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Caffeine and 2-Aminoethoxydiphenyl Borate (2-APB) Have Different Ability to Inhibit Intracellular Calcium Mobilization in Pancreatic Acinar Cell

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Inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) modulate Ca^{2+} release from intracellular Ca^{2+} store and are extensively expressed in the membrane of endoplasmic/sarcoplasmic reticulum and Golgi. Although caffeine and 2-aminoethoxydiphenyl borate (2-APB) have been widely used to block $InsP_3Rs$, the use of these is limited due to their multiple actions. In the present study, we examined and compared the ability of caffeine and 2-APB as a blocker of Ca^{2+} release from intracellular Ca^{2+} stores and Ca^{2+} entry through store-operated Ca^{2+} (SOC) channel in the mouse pancreatic acinar cell. Caffeine did not block the Ca^{2+} entry, but significantly inhibited carbamylcholine (CCh)-induced Ca^{2+} release. In contrast, 2-APB did not block CCh-induced Ca^{2+} release, but remarkably blocked SOC-mediated Ca^{2+} entry at lower concentrations. In permeabilized acinar cell, caffeine had an inhibitory effect on $InsP_3$-induced Ca^{2+} release, but 2-APB at lower concentration, which effectively blocked Ca^{2+} entry, had no inhibitory action. At higher concentrations, 2-APB has multiple paradoxical effects including inhibition of $InsP_3$-induced Ca^{2+} release and direct stimulation of Ca^{2+} release. Based on the results, we concluded that caffeine is useful as an inhibitor of $InsP_3R$, and 2-APB at lower concentration is considered a blocker of Ca^{2+} entry through SOC channels in the pancreatic acinar cell.

Key Words: Caffeine, 2-APB, $InsP_3R$, SOC, Acinar

INTRODUCTION

Intracellular Ca^{2+} controls a vast array of cellular functions like as contraction, secretion, cell growth, and proliferation [1-3]. The concentration of $[Ca^{2+}]_i$ could be raised by Ca^{2+} entry from extracellular space through membrane bound receptor-operated Ca^{2+} (ROC) channels or store-operated Ca^{2+} (SOC) channels, and Ca^{2+} release from intracellular stores through inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) or ryanodine receptors [3-6]. Widely expressed $InsP_3Rs$ in various cells can be specifically activated using a membrane-permeant $InsP_3$ ester [7], but the using of inhibiting compound is still limited because of the non-specific actions.

Heparin is one of the most commonly used $InsP_3R$ antagonists, but it has multiple actions including uncoupling G-protein signaling and activation ryanodine receptors [8]. Additionally, heparin should be used by injection or infusion into the cell due to its non-permeable characteristics even though it has been suggested that low molecular weight heparin may cross the plasma membrane and thus inhibit $InsP_3Rs$ in the intact cell. Although xestospongins, another $InsP_3R$ antagonist, have been used frequently to prove the $InsP_3$-induced Ca^{2+} release in the intact cell, the mechanism of action has not been fully elucidated at the present time. Furthermore this compound is expensive, is slow to act, and has not been universally successful [9].

Presently, the most widely used cell permeable antagonists of $InsP_3Rs$ in the intact cell are caffeine and 2-aminoethoxydiphenyl borate (2-APB) despite their some non-specific actions. Caffeine easily permeates the plasma membrane and can effectively inhibit $InsP_3Rs$ in the intact cell. However, one of the side actions of caffeine itself is an increased Ca^{2+} release from internal stores through ryanodine receptor activation [8]. 2-APB has been subsequently used to evaluate the contribution of $InsP_3Rs$ in the generation of Ca^{2+} signals. In an initial study, 2-APB evoked concentration-dependent inhibition of $InsP_3$-induced Ca^{2+} release from the mouse cerebellar membrane [10]. The inhibitory mechanism of 2-APB to $InsP_3Rs$ is unclear, since it does not directly block $InsP_3$ binding site [11-13]. Recently, it has been reported that 2-APB failed to inhibit $InsP_3$-induced Ca^{2+} release in the some cell types, and consistently blocked the Ca^{2+} entry through SOC channel [14-18].

Despite the lack of specificity of caffeine and 2-APB to $InsP_3Rs$, they remain as widely-used inhibitors of intracelluar Ca^{2+} signaling. It is still important to elucidate their cellular targets and cell specific action because different

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ABBREVIATIONS: $InsP_3Rs$, inositol 1,4,5-trisphosphate receptors; 2-APB, 2-aminoethoxydiphenyl borate; CCh, carbamylcholine; SOC channel, store-operated Ca^{2+} channel; $[Ca^{2+}]_i$, intracellular calcium.

cells have different Ca^{2+} mobilizing mechanisms. Therefore, we examined and compared the ability of caffeine and 2-APB in mouse pancreatic acinar cell in the present study. Here, we report that only caffeine potently blocks the $InsP_3$-induced Ca^{2+} release, while 2-APB in low concentration works as a blocker of store-operated Ca^{2+} entry channel in this cell.

