Proliferation and exocrine transport mechanisms in epithelial glands

Ph.D. Thesis

Dr. Szűcs Ákos

Semmelweis University

INTRODUCTION

The enzimatic procedures of the upper part of the gastrointestinal tract require specific and constant level of pH provided by the bicarbonate-rich fluid excreted from the salivary glands and the pancreas. The disorders of secretory functions – as in an inherited disorder of epithelial chloride transport (cystic fibrosis) - can cause serious malfunctions of the digestion. The advent of modern molecular biology and the introduction of sophisticated techniques in experimental cell physiology have facilitated considerable progress in our knowledge of the various transport mechanisms responsible for ductal HCO_3^- secretion. However, most of this progress has been as a result of experiments on animal tissues, therefore, rat and mouse pancreatic ducts represent poor models of human duct cell function. Similarly, the role of gastrointestinal peptides in the regulation of the exocrine secretion is not well described. However, we know, that these gastrointestinal hormones acting on G-protein coupled receptors and activating various protein kinases (ERK or MAPK) can regulate cell proliferation and differentiation in normal tissues and cancer cells as well. Identification of the mechanisms by which these key signaling pathways are modulated could provide a basis for the development of novel therapeutic interventions to treat chronic inflammatory diseases as well as cancer. Although difficult, it is possible to obtain sufficient human pancreatic tissue for both molecular biological and immunohistochemical studies. This has allowed identification and localisation of some of the key membrane components involved in human duct cell secretion and its regulation. Unfortunately, it is virtually impossible to obtain sufficient human pancreatic tissue on a regular basis to permit functional studies. However, cell lines derived from human pancreatic duct cells should permit such studies. Therefore we studied the mechanism of fluid- and bicarbonate secretion in cell lines derived from salivary galnd and pancreas (Par-C10 and Capan-1), we investigated the role of protein kinase C and the ERK cascade in the proliferation of pancreatiic adenocarcinoma cell line (Panc-1C) and we studied the involvement of gastrointestinal peptides in the regulation of these processes.

AIMS

Our aim was to:

1. establish a model for studying bicarbonate transport mechanism in epithelial cells
2. investigate the role of gastrointestinal peptides in regulation of bicarbonate secretion in epithelial cells
3. investigate the role of purinoceptors in regulation of bicarbonate secretion in epithelial cells
4. investigate the role of gastrointestinal peptides in regulation of cell proliferation in epithelial cells
5. to examine the role of different PKC isoforms in growth regulation and ERK cascade activation in human pancreatic cancer cell line
6. to study the involvement of ERK activation kinetics in the regulation of proliferation in human pancreatic cancer cell line.

METHODS

1. **Cell culture: Par-C10** cells were obtained from David Quissell, **Capan-1** from the American Type Culture. Cells were grown in standard culturing conditions. For the microfluorometry experiments, 5 x 105 cells were seeded onto collagen-coated or native PTFE membrane. Experiments were performed after the transepithelial electrical resistance of the monolayer had significantly increased as judged by measurements with an EVOM epithelial volt-ohm-meter. Panc-1 cells transfected with the human CCK-1 receptor (**Panc-1C**) were kindly provided by Dr. Craig D. Logsdon (University of Michigan, Ann Arbor, Michigan, USA). Cells were grown in Dulbecco's Modified Eagle Medium (Sigma) with 10% fetal bovine serum (FBS) in the presence of 200 μg/ml G418 at 37 °C in a humidified atmosphere containing 5% CO_2.
2. **Reverse transcriptase-polymerase chain reaction (RT-PCR):** Total RNA was isolated from Capan-1 cells using the TRIreagent. Total RNA from human pancreas, brain and kidney was used for positive controls. 5 μg of total RNA from each sample was reverse transcribed using SuperScript II with an oligo(dT) primer. First-strand cDNA was amplified with PCR primers designed, using Primer3 software, to be specific for selected transporters and receptors (online supplementary material). PCR was performed using *Taq* polymerase

(Promega, Madison, WI). As an internal concentration reference for the PCR experiments, we performed 19 cycles of amplification with primers for the acidic ribosomal phosphoprotein (XS13). The identities of the PCR products were confirmed by sequencing using an ABI Prism BigDye® Terminatorv3.1Cycle Sequencing Kit.

