ATP-consuming and ATP-generating Enzymes Secreted by Pancreas*

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Pancreatic acini release ATP in response to various stimuli, including cholecystokinin octapeptide (CCK-8), as we show in the present study. There were indications that pancreatic juice also contains enzymes that could hydrolyze ATP during its passage through the ductal system. The aim of this study was to determine which ATP-degrading and possibly ATP-generating enzymes were present in pancreatic secretion. For this purpose, pancreatic juice was collected from anesthetized rats stimulated with infusion of CCK-8. Purine-converting activities in juice samples were assayed by TLC using either [3H]ATP and [3H]ADP about equally well, i.e. CD39. Reverse-phase high-performance liquid chromatography analysis additionally shows that this enzyme has broad substrate specificity toward other nucleotides, UTP, UDP, ITP, and IDP. In addition, secretion contains ecto-5'-nucleotidase, CD73, further converting [3H]AMP to adenosine. Along with highly active hydrolytic enzymes, there were also ATP-generating enzymes in pancreatic juice, adenylyl kinase, and NDP kinase, capable of sequentially phosphorylating AMP via ADP to ATP. Activities of nonspecific phosphatases, nucleotide pyrophosphatase/ phosphodiesterases, and adenosine deaminase were negligible. Taken together, CCK-8 stimulation of pancreas causes release of both ATP-consuming and ATP-generating enzymes into pancreatic juice. This newly discovered richness of secreted enzymes underscores the importance of purine signaling between acini and pancreatic ducts lumen and implies regulation of the purine-converting enzymes release.

Extracellular ATP and other purines mediate signaling between cells by means of neural transmission, paracrine, and autocrine effects. There are several important steps in this purinergic cascade including release of ATP, interaction with specific P2 purinergic receptors on the target cells, and not the least, ATP-degrading and -converting enzymes, which determine the balance between nucleotides and nucleosides and thus activation of P2 versus P1 (adenosine) receptors.

Extracellular nucleotides can be hydrolyzed by a number of enzymes, such as ecto-nucleoside triphosphate diphosphohydrolases from the NTPDase-2 (CD39) family that hydrolyze nucleoside 5'-tri- and diphosphates (1). Ecto-nucleotide pyrophosphatase/phosphodiesterases (NPP) have broad substrate specificity and can convert ATP to AMP and PP, (2). Ecto-5'-nucleotidases (CD73) further hydrolyze AMP to adenosine, which can be taken up into the cell by specific Na+ -dependent nucleoside transporters or converted into inosine by adenosine deaminase. Ecto-alkaline phosphatases have broad action and dephosphorylate 5'-tri-, di-, and monophosphates. In addition to these degradation and inactivation pathways, there are also counteracting ATP-generating pathways, which include reverse nucleotide transphosphorylation by adenylyl kinase and nucleoside diphosphate kinase (NDPK). These kinases, which play roles in intracellular signaling and in DNA and RNA synthesis among others, are also expressed on surfaces of different cell types and can contribute to nucleotide balance (3).

In cardiovascular system and in respiratory epithelia, a number of enzymes handling nucleotides are well characterized. In human blood, soluble NPP, 5'-nucleotidase, and adenosine deaminase, as well as adenylyl and NDP kinases, contribute to “cleanup” of circulating nucleotides and adenosine (4), particularly of prothrombotic ADP, which is released from platelets and would otherwise, via P2Y1 receptor, cause further platelet activation and hemostasis (5). Endothelial cells have high activities of CD39 and CD73 (3, 6, 7) and thus take care of efficient hydrolysis of ATP/ADP to AMP and adenosine and prevent prothrombotic effect of circulating ADP. In contrast, the lymphoid cells are generally characterized by a counteracting, ATP-regenerating, and adenosine-eliminating phenotype (8). Upon adhesion, the lymphocytes inhibit endothelial ecto-5'-nucleotidase and prevent adenosine formation, thus facilitating their transmigration into the tissue (9).