METHODS

Isolation of pancreatic acinar cells

Small clusters of pancreatic acinar cells were isolated by collagenase digestion as described previously [19]. Briefly, the pancreas was removed from freely fed male Balb/C mice after CO_2 asphyxiation and cervical dislocation. The dissected tissue was enzymatically digested with type-II collagenase in DMEM containing 0.1% bovine serum albumin and 1 mg/ml soybean trypsin inhibitor for 30 min followed gentle agitation. Acinar cells were filtered using 100 μm nylon mesh and then centrifuged at 75 g. After washing twice, cells were resuspended in HEPES-buffered physiological saline solution (HEPES-PSS) containing 5.5 mM glucose, 137 mM NaCl, 0.56 mM $MgCl_2$, 4.7 mM KCl, 1 mM Na_2HPO_4, 10 mM HEPES (pH 7.4), 1.28 $CaCl_2$, and 1% (w/v) bovine serum albumin until ready for use. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

Global cytosolic Ca^{2+} measurement in intact cell

For measurement of $[Ca^{2+}]_i$, isolated acinar cells were loaded with Ca^{2+}-sesitive dye, 2 μM fura-2/AM for 30 min at room temperature. Fura-2 loaded cells were mounted on a glass coverslip at a bottom of a perfusion chamber. Cells were continuously superfused with HEPES-PSS at a flow rate of 1 ml/min using an electronic controlled perfusion system (Warner Instrument, Hamden, CT, USA). Global Ca^{2+} imaging was performed using an inverted Olympus IX71 microscope through a 40× fluorescence objective lens. Cells were excited alternately with light at 340 nm and 380 nm, using a Polychrome V monochrometer (Till Photonics, Pleasanton, CA, USA). Fluorescence images were captured at an emitted wavelength of 510 nm using a cooled charged-coupled device Cool-SNAP HQ2 camera (Photometrics, Tuscon, AZ, USA).

Store Ca^{2+} measurements in permeabilized cell

Cells were loaded with 10 μM furaptra/AM for 30 min at room temperature. Acinar cells were then allowed to adhere to Cell-Tak (BD Biosciences, San Jose, CA, USA)-coated coverslips at the bottom of a small volume perfusion chamber. Cells were permeabilized by superfusion for 1∼2 min with β-escin (40 μM) in intracellular medium containing 125 mM KCl, 19 mM NaCl, 10 mM HEPES, and 1 mM EGTA (pH 7.3). Permeabilized cells were washed in intracellular medium without β-escin for 15 min to facilitate removal of cytosolic dye. Cells were superfused in intracellular medium containing 0.650 μM $CaCl_2$ (free $[Ca^{2+}]$= 200 nM), 1.4 mM $MgCl_2$, and 3 mM Na_2ATP to activate sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and to load the intracellular Ca^{2+} stores. The free $[Ca^{2+}]$ was maintained at a constant 200 nM throughout all experimental maneuvers. The emission of the dye above 505 nm following excitation at 340 nm and 380 nm was recorded using a TILL Photonics imaging system.

Drugs

Carbamylcholine, caffeine, β-escin, and other chemicals for making buffers were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). 2-APB was purchased from Tocris Bioscience (Ballwin, MO, USA). Thapsigargin was purchased from Calbiochem (San Diego, CA, USA). Fura 2-AM and furaptra-AM were purchased from TefLabs Inc. (Austin, TX, USA). Inositol 1,4,5-trisphosphate ($InsP_3$) was purchased from Biomol Research Laboratories (Plymouth, PA, USA).

Statistical analysis of data

Results were presented as mean±S.E. Data were analyzed using the Student t test. Rates of Ca^{2+} release were estimated from these responses by fitting the initial 10 sec period of decreasing fluorescence to a single exponential function using the Origin program as described previously [20]. Differences were considered significant when the p value was less than 0.05.