3. **Immunocytochemistry:** After 10-14 days of culture on Transwell filters, the cell monolayers were fixed for 10 min at room temperature (22°C) with 4% (w/v) paraformaldehyde. After fixation, they were washed three times in Tris-buffered saline (TBS), permeabilised with 0.5% Triton-X-100 in TBS for 7 min and washed again in TBS. The monolayers were blocked in 2% normal goat serum and 2% BSA for 1 h at room temperature and then incubated overnight with the primary antibody at 4°C. The mouse monoclonal anti-occludin (Zymed Laboratories, San Francisco, CA) was used at 1:50 dilution and detected with an anti-mouse secondary antibody conjugated to FITC (Stratech Laboratories, Cambridge, UK). The filters were washed in TBS and mounted in Vectashield mounting medium containing propidium iodide as a nuclear counterstain (Vector Laboratories, Ltd., Peterborough, England). Images were captured by confocal laser scanning microscopy (Biorad MRC 1024MP mounted on a Nikon Eclipse TE300 microscope). A gallery of 40-50 optical sections (0.5 μm thick) through the *z*-plane was obtained and the composite images were processed using Confocal Assistant software (Bio-Rad, Hercules, CA).
4. **Measurement of intracellular pH:** Intracellular pH (pH_i) was measured by standard microfluorometric techniques in monolayers superfused with separate apical and basolateral solutions. For these experiments, Transwell inserts were mounted in a purpose- built, temperature-controlled chamber mounted on the stage of a Nikon Eclipse TE200 inverted microscope. The cells were loaded with the pH-sensitive fluoroprobe BCECF-AM. A small region of the epithelium was illuminated alternately at 440 and 490 nm, and the fluorescence intensities (F_{440} and F_{490} respectively) were measured at 530 nm. Intracellular pH was calculated from the F_{440}/F_{490} ratio using calibration data obtained *in situ* by the nigericin-K+ method.
5. **[^{3}H]-thymidine incorporation:** Panc-1C cells –after 24 hour-serum withdrawal - were then incubated in fresh medium with or without the specified concentrations of agonists and/or inhibitors for 24 hours. Subsequently, cells were pulse labelled with 1 μCi/ml [^{3}H]-thymidine

for 2 hours. Cells were then washed twice, fixed and then dissolved in 0.1 M NaOH and the incorporated radioactivity was determined by liquid scintillation counting.

6. **Immunoblotting:** Panc-1C cells were plated in 6-cm dishes at equal cell numbers (4×10^5 cell/dish), grown overnight and made quiescent by serum deprivation for 24 hours. Subconfluent, quiescent cells were treated with agonists and/or inhibitors as described in individual experiments. Cells were then rinsed with ice-cold phosphate-buffered saline (PBS) and directly lysed in 1×SDS-PAGE sample buffer followed by SDS-PAGE and transfer to polyvinyl difluoride membranes. Blots were then blocked, probed with primary antibodies appropriately diluted in Tris-buffered saline (TBS) containing 0.1% Tween-20 and 2% ECL Advance Blocking Reagent, washed, and incubated with secondary antibody. After additional washing, immunoreactive bands were visualized with the Amersham ECL Advance chemiluminescent detection system.

