PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(38):5997-6002
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Effect of herpesvirus infection on pancreatic duct cell secretion

Péter Hegyi, Balázs Ördög, Zoltán Rakonczai Jr, Tamás Takács, János Lonovics, Annamária Szabolcs, Réka Sári, András Tóth, Julius G Papp, András Varró, Mária K Kovács, Mike A Gray, Barry E Argent, Zsolt Boldogköi

Péter Hegyi, Zoltán Rakonczai Jr, Tamás Takács, János Lonovics, Annamária Szabolcs, Réka Sári, First Department of Medicine, Faculty of Medicine, University of Szeged, Szeged, Hungary
Péter Hegyi, Zoltán Rakonczai Jr, Mike A Gray, Barry E Argent, School of Cell and Molecular Biosciences, University Medical School, Newcastle upon Tyne NE2 4HH, UK
Balázs Ördög, Mária K Kovács, Zsolt Boldogköi, Department of Biology, Faculty of Medicine, University of Szeged, Szeged, Hungary
András Tóth, Julius G Papp, András Varró, Mária K Kovács, Division of Cardiovascular Pharmacology, Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Hungarian Academy of Sciences, University of Szeged, Szeged, Hungary
Co-first-authors: Péter Hegyi and Balázs Ördög
Supported by a Wellcome Trust IRDA Grant to P.H. (No. 022618), Hungarian Scientific Research Funds to P.H. and J.L. (No. D42188, T43066), a Wellcome Trust Travelling Fellowship to Z.R. (No. 069470), a Bolyai Postdoctoral Fellowship to P.H. (No. 00276/04) and a National Fund for Scientific Research (OTKA) to Z.B. (No. T049171)
Correspondence to: Zsolt Boldogköi, Department of Biology, Faculty of Medicine, University of Szeged, Somogyi Bela str. 4, H-6720 Szeged, Hungary. boldog@sb4.szote.u-szeged.hu
Telephone: +36-62-545595 Fax: +36-62-545131
Received: 2004-12-09 Accepted: 2005-02-18

Abstract

AIM: To examine the effect of acute infection caused by herpesvirus (pseudorabies virus, PRV) on pancreatic ductal secretion.

METHODS: The virulent Ba-DupGreen (BDG) and non-virulent Ka-RREp0lacgfp (KEG) genetically modified strains of PRV were used in this study and both of them contain the gene for green fluorescent protein (GFP). Small intra/interlobular ducts were infected with BDG virus (10^7 PFU/mL for 6 h) or with KEG virus (10^{10} PFU/mL for 6 h), while non-infected ducts were incubated only with the culture media. The ducts were then cultured for a further 18 h. The rate of HCO_3^- secretion [base efflux -$J(B^-)$] was determined from the buffering capacity of the cells and the initial rate of intracellular acidification (1) after sudden blockage of basolateral base loaders with dihydro-4,4,-diisothiocyanatostilbene-2,2,-disulfonic acid (500 μmol/L) and amiloride (200 μmol/L), and (2) after alkali loading the ducts by exposure to NH_4Cl. All the experiments were performed in HCO_3^--buffered Ringer solution at 37 ¡æ(n = 5 ducts for each experimental condition). Viral structural proteins were visualized by immunohistochemistry. Virally-encoded GFP and immunofluorescence signals were recorded by a confocal laser scanning microscope.

RESULTS: The BDG virus infected the majority of accessible cells of the duct as judged by the appearance of GFP and viral antigens in the ductal cells. KEG virus caused a similarly high efficiency of infection. After blockage of basolateral base loaders, BDG infection significantly elevated -$J(B^-)$ 24 h after the infection, compared to the non-infected group. However, KEG infection did not modify -$J(B^-)$. After alkali loading the ducts, -$J(B^-)$ was significantly elevated in the BDG group compared to the control group 24 h after the infection. As we found with the inhibitor stop method, no change was observed in the group KEG compared to the non-infected group.

CONCLUSION: Incubation with the BDG or KEG strains of PRV results in an effective infection of ductal epithelial cells. The BDG strain of PRV, which is able to initiate a lytic viral cycle, stimulates HCO_3^- secretion in guinea pig pancreatic duct by about four- to fivefold, 24 h after the infection. However, the KEG strain of PRV, which can infect, but fails to replicate, has no effect on HCO_3^- secretion. We suggest that this response of pancreatic ducts to virulent PRV infection may represent a defense mechanism against invasive pathogens to avoid pancreatic injury.

