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DIFFERENTIAL INVOLVEMENT OF PHOSPHOLIPASE A_2/ARACHIDONIC ACID AND PHOSPHOLIPASE C/PHOSPHOINOSITOL PATHWAYS DURING CHOLECYSTOKININ RECEPTOR ACTIVATED Ca^{2+} OSCILLATIONS IN PANCREATIC ACINI

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In pancreatic acini, administration of the phospholipase C inhibitor, U-73122, abolished Ca^{2+} oscillations and amylase secretion induced by CCK but had much less effect on the action of CCK analog JMV-180. In contrast, the phospholipase A_2 inhibitor, ONO-RS-082, inhibited both Ca^{2+} spikes and amylase secretion induced by JMV-180, but it had little effect on the action of CCK-8. Both arachidonic acid (AA) and a cytochrome P-450 inhibitor, SKF-96365, generated Ca^{2+} spikes from the agonist-sensitive pool. AA was capable of releasing Ca^{2+} from the endoplasmic reticulum (ER), suggesting the direct Ca^{2+} releasing pathway. There is no evidence of Ca^{2+}-induced Ca^{2+} release (CICR) since neither caffeine, a CICR potentiator, nor ryanodine, a CICR inhibitor, modulated agonist-induced Ca^{2+} oscillations and Ca^{2+} release from the ER. On the contrary, increasing concentrations of caffeine abolished agonist-induced Ca^{2+} spikes. Therefore we have demonstrated that depending on the agonists used, CCK receptor activation may result in the differential involvement of the phosphoinositol and arachidonic acid pathways to mediate calcium oscillation and amylase secretion.

It has become increasingly apparent that Ca^{2+} is an important second messenger in the majority of higher eukaryotic cells and that spatiotemporal cytoplasmic free calcium concentrations ($[Ca^{2+}]_i$) oscillate in the cell during receptor activation [1]. In pancreatic acinar cells $[Ca^{2+}]_i$ oscillates and propagates from the apical to basal surface as waves during cholecystokinin (CCK) and acetylcholine stimulation [2-4]. However, the precise mechanism(s) responsible for $[Ca^{2+}]_i$ oscillation in the cell and the functional significance of the oscillation are unknown. D-myo-inositol 1,4,5 trisphosphate (IP_3), a metabolite of the phosphoinositides/phospholipase C (PLC) pathway has been shown to release Ca^{2+} from intracellular stores [1]. It has been demonstrated that IP_3 binds to a receptor present on an intracellular Ca^{2+} store and thereby releases Ca^{2+} into the cytoplasm [1]. Therefore the presumption is that IP_3 is the principal mediator of $[Ca^{2+}]_i$ oscillation. Two models to describe Ca^{2+} oscillation mechanisms have been proposed [1]. The first model proposes that $[Ca^{2+}]_i$ oscillation is mediated by periodic production of intracellular IP_3 through repetitive activation of the Ca^{2+}-dependent PLC and via negative feedback regulation by protein kinase C. The second

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model proposes that the $[Ca^{2+}]_i$ released by IP_3 inhibits the IP_3-induced Ca^{2+} release by interfering with the IP_3 binding to its receptor (IP_3R). The sequestration of $[Ca^{2+}]_i$ into the stores permits the rebinding of IP_3 to the IP_3R and opening of the IP_3-operated Ca^{2+} channel. Thus the $[Ca^{2+}]_i$ oscillation seems to be regulated by a dynamic interplay of IP_3 and $[Ca^{2+}]_i$. Although the hypothesis appears attractive, in most cases, the IP_3 production requires a much higher concentration of agonists than that required to evoke Ca^{2+} oscillations. For instance, the CCK analog JMV-180 (1-1000 nM) generated repetitive Ca^{2+} spiking without a measurable [IP_3] in pancreatic acini [5,6]. Other studies demonstrate that repetitive Ca^{2+} spiking could be induced by a continuous injection of IP_3 in pancreatic acini, suggesting that periodic changes of [IP_3] are not needed for the Ca^{2+} oscillator to function [7]. It is, therefore, possible that the IP_3-insensitive Ca^{2+} signal transduction mechanism functions in receptor-operated Ca^{2+} signaling pathways. Several possible second messengers, such as arachidonic acid, sphingosine, cyclic ADP-ribose, and GTP, may be capable of mediating Ca^{2+} release from intracellular stores [8]. In addition, Ca^{2+} itself (released by IP_3) may mobilize Ca^{2+} from the IP_3-insensitive and probably caffeine- and ryanodine-sensitive Ca^{2+} pools (CICR) [1]. This study investigated the possibility that these IP_3-sensitive and IP_3-insensitive mechanisms may be responsible for Ca^{2+} oscillations in pancreatic acinar cells.