Airway epithelia release ATP constitutively and in response to mechanical stimulation, and all adenine nucleotides and nucleosides are detected in airway surface liquid (10). In these epithelia, it seems that the P2Y1 receptor is of central importance, and its stimulation leads to improvement of the mucociliary clearance.

*This work was supported by the Finnish Academy, the Sigrid Juselius Foundation (to G. G. Y); and the Danish Medical and Science Research Councils and Novo Nordisk Foundation (to I. N.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: NTPDase, ecto-nucleoside triphosphate diphosphohydrolase; NPP, ecto-nucleoside pyrophosphatase/phosphodiesterase; NDPK, nucleotide diphosphate kinase; CCK-8, cholecystokinin octapeptide; — BIC, bicarbonate-free physiological saline; HPLC, high-performance liquid chromatography.
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ciliary clearance by stimulation of ciliary beat frequency, Cl− and fluid secretion, and mucin secretion from goblet cells. Nucleotide levels are regulated by a number of enzymes especially active on the apical surface of various airway epithelial cell lines. These include both ATP-degrading enzymes, NPP, CD73, and alkaline phosphatase, and ATP-generating enzymes, NDPK and adenylyl kinase (10–12).

In exocrine glands, ATP and other purines are thought to be important regulators of salt and fluid transport (13). It seems that pancreatic acini have relatively few functional P2 receptors (14). However, nucleotide- and nucleoside-selective receptors may be important regulators in pancreatic ducts, which secrete bicarbonate-rich fluid. In rat pancreas, ATP is released from acini into the series of excurrent ducts that are rich in P2 receptors. Close to acini, the ATP concentrations are in the high micromolar range; however, low amounts of ATP are detected in the final pancreatic juice collected from the main duct (15, 16). CD39 is expressed in pancreatic acini and also pancreatic ducts of rats and pigs, as demonstrated by immunohistochemistry and histochemistry (16–18). Our recent study revealed that pancreatic juice also contains CD39. Relatively low levels of ADP and AMP in the juice indicated that other enzymes may be present in the juice (16). To understand the acino-ductal paracrine regulation, it is important to determine which ecto-enzymes are present in pancreatic secretions and thus estimate the prevalence of nucleotides versus nucleosides. Therefore, the aim of the present study was to determine nucleotide/nucleoside-converting enzymes secreted in pancreatic juice collected under in vivo stimulation of rat pancreas with CCK-8. To determine the whole spectrum of purine-converting enzymes in pancreatic juice, we employed TLC assay with 3H/14C-labeled nucleotide substrates, autoradiography of direct 32P transfer from γ-32P]ATP, and reverse-phase high-performance liquid chromatography (HPLC) analysis of NTP metabolism. The obtained data clearly demonstrate that pancreatic juice contains ATP-consuming enzymes, CD39 and CD73, as well as ATP-generating enzymes, adenylyl kinase and NDPK. We propose that they have a role in purinergic signaling between pancreatic acini and pancreatic ducts.

**EXPERIMENTAL PROCEDURES**

Materials—Hormones secretin and CCK-8 were obtained from Sigma. Tissue culture media and phosphate-buffered saline were from Invitrogen. Mebumal was from Nycoderm (Roskilde, Denmark). [2-3H]AMP (specific activity 24 Ci/mmol), [8-14C]ATP (57 mCi/mmol), and [2-3H]adenosine (24 Ci/mmol) were from Amersham Biosciences (Little Chalfont, UK). [γ-32P]ATP (3000 Ci/mmol) and [2,8-3H]ADP (27.5 Ci/mmol) were from PerkinElmer Life Sciences. TLC plates were Alugram SIL G/UV 254a, and Polygram CEL 300 polyethyl- eneimine types were supplied by Macherey-Nagel (Duren, Germany). Synergi Hydro-RP 80A HPLC column (4-μm, 150 × 4.6 mm) protected by a reverse-phase C18 guard cartridge were from Phenomenex (Torrance, CA). All other reagents and standard chemicals were purchased from Sigma.