RESULTS

Both caffeine and 2-APB inhibit CCh-induced $[Ca^{2+}]_i$ oscillation

Inhibitory effects of caffeine and 2-APB on lower CCh-induced $[Ca^{2+}]_i$ changes were examined in intact pancreatic acinar small clusters. A 300 nM carbamylcholine (CCh) induced a base-up $[Ca^{2+}]_i$ oscillation and this pattern was sustained continuously in the presence of 1.28 mM $[Ca^{2+}]_o$. Both caffeine (20 mM) and 2-APB (30 μM) effectively blocked sustained $[Ca^{2+}]_i$ oscillation (Fig. 1A), and its inhibitory effects were dose-dependent (Fig. 1B and 1C). However, the recovery patterns after washout of these drugs were remarkably different. The initial Ca^{2+} peak of oscillation was completely recovered after withdrawal of caffeine, while that was not fully recovered after removal of 2-APB. Since $[Ca^{2+}]_i$ oscillation could be abolished by reduced Ca^{2+} entry from extracellular space as well as reduced Ca^{2+} release from intracellular Ca^{2+} store, follow-up experiments were performed to define which pathways were blocked by caffeine and 2-APB, respectively.

Caffeine blocks initial $[Ca^{2+}]_i$ peak and 2-APB blocks sustained $[Ca^{2+}]_i$

Supraphysiological concentration of CCh has known to make two compartments of Ca^{2+} signals, initial peak and sustained plateau, in the normal $[Ca^{2+}]_o$ [3]. It is well known that the initial peak is the result of Ca^{2+} release from intracellular store, and a sustained plateau is due to the consecutive store-operated Ca^{2+} entry [21]. To define what pathways are blocked by caffeine and 2-APB, the next experiments were performed. Caffeine (30 mM) completely blocked $[Ca^{2+}]_i$ elevation by 4.60±0.75% of control value (Fig. 2A). Transient $[Ca^{2+}]_i$ rise after washout of caffeine and CCh may be due to the action of remained $InsP_3$ in the cell. The inhibitory effect of caffeine on initial $[Ca^{2+}]_i$

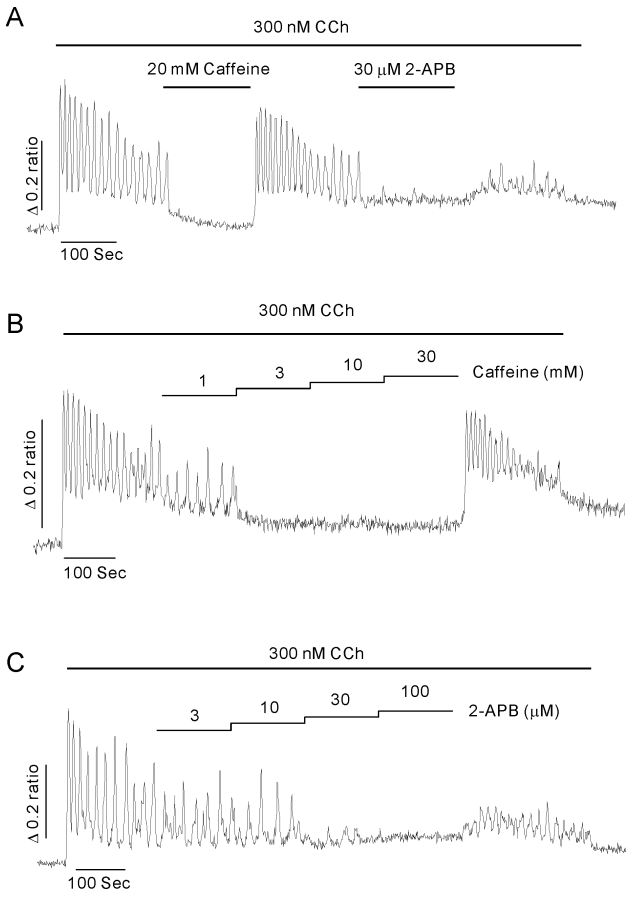

Fig. 1. Both caffeine and 2-aminoethoxydiphenyl borate (2-APB) dose-dependently inhibit lower carbamylcholine (CCh)-induced $[Ca^{2+}]_i$ oscillation in pancreatic acinar cell clusters. (A) Representative trace shows the effects of caffeine and 2-APB on CCh-induced $[Ca^{2+}]_i$ oscillation. The data were obtained from 5 separate experiments. The physiological concentration of CCh (300 nM) evoked $[Ca^{2+}]_i$ oscillation in the presence of 1.28 mM Ca^{2+}. Addition of 20 mM caffeine or 30 μM 2-APB abolished $[Ca^{2+}]_i$ oscillation completely. Representative traces of dose responses to caffeine (B) and 2-APB (C) in CCh-induced $[Ca^{2+}]_i$ oscillations obtained from at least 4 separate experiments. A gradual increase in caffeine (1~30 mM) or 2-APB (3~100 μM) effectively blocked the $[Ca^{2+}]_i$ oscillation in a dose-dependent manner. The oscillation did not fully recover after washout of 2-APB, but it completely recovered after caffeine removal.