METHODS AND MATERIALS

Chemicals were purchased from the following sources: CCK-8, arachidonic acid, sphingosine, creatine phosphate, creatine phosphokinase, ATP, and ryanodine from Sigma Chemical Co. (St. Louis, MO); JMV-180 from Novabiochem USA (La Jolla, CA); ONO-RS-082 and U-73122 from Biomol (Plymouth Meeting, PA); thapsigargin, ionomycin and IP_3 from Calbiochem (San Diego, CA); fura-2 acetoxymethyl ester (AM) from Molecular Probes (Eurgene, OR); caffeine from Aldrich (Milwaukee, WI); bovine serum albumin (BSA) from ICN (Cleveland, OH); collagenase from Worthington Biochemical Co. (Freehold, NJ); $^{45}Ca^{2+}$ (81 MBq/2.2 mCi) from Amersham (Arlington Heights, IL).

Isolated rat pancreatic acini were prepared by collagenase digestion with pancreas obtained from male Sprague-Dawley rats [2]. Acini were suspended in a physiological salt solution (PSS). The PSS contained 0.1% BSA, 0.1 mg/ml SBTI and (in mM): 137 NaCl, 4.7 KCl, 0.56 $MgCl_2$, 1.28 $CaCl_2$, 1.0 NaH_2PO_4, 10 HEPES, Eagles' minimum essential amino acid neutralized with NaOH, 2.0 L-glutamine and was adjusted to pH 7.4 and equilibrated with 100% O_2.

The $[Ca^{2+}]_i$ measurements in individual pancreatic acini were previously published [2]. In brief, isolated acini were incubated with 2 uM fura-2 AM at 37°C in a 10 ml PSS solution for 25 min. All experiments utilized a dual excitation-wavelength (340/380 nm emitted at 505 nm) modular fluorometer system (Spex Fluorolog 2) coupled to a Nikon Diaphot inverted microscope (x40). Isolated acini placed on a cover glass and mounted on the closed chamber were continuously superfused by an 8 chambered reservoir (1 ml/min). A fluorescence ratio was converted to $[Ca^{2+}]_i$ according to in vitro calibration determined with an external standard (Calcium Kit I, Molecular Probes) and 25 μM fura-2 potassium salt [2].

For a study of amylase secretion, acini obtained from one rat pancreas were preincubated for 30 min in a 10 ml PSS, washed twice, and resuspended in a 45 ml fresh PSS. Aliquots of 3 ml were then distributed into each flask and incubated with reagents for 60 min at 37°C. The incubation was terminated by centrifugation for 30s at 4°C in a Microfuge (1 mlx3 in 15 groups), and amylase released into the supernatant and remained in the pellet in each Microfuge was assayed by use of procion yellow starch as substrate. Amylase secretion was expressed as the percentage of the total content in each sample.

The endoplasmic reticulum (ER) fractions were obtained from canine pancreas (Boehringer Mannheim GmbH, Germany). 10μl of ER fractions were suspended in a 10 ml cytosol buffer (pH 7.2 at 37°C) which contained (in mM): 20 NaCl, 100 KCl, 0.5 $MgSO_4$, 0.2

NaH_2PO_4, 0.8 Na_2HPO_4, 10 HEPES, 10 glucose, 2 creatine phosphate, and 50 μg/ml creatine phosphokinase 0.2% BSA, 100 nM free Ca^{2+} prepared by Ca^{2+}/EGTA buffer (2.55 mM $CaSO_4$, 1.64 mM $MgSO_4$ and 5 mM EGTA) and 5 uCi $^{45}Ca^{2+}$. After adding 2 mM ATP, aliquots (1 ml) in each time course were aspirated, diluted by a 8 ml (2 mlx4) Ca^{2+}-free cytosol buffer at 4°C and added to a 0.2 μm pore size Millipore membrane filter (Bedford, MA) and the ER was separated from the incubation medium by vacuum filtration. Contents of $^{45}Ca^{2+}$ remaining in the ER were counted in a liquid scintillation spectrometer using a preset ^{14}C channel.

