# Can pancreatitis be treated by inhibiting $Ca^{2+}$ signaling?

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*Comment on:* Huang W, Cane MC, Mukherjee R, *et al.* Caffeine protects against experimental acute pancreatitis by inhibition of inositol 1,4,5-trisphosphate receptor-mediated Ca2+ release. Gut 2017;66:301-13.

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Ionized calcium ($Ca^{2+}$) has long been known to be a major controller of cell function including pancreatic acinar cell secretion; in fact, research on pancreatic acinar cells in the 1980's was instrumental in establishing the mechanisms by which sequestered calcium is released into the cytoplasm to act as an intracellular messenger in response to hormones and neurotransmitters (1). In acinar cells this signaling consists of transient $Ca^{2+}$ elevations in the apical pole of the cell that triggers exocytosis of zymogen granules (2). Hyperstimulation of acinar cells especially by CCK led to a large single peak of $Ca^{2+}$ followed by a plateau of elevated calcium. Since hyperstimulation by the CCK analog cerulein has long been known to induce experimental pancreatitis (3), it was a logical step forward to show using isolated pancreatic acini that a high level of intracellular $Ca^{2+}$ was associated with and probably caused premature trypsin activation, cell vacuolization, and necrosis which are considered the *in vitro* equivalent of acute pancreatitis (AP) (4-6).

Animal models of AP have been shown to involve two major pathways, the premature activation of trypsin and the development of local and systemic inflammation triggered by activation of NFκ-B in the acinar cell (7,8). Both pathways are activated in part by $Ca^{2+}$ but targeting $Ca^{2+}$ signaling to treat pancreatitis had to await a detailed knowledge of $Ca^{2+}$ signaling (2,9,10).

*Figure 1* shows some of the molecules involved in regulating intracellular $Ca^{2+}$ in acinar cells. The inositol 1, 4, 5-trisphosphate receptor ($IP_3R$) and the ryanodine receptor (RyR) release $Ca^{2+}$ from the ER into the cytoplasm while PMCA and SERCA are ATPases pumping $Ca^{2+}$ out of the cytoplasm and into extracellular space or back into the ER respectively. When the ER calcium store is depleted, the protein stromal interaction molecule (STIM) 1 and 2 present in the ER membrane aggregates and moves to the plasma membrane to activate calcium influx through the calcium channels Orai1 and TRPC3. Agents targeting the $IP_3$ receptor, the RyR, the $Ca^{2+}$ influx mechanism mediated by Orai1, the calcium activated phosphatase calcineurin, and the plasma membrane calcium efflux pump PMCA are all under study as ways to diminish pancreatitis. Because of its central position in $Ca^{2+}$ signaling and relevance to this Editorial some additional information on the $IP_3R$ will be presented. $IP_3Rs$ are coded for by 3 genes (Type 1, 2 and 3) in vertebrates that are closely related. Each $IP_3R$ is a large protein of about 2,700 amino acids and they assemble into homo or heterotetramers which contain a central pore that functions as a gated $Ca^{2+}$ channel (11). $IP_3$ binds to a segment termed the $IP_3$-binding core in the N terminal portion which leads to an opening of the transmembrane channel. This event is also regulated by $Ca^{2+}$, ATP, phosphorylation of the $IP_3R$, by various kinases but most importantly the cyclic adenosine monophosphate (cAMP) activated kinase, and the binding of a large number of proteins (12,13). The $Ca^{2+}$ released by the $IP_3R$ channel can also activate the RyR, a related calcium channel prominent in muscle that is activated by $Ca^{2+}$ but not $IP_3$. RyRs are also present in acinar cells and play a supporting role in $Ca^{2+}$