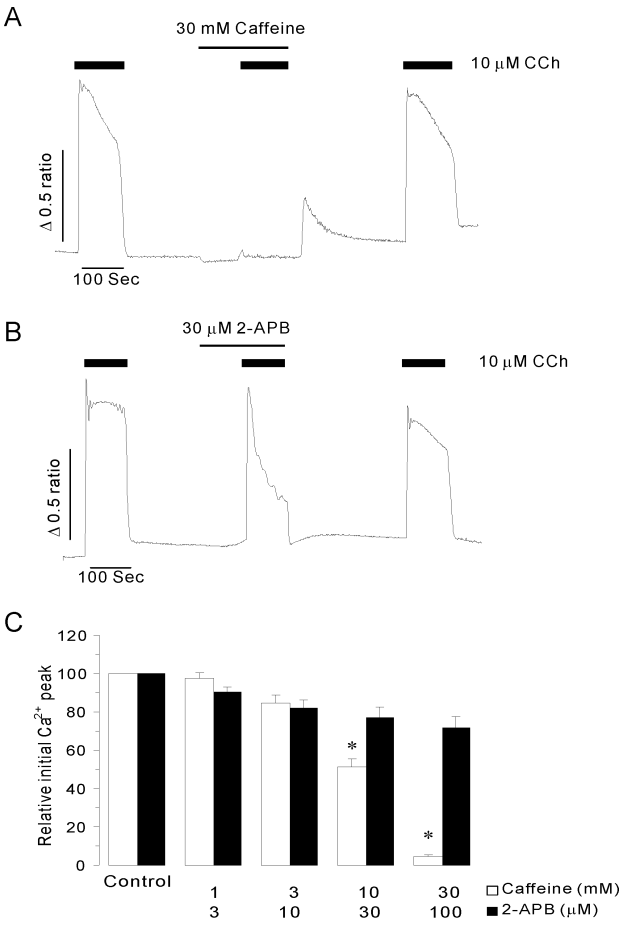

Fig. 2. Caffeine and 2-APB have different effects on higher carbamylcholine (CCh)-induced intracellular Ca^{2+} mobilization in pancreatic acinar cell clusters. (A) Representative trace shows the effect of caffeine on CCh-induced $[Ca^{2+}]_i$ rise. The data were obtained from 5 separate experiments. Caffeine was added 100 sec prior to CCh perfusion. CCh (10 μM) evoked initial $[Ca^{2+}]_i$ peak followed by sustained plateau in the presence of 1.28 mM Ca^{2+}. Caffeine (30 mM) blocked $[Ca^{2+}]_i$ rise completely. (B) Representative trace shows the effect of 2-APB on CCh-induced $[Ca^{2+}]_i$ rise. The data were obtained from 5 separate experiments. 2-APB had no effect on initial $[Ca^{2+}]_i$ peak, but it markedly blocked the sustained plateau. (C) Summarized dose responses to caffeine and 2-APB in CCh-induced initial $[Ca^{2+}]_i$ peak. Data were represented as % of initial levels, and expressed as mean±S.E. Asterisks indicate the value is significantly different from the corresponding value of control ($p<0.05$). Only caffeine inhibited CCh-induced initial $[Ca^{2+}]_i$ peak, dose dependently.

peak was dose dependent (Fig. 2C). On the other hand, 2-APB (30 μM) has no effect on initial Ca^{2+} peak, but it significantly blocked sustained Ca^{2+} plateau (Fig. 2B and 2C). These above results suggest that the inhibitory effect of caffeine on $[Ca^{2+}]_i$ signaling may be resulted from inhibiting Ca^{2+} release and that of 2-APB due to Ca^{2+} entry block. To further clarify this issue, we tested the effects of caffeine and 2-APB on thapsigargin-induced Ca^{2+} entry.

2-APB blocks thapsigargin-induced Ca^{2+} entry

Perfusion of thapsigargin, a sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor, resulted in marked $[Ca^{2+}]_i$ rise followed by decline to basal value without $[Ca^{2+}]_o$. Reintroduction of 1.28 mM of Ca^{2+} elevated $[Ca^{2+}]_i$, which may be due to activation of store-operated Ca^{2+} (SOC) channel. This SOC channel-mediated Ca^{2+} entry was not blocked by caffeine, but dose-dependently blocked by 2-APB (Fig. 3A and 3B). As shown in Fig. 3C, 30 μM 2-APB maximally inhibited SOC channel-medicated Ca^{2+} entry (46.21±6.06% of control value). The recovery of Ca^{2+} entry was not observed after removal of 2-APB.