RESULTS

1. **RT-PCR analysis of transporter and receptor expression:** The expression of mRNA for key electrolyte transporters was evaluated by PCR using cDNAs obtained from normal human pancreas and **Capan-1** cells. Transcripts of the expected size were detected for CFTR (cystic fibrosis transmembrane conductance regulator), NHE1 (but not NHE3), pNBC1 and NKCC1 (but not NKCC2) in both normal pancreas and in the cell line. Transcripts were also detected for secretin, VIP/PACAP (VPAC1 and VPAC2) receptors, but not for PACAP (pituitary adenylate cyclase-activating polypeptide) receptors. Of the purinoceptors examined, positive results were obtained for P2Y1, P2Y2, P2Y4 and P2Y6, and for P2X1, P2X4 and P2X5, but not P2X2, P2X6 or P2X7.
2. **Polarization of Capan-1 monolayers grown on permeable supports:** The tight-junction protein occludin labelling was localised to the plasma membrane and showed a characteristic 'chicken wire' pattern at the apical border of the cells, consistent with its expression at the tight junctions. Measurements of transepithelial electrical resistance during the culture period showed a steady increase to a plateau value of between 50 and 150 Ω cm2 after 8-12 days, consistent with the relatively leaky phenotype previously described.
3. **Evidence for basolateral Na^+/H^+ exchange:** In **Capan-1** cells int he absence of HCO_3^-/CO_2 after acid load there was no subsequent recovery of pH_i in the absence of extracellular Na^+.

Thus there was no evidence for any Na^+-independent mechanism, such as an H^+-ATPase, under the conditions of these experiments. When Na^+ was restored to the basolateral bathing fluid, however, there was a swift recovery of pH_i, which was completely abolished by 3 μM EIPA. So the basolateral extrusion of H^+ is most likely mediated by an Na^+/H^+ exchanger, probably the ubiquitous NHE1.

4. **Evidence for basolateral Na^+-HCO_3^- cotransport:** In the presence of HCO_3^-/CO_2, a very slow but significant recovery of pH_i was observed after the NH_4^+ pulse despite the absence of extracellular Na^+ which was not inhibited by 1 μM bafilomycin A1, suggesting that it was probably not due to H^+ extrusion by a vesicular-type H^+-ATPase (V-ATPase). The identity of the transporter responsible for this phenomenon remains to be determined. Restoring Na^+ to the basolateral bathing solution led to a rapid recovery of pH_i which was abolished by the combined basolateral application of 3μM EIPA and 500 μM H_2DIDS. However, after withdrawal of the H_2DIDS, but in the continued presence of EIPA, there was a marked increase in pH_i. showing the presence of an H_2DIDS-sensitive, Na^+- dependent HCO_3^- uptake mechanism at the basolateral membrane, most probably the Na^+-HCO_3^- cotransporter NBC1 that was detected by RT-PCR.
5. **Evidence for basolateral Na^+-K^+-$2Cl^-$ cotransport: B**asolateral application of 500 μM bumetanide, an NKCC1 inhibitor, slowed the re-acidification that occurred during the NH_4^+ pulse by about 60%. This suggests that there may be some NKCC1 activity in these cells, which would be consistent with the RT-PCR data described above.
6. **Measurement of transepithelial HCO_3^- secretion:** Intracellular pH is maintained during HCO_3^- secretion by a balance between the alkalinizing effect of basolateral HCO_3^- uptake (and H^+ extrusion) and the acidifying effect of HCO_3^- efflux across the apical membrane. Therefore when basolateral HCO_3^- uptake is blocked in this way, the continuing efflux of HCO_3^- across the apical membrane leads to a fall in pH_i. The initial rate of decrease in pH_i is therefore believed to be a good indirect measure of apical HCO_3^- secretion. In **Par-C10** cells application of forskolin did not in itself cause any change in pH_i, but when EIPA and H_2DIDS were subsequently applied, the rate of decrease in pH_i was approximately doubled when compared with the corresponding values in unstimulated cells. The same stimulatory effect of forskolin was obtained after the inhibition of basolateral HCO_3^- uptake in **Capan-1** cells.