Key words: Pancreas; Hypersecretion; Ductal cells; Infection; Pseudorabies virus

Hegyi P, Ördög B, Rakonczai Z Jr, Takács T, Lonovics J, Szabolcs A, Sári R, Tóth A, Papp JG, Varró A, Kovács MK, Gray MA, Argent BE, Boldogköi Z. Effect of herpesvirus infection on pancreatic duct cell secretion. *World J Gastroenterol* 2005; 11(38): 5997-6002
http://www.wjgnet.com/1007-9327/11/5997.asp

INTRODUCTION

Pseudorabies virus (PRV) is an ¦Áherpesvirus closely related to the herpes simplex virus (HSV), a well-known human pathogen[1,2]. The natural host of PRV is the pig, but it has a very wide host range including several mammalian families, such as carnivores, ungulates and rodents, but humans are resistant to PRV infection[1]. PRV infection is a multistep process initiated by the receptor-mediated attachment of the virus to the cell surface. Upon entering the cells, the PRV nucleocapsid approaches the nuclear membrane and the viral DNA is released into the nuclei by a poorly understood mechanism. The lytic cycle of the virus is controlled by a transcriptional cascade mechanism[2].

Acute pancreatitis is most commonly associated with

biliary stone and alcoholism. Other causes, including infections, drugs, lipid abnormalities, congenital anomalies, trauma, tumors, and idiopathic form, account for at least 10-20% of the total number of cases[3]. The incidence of pancreatitis may even be higher since the diagnosis of acute pancreatitis may go undetected in many patients. Several viruses have been reported to cause acute pancreatitis, including coxsackievirus B3[4] and B4[5], mumpsvirus[6], varicella[7], hepatitis A[8] and B[9], cytomegalovirus[10], varicella-zoster virus[11] and HSV[12].

We have previously shown that hypersecretion can be observed during the early phase of experimental edematous and necrotizing pancreatitis[13]. However, the source and the role of this hypersecretion are unknown. Either acinar cells and/or the ductal epithelium could be responsible for hypersecretion.

Our aim in this study was to examine the effect of acute infection caused by PRV on pancreatic ductal secretion.

MATERIALS AND METHODS

Virus strain

Ba-Dup Green (BDG), a genetically modified replicating strain of PRV, was used in this study. The Bartha virus[14], which is an attenuated live vaccine strain of PRV, was used as the parental virus for the generation of BDG. Two copies of a genetically modified green fluorescent protein (GFP) gene-containing expression cassettes were inserted into the PRV antisense promoter region located in the inverted repeat segment of the virus[15,16]. Ka-EP0lacgfp (KEG) strain of PRV was used as a non-replicating control virus for these experiments. KEG was constructed by deleting the small subunit of ribonucleotide reductase[17] and the early protein 0 (EP0)[18] genes. An expression cassette containing a GFP and a lacZ gene was inserted in place of the EP0 gene[19]. These mutations render the virus incapable of replication in non-dividing cells[19].

Isolation and infection of pancreatic ducts

Small intra/interlobular ducts were isolated from the pancreas of guinea pigs weighing 150-250 g. The guinea pig was humanely killed by cervical dislocation, the pancreas was removed and intra/interlobular ducts were isolated by enzymatic dissociation, microdissection and then cultured at 37 ¡æin 50 mL/L CO_2[20]. Ducts were incubated in McCoy's-based culture medium containing BDG viruses at a dose of 10^7 PFU/mL for 6 h or KEG virus (10^{10} PFU/mL for 6 h), while the non-infected ducts were exposed to culture media only. The ducts were then cultured for a further 18 h as described earlier[20], during which time the ducts seal to form a closed sac that swells due to accumulation of ions and water secretions within the duct lumen[20].

Immunohistochemistry

The immunofluorescent detection of structural viral proteins was performed as follows. Isolated pancreatic ducts were fixed in 40 g/L paraformaldehyde in PBS, pH 7.4 for 1 h at room temperature. Following three rinses in PBS, the ducts were blocked with 10 g/L bovine serum albumin and 1 mL/L Triton X-100 in PBS for 1 h at room temperature. Thereafter, a rabbit polyclonal antiviral antibody (Affinity Bioreagents, Golden, USA) diluted at 1:500 (v/v) in blocking solution was applied for 24 h at 4 ¡æ After being washed with PBS, the ducts were incubated with an anti-rabbit antibody conjugated with a red light-emitting dye (Alexa Fluor® 633; Molecular probes, USA) diluted at 1:1 000 (v/v) in a blocking solution for 24 h at 4 ¡æ After further washing in PBS, the ducts were mounted on slides using Aqua-Poly/ Mount (Polysciences Inc., Niles, USA). The specificity of immunohistochemical procedure was assessed by simultaneous staining of non-infected ducts. No immunohistochemical labeling was observed in control experiments.

Microscopy

For monitoring virally expressed GFP and immunofluorescence signals, fluorescent images and optical sections of the infected ducts were recorded by a confocal laser scanning microscope (Zeiss, LSM 400).