Caffeine inhibits $InsP_3$-induced calcium release in permeabilized acinar cell

To determine the inhibitory effects of caffeine and 2-APB on $InsP_3$-induced calcium release, we performed the experi-

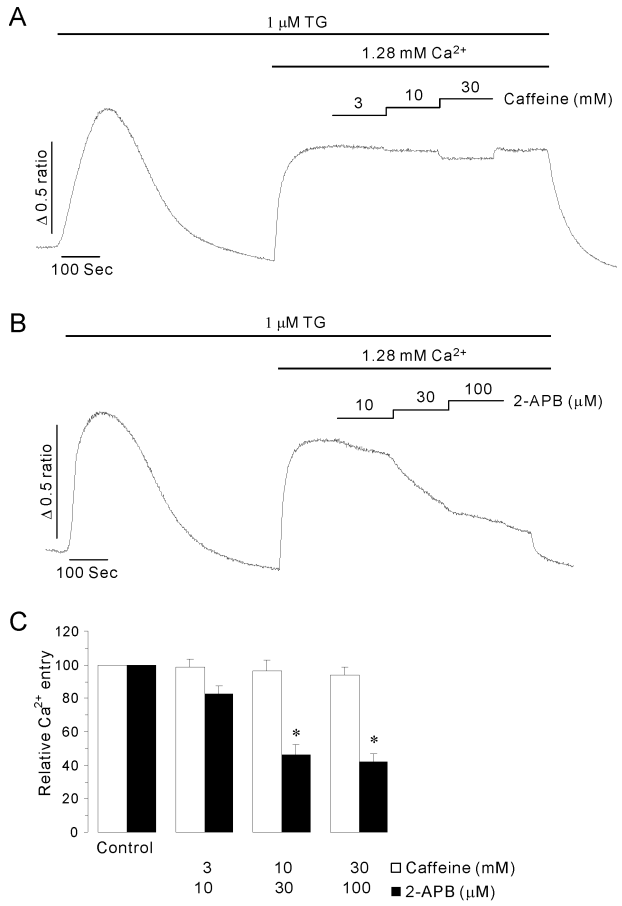

Fig. 3. 2-APB blocks thapsigargin (TG)-induced Ca^{2+} entry in a dose-dependent manner in pancreatic acinar cell clusters. Representative traces show the effects of caffeine (A) and 2-APB (B) on TG-induced Ca^{2+} entry. The data were obtained from at least 6 separate experiments. TG raised $[Ca^{2+}]_i$ transiently in the absence of $[Ca^{2+}]_o$, and reintroduction of 1.28 mM Ca^{2+} resulted in a marked increase of Ca^{2+} entry. The Ca^{2+} entry was not reduced by caffeine, but it was blocked by 2-APB dose-dependently. (C) Summarized dose responses to caffeine and 2-APB in TG-induced Ca^{2+} entry. Data were represented as % of maximal levels. Asterisks indicate the value is significantly different from the corresponding values of control ($p<0.05$). Only 2-APB inhibited SOC-mediated Ca^{2+} entry, dose-dependently.

