

Short Communication

The Endothelium-Dependent Effects of Thimerosal on Mouse Pial Arterioles In Vivo: Evidence for Control of Microvascular Events by EDRF as well as Prostaglandins

William I. Rosenblum, Hiroyuki Nishimura, *Earl F. Ellis, and Guy H. Nelson

*Department of Pathology (Neuropathology), Medical College of Virginia, and *Department of Pharmacology, School of Basic Health Sciences, Virginia Commonwealth University, Richmond, Virginia, U.S.A.*

Summary: Thimerosal causes synthesis and/or release of both endothelium-derived relaxing factor (EDRF) and prostaglandins from conductance vessels in vitro. We tested its effects and mechanism of action on mouse pial arterioles in vivo using intravital microscopic techniques. Topical thimerosal dilated pial arterioles. This effect was eliminated by endothelial injury produced by a laser/ Evans blue technique. Dilation was also eliminated by topical L-NMMA, a reported inhibitor of EDRF synthesis. Topical thimerosal also reduced the incidence of platelet adhesion/aggregation (“capture”) at a site of minimal endothelial damage. This effect was eliminated by L-NMMA pretreatment. The ability of thimerosal to dilate arterioles was eliminated not only by treatments

thought to eliminate synthesis/release of EDRF, but also by cyclooxygenase inhibitors. However, inhibition of platelet adhesion/aggregation was not affected by cyclooxygenase inhibition. Thimerosal significantly increased production of prostaglandin E_2 recovered from a closed cranial window. We conclude that the dilating effects of thimerosal on diameter require two endothelium-derived agents: EDRF and one or more prostaglandins acting in concert. However, the inhibiting effect of thimerosal on local platelet adhesion/aggregation appears to be caused only by an increase in EDRF at the injured site. **Key Words:** Thimerosal—EDRF—Prostaglandins—Vasodilation—Brain microcirculation—Endothelial injury—Platelet adhesion/aggregation.

We have published evidence that “classical” endothelium-dependent relaxing factor (EDRF) plays a role both in modulating the tone of mouse pial arterioles in vivo and in modifying the ability of the endothelium of these vessels to attract platelets or initiate platelet aggregation (Rosenblum, 1986, 1988; Rosenblum et al., 1987, 1990b; Nishimura et al., 1991). We designate this EDRF as $EDRF_{ACh}$ to signify that it was originally shown to mediate relaxation by acetylcholine (ACh) (Furchgott, 1983).

Thimerosal (sodium ethylmercurithiosalicylate) activates the synthesis and/or release of both “clas-

sical” endothelium-dependent relaxing factor and prostaglandins (PG) (Forstermann et al., 1986). In the present studies, we took advantage of these properties of thimerosal to investigate the relative importance of $EDRF_{ACh}$ and PGs in modulating microvascular events in the mouse brain in vivo.

METHODS

Our methods have been described in great detail in numerous publications (Rosenblum and Zweifach, 1963; Rosenblum, 1971; Rosenblum and Nelson, 1988a,b; 1990; Rosenblum et al., 1990b). In brief, ICR male mice were anesthetized with urethane and their cerebral surface arterioles (pial arterioles) exposed by craniotomy. The mice were maintained at 37°C and the cerebral surface was continuously suffused with mock cerebrospinal fluid (Elliott and Jasper, 1949) at pH 7.3–7.4. All solutions applied to the surface are maintained within this pH range. Television microscopy and an image splitter were used to monitor and measure the selected segment (Baez, 1966).

Selective injury of the endothelium was produced by a

Received September 23, 1991; final revision received December 2, 1991; accepted January 15, 1992.

Address correspondence and reprint requests to Dr. W. I. Rosenblum at Neuropathology, Medical College of Virginia, Box 17, MCV Station, Richmond, VA 23298-0017, U.S.A.

Abbreviations used: ASA, acetylsalicylic acid; EDRF, endothelium-derived relaxing factor; INDO, indomethacin; L-NMMA, N-guanidino-L-monomethyl arginine; PG, prostaglandin.