URSODEOXYCHOLATE (UDCA) POTENTIATES CHOLECYSTOKININ (CCK)-INDUCED $[Ca^{2+}]_i$ DYNAMICS AND SECRETORY RESPONSE BUT NOT CCK-JMV-180-INDUCED RESPONSES IN DISPERSED PERIFUSED ACINI OF THE RAT PANCREAS

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ABSTRACT

Ursodeoxycholate (UDCA), which has been widely applied for the treatment of hepatic diseases, increases amylase secretion from dispersed pancreatic acini *in vitro*. We examined the stimulatory mechanism in this study. UDCA (300 μ M) significantly enhanced CCK-8-induced amylase secretion and intracellular cytosolic Ca^{2+} movement but caused slight and insignificant potentiation of the CCK-JMV-180-induced amylase secretion and intracellular cytosolic Ca^{2+} movement from isolated rat pancreatic acini. These results are compatible with the notion that UDCA enhances amylase secretion by the mechanism which potentiates the CCK-8-induced formation of inositol[1,4,5] trisphosphate (IP_3), in contrast to its ineffectiveness upon the IP_3 -independent process activated by CCK-JMV-180.

Ursodeoxycholic acid (UDCA), a hydrophilic dihydroxy bile acid which is the 7β -OH epimer of chenodeoxycholic acid, improves cholestatic and hepatic disorders, including primary biliary cirrhosis. The mechanism has not been clarified but it is speculated that multiple bioactivities are involved. UDCA displaces more hydrophobic bile acids from hepatocyte membranes, reducing cholestasis and protecting hepatocytes (4). It possesses immunomodulating properties and activates the glucocorticoid receptor (17). On the other hand, we reported that UDCA stimulates the exocrine pancreas. The intraduodenal administration of UDCA stimulated pancreatic exocrine secretion *in vivo* and UDCA increased amylase release from dispersed acini *in vitro* (7, 8, 12, 14). UDCA at concentrations above 300 μ M impaired acinar cells due to a toxic detergent action (12, 14), although lower concentrations enhanced amylase release with or without cholecystokinin (CCK). However, the exact mechanism of this stimulatory effect of UDCA on

pancreatic acinar cells remains unknown.

The stimulatory action of CCK on amylase secretion is mediated by the mobilization of intracellular Ca^{2+} , and this Ca^{2+} mobilization is reported to be caused by the occupation of high affinity CCK receptors through the formation of inositol[1, 4, 5] trisphosphate (IP_3). CCK-JMV-180, an agonist of the high-affinity CCK receptor and an antagonist of the low-affinity CCK receptor (10), stimulated amylase secretion and the release of Ca^{2+} from non- IP_3 -sensitive storage sites in contrast to stimulation by CCK (10). This study was designed to examine the mechanism of UDCA enhancement of CCK- and CCK-JMV-180-stimulated amylase release from dispersed acini *in vitro*. We examined dynamic amylase secretion and Ca^{2+} movement using a perfusion apparatus and investigated the role of UDCA on amylase secretion and intracellular Ca^{2+} mobilization.

MATERIALS AND METHODS

UDCA was a generous gift from Tokyo Tanabe,

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Tokyo, Japan. CCK-8 and CCK-JMV-180 were purchased from the Peptide Institute, Osaka, Japan and Research Plus, Bayonne, NJ, U.S.A., respectively.