7. **Effects of secretin and vasoactive intestinal peptide (VIP) on transepithelial HCO_3^- secretion:** As observed with forskolin, in **Capan-1** cells the intracellular acidification resulting from subsequent application of EIPA and H_2DIDS was accelerated by secretin and VIP in a dose-dependent manner. Both secretin and VIP had similar potencies, both inducing 50% of maximal stimulation in the 10 nM range. Secretin was approximately three times less potent than VIP.
8. **Effects of ATP and UTP on transepithelial HCO_3^- secretion:** In **Par-C10** cells acidification evoked by the inhibition of basolateral bicarbonate uptake was strongly accelerated by luminal application of ATP. However, the basolateral application of ATP resulted in a same acceleration in the rate of intracellular acidification caused by the inhibition of basolateral bicarbonate supply compared to the control. UTP increased the rate of acidification only from the luminal side, application to the basolateral side was without effect.In **Capan-1** cells apical application of either 1 μM ATP or UTP had no direct effect on the pH_i but the acidification evoked by the subsequent application of EIPA and H_2DIDS was significantly accelerated when compared with the unstimulated controls. Applied to the basolateral side, however, ATP and UTP both reduced the rate of intracellular acidification evoked by EIPA and H_2DIDS.
9. **Protein kinase Cε can mediate proliferation or growth arrest in Panc-1 human pancreatic cancer cells:** NT stimulated, whereas CCK moderately and PMA dramatically inhibited DNA synthesis. To study the involvement of different PKC isoforms, we used isoform-selective PKC inhibitor 20 nM Gö6983 to inhibit PKCα, -β, -γ and -δ, 200 nM Ro 32-0432 to inhibit PKCα, -βI and -ε and 40 nM Gö6976 to inhibit PKCα, -βI and -μ. While Ro 32-0432 prevented the effects of PMA, CCK and NT on the proliferation of Panc-1C cells, Gö6983 was without effect. Like Gö6983, Gö6976 also did not prevent the effects of PMA, CCK and NT. These results suggest that PKCε is responsible for the growth regulatory effect of PKC activation in Panc-1 pancreatic cancer cells.
10. **PMA, CCK and NT stimulate the ERK cascade and regulate growth in an ERK-dependent manner in Panc-1C cells:** Cells were made quiescent by serum deprivation for 24 hours, treated with 100 nM PMA, 10 nM CCK or 50 nM NT over a time course ranging from 2 min to 6 h and then processed for immunoblotting. The ERK cascade was activated with different kinetics: an increase in the level of phospho-ERK was induced for at least 6 h

by PMA, for up to 30 min by CCK and for as little as 2 min by NT. The level of pan ERK, on the other hand, remained constant. Our findings suggest that the kinetics of ERK activation correlates with the effects on DNA synthesis. Pretreatment of cells with 20 μM PD98059 (an inhibitor of MEK that prevents ERK phosphorylation) in itself reduced DNA synthesis by more than 50%, whereas it prevented the stimulatory effect of NT and the inhibitory effect of PMA, restoring [^{3}H]-thymidine incorporation to levels close to what was obtained by incubation with PD98059 alone. However, PD98059 did not prevent the moderate inhibitory effect of CCK, presumably because in itself it had a stronger inhibitory effect on DNA synthesis. The expression of key cell cycle gene cyclin D1 was upregulated by PMA, CCK and NT, whereas p21Cip1 expression was increased by PMA and CCK but not by NT. Furthermore, pretreatment with PD98059 reduced the above effects of PMA, CCK and NT on the expression of the cell cycle genes. These results further confirm that direct or indirect PKC activation regulates the growth of Panc-1 pancreatic cancer cells via the ERK cascade.

11. **PMA, CCK and NT stimulates the ERK cascade in Panc-1C cells in a PKC-dependent manner:** Pretreatment with 200 nM Ro-32-0432, but not with 20 nM Gö6983 or 40 nM Gö6976, reduced the effect of PMA, CCK and NT on ERK activation. This suggests that the novel isoform PKCε may be reponsible for the activation of ERK cascade.

DISCUSSION

Par-C10 and Capan-1 cells grown on permeable filter form polarized monolayers

Previous studies using **Capan-1** cells grown on permeable filters have reported widely divergent values for the transepithelial electrical resistance. However, our data are more in line with the 'leakier' phenotype and are also consistent with previous evidence that native pancreatic ducts have a substantial paracellular permeability. Despite the low resistance of the Capan-1 monolayers, immunolabelling with an occludin antibody shaw that the cells form polarized monolayers with well developed tight junctions. In **Par-C10** cells our results correlate with the findings of Turner et coworkers that Par-C10 cells typically displayed the high transcellular resistance values and exhibited the most polarized phenotype.