Collection of Pancreatic Juice—The necessary permission for animal experiments was obtained from the Danish Animal Ethical Committee. In vivo collection of pancreatic juice was undertaken on female Wistar rats weighing 160–300 g. The animals, fasted overnight, were anesthetized with mebumal (pentobarbital, 40 mg/kg, intraperitoneal). Anesthesia was maintained during the experiments by additional, intravenous injections of mebumal. The body temperature of animals was maintained at 38 °C by means of a thermostatically controlled heating. The animals were tracheostomized, and the facial vein was cannulated for infusions. The abdomen was opened by a midline incision, and the pylorus and the proximal ends of the bile duct were ligated. The common pancreatic bile duct was cannulated with an ~2-cm-long polyethylene tube, and collection of pancreatic juice was started with a control period of 30–60 min during which medium (Dulbecco’s modified Eagle’s medium 1000/Ham’s F12 medium) was infused into the facial vein. Secretion was then stimulated by infusion CCK-8 (5.6 pmol/min/200 g of animal), for about 60 min. The infusion rate (0.03 ml/min/animal) was held constant with a syringe pump (Cole-Parmer). Pancreatic juice was collected on ice over 10–15-min periods and stored at −80 °C or on dry ice for transport. Blood was collected in heparinized syringes, and plasma was separated by centrifugation. After experiments, animals were killed by overdose of mebumal. For estimations of ATP in pancreatic juice, 5–10-μl samples were quickly thawed and immediately assayed with an SL kit using internal and external standards according to the manufacturer’s instructions (BioThema, Haringe, Sweden). Luminescence was detected in a FLUOstar Optima microtiter plate reader (BMG Labtech, Offenburg, Germany).

Preparation of Pancreatic Acini—Acini were prepared by collagenase digestion as described earlier (15). After filtering through nylon mesh, cells were gently washed in physiological HCO3−-free buffer (−BIC) of the following composition (in mmol/liter): 145 Na+, 3.6 K+, 1.5 Ca2+, 1 Mg2+, 145 Cl−, 2.0 phosphate, 5 glucose, and 10 HEPES. Finally, cells were suspended in −BIC solution, and 50-μl aliquots were pipetted into 96-well microtiter plates followed by 50 μl of luciferin/luciferase mix HSII (Roche Diagnostics, Manheim, Germany), which was dissolved in −BIC. Acini were allowed to rest for 45–60 min, but most of them did not attach to substrate. Subsequently, luminescence was monitored after injection of 5-μl volumes of −BIC and CCK-8 made up in −BIC. Luminescence was monitored in 1-s intervals in the microtiter plate reader. Temperature was 25 °C to slow down ATP hydrolysis by enzymes. ATP standards were treated as samples, and standard curves were constructed for each experiment. Under the given experimental conditions, ATP standards gave stable luminescence signals. ATP release monitored in arbitrary luminescence units was recalculated as ATP concentration and corrected for 1 million cells/ml. Cell numbers were estimated by cell counting and from cellular ATP freed following cell lysis.

Protein Measurement—Total protein concentration in pancreatic juice was determined by using BCA Protein Assay Kit (Pierce).

HPLC Analysis of Nucleotide Metabolism—Pancreatic juice (1.5–2 μl, ~250 μg of protein) was incubated with 20 μM ITP/UTP in a final volume of 300 μl of phosphate-buffered saline supplemented with 0.4 μM MgCl2. Aliquots of the mixture (100 μl) were collected at the beginning (zero point) and after a
60-min incubation at 37 °C, and nucleotides were extracted by adding 20 μl of 4 M perchloric acid. After centrifugation, the supernatant was adjusted to neutral pH by 4 N KOH (~28 μl), clarified again by centrifugation, and stored at −70 °C. The samples (20 μl) were then injected onto a Synergy Hydro-RP 80A column and separated by reverse-phase HPLC as described previously (4).