RESULTS

EFFECTS OF PLA_2 AND PLC INHIBITORS ON JMV-180 AND CCK-8 STIMULATED Ca^{2+} OSCILLATIONS AND AMYLASE SECRETION. Application of the CCK analog JMV-180 (10 nM) to fura-2 loaded individual pancreatic acini resulted in the generation of repetitive Ca^{2+} spiking. The JMV-180 stimulated $[Ca^{2+}]_i$ oscillation was inhibited by the phospholipase A_2 (PLA_2) inhibitor, ONO-RS-082 (10 μM) (Figure 1A). A 75% and 70% reduction in the frequency and amplitude of oscillations were observed respectively (Table I). In contrast, the PLC inhibitor U-73122 (10 μM) showed a much lesser effect on JMV-180 induced Ca^{2+} oscillations (Figure 1B). Although there is individual variation with respect to the sensitivity to these compounds, ONO-RS-082 demonstrated a much more potent effect than U-73122 in inhibiting JMV-180 stimulated Ca^{2+} spikes in all cases (Table I). As reported previously [9], opposite findings were observed with CCK-8 stimulation. 10 pM CCK-8, an equivalent EC_{50} to 10 nM JMV-180 [5], caused relatively slow Ca^{2+} spikes compared to JMV-180. The CCK-8 stimulated Ca^{2+} oscillation was not inhibited by ONO-RS-082 (Figure 1C). In contrast, application of U-73122 resulted in a significant inhibition of CCK-8 stimulated Ca^{2+} spikes (Figure 1D). There was a 50% and 81% reduction in frequency and amplitude respectively (Table I). These observations suggest that CCK-8 stimulated Ca^{2+} oscillations are mediated by

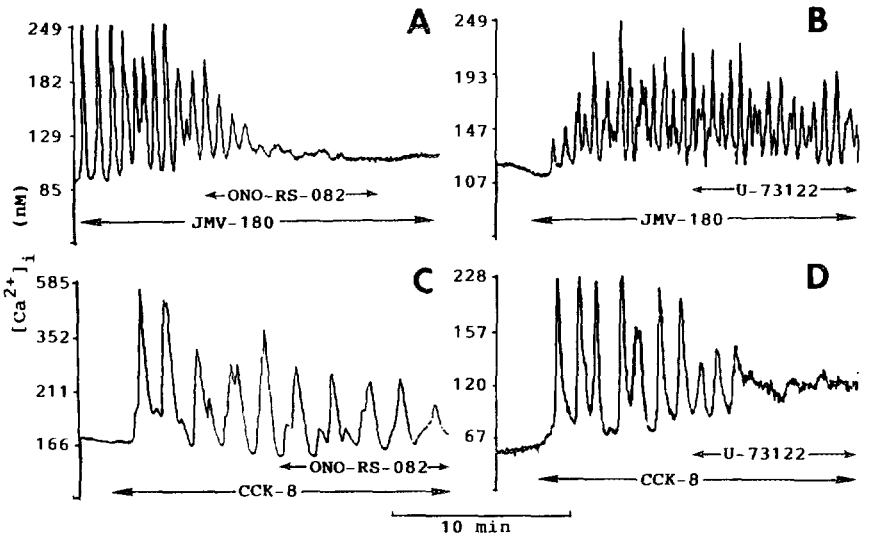

FIGURE 1. Effects of the phospholipase A_2 inhibitor (10 μM ONO-RS-082) and phospholipase C inhibitor (10 μM U-73122) on JMV-180 (10 nM)- and CCK-8 (10 pM)-induced Ca^{2+} oscillations in individual rat pancreatic acini. Each panel is representative of 5-7 separate determinations.