Functional polarization of the Capan-1 cells

In the absence of HCO_3^-/CO_2, the recovery of pH_i following an acid load was dependent on basolateral, but not apical, Na^+ and was completely blocked by basolateral application of EIPA. The presence of a basolateral Na^+/H^+ exchanger, usually NHE1, is an almost universal feature of mammalian epithelia. The absence of apical NHE activity, which has been reported in the larger ducts of some species, suggests that the Capan-1 cells do not reabsorb HCO_3^- but rather have a predominantly secretory phenotype more typical of smaller ducts. The presence of a basolateral Na^+-HCO_3^- cotransporter was revealed in acid loading experiments performed in the presence of HCO_3^-/CO_2. We found that the recovery of pH_i when Na^+ was restored to the basolateral bathing solution was blocked by combined treatment with EIPA and H_2DIDS but not by EIPA alone. This is consistent both with our RT-PCR evidence for pNBC1 expression and with a previous report of Na^+-HCO_3^- cotransporter activity in Capan-1 cells. However, this is the first demonstration of its basolateral localization in these cells - a finding that is consistent with previous observations in rat and guinea-pig ducts and in CFPAC-WT cells. When the acid-load experiments were performed in the presence of HCO_3^-/CO_2, we found evidence for a small additional contribution from an Na^+-independent acid extruder. The failure of bafilomycin A_1 to inhibit its activity argues against it being a V-ATPase, and its identity has still to be determined. Interestingly, a similar phenomenon has been observed in guinea-pig ducts. The pH_i changes that occur during NH_4^+ pulse experiments can also reveal the presence of an Na^+-K^+-$2Cl^-$ cotransporter. Given that we could detect NKCC1 expression in Capan-1 cells by RT-PCR, we

showed a bumetanide-sensitive NH_4^+ uptake via a basolateral NKCC1. The presence of NKCC1 in these cells is somewhat surprising given that Na^+-coupled Cl^- uptake at the basolateral membrane would provide a driving force for apical secretion of Cl^- and this is thought to be incompatible with the secretion of a HCO_3^--rich juice. Its presence in Capan-1 cells may, however, reflect the origin of these cells in the larger ducts of the human pancreas, where the expression levels of the basolateral transporters may differ from those in the smaller intercalated and intralobular ducts that are thought to secrete most of the HCO_3^--rich fluid.

Transepithelial bicarbonate secretion – the role of the gastroitestinal peptides

We have shown that in Capan-1 cells the basolateral NHE and NBC are the main transporters involved in the accumulation of intracellular HCO_3^- for subsequent secretion across the apical membrane. The fact that EIPA and H_2DIDS applied together during stimulation with forskolin caused a faster decline in pH_i than EIPA alone suggests that the NBC makes a substantial contribution to basolateral HCO_3^- uptake, as it does in guinea-pig ducts. However, our results appear to contradict the findings of Cheng *et al.* who reported a negligible role for the NBC in HCO_3^- accumulation in Capan-1 cells. Previous work has suggested that both secretin and VIP receptors may be expressed in Capan-1 cells. Consistent with this we have found evidence for the expression of secretin, $VPAC_1$ and $VPAC_2$ receptors by RT-PCR, and our functional studies also indicate that both secretin and VIP receptors are active in these cells. Although the two peptides clearly stimulated HCO_3^- secretion.

Transepithelial bicarbonate secretion – the role of the purinoceptors

In **Par-C10** cells using inhibitory stop method the application of ATP to the luminal and basolateral membrane resulted in a same accelerated fall in pH_i caused by the inhibitors. However, UTP stimulated HCO_3^- secretion when applied to the luminal side, basolateral administration was without effect. Lee and coworkers – partially correlated with our findings - published the evidence that in isolated submandibular gland acinar and duct cells express at least two different separate types of P_2 receptors in the luminal and basolateral membrane: ATP stimulated $[Ca^{2+}]$ increase, when aplied to the luminal or the basolateral sides, bzATP increased $[Ca^{2+}]_i$ only when applied to the luminal side, UTP only when applied to the basolateral side. Our result suggest that Par-C10 cells express the P_2Y_2 receptors in the luminal membrane.