Measurement of Purine-converting Activities in Pancreatic Juice—Nucleotide-converting activities were determined by incubating 1 μl of juice samples (150–200 μg of protein) for 45–60 min at 37 °C in a final volume of 80 μl of RPMI 1640 medium containing 2 mM β-glycerophosphate in the following ways. (i) For evaluation of ATPase activity, juice was incubated with 300 μM [14C]ATP. (ii) ADPase was assayed with 300 μM [3H]ADP in the presence of adenylyl kinase inhibitor Ap5A (50 μM). (iii) 5’-Nucleotidase activity was measured with 300 μM [3H]AMP. (iv) For adenylyl kinase and NDPK activities, the assay medium contained 300 μM [3H]AMP or [3H]ADP as respective phosphate acceptors and 800 μmol/liter γ-phosphate-donating ATP/NTP. In the case of time-dependent studies, juice was incubated with 20 μM tracer nucleotides in a starting volume of 120 μl of RPMI 1640, and aliquots of the mixture were periodically applied onto Alugram sheets. Radiolabeled nucleotide substrates and their products were separated by TLC, visualized in UV light, and quantified by scintillation counting, as described earlier (3). Likewise, for measurement of adenosine deaminase, juice was incubated with 300 μM [3H]adenosine for 60 min, and the amount of generated [3H]inosine/hypoxanthine was quantified by TLC using an appropriate solvent mixture (4).

Autoradiographic Analysis of [γ-32P]ATP Metabolism by Pancreatic Juice—Samples (50 μg of protein) were incubated for 40 min at 37 °C in a final volume of 60 μl of RPMI 1640 containing 2 mM β-glycerophosphate, 10 μM ATP with tracer [γ-32P]ATP, and 250 μM unlabeled nucleotides. Aliquots of the mixture were spotted onto Polygram sheets, separated by TLC with 0.75 mol/liter KH2PO4 (pH 3.5), and developed by autoradiography.

Data Presentation—Data are presented as original recordings and summaries showing the mean values ± S.E. Data were analyzed in Origin (Microcal Software, Inc).

RESULTS

From isolated preparations of pancreatic acini and ducts, we have learned that in response to cholinergic stimulation, pancreatic acini secrete ATP (15). This ATP might be destined for the adjoining pancreatic ducts, which express functional P2 receptors (see the Introduction). In the present study, we wished to determine whether similar signaling functions with the classical acinar agonist CCK-8, both at the acinar cell level and at the whole organ level. In the first series of experiments, ATP release was monitored in situ in a suspension of pancreatic acini. In the first part of the recording in Fig. 1A, one can observe that mechanical disturbance caused by pump injection of −BIC results in a small ATP release. Repeated injection of −BIC has no further effect (results not shown). However, injection of CCK-8 (10−11 M) resulted in a transient increase in ATP. The peak agonist-induced ATP release is expressed per 1 million cells/ml (Fig. 1B). The decay in the ATP signal was most likely due to the presence of enzymes hydrolyzing ATP since ATP standards gave sustained luminescence signal under our conditions. In addition, supernatant collected from acinar incubation medium was able to degrade ATP standards, similar to what we have also seen with whole pancreas juice (see below).

