

RESEARCH INTERESTS - SANDO

Involvement of Protein Kinase C in signaling pathways affected by anesthetics

General anesthetics have been used clinically for over 100 years but their mechanism of action is not yet defined. The Myer-Overton correlation of anesthetic potency with oil-water partitioning has suggested interaction with both membrane lipids and with hydrophobic domains of proteins. We have found that Protein kinase C (PKC) isozymes are activated reversibly by membrane lipids in a manner that depends on physical properties of the membrane and that PKC activity is affected by anesthetic alcohols in a manner that correlates with alcohol effects on lipid properties. Recently, Das et al. (J. Biol. Chem. 279:37964, 2004) have identified an alcohol binding site in the membrane-binding C1b domain of PKC ϵ . Thus, this enzyme family may constitute a major class of general anesthetic receptors. The major interest of my laboratory is to understand the structure and activation of individual PKC isozymes and the roles each plays in specific cellular processes. This understanding should facilitate design of more specific PKC activators and inhibitors for clinical use.

Anesthetic effects are reversible at high pressure. In a past collaboration with Drs. Steers and Tuttle (Urology), we examined the hypothesis that PKC may transduce physical stimuli such as cell stretch or pressure changes that occur in the urinary tract or the vasculature. Properties of membrane lipids that activate PKC have been studied using biochemical and biophysical techniques. Structural analysis of PKC is conducted in collaboration with Dr. Kretsinger (Biology) via generation of 2-dimensional crystals on defined lipid monolayers and with Drs. Grisham and Cafiso (Chemistry) using NMR and EPR.

Among the proteins regulated by PKC are many ion channels and receptors that also are affected by anesthetics. In collaboration with Drs. Lynch, Kamatchi, Patel, Zuo and Bayliss (Anesthesiology), we are analyzing the regulation by PKC of calcium, sodium, and potassium channels and of glutamate transporters involved in anesthetic actions. In collaboration with Dr. Hussaini (Pathology), we also have studied the role of PKC isozymes in development of invasive glioblastomas. A newer project involving collaborations with Drs. Gaylann (Endocrinology), Stevenson and Somlyo (Physiology) and Patel (Anesthesiology) addresses the regulation by PKC of a peptide hormone that has effects on several ion channels and on lymphocyte, cardiovascular, adipocyte, and osteoblast functions.

GRANT SUPPORT

Sando % effort - 20% 3 RO1 GM31184-17S1 Sando (PI) 9-30-03 – 8-30-07, NIH/NIGMS Structure and Activation of Protein kinase C isozymes. The aims of this grant are I) to determine the structures of PKC isozymes on a lipid surface in the presence of various activators, inhibitors and substrates using 2D, crystallography and II) to determine whether lipid modulators affect PKC by altering domain formation in the membrane in vitro.

1 RO1 CA90851-01 Hussaini (PI) 7-01-02 – 6-30-07, Sando % effort - 10 % NIH/NCI The role of PKCh in regulating astrocytoma invasive growth. The specific aims of this grant are to determine I) whether PKCh expression or activation differs between neoplastic and non-neoplastic human astrocytes, II) how PKCh expression is controlled in astrocytic tumors, and III) whether manipulation of PKCh expression alters growth, migration/invasion or apoptotic properties of neoplastic astrocytes.

1 RO1 GM065211-01A Zuo (PI) 2-01-03 – 1-31-08, Sando % effort - 12 % NIH/NIGMS Volatile anesthetic modulation of glutamate transporters. The specific aims are to determine I) whether volatile anesthetics affect trafficking, phosphorylation and activity of glutamate transporters, and II) whether volatile anesthetics reduce ischemia/hypoxia-induced reversed transport of glutamate.

1 RO1 GM65214-01A Kamatchi (PI) 8-01-03 – 7-30-08, Sando % effort - 15% NIH/NIGMS PKC modulation of calcium current and anesthetic action. The aims are I) to identify PKC-induced phosphorylation sites in the $\alpha 1$ subunits of Cav channels in *Xenopus* oocytes expressing Cav channels and muscarinic M1 or angiotensin AT 1A receptors, II) to identify PKC isozymes involved in the actions of each agonist, and III) to identify the PKC isozymes through which volatile anesthetics affect the Cav channels

KEY WORDS

Protein kinase C (PKC)

Anesthetic mechanisms

Signal transduction

Cell regulation

Cellular & molecular endocrinology

Cellular & molecular immunology