Table I

Effects of the PLA_2 inhibitor (ONO-RS-082) and PLC inhibitor (U-73122) on JMV-180- and CCK-8-induced Ca^{2+} oscillations in individual rat ancreatic acini

		Control	ONO-RS-082 (10^{-5}M)	U-73122 (10^{-5}M)
JMV-180 (10^{-8}M)	Frequency	12±2 (9)	3±0.7[a] (5)	11±3 (6)
	$\Delta [Ca^{2+}]_i$	118±7	36±8[b]	60±20[c]
CCK-8 (10^{-11}M)	Frequency	9±1 (13)	11±0.8 (5)	4.5±1.4[d] (10)
	$\Delta[Ca^{2+}]_i$	180±38	120±6	34.5±7[e]

The frequency was expressed as Ca^{2+} spiking cycles/10 min. $\Delta[Ca^{2+}]_i$ = peak-basal. Basal $[Ca^{2+}]_i$ was 86±8 nM (n=22). a: p=0.005, b: p<0.0001, c: p=0.01, d: p=0.05, e: p=0.01 against each control (by two-tailed unpaired t tests). Data are the mean ± S.E.M. from five to seven separate experiments. Number in parentheses indicates the number of single cells examined.

the PLC pathway, whereas JMV-180 mainly acts via the PLA_2 pathway. In accordance with Ca^{2+} signaling data, JMV-180 stimulated amylase secretion was significantly inhibited by ONO-RS-082, but not by U-73122 (Table II). In contrast, CCK-8 stimulated secretion was inhibited by U-73122, but not by ONO-RS-082. Although U-73122 inhibited JMV-induced Ca^{2+} spike amplitude and ONO-RS-082 decreased that evoked by CCK-8, they did not affect the Ca^{2+} spike frequency (Table I). This may explain little or no effects of U-73122 and ONO-RS-082 on amylase secretion stimulated by JMV-180 and CCK-8 respectively (frequency-encoded signal).

INVOLVEMENTS OF ARACHIDONIC ACID IN INDUCING Ca^{2+} SPIKING. PLA_2 catalyzes the phosphatidylcholine hydrolysis, resulting in the production of arachidonic acid

Table II

Effects of ONO-RS-082 and U-73122 on JMV-180- and CCK-8-induced amylase secretion

Reagents (M)	Amylase secretion (% of total/60 min)	N
Basal	7.8±0.7	5
+ ONO-RS-082 (10^{-5}M)	10.8±1.4	5
+ U-73122 (10^{-5}M)	8.9±2.2	5
JMV-180 (10^{-8}M)	28.6±1.5	6
+ONO-RS-082	18.4±2.2[a]	6
+U-73122	23.3±2.2	6
CCK-8 (10^{-11}M)	23.0±5.7	3
+ONO-RS-082	29.4±5.6	3
+U-73122	14.5±0.6[b]	3

a: p=0.004 against JMV-180 alone; b: p<0.05 against CCK-8 alone. Data are the mean ± S.E.M. from three separate experiments. N indicates the number of determinations.

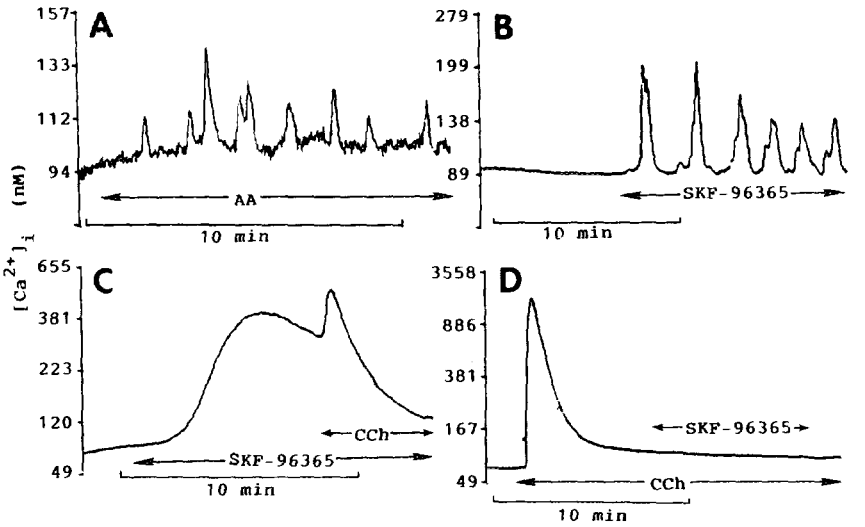

FIGURE 2. Arachidonic acid (50 μM AA)- and the cytochrome P-450 inhibitor, SKF-96365 (10 μM)-induced Ca^{2+} oscillations in individual rat pancreatic acini. Carbachol (CCh) concentration was 10 μM. Each panel is representative of 5-12 separate determinations.