In microperfused guinea-pig ducts, application of ATP and UTP to the luminal and basolateral membranes evoked opposite effects: luminal nucleotides stimulated HCO_3^- secretion while basolateral nucleotides were inhibitory. In the present study we have observed similar effects in Capan-1 cells. Although our RT-PCR analysis showed that Capan-1 cells express mRNAs for P2Y and P2X receptors, the similarity of the responses to ATP and UTP suggests that purinergic activation at both apical and basolateral locations is mediated by P2Y receptors, which are equally responsive to ATP and UTP. Previous studies on Capan-1 cells have shown that ATP stimulates Cl^-/HCO_3^- exchange at the apical membrane by a Ca^{2+}-dependent process that is also dependent on the presence of CFTR. Furthermore, our finding that human pancreatic juice contains significant amounts of ATP supports the notion that luminal ATP may be an important paracrine signal in the pancreatic ductal system. The inhibitory effect of ATP applied to the basolateral membrane is more difficult to explain. One factor that might be important is the observation that, in rat pancreatic duct cells, stimulation of $P2Y_2$ (and possibly $P2Y_4$) receptors raises intracellular Ca^{2+} but at the same time reduces the whole-cell K^+ conductance. If the latter effect is mediated by local signalling interactions confined to the basolateral membrane, where the K^+ channels are predominantly located, the resulting depolarization would certainly have an inhibitory effect on HCO_3^- secretion.

Effect of PKC isoforms on cell proliferation

In accordance with previous observations, phorbol ester-mediated direct activation of PKC leads to a striking decrease in DNA synthesis, while activation of transfected CCK-1 receptors moderately inhibits, and stimulation of natively expressed NT receptors stimulates, cell growth. To examine whether different PKC isoforms are responsible for the opposite effects of PMA, CCK and NT on cell growth, we used isoform-selective PKC inhibitors. We showed that inhibition of classical PKC isoforms (cPKCs) *and* nPKCε, but not cPKCs *and* nPKCδ, prevents the effects of PMA, CCK and NT on the DNA synthesis of Panc-1C cells. Our results therefore suggest that the effects on proliferation are mediated by PKCε.

The role of ERK activation in cell proliferation

Our results show that PMA, CCK and NT all stimulate the ERK cascade, but with strikingly different kinetics. The growth regulatory effects thus appear to correlate with the duration of ERK activation in Panc-1C cells: transient activation is associated with proliferation, whereas sustained ERK activation is associated with growth arrest. These results are apparently at odds

with those of Kisfalvi and coworkers. Thus, it is possible that there is an optimal duration of ERK activation that results in the highest increase in DNA synthesis, and that the differences between our study and that of Kisfalvi et al. reflect the slightly different choice of time points for measuring ERK activation. Pretreatment of the cells with this inhibitor before applying PMA, CCK or NT restored DNA synthesis to levels close to what was obtained with PD98059 alone. At least in the case of PMA and NT, these results indicate that ERK mediates the effects on proliferation. PMA, CCK and NT regulate the level of cyclin D1 and p21Cip1 in an ERK-dependent manner. These findings are in accordance with results obtained in colorectal cancer cells and fibroblasts, where the duration of the ERK signal determines whether cyclin D1 alone is induced, leading to proliferation, or whether cylin D1 expression is accompanied by the induction of p21Cip1, which results in growth arrest.

The role of PKC isoforms in the activation of ERK cascade

By detecting phosphorylated ERK in Panc-1C cells pretreated with isoform-selective inhibitors and subsequently treated with PMA, CCK and NT, we have found that ERK phosphorylation depends on PKCε but not on classical PKC isoforms or PKCδ. We therefore conclude that the ERK cascade is activated by PKCε in Panc-1C human pancreatic cancer cells. These findings are in line with previous results of others found that PKCε activated Raf-1 in mouse and rat fibroblasts. We propose the following model for the growth regulatory effect of PKC activation in Panc-1C human pancreatic cancer cells. Occupation of native NTR-1 or transfected CCK-1 receptors, as well as direct stimulation by phorbol esters results in the activation of several PKC isoforms, including PKCε, which is associated with Raf-1. PKCε then stimulates Raf-1, leading to the activation of the ERK cascade. The duration of the ERK signal is different in response to PMA, CCK and NT, which results in differential induction of cell cycle genes in order to either stimulate or inhibit proliferation. The mechanisms by which the ERK signal is modulated in response to different stimuli remain to be determined.

