Title: Lidocaine Alters Phosphatidylinositol Hydrolysis in Vascular Endothelial Cells

Authors: Deborah S. Brown, M.D., and Audrey J. Robinson-White, Ph.D.,

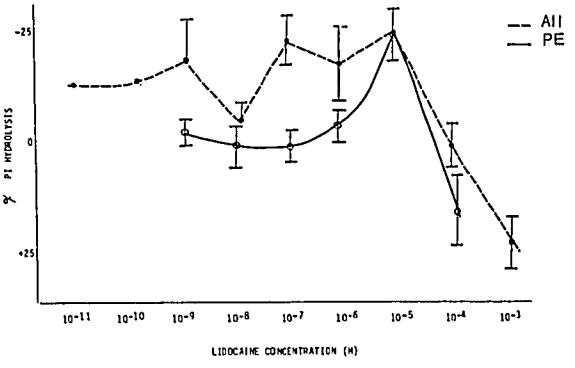
Affiliation: Department of Anesthesiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799

Introduction. Phosphatidylinositol (PI), a plasma membrane phospholipid, acts as a second messenger to regulate intracellular Ca^{+2} levels and initate a cellular response. Previously it has been reported that lidocaine, depending on the concentration[1], can cause both vasoconstriction and vasodilation[1,2]. However, these studies were indirect measurements that did not attempt to establish a biochemical mechanism for the effect. Recently, barbiturates have been shown to competitively inhibit PI hydrolysis in cultured vascular smooth muscle cells[3]. Therefore, we sought to determine whether the vascular effects of lidocaine were related to PI hydrolysis in cultured endothelial cells.

Methods. a) Rat aortic endothelial cells were obtained by explant of aortic tissue[4]. Briefly, rat thoracic aortas were excised, cleaned of fat and adventitia, and cut into 1 mm^2 sections. Sections were planted into culture flasks and cells were allowed to grow out from the sections in supplemented M199 plus 20% FBS, in 5% CO_2/95% air at 37°C. Cells were subcultured as a homogeneous population of endothelial cells as evidenced by light and electron microscopy. b) Phosphatidylinositol breakdown was measured by incubating cultures with ^3H inositol and then replacing culture media with buffered salt that contained $LiCl^+$(10mM). Cultures were then incubated (water bath, 37°C). Anesthetics were added 10 min later, and vasoactive stimulators and anesthetics/ or buffer 10 min thereafter. Reactions were terminated by the addition of a chloroform/ methanol mixture (1:2) The ^3H labelled metabolites were separated as water soluble inositol phosphates and chloroform inositol phospholipids. The inositol phosphate metabolites were separated by dowex-1-formate columns. The accumulation of inositol was expressed as the percent of ^3H phosphatidylinositol in unstimulated cultures[5]. Values were mean + S.E.M. of 2 to 8 experiments performed in triplicate.

Results. Endothelial cells were induced to undergo PI hydrolysis by angiotensin II (AII, at 5nM) and phenylephrine (PE, at 10uM). Percent hydrolysis was 11.9 and 5.0 respectively. The order of potency (AII>PE) was apparent with respect to ED_{50} and the maximum extent of hydrolysis induced by optimum concentrations of both agonists. Studies with lidocaine (see figure) showed both inhibition as well as stimulation of PI hydrolysis. With AII (5nM) and PE (10uM), lidocaine inhibited PI hydrolysis (0 to 25%) at concentrations of 10^{-9}M to 10^{-5}M. The inhibition was greater with AII than with PE. However, with both AII and PE, with concentrations of lidocaine between 10^{-5}M and 10^{-3}M, there was a sharp reversal of the inhibition resulting in stimulation of PI hydrolysis (up to 25%). No differences were observed when commercial preparations of lidocaine, with and without preservatives, were employed.

Discussion. The data demonstrates that lidocaine may alter PI hydrolysis in cultured aortic endothelial cells in a biphasic manner. Lower concentrations of lidocaine (10^{-10}M to 10^{-5}M), which inhibit PI hydrolysis, correspond to concentrations seen in the plasma of patients undergoing intravenous infusion or nerve blocks (10 ug/ml or $1x10^{-5}$M). Higher concentrations (10^{-5}M to 10^{-3}M) which stimulate PI hydrolysis are similar to concentrations occurring at the site of injection (10^4 ug/ml or 3.7×10^{-2}M). These concentrations of lidocaine that exert a biphasic effect on PI hydrolysis in cultured vascular endothelial cells have also been shown to produce both vasodilation and vasoconstriction of arterioles in rat cremaster muscle[1]. Endothelial cells are known to regulate the tonic state of the vessel by release of factors that relax vascular smooth muscle cells, and since both vasodilatory and vasoconstrictor[1,2] effects have been observed with local anesthetics, it is possible that local anesthetics exert their vasodilating and vasoconstricting actions through an alteration of endothelial cell phosphatidylinositol hydrolysis.



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