Preparation of Dispersed Acini

Pancreatic acini were isolated from male Wistar albino rats, by means of enzymatic digestion with collagenase and mechanical dissociation, according to Amsterdam and Jamieson (1), with some modifications (5, 11, 13). Briefly, rats were fasted overnight and then killed by cervical dislocation. Thereafter, 5 ml of standard medium containing 200 U/ml collagenase (Worthington, type IV) was injected into the pancreas, which was shaken at 37°C in standard medium for 15 min. The standard medium consisted of 20 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 5 mM glucose, 0.2% bovine serum albumin (BSA), 0.01% soybean trypsin inhibitor (Type 1-S, Sigma Chemical, St. Louis, MO), and Eagle's minimum essential amino acids and vitamins (Nissui Pharmaceutical, Tokyo, Japan). The solution was adjusted to pH 7.4 and equilibrated with 100% O₂. After the first digestion, the pancreas was washed with the standard medium, and then incubated with collagenase again for 32 min in the same medium. Acini were dissociated by pipetting through 4 pipettes of decreasing bore, filtered through one layer of gauze, layered over 10 ml of standard medium containing 4% BSA, and centrifuged for 2 min at 20 g. The dispersed acini were washed three times in the same buffer with centrifugation for 30 s at 20 g. The pellet was resuspended in 10 ml of the standard medium. The viability of the acini obtained by this procedure was assessed by trypan blue exclusion.

Perfusion Procedure

To examine the time course of enzyme secretion in detail, we used a perfusion system of dispersed rat pancreatic acini. The system consisted of a peristaltic minipump, a perfusion chamber and a fraction collector as described by Imamura *et al.*, (5, 11–13). Pancreatic acini were injected into the perfusion chamber equipped with a filter (5.0 µm pore size, SNWP, Millipore). After an initial wash and equilibration with the perfusion buffer for 90 min, 10 pM or 100 pM CCK-8, or 5 nM or 50 nM CCK-JMV-180 dissolved in the perfusion buffer, were perfused for 30 min. UDCA (300 µM) dissolved in the perfusion buffer was added to the perfusates

30 min before the stimulants. The medium was perfused at a rate of 0.6 ml/min through 1 mm diameter silicon tubing and gassed continuously with 100% O₂. The medium was changed by switching the stopcock. The perfusion chamber was immersed in a water bath at 37°C. Fractions of the perfusion medium were collected every 1 min in a fraction collector and their amylase activity was assayed. In each experiment, a sample of the acini suspension was taken at the start of perfusion, centrifuged, and assayed for total amylase activity. The amylase release of each fraction was calculated as the percentage of the total amylase activity in the acini suspension. The total amylase content was measured after sonication. Amylase activity was measured by the method of Ceska *et al.* with the Phadebas reagent.

Fura-2/AM Loading and Digital Image Analysis

Fura-2/AM was loaded and the fluorescence ratio was measured with a double excitation wavelength as reported (2, 6). In brief, the acini were loaded with a final concentration of 10 mM Fura-2/AM for 40 min at 37°C with mild shaking and transferred onto a Cell-Tak-coated nonfluorescent glass cover slide mounted in a Sykus-Moore chamber and allowed to attach to the cover slide for several minutes. The chamber was then installed on the stage of an inverted microscope (TMD-2, Nikon, Tokyo, Japan) equipped with an inlet of perfusing solution and an outlet by suction. At 15 s intervals, a pair of fluorescence intensities emitted (510 nm) by excitation wavelengths of 340 and 380 nm were focused on a SIT (silicon intensified target) camera. The signals from the camera were analyzed by a digitized image processor (Argus-100/Ca, Hamamatsu Photonics, Hamamatsu, Japan).

Results were analyzed by Student's *t*-test or the multiple analysis of variance (MANOVA) with repeated measures, followed by Newman-Keul's multiple comparison test. The difference was judged to be significant when $P < 0.05$.

RESULTS

The Effect of UDCA on Secretory Response to CCK-8 or CCK-JMV-180

Continuous stimulation with 10 pM CCK-8 caused amylase release, which consisted of an initial peak followed by gradual decay (Fig. 1, upper panel). When 300 µM UDCA was added 30 min before and during continuous stimulation with 10 pM

Fig. 1 Effect of 300 μ M UDCA on 10 pM (upper panel) or 100 pM (lower panel) CCK-8-induced amylase release from perfused rat pancreatic acini. Values are means $\pm$ SE ($n = 4$). UDCA potentiated 10 pM CCK-8-induced amylase release ($F = 1.924$, $P < 0.05$). The secretory response to 100 pM CCK-8 was not significantly potentiated by UDCA ($F = 0.861$, $P < 0.6142$), but the response after stopping CCK-8 stimulation was significantly enhanced by UDCA ($F = 3.125$, $P < 0.05$).