CONCLUSIONS

We conclude, that

1. **Par-C10** and **Capan-1** cells grown to confluence on permeable filter offer a good modell for studying fluid- and electrolyte secretion in epithelial cells.
2. these cells achieve vectorial bicarbonate transport from basolateral to apical side in a regulated manner.
3. in **Capan-1** cells basolateral NBC (presumably pNBC1) and NHE (presumably NHE1) are the primary transporters involved both in pH_i maintenance while Capan-1 cells loose bicarbonate apically.
4. in **Capan-1** cells, the driving force for Cl^- secretion is maintained by the Na^+-K^+-$2Cl^-$ cotransporter (presumably NKCC1) located in the basolateral membrane.
5. in **Capan-1** cells he activity of NBC and NHE is stimulated by both forskolin, secretin and VIP in cAMP-dependent manner, resulting in an increased apical bicarbonate secretion.
6. in **Par-C10** and **Capan-1** cells, activation of basolateral and apical purinoceptors can regulate transepithelial bicarbonate secretion, activating different receptor isoforms and signalling pathways.
7. gastrointestinal peptides regulate not only the exocrine secretion, but the cell proliferation in eithelial cells: in **Panc-1C** cells, phorbol ester-mediated direct activation of PKC leads to a striking decrease in DNA synthesis, while activation of transfected CCK-1 receptors moderately inhibits, and stimulation of natively expressed NT receptors stimulates cell growth.
8. the effects of NT, CCK and PMA on cell proliferation are mediated by PKCε.
9. PMA, CCK and NT all stimulate the ERK cascade, but with strikingly different kinetics in **Panc-1C** cells.
10. ERK cascade is activated by PKCε in **Panc-1C** human pancreatic cancer cells.

ACKNOWLEDGEMENTS

I would like to thank my supervisor from the University, prof. Gábor Varga, Ph.D.

I also thank all my colleagues for their help and advice: Kornélia Barabás, dr. Beáta Burghardt, Irma Demeter, Gabriella Óvári and Vanda Szlávik.

Special thanks to dr. Gábor Rácz, for the possibility of joint publication

Many thanks to the Department of Oral Biology, especially to prof. György Simon for his warm friendship and muchappreciated support, not just in the work but in spirit also.

I would like to thank Martin Steward, Ph.D. for their understanding and critical help.

Special thank to Gábor Szalmay for introducing me to my latter supervisor.

Last, but not least, I thank to my family and to my friends, who created the conditions that enable me to pursue a scientific career.

THE THESIS IS BASED ON THESE ARTICLES:

Szucs A, Demeter I, Burghardt B, Ovari G, Case RM, Steward MC, Varga G.
Vectorial bicarbonate transport by Capan-1 cells: a model for human pancreatic ductal secretion.
Cell Physiol Biochem. 2006;18(4-5):253-64.

Racz GZ, **Szucs A**, Szlavik V, Vag J, Burghardt B, Elliott AC, Varga G.
Possible role of duration of PKC-induced ERK activation in the effects of agonists and phorbol esters on DNA synthesis in Panc-1 cells.
J Cell Biochem. 2006 Aug 15;98(6):1667-80.

Demeter I., **Szűcs Á.,** Óvári G., Varga G.
Capan-1 sejtek HCO_3^- szekréciójában szerepet játszó transzportfolyamatok vizsgálata
Orvostudományi Értesítő, 2005, 78 (1): 56-60;

Szűcs Á, Demeter I, Hegyesi O, Nagy Á, Burghardt B, Steward MC, Varga G
Vectorial bicarbonate transport by Par- C10 cells: a model for human parotid secretion
Prepared for publication