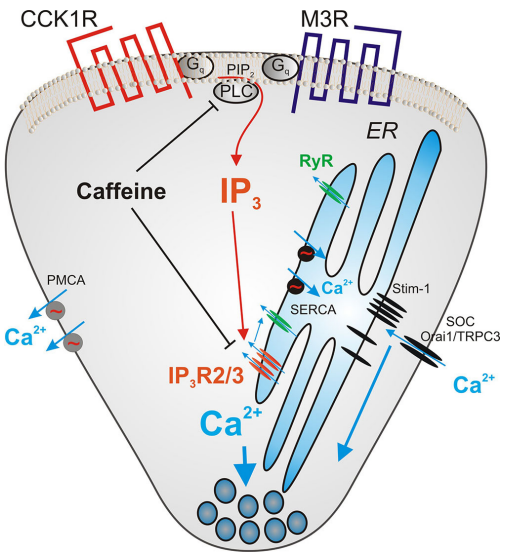


**Figure 1** Pathways of calcium signaling through $IP_3$ in pancreatic acinar cells initiated by CCK and acetylcholine. The sites at which caffeine has been shown to inhibit signaling, the $G_q$ activated phospholipase C (PLC) that produces $IP_3$ and the $IP_3$ R are shown. Figure simplified from the Pancreapedia with permission. For more detail on acinar cell $Ca^{2+}$ signaling see the Pancreapedia review (10).

mobilization under physiological conditions.

A study recently published in *Gut* by Huang, Sutton and colleagues on the use of caffeine to protect against experimental pancreatitis provides hope that targeting $Ca^{2+}$ may provide a therapeutic mechanism for human pancreatitis (14). Caffeine had previously been shown to inhibit intracellular $Ca^{2+}$ signaling mediated by $IP_3$ (1,15). In pancreatic acinar cells it is not clear whether caffeine was blocking $IP_3$ production or action (16). Huang *et al.* utilized both isolated acini and intact mice as well as measuring caffeine metabolites and the intracellular concentration of caffeine (14). They showed that when intracellular caffeine levels reached 2 mM in mouse acinar cells that $Ca^{2+}$ signaling induced by acetylcholine was inhibited and that dosage of 25 mg/kg caffeine every hour to mice led to similar serum levels of caffeine. They also showed that caffeine blocked the effect of uncaged $IP_3$ although higher concentrations of caffeine seem to have been required. Most importantly, they showed that caffeine given after the toxic insult significantly ameliorated three models of experimental AP, those induced by cerulein, bile salts and ethanol plus fatty acid. While related xanthines and caffeine metabolites could inhibit $Ca^{2+}$ signaling, only caffeine inhibited pancreatitis. The work is comprehensive, well carried out and a significant step forward in developing a therapeutic protocol that can be applied to human disease.

Caffeine is chemically 1, 3, 7-trimethylxanthine and is structurally related to adenosine (*Figure 2*). Its major actions are as a CNS stimulant, a relaxer of smooth muscle, a stimulant of cardiac muscle and a stimulant of urinary diuresis. These and other overall effects result from a number of biochemical actions which include inhibition of adenosine receptors, inhibition of cyclic nucleotide phosphodiesterase which increases cAMP levels and as a modulator of intracellular $Ca^{2+}$ handling. Caffeine is metabolized primarily in the liver and its major metabolites are paraxanthine, theobromine and theophylline, each of which lacks one specific methyl group. The metabolism is carried out by cytochrome P450 enzymes particularly CYP1A2 and the metabolites are excreted in the urine (17). The plasma half-life of caffeine is 4–6 hours in humans and 1 hour in mouse and can be affected by polymorphisms in CYP enzymes. It is important to note that drinking a cup of coffee results in plasma caffeine of at most 10 μM which is a hundred times lower than the concentrations studied by Huang *et al.* (14).

Caffeine has both advantages and disadvantages as a potential therapeutic agent. It is cell permeant, inexpensive and acts orally as attested by millions of users. A disadvantage is that the therapeutic dose may be close to the toxic dose. The other disadvantage is that caffeine has multiple actions throughout the body and the actual target affecting pancreatitis is unclear. Huang *et al.* assume that caffeine is an $IP_3R$ antagonist (14). The current state-of-the-art for studying $IP_3$ receptors is to express normal or modified $IP_3R$ in DT40 cells which lack endogenous $IP_3R$. Saleem *et al.* studied DT40 cells expressing $IP_3R$ type 1, 2 and 3 individually and found that caffeine was a low affinity antagonist of $IP_3R1$ without affecting binding of $IP_3$ (18). However, caffeine had no effect on $IP_3$ induced calcium release by $IP_3R$ type 2 and 3, the most abundant forms in pancreatic acinar cells and whose compound gene deletion blocks acinar cell secretion (19). Thus caffeine may have other and possibly multiple targets in acinar cells. Epidemiological data on caffeine consumption and the occurrence of pancreatitis is mixed (20,21) and such chronic consumption almost certainly leads to lower plasma levels of caffeine then used by Huang *et al.*