CCK-8, the secretory responses were significantly larger than those to 10 pM CCK-8 alone (Fig. 1, upper panel). The secretory response to 100 pM CCK-8 was not significantly potentiated by UDCA, but the response after CCK-8 stimulation was significantly enhanced by UDCA (Fig. 1, lower panel).

CCK-JMV-180 (0.001–1 μ M) caused a dose-dependent increase in amylase release. Continuous stimulation of the dispersed perfused rat pancreas

with 50 nM CCK-JMV-180 induced the maximum secretory response, which consisted of two phases: an initial peak followed by a second gradual decay (Fig. 2, lower panel). A further increase in the CCK-JMV-180 concentration caused a secretory response similar to the maximum level as described (15). When 300 μ M UDCA was added 30 min before and during continuous stimulation with 50 nM CCK-JMV-180, the secretory response was slightly and insignificantly larger than that to

Fig. 2 Effect of 300 μ M UDCA on 5 nM (upper panel) or 50 nM (lower panel) of CCK-JMV-180-induced amylase release from perfused rat pancreatic acini. Values are means $\pm$ SE ($n = 4$). UDCA did not affect 5 nM ($F = 0.571$, $P < 0.898$) or 50 nM ($F = 1.74$, $P = 0.0516$) CCK-JMV-180-induced amylase release.

50 nM CCK-JMV-180 alone (Fig. 2, lower panel). The ineffectiveness of the UDCA treatment was also confirmed in the secretory response to 5 nM CCK-JMV-180 (Fig. 2, upper panel).

The Effect of UDCA upon CCK-8 or CCK-JMV-180-Induced $[Ca^{2+}]_c$ Dynamics in Isolated Pancreatic Acini

The effect of UDCA upon $[Ca^{2+}]_c$ dynamics was examined in Fura-2-loaded isolated pancreatic acini which were continuously stimulated with CCK-8

(10–100 pM) or CCK-JMV-180 (5–50 nM).

Continuous stimulation with 10 pM CCK-8 evoked recurrent Ca^{2+} spikes in the dispersed perfused acini (Fig. 3, upper panel). When 300 μ M UDCA was added 10 min before and during the continuous stimulation with 10 pM CCK-8, there was a rapid increase in $[Ca^{2+}]_c$ followed by a plateau, upon which small fluctuations of $[Ca^{2+}]_c$ were superimposed (Fig. 3, lower panel). The $[Ca^{2+}]_c$ dynamics recorded from 10 pM CCK-8 in combination with UDCA resembled that induced by 50 pM CCK-8 alone (3). The integrated value of the

Fig. 3 Changes in $[Ca^{2+}]_i$ before, during and after continuous stimulation with 10 pM CCK-8 in the presence (lower panel) or absence of 300 μ M UDCA (upper panel)

$[Ca^{2+}]_i$ elevation in the presence of 10 pM CCK-8 and 300 μ M UDCA ($531.7 \pm 104.9 \mu\text{M}/20 \text{ min}$, $n = 5$) was significantly larger than that in the presence of 10 pM CCK-8 alone ($263.7 \pm 52.4 \mu\text{M}/20 \text{ min}$, $n = 5$).

Continuous stimulation with 100 pM CCK-8 caused an increase in the $[Ca^{2+}]_i$, which consisted of an initial peak followed by a second gradual decay (Fig. 4, upper panel). When 300 μ M UDCA was added 10 min before and during continuous stimulation with 100 pM CCK-8, the secretory response was significantly prolonged. The integrat-

ed value of $[Ca^{2+}]_i$ in the presence of these two drugs ($665.1 \pm 55.6 \mu\text{M}/20 \text{ min}$, $n = 5$) was larger than the corresponding value obtained in the presence of 100 pM CCK-8 alone ($490.6 \pm 97.4 \mu\text{M}/20 \text{ min}$, $n = 5$) (Fig. 4, lower panel), although these responses were not significant.