(AA) [10]. Since our results suggest that the JMV-180 stimulated Ca^{2+} oscillation may be mediated by the PLA_2 pathway, we examined the effect of AA in fura-2 loaded intact acini. As show in Figure 2A, 50 μM AA produced either repetitive Ca^{2+} spiking (3/5 single cells) or a monophasic Ca^{2+} spike (2/5) resulting in an increase of 61±8 nM of $[Ca^{2+}]_i$ from basal. Although SKF-96365 has been used as a receptor-oparated Ca^{2+} channel blocker in platelets [11], it is also an inhibitor of the microsomal enzyme, cytochrome P-450, which catalyzes AA metabolism [12]. SKF-96365 (10 μM) caused either repetitive Ca^{2+} spikes (4/12) or a monophasic spike (8/12), probably due to an accumulation of intracellular AA levels. This resulted in an increase of 108±24 nM of $[Ca^{2+}]_i$ from basal (Figure 2B). The source of Ca^{2+} utilized by SKF-96365 for Ca^{2+} spikes is the agonist-sensitive pool since subsequent applications of carbachol resulted in a decrease of the spike amplitude (Figure 2C). Unlike in platelets [11], this compound did not inhibit the carbachol-induced sustained $[Ca^{2+}]_i$ plateau (Figure 2D). Our results suggest that AA itself, but not its metabolites, is capable of generating Ca^{2+} spikes.

ARACHIDONIC ACID-INDUCED $^{45}Ca^{2+}$ RELEASE FROM THE ER. To further examine the effect of AA, $^{45}Ca^{2+}$ flux studies using canine pancreatic ER fractions were performed. First, we examined whether IP3 and the ER Ca^{2+} pump inhibitor, thapsigargin [13], are capable of releasing Ca^{2+} from the ER fractions. Both IP3 (4 μM) and thapsigargin (1 μM) released $^{45}Ca^{2+}$ from the ER in an ATP-dependent manner (Figure 3A). The maximum IP3-induced $^{45}Ca^{2+}$ release was approximately 50% of the total amount in the ER and subsequent $^{45}Ca^{2+}$ reuptake into the ER occurred within 5-10 min in the presence of ATP regenerating system. Since the thapsigargin-induced $^{45}Ca^{2+}$ release (~80%) was due to inhibition of the ER Ca^{2+} pump activity and subsequent leak of $^{45}Ca^{2+}$ [13], there was no reuptake of $^{45}Ca^{2+}$ into the

