles); (C) Blockade of endothelial NO synthase with L-NNA (10^{-4} mol/l) (dotted line) or inhibition of guanylate cyclase with methylene blue (10^{-5} mol/l) (open circles) partially inhibits the relaxation response to ceramide compared to untreated controls (closed circles); (D) Blockade of endothelial NO synthase with L-NNA (10^{-4} mol/l) (dotted line) or inhibition of guanylate cyclase with methylene blue (10^{-5} mol/l) (open circles) completely blocks the relaxation response to ceramide compared to untreated controls (closed circles). $*$ $P < 0.05$ vs. control (closed circles).

C2-ceramide (Fig. 1C), and completely blocked the relaxation response to acetylcholine (Fig. 1D). Similar results were observed with the guanylate cyclase inhibitor methylene blue (10^{-5} mol/l). The relaxation response of ceramides with varying hydrocarbon chain lengths (i.e., C6-, C8-, C16-ceramide) were not significantly different from those of the C2 form (data not shown).

We tested the hypothesis that the endothelium may contribute to ceramide-induced vasodilation in contracted rat thoracic aortic rings. Previously, we have shown that ceramide elicits concentration-dependent vasodilation in contracted rat thoracic aortic rings devoid of endothelium (Johns et al., 1997). Here, we describe an augmentation of ceramide-induced relaxation in the presence of an intact endothelium. Inhibition of endothelial NO synthase with L-NNA and blockade of guanylate cyclase with methylene blue attenuated the relaxation response to ceramide. Therefore a portion of the relaxation to ceramide is dependent upon endothelium-derived nitric oxide.

TNF-α is an inflammatory cytokine known to cause vasodilation through endothelium-dependent and -independent mechanisms (Hollenberg et al., 1991; Takahashi et al., 1992). Because TNF-α causes ceramide generation in other cell types, it is possible that the mechanism for both endothelium-dependent and independent vasodilation occurs via ceramide signaling. Another interesting and important aspect of ceramide signaling is that the caveolae of endothelial cells are enriched in sphingomyelin, endothelial NOS, and other regulatory proteins (Okamoto et al., 1998). Liu and Anderson (1995), showed that in human fibroblasts, cytokines elicit ceramide generation localized to caveolae. Therefore, this study provides novel pharmacological observations linking the second messenger, ceramide with its potential activity in the caveolar subdomain of the membrane where sphingomyelin and endothelial NO synthase are co-localized.

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