Continuous stimulation with 5–50 nM CCK-JMV-180 evoked the recurrent Ca^{2+} spikes in the Fura-2-loaded isolated pancreatic acini. When 300 μ M UDCA was added 30 min before and during continuous stimulation with 5–50 nM CCK-JMV-180, the $[Ca^{2+}]_i$ dynamics were simi-

Fig. 4 Changes in $[Ca^{2+}]_e$ before, during and after continuous stimulation with 100 pM CCK-8 in the presence (lower panel) or absence of 300 μ M UDCA (upper panel)

lar to those induced by CCK-JMV-180 alone (Fig. 5).

DISCUSSION

The present data show that the perfusion of 300 μ M UDCA significantly enhanced both CCK-8-induced amylase secretion and CCK-8-induced $[Ca^{2+}]_e$ dynamics in isolated rat pancreatic acini. However, UDCA did not enhance CCK-JMV-180-induced amylase secretion and $[Ca^{2+}]_e$. The stimulus-secretion coupling of digestive enzyme secretion from the exocrine pancreas stimulated by

CCK was accelerated by two intracellular messengers: IP_3 which releases Ca^{2+} from intracellular stores to the cytoplasm, and diacylglycerol (DG), which activates protein kinase C. In contrast to the CCK-activated IP_3 -dependent process, CCK-JMV-180 stimulates amylase secretion and the release of Ca^{2+} from non- IP_3 -sensitive storage sites (10). We showed that UDCA alone directly acted upon pancreatic acini and stimulated amylase secretion by stimulating an influx of extracellular Ca^{2+} , and not through IP_3 and DG formation or Ca^{2+} mobilization (12, 14). This study showed that under combined stimulation with UDCA and CCK-8, UDCA

Fig. 5 Changes in $[Ca^{2+}]_e$ before, during and after continuous stimulation with 5 nM or 50 nM CCK-JMV-180, in the presence or absence of 300 μ M UDCA

enhanced CCK-8-induced amylase release through IP_3 formation and Ca^{2+} mobilization.

By means of the perfusion system, we showed that CCK-8 or CCK-JMV-180 caused biphasic amylase secretion. This consisted of an initial burst of secretion which increased the intracellular Ca^{2+} stimulated by the secretagogues, and the sustained secretion which depended upon extracellular Ca^{2+} (5); the initial burst phase of CCK-JMV-180 was lower than that of CCK-8 stimulation. Therefore, the results suggested that the initial burst secretion revealed the release of intracellular Ca^{2+} mediated by IP_3 (17). Extracellular Ca^{2+} also plays an important role in the action of UDCA on pancreatic acini. UDCA enhanced the initial burst phase of amylase secretion probably by increasing the sensitivity of the membrane receptor and by potentiating receptor-mediated Ca^{2+} entry. Deoxycholate (DCA) (250 μ M) affects the cell membrane and increases the sensitivity to secretagogues (16). DCA is thought to activate IP_3 formation and stimu-

late amylase release from pancreatic acini (9). We therefore consider that the mechanism of DCA and UDCA on amylase release may be the same.

UDCA activates latent glucocorticoid receptor in cytosol into a DNA-binding species *in vitro* (17). It is therefore possible that UDCA modifies intracytosolic receptor proteins for example, via phosphorylation, and alters the receptor function. Further studies are needed to understand the mechanism by which amylase release is enhanced by UDCA.

In conclusion, UDCA acts not only directly to pancreatic acini and potentiates amylase secretion by a mechanism that stimulates influx of extracellular Ca^{2+} , but it also activates IP_3 formation.

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