Of the other mechanisms to ameliorate pancreatitis by inhibiting $Ca^{2+}$ signaling, the best studied is to block the

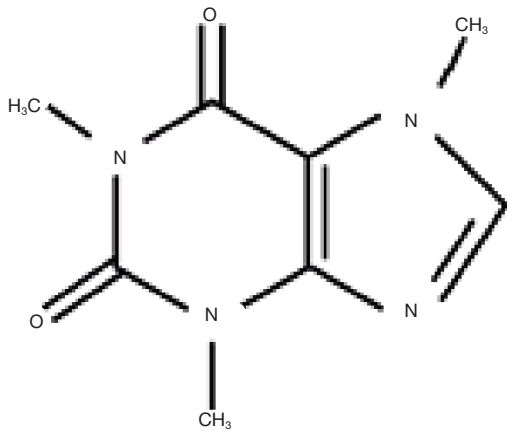


**Figure 2** Structure of the caffeine molecule. The core is xanthine while the methyl groups are on nitrogen atoms in position 1, 3, and 7.

calcium release-activated calcium channel whose major channel component is the protein Orai1. This process of $Ca^{2+}$ entry is also known as store-operated calcium entry and is activated by decreases in endoplasmic reticulum calcium stores and mediated by STIM1 and STIM2 which activate distinct channels composed of Orail1 and TRPC3 by physical interaction (22,23). Two Orai channel blockers have been developed, GSK-7975A by GlaxoSmithKline and CM_128 by CalciMedica. Gerasimenko *et al.* showed in isolated mouse acini that GSK-7975A inhibited store-operated calcium entry induced by depleting ER calcium stores with thapsigargin, a SERCA inhibitor and by palmitoleic ethyl ester, a toxic metabolite of alcohol induced pancreatitis (24). Wen *et al.* then showed that both of the Orai1 inhibitors reduced pancreatitis in mice induced by cerulein, bile salts or ethanol plus palmitoleic acid when given 1 hour after induction of pancreatitis and to a lesser extent after 6 hours (25). The Orai inhibitors were also shown to prevent necrosis *in vitro* in isolated human as well as mouse pancreatic cells. Another approach to inhibit $Ca^{2+}$ signaling is to increase $Mg^{2+}$ as it can act to attenuate $Ca^{2+}$ signaling in some physiological situations. In particular, high extracellular $Mg^{2+}$ has been reported to reduce store operated $Ca^{2+}$ entry in isolated mouse acini (26). $Mg^{2+}$ administration to rats and mice has been shown to reduce experimental pancreatitis (27) and is under study in clinical trials. $Mg^{2+}$ has the advantage that it is safe and easy to administer although the exact mechanism of action is not fully understood.

Several issues need to be overcome before inhibiting $Ca^{2+}$ signaling will become a useful therapeutic protocol. First, inhibiting $Ca^{2+}$ signaling is expected to have effects throughout the body. At this point there is no way to target only pancreas. Second, all *in vivo* studies to date have been carried out in mice. While there are good reasons for the ascendancy of mice as experimental animals, larger animals show a slower time course of events and sometimes a qualitative difference. To date the success of translating therapeutic approaches for pancreatitis from mice to humans has been poor. Current research standards require multiple experimental models but they are all generally in mice. Whether studies in large animals such as pigs would help is unknown. Finally, the calcium signaling events occur early in the course of pancreatitis and patients, especially in referral centers, come with established disease. It will be important to compare efficacy in rapidly treated cases seen in the ER within a limited number of hours after the initiation of symptoms with patients seen after 24–48 hours. In this regard, studies evaluating ERCP induced pancreatitis have an advantage even though the pancreatitis is usually mild. Patients requiring treatment after pancreatitis is established may not benefit as much and may require other treatments.

Overall, blocking the sustained increase in intracellular free $Ca^{2+}$ has the potential to reduce the cellular damage in pancreatitis. Both caffeine and the calcium influx blockers are worthy of further study.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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