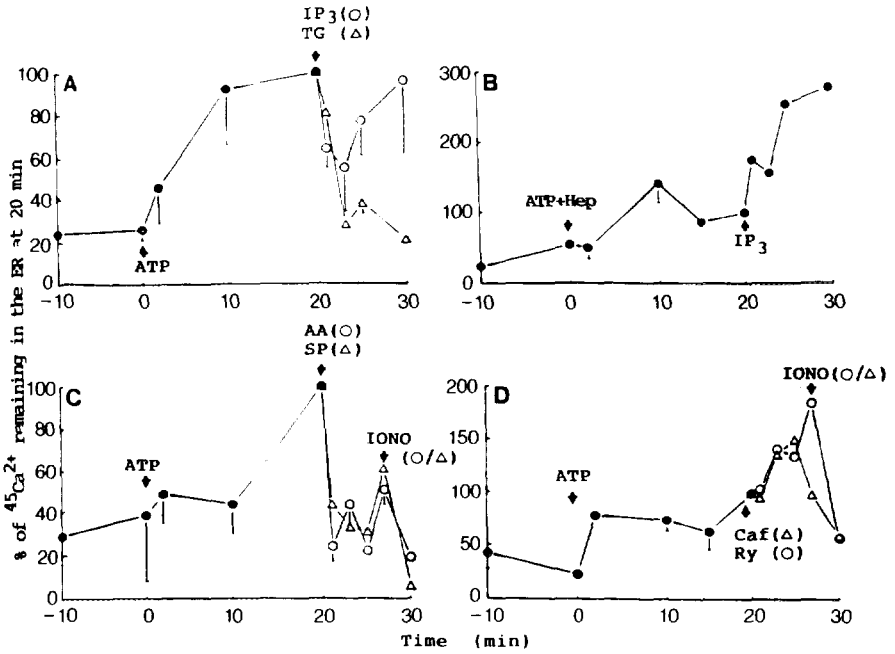

FIGURE 3. Effects of IP3, thapsigargin, sphingosine and arachidonic acid (AA) on $^{45}Ca^{2+}$ release from canine pancreatic microsomal fractions. Note that caffeine and ryanodine were ineffective to affect $^{45}Ca^{2+}$ flux. Data are the mean ± S.E.M. from twelve separate experiments. Abbreviations and concentrations used: ATP (2 mM); adenosine 5'-triphosphate, IP3 (4 μM), TG (1 μM); thapsigargin, Hep (2 μM); heparin, AA (10 μM); arachidonic acid, SP (30 μM); sphingosine, Iono (10 μM); ionomycin, Caf (2 mM); caffeine, Ry (10 μM); ryanodine.

ER. Heparin, an inhibitor of the IP3-binding to the IP3 receptor, inhibited the IP3-induced Ca^{2+} release but it potentiated the ATP-dependent $^{45}Ca^{2+}$ uptake into the ER (Figure 3B). Our results indicate that these pancreatic microsomes contain both the IP3 receptor and ER Ca^{2+} pump and should be useful for studying intracellular Ca^{2+} release mechanisms. Using these fractions, we demonstrated that AA (10 μM) was capable of stimulating ~80% $^{45}Ca^{2+}$ release followed by subsequent partial reuptake of $^{45}Ca^{2+}$ into the ER (Figure 3C). These data indicate that AA directly acts upon the ER to release Ca^{2+}. Another major unsaturated fatty acid, sphingosine, also released $^{45}Ca^{2+}$ from the ER. Since several lines of evidence suggest that the CICR is involved in the Ca^{2+} releasing mechanism in the pancreatic acini [14], we examined the effects of caffeine and ryanodine to release Ca^{2+} from the ER. In our preparations, both caffeine (2 mM) and ryanodine (10 μM) were unable to affect $^{45}Ca^{2+}$ flux in the ER fraction. Application of ionomycin (10 μM) resulted in release of $^{45}Ca^{2+}$ from the ER (Figure 3D).

EFFECTS OF CAFFEINE AND RYANODINE ON Ca^{2+} OSCILLATIONS. If the CICR pathway is operating in pancreatic acini, a potentiator of CICR (caffeine) and a blocker of the SR Ca^{2+} channel (ryanodine) should accelerate or abolish Ca^{2+} spiking, respectively [1]. However, 2 mM caffeine had no effect on JMV-180-induced Ca^{2+} spiking and high concentration of caffeine (20 mM) resulted in a significant inhibition of JMV-180-induced Ca^{2+} oscillations (Figure 4A, B and Table III). Ryanodine (10 μM) also had no effect on JMV-180-induced Ca^{2+} spikes (Figure 4C). Caffeine plus ryanodine abolished the Ca^{2+} spike induced by JMV-180.

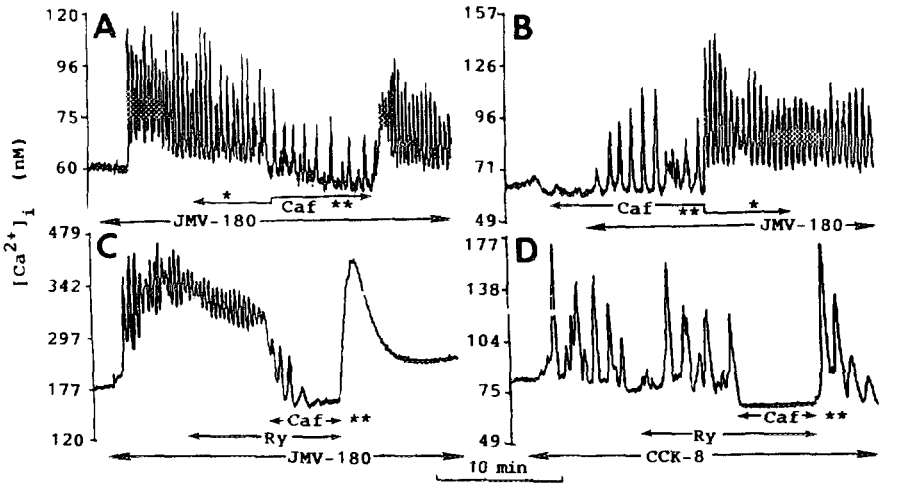

FIGURE 4. Effects of caffeine (Caf) (2* and 20** mM) and ryanodine (Ry) (10 μM) on JMV-180 (10 nM)- and CCK-8 (20 pM)-induced Ca^{2+} oscillations in individual rat pancreatic acini. Each panel is representative of 3-6 separate determinations.