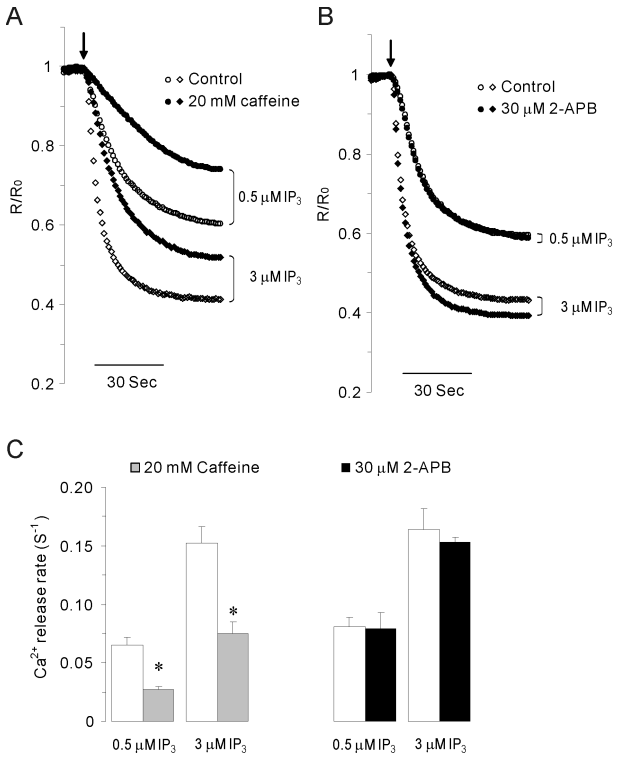

Fig. 4. Caffeine inhibits inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release from internal store in the permeabilized pancreatic acinar cell. The effects of caffeine (A) and 2-APB (B) on IP_3-induced Ca^{2+} release were obtained from at least 5 experiments. Arrows indicate the starting point of IP_3 perfusion. The fluorescence ratio was normalized to an initial 10 sec period prior to IP_3 perfusion. Caffeine (20 mM) inhibited submaximal (0.5 μM) and maximal (3 μM) IP_3-induced Ca^{2+} release, whereas 2-APB (30 μM) failed to inhibit both concentrations of IP_3-induced Ca^{2+} release. (C) Summarized data show the effects of caffeine and 2-APB on Ca^{2+} release rates in the permeabilized cell. Rates of Ca^{2+} release were fitted by a single exponential function. Asterisks indicate the value is significantly different from the corresponding values for control ($p<0.05$). Only caffeine had an inhibitory effect on submaximal and maximal IP_3-induced Ca^{2+} release.

ment in the permeabilized pancreatic acinar cell using β-escin. After re-filling of internal Ca^{2+} stores by MgATP in the presence of 200 nM of Ca^{2+}, submaximal (0.5 μM) or maximal (3 μM) concentrations of $InsP_3$ were perfused with or without caffeine and 2-APB, respectively. Caffeine (20 mM) significantly inhibited submaximal and maximal $InsP_3$-induced Ca^{2+} release rate by 65.0% and 51.6% of control values, respectively (Fig. 4A and 4C), while 2-APB (30 μM) failed to inhibit Ca^{2+} release induced by both concentrations of $InsP_3$ (Fig. 4B and 4C). These results strongly indicate that caffeine is a useful blocker of $InsP_3$-induced Ca^{2+} release in the mouse pancreatic acinar cell.

Higher 2-APB has multiple effects on Ca^{2+} release from internal store

Although low concentrations of 2-APB (below 30 μM) had no effect on CCh-induced initial Ca^{2+} peak in intact cell and $InsP_3$-induced calcium release in permeabilized cell, high concentrations of 2-APB (above 100 μM) itself at markedly increased $[Ca^{2+}]_i$ in the intact cell. As shown in Fig. 5A, the patterns of $[Ca^{2+}]_i$ elevation are very different according to the perfused concentrations of 2-APB; 100 μM of 2-APB produced a slightly progressive $[Ca^{2+}]_i$ increase, 300 μM of 2-APB produced a transient peak-type $[Ca^{2+}]_i$ increase, and 1 mM of 2-APB produced a sustained $[Ca^{2+}]_i$ increase. In the permeabilized cell, 100 μM of 2-APB directly enhanced Ca^{2+} release from internal stores by itself (Fig. 5B). Paradoxically, 2-APB reduced $InsP_3$-induced Ca^{2+} release by 74.7% of control value (Fig. 5B and 5C). We could not test the inhibitory effect of 300 μM of 2-APB on $InsP_3$-induced Ca^{2+} release in permeabilized cell, because it rapidly accelerated Ca^{2+} release from internal stores in the present study (data not shown). From the above results, we suggest that high (~100 μM) concentrations of 2-APB may need careful use, because this compound has paradoxical, multiple effects on intracellular Ca^{2+} mobilization.

