

# ENDOGENOUS PKI $\gamma$ INHIBITS THE ANTI-APOPTOTIC EFFECTS OF PTH AND $\beta$ -ADRENERGIC AGONISTS IN OSTEOBLASTS

Chen, X; Song, I H; Dennis, J E; Greenfield, E M

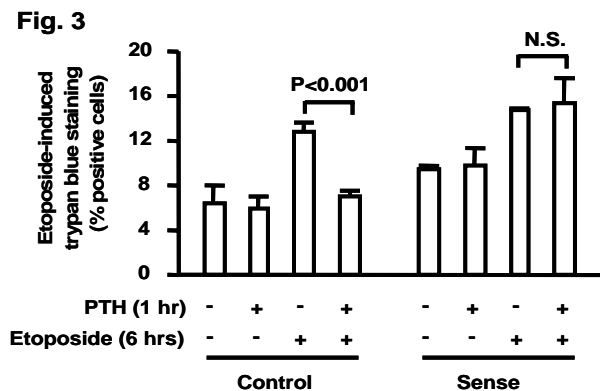
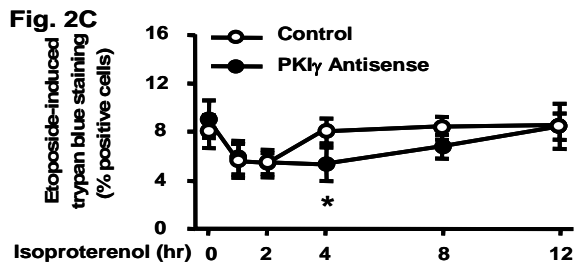
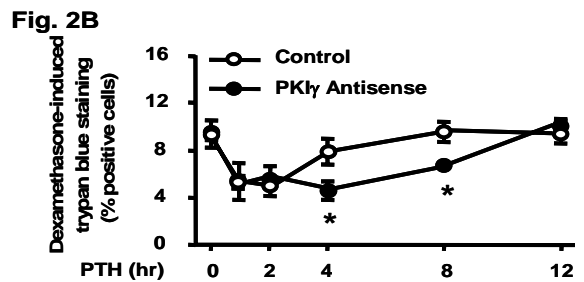
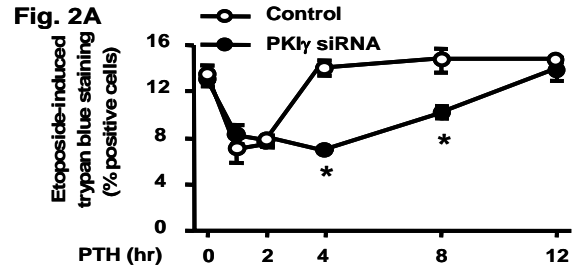
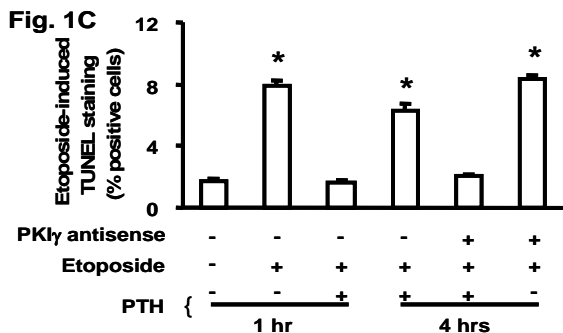
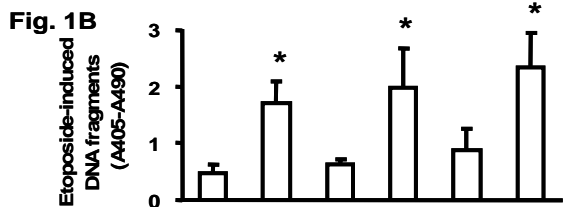
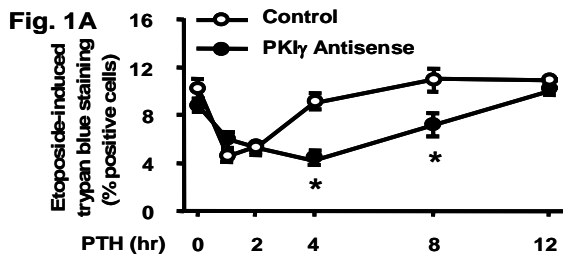
Departments of Orthopaedics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio  
216-368-1373; Fax: 216-368-1332; sxc107@po.cwru.edu

**BACKGROUND:** Parathyroid hormone (PTH) has both catabolic and anabolic effects on bone, which are primarily due to cAMP/PKA signaling and regulation of gene expression. The anabolic effect of PTH is primarily mediated by immediate-early genes that are upregulated in a rapid and transient fashion. We previously showed that efficient termination of PTH-induced immediate-early gene expression depends on protein kinase inhibitor- $\gamma$  (PKI $\gamma$ ) (1). Inhibition of osteoblast apoptosis is thought to be an important, but transient, mechanism partly responsible for the anabolic effects of intermittent PTH. Therefore, we hypothesized that endogenous PKI $\gamma$  also terminates the anti-apoptotic effect of PTH.

**METHODS:** PKI $\gamma$  mRNA and protein levels in ROS 17/2.8 cells were knocked down by induction of a transfected antisense construct or by siRNA as described in (1). These cells were pretreated with PTH or isoproterenol for the indicated time periods and apoptosis was then induced with etoposide or dexamethasone. Apoptosis was assessed by TUNEL staining, ELISA measurement of histone-associated DNA fragments, and trypan blue staining.

**RESULTS:** The anti-apoptotic effect of PTH pretreatment lasted for 2hr in control cells (open symbols in Fig. 1A) and PKI $\gamma$  knockdown by antisense substantially extended the inhibition by PTH of etoposide-induced apoptosis to 8hr (closed symbols in Fig. 1A). Measurement of DNA fragments (Fig. 1B) and TUNEL staining (Fig. 1C) confirmed that etoposide induced apoptosis (1<sup>st</sup> and 2<sup>nd</sup> bars), that PTH pretreatment for 1hr blocked the apoptosis (3<sup>rd</sup> bars), that PTH pretreatment for 4hr had no effect (4<sup>th</sup> bars), and that PKI $\gamma$  knockdown extended the effects of PTH to 4hr (5<sup>th</sup> bars) but did not alter apoptosis in the absence of PTH (6<sup>th</sup> bars). Similar results to those shown in Fig. 1 were obtained when siRNA was used to knockdown PKI $\gamma$  instead of antisense (Fig. 2A), when dexamethasone was used as an apoptosis inducer instead of etoposide (Fig. 2B), and when isoproterenol was used as a stimulator of

PKA signaling instead of PTH (Fig. 2C). Also consistent with our hypothesis, over-expression of PKI $\gamma$  by sense transfection abolished the anti-apoptotic effect of PTH (Fig. 3).



**DISCUSSION:** These results demonstrate that endogenous PKI $\gamma$  terminates the anti-apoptotic effects of cAMP/PKA signaling in osteoblasts. Since significant individual variability exists in the anabolic responses to PTH therapy in current clinical treatment of osteoporosis, inhibition of PKI $\gamma$  activity may provide a useful co-therapy in combination with intermittent PTH or  $\beta$ -adrenergic agonists for bone loss in conditions such as osteoporosis. This study is also the first demonstration that  $\beta$ -adrenergic agonists, such as isoproterenol, mimic the anti-apoptotic effects of PTH in osteoblasts.

**ACKNOWLEDGEMENTS:** NIH grant DK064963 to EMG

**REFERENCE:** (1) Chen, et al. JBC 2005; 280:2700-7