Next we studied pancreatic secretion of intact pancreas stimulated to secrete with CCK-8, which when infused at 5.6 pmol/min/200 g of animal, would be 10−11 to 10−10 M in the interstitium. Fig. 1C shows the secretory response of rat pancreas with time and ATP concentrations in pancreatic juice. Pancreatic juice collected from cannulated common pancreatic bile duct was analyzed for ATP, and the concentrations were 4.5 ± 1.2 nmol/liter (n = 7)(Fig. 1D). These ATP concentrations are higher than detected earlier, most likely due to improved techniques for juice collection (16, 19). Nevertheless, ATP concentrations in juice are significantly lower than estimated from acini preparations. In fact, if we assume that all acinar cells that comprise the bulk of pancreatic tissue would release ATP into the juice, then disparity between proximal and distal ATP concentrations would be even larger. We postulated that the diminished ATP levels as secretion progressed from acini
through the whole duct tree were indicative that the pancreatic juice also contained ATP-hydrolyzing enzymes. Therefore, pancreatic juice was collected between 60 and 80 min of stimulation, when secretion was well established, not contaminated by bile and possible cellular debris from cannulation processes. The collected samples were used for enzyme analysis, which was the primary focus of this study.

Fig. 2 shows the time courses of hydrolysis of 20 μM [14C]ATP by pancreatic juice (Fig. 2A) and formation of its dephosphorylated 14C metabolites (Fig. 2B). Gradual decay in ATP concentration was accompanied by an immediate rise in AMP and, after a time lag, there was progressive production of ADP, AMP, adenosine (Ado), and inosine/hypoxanthine (Ino/Hyp) (B). The ordinate shows the relative content of radioactive metabolites expressed as percentage of initial concentrations. The graphs show mean ± S.E. data of five independent experiments performed with different juice samples.

Little ADP formation in the assay medium may be explained by immediate breakdown of the ATP-derived ADP via high NTPDase activity or, alternatively, due to direct ATP conversion into AMP through NPP reaction. Nevertheless, the latter suggestion seems highly unlikely for the following reasons. Firstly, in the presence of 300 μM unlabeled ADP, hydrolysis of 20 μM [14C]ATP by juice samples was markedly diminished (Fig. 2A), suggesting that ADP and ATP compete for the same catalytic site of NTPDase. Secondly, Fig. 3 unequivocally shows that pancreatic juice directly converts [γ-32P]ATP to 32P, without any detectable formation of 32PP. This indicates that ATP-hydrolyzing activity in pancreatic juice is mainly represented by NTPDase rather than NPP. EDTA eliminated this nucleotidase reaction (lane 7), showing the Ca2+/Mg2+ dependence of NTP-Dases. Upon incubation of [γ-32P]ATP with AMP or nucleotide diphosphates GDP and UDP, there was a slight generation of [32P]ADP and [32P]NTPs, respectively (lanes 3–5), indicating that pancreatic juice also contains adenylate kinase and NDPK, which transfers [γ-32P]. Adenylate kinase inhibitor, Ap5A, inhibited 32P transfer from ATP to AMP (lane 4).

The levels of nonspecific phosphatases were also negligible as similar patterns of [14C]ATP hydrolyses were observed with and without the excess of alternative phosphorylated substrate, β-glycerophosphate (data not shown). For further evaluation of the nucleotidase substrate specificity, pancreatic juice was incubated with 20 μM ITP or GTP, and the reaction products were separated by reverse-phase HPLC. Clearly, significant portions of ITP (Fig. 4A) and UTP (Fig. 4B) were hydrolyzed into respective nucleoside di- and monophosphates after a 60-min incubation, showing the broad specificity of pancreatic nucleotidase for various NTPs and NDPs.

The pattern of subsequent nucleotide hydrolysis by juice samples was then evaluated with [3H]AMP and ADP as initial tracer substrates, and the results are shown in Fig. 5. Pancreatic juice progressively hydrolyzed [3H]AMP into [3H]AMP/adenosine, and this catalytic reaction was markedly attenuated in the presence of 300 μM ATP (Fig. 5, A and B). The addition of [3H]AMP to pancreatic juice was also accompanied...
by its gradual hydrolysis to [3H]adenosine through 5′-nucleotidase reaction (Fig. 5C). Unlabeled ATP efficiently blocks the [3H]AMP hydrolysis, presumably due to feed-forward inhibition of 5′-nucleotidase activity and/or concurrent activation of backward phosphotransfer reactions (3).