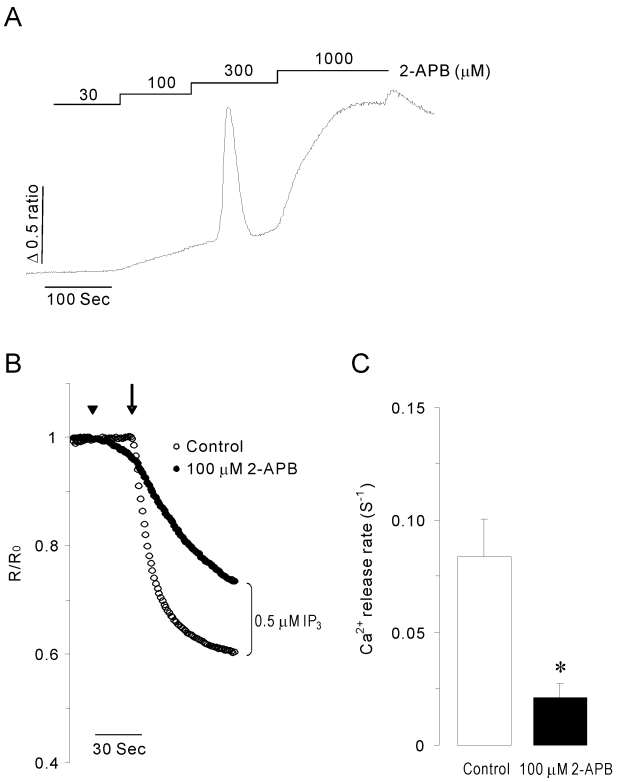

Fig. 5. Higher 2-APB has multiple effects on Ca^{2+} mobilization. (A) Representative trace shows the effects of higher 2-APB on Ca^{2+} mobilization in intact pancreatic acinar cell clusters. The data were obtained from 4 separate experiments. Gradual increase of 2-APB (30 μM~1 mM) enhanced $[Ca^{2+}]_i$ dose-dependently. However, patterns of $[Ca^{2+}]_i$ rise were variable according to dosage used. (B) Dual effects of 2-APB on Ca^{2+} release in the permeabilized cells were obtained from 4 separate experiments. 2-APB was perfused (arrow head) 30 sec prior to IP_3 perfusion (arrow). The fluorescence ratio was normalized to initial 10 sec period prior to 2-APB perfusion. 2-APB (100 μM) itself elicited Ca^{2+} release, but reduced the submaximal (0.5 μM) IP_3-induced Ca^{2+} release. (C) Summarized data of 2-APB effect on Ca^{2+} release rates in permeabilized cell. Asterisks indicate the value is significantly different from the corresponding values of control ($p<0.05$). 2-APB (100 μM) markedly inhibited submaximal (0.5 μM) IP_3-induced Ca^{2+} release.

DISCUSSION

Caffeine has been widely used in studies to demonstrate the mechanism of $InsP_3Rs$-mediated Ca^{2+} release from internal store in various cells, including human B lymphocytes, retinal ganglionic cells, pyramidal neurons, and pancreatic acinar cells [22-25]. Its high plasma membrane permeability, relatively low cost, and lack of effect on Ca^{2+} entry from extracellular space make it more useful than other antagonists, such as xestospongins or heparin. CCh, an acetylcholine analogue, has known to make specific $[Ca^{2+}]_i$ signals in pancreatic acinar cells in a dose-dependent manner. Lower CCh evoked continuous Ca^{2+} oscillation, and higher CCh induced initial Ca^{2+} peak and sustained plateau in the presence of normal extracellular Ca^{2+}. CCh-induced initial Ca^{2+} mobilization has been recognized as a result of Ca^{2+} release from internal stores, because those are essentially unaffected by removal of extracellular Ca^{2+}, and those are not observed by store depletion using thapsigargin or cyclopiazonic acid (CPA), SERCA inhibitors [3]. We found that caffeine blocked lower CCh-induced Ca^{2+} oscillation, as well as higher CCh-induced initial $[Ca^{2+}]_i$ peak on a dose-dependent in intact cell. In addition, caffeine inhibited $InsP_3$-induced Ca^{2+} release from internal store in permeabilized cell. Present data provide that caffeine is very effective blocker of $InsP_3$-induced Ca^{2+} release from internal store in pancreatic acinar cell. A different pathway that increases $[Ca^{2+}]_i$ is SOC channel-mediated Ca^{2+} entry. Many underlying mechanisms linking intracellular Ca^{2+} store and Ca^{2+} entry have been investigated. A proposed mechanism is conformational coupling between the ER membrane and the plasma membrane by Orai and TRP-family [25-28]. In this study, $[Ca^{2+}]_i$ was increased when Ca^{2+} was reintroduced after thapsigargin treatment within free extracellular Ca^{2+}. This $[Ca^{2+}]_i$ elevation may be due to Ca^{2+} entry *via* the SOC channel. In contrary to a blocking effect of $InsP_3$-induced Ca^{2+} release, caffeine failed to inhibit SOC channel-mediated Ca^{2+} entry. These results indicate that caffeine effectively antagonizes the $InsP_3R$-medicated Ca^{2+} release from intracellular stores without an effect on the SOC channel- mediated Ca^{2+} entry in this cell.