Similarly, ryanodine also had no effect on CCK-8 stimulated Ca^{2+} oscillations, whereas caffeine plus ryanodine totally inhibited them. Removal of these reagents resulted in the return of Ca^{2+} spiking (Figure 4C, D). These observations suggest that the CICR is not involved in the agonist mediated Ca^{2+} oscillation mechanism in pancreatic acini.

DISCUSSION

In this study we investigated the mechanisms responsible for generating and maintaining Ca^{2+} oscillation in pancreatic acini. Similar to previous reports [9] we demonstrated that administration of the phospholipase C inhibitor, U-73122, abolished Ca^{2+} oscillations and amylase secretion induced by CCK-8 but had significantly less effect on the actions of JMV-180.

Table III

Effects of caffeine and ryanodine on JMV-180-induced Ca^{2+} oscillations

Reagents (M)	Frequency (cycles/10 min)	$\Delta[Ca^{2+}]_i$ (nM)	N
JMV-180 (10^{-8}M)	14.4±2.3	91±15	10
+ Caffeine ($2x10^{-2}$M)	5.2±1.1[a]	23±9[b]	6
+ Ryanodine (10^{-5}M)	18.4±4.1	73±18	5
+ Caffeine + Ryanodine	4.0±0.6[c]	-(31±10)[d]	3

Basal $[Ca^{2+}]_i$ was 83±26 nM (n=10). $\Delta[Ca^{2+}]_i$ = peak-basal. a: p=0.001, b: p=0.006, c: p=0.02, d: p<0,0001 against JMV-180 alone. Data are the mean ± S.E.M. from three to four separate experiments.

In addition, we showed that the phospholipase A_2 inhibitor, ONO-RS-082 inhibited both Ca^{2+} spikes and amylase secretion evoked by JMV-180, but it had little effects on the action of CCK-8. This suggests that CCK-8 induced Ca^{2+} oscillation is mediated mainly by metabolites of the phosphoinositol pathway, whereas JMV-180 acts via the arachidonic acid (AA) cascade. The CCK receptor in pancreatic acini has recently been cloned (444 amino acids and seven hydrophobic transmembrane domains) [17]. Functional and binding studies suggest that the CCK receptor may exist in different affinity states [5,25]. JMV-180, an analogue of CCK receptor [16], stimulates amylase secretion to the same maximal value as is observed with CCK-8 but does not cause a decrease in secretion when supramaximal concentrations of the analogue are used [23,25]. Furthermore, JMV-180 prevents IP_3 production and subsequent Ca^{2+} mobilization evoked by supramaximal concentrations of CCK-8 [5], suggesting that JMV-180 functions as an agonist at high affinity CCK receptors and as a competitive antagonist at low affinity CCK receptors. Therefore, it is conceivable that the different actions of JMV-180 and CCK-8 may reflect the coupling of high and low affinity states of the CCK receptor to different intracellular messengers. To further investigate the role of the PLA_2/AA pathway in the mediation of Ca^{2+} oscillation, we demonstrated that similar to JMV-180, AA and SKF-96365 evoked Ca^{2+} oscillation from the agonist sensitive pool. Furthermore AA is capable of directly releasing $^{45}Ca^{2+}$ from the ER. Similarly, AA stimulates Ca^{2+} release from the ER with a similar potency as IP_3 in permeabilized islets [18]. Thus AA may function as a second messenger to mediate Ca^{2+} oscillation. As reported in this and other studies [19-21], it is unlikely that in pancreatic acini, metabolites of AA such as prostaglandins, induce Ca^{2+} spikes and amylase secretion. Since the half life of AA is much longer than that of IP_3 [22], this may explain the sustained Ca^{2+} oscillations evoked by AA which may continue for more than 60 min [2]. Although AA itself may inhibit the IP_3-induced Ca^{2+} current and an inhibitor of PLA_2 may potentiate the IP_3 response in pancreatic acini [19], the precise mechanism underlying the interaction between AA and IP_3 is unclear. To investigate additional mechanisms responsible for IP_3-insensitive Ca^{2+} release we examined if Ca^{2+} released by IP_3 can mobilize Ca^{2+} from the caffeine- and ryanodine-sensitive Ca^{2+} pools [1]. In pancreatic acini intracellular Ca^{2+} infusion has been shown to induce a repetitive Ca^{2+}-activated Cl^- current by a caffeine sensitive mechanism [14]. Our study indicated that there is no evidence of CICR in both intact acini and the ER fraction. Similar observations have been reported in permeabilized acini where caffeine has little or no effect on Ca^{2+} release [15]. In fact our study and others [4,24] showed that 10-20 mM caffeine actually decreased the agonist-induced Ca^{2+} spikes and waves, and IP_3 production.