Quantitative radio-TLC analysis of pancreatic purine-converting enzymes was then performed using saturated concentrations of radiolabeled and unlabeled nucleotides/adenosine, and these results are summarized in Fig. 6. The data show the ability of rat pancreatic juice to efficiently hydrolyze [3H]ADP and to about a similar extent as [14C]ATP. Pancreatic juice also efficiently hydrolyzes [3H]AMP by means of the 5′-nucleotidase activity. In contrast, incubation of juice with [3H]adenosine did not cause its significant deamination to [3H]nucleosides (Fig. 6A), indicating negligible activity of adenosine deaminase (also see above). Pancreatic adenylate kinase and NDPK activities were also determined by incubating juice samples with [3H]AMP or [3H]ADP as corresponding phosphate-accepting substrates in the presence of unlabeled γ-phosphate-donating ATP/NTP (Fig. 6B). In support of qualitative autoradiographic data shown in Fig. 3, these enzyme assays confirmed moderate ATP-dependent [3H]AMP conversion into high energy [3H]phosphoryls through the adenylate kinase reaction. Unlike [3H]AMP phosphorylation, subsequent NDP kinase-mediated [3H]ADP conversion into [3H]ATP is characterized by a higher rate and can be activated not only by ATP but also by other NTPs (Fig. 6B).

For comparative analysis, the major pancreatic activities shown in Fig. 6 were also expressed as nmol/ml of juice/hour and further correlated with soluble activities determined for two plasma samples. Mean activities for the following enzymes, ATPase, 5′-nucleotidase, adenosine deaminase, and adenylate kinase, were 2.1 and 0.29, 1.42 and 2.22, 0.07 and 0.20, 0.45 and 0.86 nmol/ml/hour for rat pancreatic juice and plasma, respectively.

**DISCUSSION**

The present study shows that pancreatic juice contains both ATP-consuming and ATP-generating enzymes. The following groups of enzymes were identified: NTPDase with broad substrate specificity, 5′-nucleotidase, NDPK, and adenylate kinase. No significant activities of NPP, nonspecific phosphatases, or adenosine deaminase were detected. The fact that these purinergic enzymes are secreted into pancreatic juice is a new observation, which indicates that the release of these enzymes may be regulated.
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FIGURE 6. Purine-converting activities in rat pancreatic juice. A, nucleotide hydrolase and adenosine deaminase activities (ADA) were determined by incubation of juice samples with nearly saturating (300 μM) concentrations of radiolabeled nucleotides and adenosine as preferential enzyme substrates. 5-NT, 5'-nucleotidase. B, the counteracting NDPK and adenylate kinase (AK) activities were assayed by juice co-incubation with 300 μM [3H]AMP or [3H]ADP as respective phosphate acceptors and 800 μM γ-phosphate donating ATP/NTP. The ordinate shows specific enzyme activities expressed as nmol of tracer nucleotide substrates inactivated (A) or phosphorylated (B) by mg of juice protein per hour (mean ± S.E.; n = 3–6).

Mammalian NTPases are a family of enzymes that catalyze hydrolysis of γ and β residues of nucleotides with different specificities. To date, there are eight members of this family. The catalytic site is facing the extracellular milieu as in NTPases 1–3 and 8 and/or intracellular organelles as in NTPases 4–7, and further, NTPases 5 and 6 can be proteolytically cleaved from the plasma membrane and secreted (1, 20–22). The NTPases 1, 2, 3, and 8 catalyze hydrolysis of triphospho- and diphosphonucleotides with ATP:ADP rates of hydrolysis of about 1:1, 1:0.03, 3:1, and 2:1. The enzyme that we find in pancreatic juice can clearly hydrolyze ATP and ADP efficiently (Figs. 2, 5, and 6); ADPase activity is slightly higher and similar to the endothelial enzyme (3). There is no significant buildup of intermediate product ADP during ATP degradation (Fig. 2B). ATP and ADP compete for the same site (Figs. 2A and 5A), the enzyme has relatively broad specificity for NTP (Fig. 4), and it requires divalent cations (Fig. 3). Thus, the present study shows that the most likely candidate for pancreatic ATPase/ADPase is NTPDase 1, that is, CD39. This conclusion supports our earlier study where we detected CD39 by Western blotting on pancreatic juice collected from rats stimulated with CCK-8 (16).

