

TITLE: ETOMIDATE PRODUCES VASODILATION BY MIXED ENDOTHELIUM-DEPENDENT AND INDEPENDENT MECHANISMS
AUTHORS: S. Dozaki, M.D., Ph.D.; K.S.K. Chang, M.D., Ph.D.; R.F. Davis, M.D.
AFFILIATION: Dept. of Anesthesiology, Oregon Health Sciences University, and V.A. Medical Center, Portland, OR 97201

Introduction: Vascular endothelium (ED) mediates vasodilation by releasing endothelium-derived relaxing factor (EDRF). Some pharmacologic vasodilation depends upon EDRF release (i.e., acetylcholine) while other drugs act directly on the smooth muscle (i.e., nitroprusside)¹. Hypertension, diabetes and atherosclerosis alter normal ED function². Accordingly it is important to understand the direct vascular effects of anesthetic drugs that may be administered to patients with potentially abnormal ED function. This protocol examines the vascular response to etomidate (ET) utilizing the isolated rat thoracic aortic ring preparation with and without ED.

Methods: Thoracic aortic rings (3 mm width) were obtained from male Sprague-Dawley rats (N=16) and suspended in an organ bath in Krebs-Henseleit solution (37°) aerated with 95% O₂/5% CO₂. The force of isometric contraction was recorded using 2 gm tension. Randomly selected rings were denuded of ED by gently rubbing the intimal surface with forceps. Submaximal contraction (50-70%) was induced using either KCl (40 mM) or phenylephrine (PE, 3x10⁻⁷ M). ET was then added cumulatively to the bath.

Relaxation responses with increasing ET concentration were expressed as the percentage tension decrement from the contractile force elicited by KCl or PE. Thus, cumulative ET concentration (3x10⁻⁶-10⁻⁴ M) vs relaxation response data with and without ED were obtained. Data are expressed as mean ± SEM. Statistical analysis was performed by ANOVA with a significance level of p < 0.05.

Results: ET produced concentration-dependent relaxation that was significantly attenuated in the ED denuded rings, compared to the ED intact rings, with both KCl and PE precontractions (Fig. 1 and 2). There was no difference in the degree of relaxation with ET comparing KCl and PE contracted rings.

Discussion: The data indicate that ET produced vascular relaxation in the ED denuded as well as ED intact rings. However, in the ED denuded rings the relaxation response to ET was significantly less compared to those with intact ED, whether precontracted with KCl or PE. Thus, the ET relaxation is partially dependent on ED. KCl and PE produce contraction by different mechanisms; extracellular Ca²⁺ influx through voltage gated Ca²⁺ channels for KCl and receptor operated intracellular Ca²⁺ release and Ca²⁺ channels for PE. Nevertheless, the relaxation response to ET was not different comparing KCl to PE contracted rings, suggesting nonspecific vascular relaxation. The relaxation response began to be observed at 10⁻⁵ M (2.4 µg/cc) which is within the clinical range. Because ET produced less relaxation in ED denuded rings, one might expect a less pronounced depressor effect clinically with diseases such as diabetes, hypertension or atherosclerosis, which adversely affect ED function.

References:

1. Ann Rev Pharmacol Toxicol 24:175-197, 1984.
2. New Eng J Med 323:27-36, 1990.

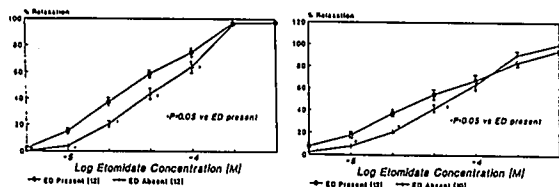


Fig. 1 - KCl Contracted Aorta

Fig. 2 - PE Contracted Aorta

TITLE: MECHANISM OF INHIBITION OF EDRF SYNTHASE ACTIVITY BY HIGH AND LOW OXYGEN TENSIONS.
AUTHORS: R.A. Johns, M.D. and A. Rengasamy, Ph.D.
AFFILIATION: Dept. of Anesthesiology, University of Virginia Health Science Center, Box 238, Charlottesville, Virginia 22908.

OBJECTIVES: Endothelium-derived relaxing factor (EDRF), first discovered as a potent vasodilator, is now recognized as an important messenger for the activation of soluble guanylate cyclase in a wide variety of cell types including vascular smooth muscle (VSM), central and peripheral neurons, adrenocytes, macrophages and PMN's. EDRF is now known to be synthesized from the guanido-nitrogen atom of L-arginine by EDRF synthase, a calcium, calmodulin and NADPH requiring enzyme. We previously reported that EDRF-induced pulmonary vascular relaxation and cyclic GMP accumulation are markedly inhibited by hypoxia. Hyperoxia also inhibits EDRF-dependent vasodilation. We have now employed an EDRF synthase preparation from bovine cerebellum to investigate the mechanism of hypoxia- and hyperoxia-induced inhibition of EDRF activity.

METHODS: Cerebellum (10g) was homogenized in 50 ml buffer (0.32 M sucrose, 20 mM HEPES (pH 7.4), 0.5 mM EDTA, 1 mM dithiothreitol, 5 mg phenylmethylsulfonylfluoride), centrifuged at 20,000 X g for 60 min and the supernatant passed through a Dowex AG50W-X8 (Na⁺ form) column to remove endogenous arginine. EDRF synthase activity was measured by determining the production of [³H]-citrulline, a stable byproduct of the reaction or by performing the reaction in wells of cultured VSM and measuring EDRF production as assayed by cyclic GMP accumulation in the VSM. Enzyme activity was determined under hypoxic (pO₂=25-30 mm Hg), normoxic (pO₂=130-135 mm Hg) and hyperoxic (pO₂= 450-460 mm Hg) conditions. In some experiments, hypoxia was replaced by normoxia or hyperoxia after 30 min. Recovery of [³H]-citrulline was 96% and nonspecific elution of [³H]-L-arginine was 3%.

RESULTS: The enzyme preparation employed was confirmed to be calcium, calmodulin, and NADPH dependent and was inhibited by nitro-L-arginine, a specific inhibitor of EDRF synthase. Using [³H]-citrulline as a measure of activity, the Km values for arginine in hypoxia, normoxia and hyperoxia were 4.1±0.7, 4.3±0.6 and 5.5±0.7 µM while the Vmax values were 27±5, 76±5, and 100±5 pmol/min per mg protein, respectively. The inhibition of EDRF synthase activity by hypoxia was reversed by reexposure to normoxia or hyperoxia. The effect of oxygen tension on EDRF produced by the enzyme was assessed by the accumulation of cyclic GMP in cultured VSM. Hypoxia almost completely inhibited the production of cyclic GMP which was comparable to its inhibition of [³H]-citrulline production. Hyperoxia, however, showed partial inhibition of cyclic GMP production despite the fact that [³H]-citrulline production was not different from that seen with normoxia. This cyclic GMP inhibition by hyperoxia was reversed by superoxide dismutase.

CONCLUSION: We conclude that hypoxia reversibly inhibits EDRF synthase activity primarily through depletion of oxygen, one of the substrates for the enzyme. Hyperoxia enhances the activity of EDRF synthase but EDRF, once formed, is partially inactivated by superoxide radical. EDRF production appears to be tightly regulated by oxygen tension.