In highly RyRs-expressed cells, such as skeletal muscle, cardiac muscle, and neurons, caffeine is known as an activator of RyRs. Caffeine itself increases $[Ca^{2+}]_i$ and this $[Ca^{2+}]_i$ rise is blocked by RyR antagonist [8,29]. Caffeine increases both the open time and open probability of RyR in a cooperative manner with both Ca^{2+} and ATP, and therefore enhances the affinity of RyR for the physiological activator [30]. However, caffeine does not elicit Ca^{2+} release in the pancreatic acinar cell, actually inhibits the secretagogue-induced Ca^{2+} signals through an inhibitory action to $InsP_3$-medicated Ca^{2+} release from internal store like the observed results in the present study [31,32]. $InsP_3Rs$ have a well-defined localization in the pancreatic acinar cell, but a few RyRs distribute diffusely in the basal aspect of the cell. These studies suggest that RyRs play a limited role in the propagation of Ca^{2+} signals from the initially released trigger zone to basal aspects of the pancreatic acinar cell [33]. In the present study, caffeine at any dose did not enhance $[Ca^{2+}]_i$ when treated alone. This result, together with presumably much lower numbers of RyRs in pancreatic acinar cell, probably explains the absence of caffeine-induced Ca^{2+} release in this cell. Therefore, caffeine could be a very useful tool in the study of $InsP_3Rs$-mediated Ca^{2+} release in the pancreatic acinar cell due to lack of effects on RyR-medicated Ca^{2+} release and on SOC channel-mediated Ca^{2+} entry.

2-Aminoethoxydiphenyl borate (2-APB) was first introduced as an $InsP_3R$ antagonist. Unlike xestospongins or caffeine, which can modulate both $InsP_3Rs$ and RyRs, 2-APB may not have any effects on RyRs-mediated Ca^{2+} release from internal store [10]. Therefore, 2-APB has been subsequently used in many studies to prove the contribution of $InsP_3Rs$ in the generation of $[Ca^{2+}]_i$ signals. Although 2-APB has been used as an $InsP_3R$ antagonist, there are some reports that 2-APB has different effects according to the cell types and concentrations used. In the smooth muscle cell, 2-APB inhibits contractile response to $InsP_3$-generating stimulus, whereas that triggered by KCl-induced depolarization was unaffected, suggesting that there is no effect on voltage-operated Ca^{2+} (VOC) entry [34]. 2-APB has been also known as an inhibitor of SOC

channels, which is important to internal Ca^{2+} store refilling [14-18]. Although many results suggest that 2-APB reduces $[Ca^{2+}]_i$ through blockage of stored Ca^{2+} release or Ca^{2+} entry, there are several reports that 2-APB enhances $[Ca^{2+}]_i$ through inhibition of SERCA activity or activation of Ca^{2+} leak from internal stores [11,12,35]. It remains controversial as to the active mechanism of 2-APB according to the experiments. In our study, 30 μM of 2-APB, which effectively blocked SOC channel-mediated Ca^{2+} entry, but showed no effect on higher CCh-induced initial Ca^{2+} peak in intact cell and on $InsP_3$-induced Ca^{2+} release in permeabilized cell. On the other hand, 100 μM of 2-APB, had paradoxical multiple effects that enhance $[Ca^{2+}]_i$ in the intact cell, stimulate Ca^{2+} release from stores in the permeabilized cell, and inhibit $InsP_3$-induced Ca^{2+} release in the permeabilized cell. These findings indicate that low dose (~30 μM) of 2-APB could be used as a SOC inhibitor in this cell without any effect on $InsP_3R$, but high dose of (100~ μM) of 2-APB must be used with caution due to the multiple actions on SOC channel, SERCA, and $InsP_3R$.

Cytosolic Ca^{2+} is mobilized from two closely coupled components, rapid release of Ca^{2+} stored in the endoplasmic reticulum followed by slowly developing extracellular Ca^{2+} entry [26,27]. G protein-coupled receptors, through activation of phospholipase C, generate $InsP_3$ which interact with $InsP_3Rs$ on the ER. The $InsP_3Rs$ serve as Ca^{2+} channels to release stored Ca^{2+} and generate the initial Ca^{2+} signal in pancreatic acinar cell [3]. Pharmacological discrimination of these channels activity is important because a number of reports show that each channel influences the activity of the others. Unfortunately, common blockers have non-selective ability on these channels, which makes the interpretion of their roles and activities difficult. Although caffeine and 2-APB have a benefit due to the high plasma membrane permeability and the relatively low cost, their utility as universal inhibitors of $InsP_3R$ is limited due to the multiple responses according to cell types. From the present study, we could suggest that caffeine is useful as a reversible inhibitor of $InsP_3$-mediated Ca^{2+} release channel, and at lower concentrations; 2-APB is a considerable tool as a poorly reversible inhibitor of SOC-mediated Ca^{2+} entry channels in the pancreatic acinar cell. Moreover, high concentration of 2-APB must be used with caution due to the actions on multiple targets and the paradoxical responses of $[Ca^{2+}]_i$ signals.

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