Although CD39 is mainly regarded as a vascular enzyme, it has been originally identified in rat pancreatic tissue (23) (see Ref. 16) and isolated from membranes of zymogen granule of pig pancreas (24, 25). There is ongoing discussion about whether CD39 and/or other members of the CD39 family are present in pancreatic tissue and whether distribution is species-dependent, and most importantly, about possible function of these enzymes. In human pancreas, immuno- and histochemistry indicate that active NTPDase 1 (CD39) is not detected in acini or ducts (26). In recent studies on pancreatic tissue from mice, ATPase and ADPase activities determined by inorganic phosphate assays were significantly lower in CD39−/− mice when compared with control mice (17). Immuno- and histochemistry revealed that acini and ducts express both CD39 and CD39L1, i.e. NTPDases 1 and 2, but zymogen granules were not stained consistently. Nevertheless, earlier biochemical studies showed NTPDase activity on granule membranes (18, 27, 28).

In our study where rat pancreas was prestimulated with CCK-8 before fixation, CD39 was redistributed to the secretory pole of acini (16), clearly ready to be secreted into pancreatic juice, as demonstrated by enzymatic analyses in the present study.

In addition to CD39, the enzyme hydrolyzing AMP to adenosine, i.e. 5'-nucleotidase, appears in pancreatic juice (Figs. 5 and 6A). This finding casts a light on an older study on rat pancreas AMPase activity that was detected histochemically, which showed localization of enzyme activity to different regions of acinar cells during the 24-h period (29). During day time, when secretory granules were accumulated, activity was seen in luminal and basolateral plasma membrane, as well as in intracellular organelles. During feeding/secretory phases, only basolateral marking was retained, suggesting that the enzyme was secreted or shed. Our study indicates that 5'-nucleotidase distribution within pancreas could be regulated since CCK-8 stimulation leads to secretion of 5'-nucleotidase into pancreatic juice. We propose that pancreatic 5'-nucleotidase, most likely CD73, serves a physiological role in epithelial function by providing adenosine for pancreatic ducts.

In contrast to airway epithelia and the cardiovascular system (see the Introduction), no significant activities of nonspecific alkaline phosphatase, NPP, and adenosine deaminase were detected in pancreatic juice. This would indicate that pancreas has a relatively specific set of nucleotide-handling enzymes destined for secretion. Some enzymes may also localize within pancreatic tissue.

Pancreatic juice also contained moderate adenylate kinase and NDPK activities (Figs. 3 and 6B), potentially interconverting extracellular nucleotides via backward phosphotransfer reactions. Our finding on regulated secretion of these kinases is supported by findings in another exocrine gland. Namely, cholinergically stimulated submucosal glands in airway epithelia also secrete adenylate kinase and NDPK (30), and on apical surfaces their avid activities would counteract ATP hydrolysis and thus propagate purine-mediated mucociliary clearance. In pancreatic juice, these ATP-generating enzymes have lower activities than ATP-hydrolyzing enzymes (Fig. 6), suggesting that adenosine receptor-mediated signaling would be important (see below).

