**SUPPLEMENTAL DATA**

# Protein kinase D1 mediates NF-κB activation induced by cholecystokinin and cholinergic signaling in pancreatic acinar cells

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## Supplemental results

*PKD1 is the predominant PKD isoform in rat pancreatic acinar cells and is phosphorylated by CCK-8 and CCh.* To determine which PKD isoform(s) is expressed in rat pancreatic acinar cells, acinar cell lysates were first analyzed by Western blot analysis with a polyclonal antibody that recognizes the COOH-terminal region of both PKD1 and PKD2 (**Fig. S1 *A***). Lysates from rat IEC-18 cells known to express all three PKD isoforms, AR42J cells transfected with pcDNA3-PKD1, and pancreatic cancer MiaPaCa-2 cells known to predominantly express PKD2 were analyzed in parallel as positive markers. As shown in this panel, rat pancreatic acinar cells yielded two PKD bands (*Lanes 3* & *4*). The predominant upper bands migrated with an apparent molecular mass (115 kDa) and corresponded to that of overexpressed PKD1 in transfected AR42J cell (*Lane 2*, PKD1/AR42J), whereas the minor lower bands in *Lanes 3* and *4* corresponded to the PKD2 bands of MiaPaCa-2 cells (*Lane 5*) and IEC18 cells (*Lane 1*).

We further used a specific PKD2 antibody (Bethyl Laboratories, Montgomery, TX) to determine if PKD2 is present in rat pancreatic acinar cells. The same cell lysates (with same amount of protein) were analyzed by Western blot (**Fig. S1 *C***). Only a very faint band, migrating

with an apparent molecular mass of 105 kDa, corresponding to PKD2, was visualized (*Lanes 4&5*) after prolonged exposure. However, in the positive controls (*Lanes 1-3*), the same antibody detected a high expression of both the endogenous PKD2 protein in IEC18 and MiaPaCa-2 cells and exogenous GFP-PKD2 in transfected AR42J. Similar results were also obtained by Western blot with PKD2 antibody from Upstate, Lake Placid, NY (data not shown). These results indicate that there is little PKD2 present in rat pancreatic acinar cells. Similarly, as shown in **Fig. S1 *D***, we detected a very weak immunoreactive signal corresponding to PKD3 protein (110 kDa) in rat pancreatic acinar cell lysate (*Lanes 3&4*) using a specific PKD3 antibody that readily recognized the PKD3 protein in IEC18 cells (*Lane 1*) and GFP-PKD3 (*Lane 2*) in transfected AR42J cells. Finally, lysates from rat pancreatic acini simulated with and without CCK-8 or CCh were analyzed by Western blot using a site-specific antibody recognizing phosphorylated activation loop Ser744/748 of PKD (**Fig. S1 *B***). Although the anti-pS744/748 PKD antibody recognizes the phosphorylated serines in the activation loop of all three PKD isoforms, we detected a single phosphorylated PKD band that co-migrated with PKD1, but not with PKD2 or PKD3 (*Lanes 2&3*). Our results indicate that PKD1 protein is the predominant PKD isoform endogenously expressed in rat pancreatic acinar cells. Thus, the rat pancreatic acinar cells provide a model system to study the regulation and function of PKD1 without significant contribution of the other PKD isoforms.

## Supplemental Figure Lengents

**Fig. S1. PKD1 is the predominant PKD isoform in rat pancreatic acinar cells.** ***A***, Lysates from rat IEC-18 cells which express all three PKD isoforms, rat pancreatic AR42J cells transfected with PKD1 (PKD1/AR42J), rat pancreatic acinar cells (rat PAC), and pancreatic cancer MiaPaCa-2 cells in which PKD2 is the predominant PKD isoform were analyzed by

Western blot analysis with a polyclonal antibody that recognizes the COOH-terminal region of both PKD1 and PKD2 (PKD C-20). ***B***, Western blot analysis using anti-pS744/748 PKD antibody for the lysate samples from rat PAC incubated without or with 100 nM CCK-8 (CCK) or 100 μM CCh for 10 min at 37°. ***C***, Western blot analysis of lysates from rat IEC-18, MiaPaCa-2, AR42J transfected with green fluorescent protein (GFP)-PKD2 (GFP-PKD2 /AR42J), and rat PAC using a specific anti-PKD2 antibody. ***D***, Western blot with a specific anti-PKD3 antibody for the lysates from IEC-18, AR42J transfected with GFP-PKD3 (GFP-PKD3/AR42J), and rat PAC. Representatives of at least 3 Western blots.

**Fig. S2. CCK-8 and CCh dose dependently induce PKD1 phosphorylation and activation in rat pancreatic acinar cells.** Rat pancreatic acinar cells were incubated with increasing concentrations of CCK-8 (CCK) or CCh for 10 min. ***A*****,** Western blotting analyses of the dose response of CCK- or CCh-induced PKD1 phosphorylation using PKD1 pS744/748 antibody or PKD1 pS916 antibody. The blots were reblotted for PKD1 expression using PKD C-20 antibody and then for GAPDH to verify equal protein loading. All Western blots shown are representative of 3 independent experiments. ***B,*** PKD-1 catalytic activity in the acinar cell lysates. The lysates were immunoprecipitated using PKD C-20 antibody and PKD1 activity in the immunocomplexes was determined by *in vitro* kinase assay as described in "Materials and Methods," The results are expressed as an increased fold over unstimulated control in activity. Values are means $\pm$ S.E. (n=3).