Title: Pentobarbital Selectively Inhibits Phosphatidylinositol Hydrolysis In Cultured Vascular Endothelial Cells

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INTRODUCTION: Barbiturates cause major changes in cardiovascular function which lead to hypotension and vasodilation. The underlying biochemical mechanism of the effects remain unknown. One recent hypothesis proposes that barbiturates interact in a specific manner with membrane proteins[1] (i.e. receptors, binding proteins) and thereby inhibit or stimulate transmembrane signaling such as phosphatidylinositol (PI) or C'AMP and C'GMP pathways. To determine the effect of barbiturates on cardiovascular function at a cellular biochemical level, we investigated the effect of pentobarbital (Pb) on the PI pathway in cultured rat aortic endothelial cells.

METHODS: Endothelial Cell Culture: Endothelial cells were cultured by a modification of a previously described procedure.[2] Briefly, rat thoracic aortas were excised, cleaned of fat and adventitia and minced into 1 mm^2 sections. Sections were planted into culture flasks. Cells that grew out from the sections were subcultured as a homogenous population as shown by light, electron, and fluorescent microscopy. PI Breakdown: PI breakdown was measured by incubating cultures with [^{3}H]inositol, washing, and replacing medium with a $LiCl^+$ (10mM) containing buffer. Cultures were incubated at 37°C for 10 min. Pb was added (10 min) and stimulators and Pb/ or buffer added thereafter. Reactions were terminated by the addition of chloroform/methanol (1:2). The [^{3}H]-labelled metabolites were separated as water soluble inositol phosphates and chloroform inositol phospholipids. The inositol phosphate metabolites were separated by Dowex-1-formate columns.[3] The data is expressed as % inhibition of PI hydrolysis in the presence of Pb. Values are mean + SEM of 2-3 experiments performed in triplicate. [^{125}I]- Angiotensin II ([^{125}I]-AII) Binding Assay: [^{125}I]- AII binding was measured in cells grown in 6-well culture plates. Cultures were washed and medium replaced with buffer. Pb was added (22°C), and [^{125}I]-AII and Pb/or buffer added thereafter. Binding was halted with ice-cold buffer. Cells were scraped from wells and filtered. Filters were washed, dried, and ^{125}I content determined by gamma count. Values were mean ± SEM of 3 experiments performed in triplicate.

RESULTS: PI hydrolysis in vascular endothelial cells was stimulated by angiotensin II (AII), norepinephrine (NE) and phenylephrine (PE) in a time and dose-dependent manner. The order of potency was AII> NE>PE with respect to the maximal % hydrolysis induced by each agonist. Pb, at clinically relevant concentrations (0.1 to 1mM), inhibited PI hydrolysis caused by all 3 agonists. The inhibition was inversely related to the concentration and directly related to the potency of the agonists. Kinetic analysis of the inhibition of stimulation by the most potent agonist (AII) indicated competitive inhibition of a high affinity component (Km 1.5 nM, Figure). Endothelial cells also bound [^{125}I]-AII to a high affinity receptor site (KD 1.6 nM), and binding was inhibited by Pb (0.1 to 1.0 mM). In order to examine the action of Pb on a GTP-regulatory binding protein (s), PI hydrolysis was examined in permeablized $2H_3$ cells stimulated with GTP-γ-S. Hydrolysis was inhibited by 20-80% in the presence of Pb.

DISCUSSION: The present results demonstrate that Pb selectively inhibits AII stimulated PI hydrolhydrolysis in cultured endothelial cells, and alters the affinity of [^{125}I]-AII for the receptor. Although these studies suggested competitive inhibition at the AII receptor site, we propose that the site of inhibition is at a GTP-regulatory binding protein (s). PI breakdown is reported to be initiated by the coupling of receptors to phospholipase C by a plasma membrane located GTP-regulatory binding protein (s). Chemicals such as GTP-γ-S and NaF have been shown to act directly on GTP-regulatory binding protein (s) and thereby alter the affinity of the receptor for the ligand[4]. The effects observed in this study with Pb could be explained by a similar direct action of Pb on GTP-regulatory binding protein (s). This hypothesis is supported by our observation that Pb inhibits PI hydrolysis following receptor activation and directly inhibits GTP-γ-S activation of GTP-regulating binding protein in $2H_3$ cells. However, it remains to be determined if the inhibition of PI hydrolysis by Pb is through its action on GTP-regulatory binding protein(s) in vascular cells. Since endothelial cells regulate the tonic state of the vessel, these findings may not only have broad implications on the action of barbiturates on GTP-regulating binding proteins in general, but may help to elucidate the many effects of barbiturates on the cardiovascular system.

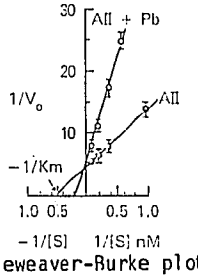

Figure. Lineweaver-Burke plot of PI hydrolysis

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