Where do the enzymes come from, and are they really active in situ? Immunohistochemical studies showed that CD39 is localized in acini, mainly in granular compartments, and also in ducts. Since CCK-8 is the major stimulant of acini, presumably it is secreted from acini. Secretin, the ductal agonist, did not cause release of CD39 (16). Very likely then, other enzymes determined in this study (NTPDase1/CD39, 5'-nucleotidase, adenylate kinase, and NDPK) could also have originated in the acini. Since at least CD39 is secreted as a full enzyme and not cleaved one, the question is whether we can call these enzymes “soluble enzymes” or whether they are associated with postulated microvesicles (16), such as those known particularly in immune and hemostatic systems (31, 32). Both types of release could be associated with zymogen granules, where at least CD39 immunolocalization is strong (16). In any case, enzyme release, however it happens, seems to be regulated. In pancreatic juice, where normal concentrations of free Ca2+ and Mg2+ are submillimolar (33, 34), the conditions for enzymes would be suboptimal when compared with in vitro enzymatic assays.
Nevertheless, since the juice emerging out of the organ/cannula has relatively low ATP concentrations when compared with estimated concentrations released from acini (Fig. 1), some ATP is degraded either by secreted enzymes or possibly also by ecto-enzymes lining ducts. We estimate that in the rat pancreas, the ductal system plus cannula would occupy a volume of around $50 \mu l$. Thus, with secretion rates of about $3 \mu l/min$, there would be sufficient “contact time” for enzymes to modify secretion.

The physiological implications for pancreas are as follows. CCK-8 stimulation of pancreatic acini leads to secretion of ATP (Fig. 1). It is not excluded that there are also other sites for ATP release within pancreas. In addition, CCK-8-stimulated secretion also contains CD39, and kinases, as shown by the present study. On one hand, relatively large activities of CD39 and $5'\text{-nucleotidase}$ would favor ATP hydrolysis and production of adenosine. On the other hand, there is also a possibility to generate ATP by adenylate kinase and by trans-phosphorylation of ADP to ATP, where other NTPs can be used as phosphate donors. Thus, P2 receptors with a preference for NTP over NDP, and P1 receptors, would have possibilities to be stimulated. Indeed, ducts from adult rat pancreas express the ATP/UTP-prefering receptors, P2X$_4$, P2X$_7$, P2Y$_2$, and P2Y$_9$, but not the ADP/UDP-prefering receptors, P2Y$_1$, or P2Y$_6$ (35). In addition, ducts also express a number of adenosine receptors (preliminary studies). The nucleotide- and nucleoside-selective receptors are most likely involved in regulation of bicarbonate and fluid secretion occurring in pancreatic ducts (13, 16). This newly discovered richness in secreted purine-handling enzymes underscores the importance of acini-to-duct communication and of P2 and P1 receptor signaling along pancreatic duct lumen. Most likely, the secretary profiles of ATP-generating versus ATP-hydrolyzing enzymes, as well as ATP release, may depend on the extent and duration of stimulation in this complex organ. Accordingly, P2 and P1 receptor distribution may vary with the generation of ducts.

In conclusion, enzyme assays on pancreatic juice in this study, together with our previous Western blotting data (16), demonstrate the presence of specific $Ca^{2+}$-dependent soluble enzyme with hallmark characteristics of NTPDase/CD39, which has a broad substrate specificity toward various nucleoside tri- and diphosphates. We have shown the presence of yet another soluble pancreatic nucleotide-hydrolyzing enzyme, $5'\text{-nucleotidase}$, and in addition, provided kinetic evidence for the existence of moderate adenylate kinase and NDPK activities potentially interconverting extracellular nucleotides via backward phosphotransfer reactions. Soluble adenosine deaminase, nucleotide pyrophosphatase, and non-specific phosphatase do not seem to contribute to the purine metabolism in the rat pancreatic juice. The given complement of enzymes may be important in purine signaling within pancreas and thus coordination of pancreatic secretion on the whole organ level.

Acknowledgments—The technical assistance of A. V. Olsen and A. Q. C. Scheuer is greatly acknowledged.

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