REFERENCES

1. Berridge, M.J. (1990) J. Biol. Chem. 265:9583-9586.
2. Tsunoda, Y., Stuenkel, E.L., and Williams, J.A. (1990) Am. J. Physiol. 258:C147-C155.
3. Kasai, H., and Augustine, J.A. (1990) Nature 348:735-738.
4. Nathanson, M.H., Padfield, P.J., O'Sullivan, A.J., Burgstahler, A.D., and Jamieson, J.D. (1992) J. Biol. Chem. 267:18118-18121.
5. Matozaki, T., Goke, B., Tsunoda, Y., Rodrignez, M., Martines, J., and Williams, J.A. (1990) J. Biol. Chem. 265:6247-6254.
6. Saluja, A.K., Powers, R.E., and Steer, M.L. (1989) Biochem. Biophys. Res. Commun. 164:8-13.

7. Wakui, M., Potter, B.V.L., and Petersen, O.H. (1989) Nature 339:317-320.
8. Tsien, R.W., and Tsien, R.Y. (1990) Ann. Rev. Cell Biol. 6:715-760.
9. Yule, D.I., and Williams, J.A. (1992) J. Biol. Chem. 267:13830-13835.
10. Axelrod, J., Burch, R.M., and Jelsema, C.L. (1988) Trends Neurosci. 11:117-123.
11. Merritt, J.E., Armstrong, W.P., Benham, C.D., Hallam, T.J., Jacob, R., Jaxa-Chamiec, A., Leigh, B.K., McCarthy, S.A., Moores, K.E., and Rink, T.J. (1990) Biochem. J. 271:515-522.
12. Alonso, M.T., Alvarez, J., Montero, M., Sanchez, A., and Garcia-Sancho, J. (1991) Biochem. J. 280:783-789.
13. Thastrup, O., Cullen, P.J., Drobak, B.K., Hanley, M.R., and Dawson, A.P. (1990) Proc. Natl. Acad. Sci. USA 87:2466-2470.
14. Wakui, M., Osipchuk, Y.V., and Petersen, O.H. (1990) Cell 63:1025-1032.
15. Saluja, A.K., Dawra, R.J., Lerch, MM., and Steer, M.L. (1992) J. Biol. Chem. 267:11202-11207.
16. Martinez, J., Rodriguez, M., Lington, M.F., and Galas, M.C. (1988) In Neurology and Neurobiology of CCK Antagonists (P. Wang and R Schoanfeld, Eds) pp. 29-51. Alan R. Liss, N.Y.
17. Wank, S.A., Harkins, R., Jensen, R.T., Shapira, H., Weerth, A.D., and Slattery, T. (1992) Proc. Natl. Acad. Sci. USA 89:3125-3129.
18. Wolf, B.A., Turk, J., Sherman, W.R., and McDaniel, M.L. (1986) J. Biol. Chem. 261:3501-3511.
19. Maruyama, Y. (1990) J. Physiol. (Lond.) 430:471-482.
20. Chauvelot, L., Heisler, S., Hout, J., and Gagnon, D. (1979) Life Sci. 25:913-920.
21. Stenson, W.F., and Lobos, E. (1982) Am. J. Physiol. 242:G493-G497.
22. Liscovitch, M. (1992) Trends Biochem. 17:393-401.
23. Sato, S., Stark, H.A., Martinez, J., Beaven, M.A., Jensen, R.T., and Gardner, J.D. (1989) Am. J. Physiol. 257:G202-G209.
24. Toescu, E.C., O'Neil, S.C., Petersen, O.H., and Eisner, D.A. (1992) J. Biol. Chem. 267:23467-23470.
25. Matozaki, T., Martinez, J., and Williams, J.A. (1989) Am. J. Physiol. 257:G594-G600.