Measurement of intracellular pH

Cultured ducts were attached, using Cell-Tak, to a coverslip (24 mmol/L) forming the base of a perfusion chamber mounted on a Nikon Diaphot microscope (Nikon UK Ltd, Budapest, Hungary). The ducts were bathed in the standard Hepes solution at 37 ¡æand loaded with the pH-sensitive fluorescent dye 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) by exposure to 2 μmol/L BCECF-AM for 20-30 min. After loading, the ducts were continuously perfused with solutions at a rate of 4-5 mL/min. Intracellular pH (pH_i) was measured using a microspectrofluorimeter system (Cairn, Kent, UK). A small area of 5-10 cells was excited with light at wavelengths of 490 and 440 nm, and the 490/440 fluorescence emission ratio was measured at 535 nm. Four pH_i measurements were obtained per second. *In situ* calibration of the fluorescence signal was performed using the high K^+-nigericin technique[21,22]. During calibration, the ducts were bathed in high K^+ HEPES solution and extracellular pH stepped between 5.95 and 8.46.

Determination of base efflux

The intrinsic buffering capacity (β_i) of duct cells was estimated according to the NH_4^+ pre-pulse technique[23,24]. β_i refers to the ability of intrinsic cellular components (excluding HCO_3^-/CO_2) to buffer changes of pH_i. Briefly, pancreatic duct cells were exposed to various concentrations of NH_4Cl, while Na^+ and HCO_3^- were omitted from the solution in order to block the Na^+-dependent pH regulatory mechanisms. β_i was estimated by the Henderson-Hasselbach equation. The total buffering capacity (β_{total}) was calculated from: $\beta_{total} = \beta_i+\beta_{HCO3^-} = \beta_i+2.3\times[HCO_3^-]_i$, where β_{HCO3}- is the buffering capacity of the HCO_3^-/CO_2 system and $[HCO_3^-]_i$ is the intracellular HCO_3^- concentration[24].

Measurement of HCO_3^- efflux

Inhibitor stop method Exposing the ducts to dihydro-4, 4,-diisothiocyanatostilbene-2,2,-disulfonic acid (H_2DIDS, 0.5 mmol/L) and amiloride (0.2 mmol/L) for 5 min caused a marked acidification of pH_i (Figure 2A). This acidification occurred due to inhibition of the basolateral Na^+/HCO_3^- co-transporters and Na^+/H^+ exchangers, which normally

act to transport HCO_3^- into the duct cell from the blood[25,26]. The rate of pH_i acidification after the exposure to H_2DIDS and amiloride could reflect the intracellular buffering capacity and the rate at which HCO_3^- effluxes (i.e., secreted) across the apical membrane via Cl^-/HCO_3^- exchangers and CFTR channels[24,25].

The initial rate of intracellular acidification (dpH/dt), over the first 60 s of exposure to amiloride and H_2DIDS, was calculated by linear regression analysis using 240 data points (four pH_i measurements per second)[24].

Alkali load method Exposing the ducts to 20 mol/L NH_4Cl caused an alkalization of pH_i due to the rapid influx of NH_3 into cells (Figure 3A). Recently, we demonstrated that the recovery of pH_i under these conditions was dependent on the presence of HCO_3^- in the bathing solution, suggesting that it results from HCO_3^- efflux (i.e., secretion) out of the duct cells[24]. In the present study, the initial rate of recovery from alkalosis (dpH/dt) over the first 30 s (120 pH_i measurements) in the continued presence of NH_4Cl was calculated as described previously[24].

The rates of pH_i change measured in these inhibitors stopped and alkali load experiments were converted to transmembrane base flux $J(B^-)$ using the equation: $J(B^-)= dpH/dt \times \beta_{total}$. We denoted base influx as $J(B^-)$ and base efflux (secretion) as $-J(B^-)$.

Solutions and chemicals

The standard Hepes-buffered solution contained (mmol/L) 130 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, and 10 Na-Hepes. The high K^+ Hepes-buffered solution contained (mmol/L) 130 KCl, 5 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 D-glucose, and 10 Na-Hepes. Hepes-buffered solutions were gassed with 100% O_2 and their pH was set to 7.4 at 37 ¡æwith HCl. The standard HCO_3^--buffered solution contained (mmol/L) 115 NaCl, 25 $NaHCO_3$, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 D-glucose. The ammonia pulse solution contained (mmol/L) 95 NaCl, 20 NH_4Cl, 25 $NaHCO_3$, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 D-glucose. HCO_3^--buffered solutions were gassed with 95% O_2/50 mL/L CO_2 to set pH to 7.4 at 37 ¡æ

Chromatographically pure collagenase was obtained from Worthington (Lakewood, NJ, USA), culture media from Sigma (Budapest, Hungary). Nigericin (Sigma, Budapest, Hungary) was dissolved in absolute ethanol, H_2DIDS (from Molecular Probes,Eugene, OR, USA) and amiloride in dimethyl sulfoxide (DMSO). CellTak was obtained from Becton Dickinson Labware (Bedford, MA, USA). BCECF-AM was obtained from Molecular Probes (Eugene, OR, USA) and was made up as a 2 mmol/L stock solution using DMSO. The 10-fold concentrated PBS stock solution contained (g/L) 80 NaCl, 2 KCl, 2.4 NaH_2PO_4, 14.4 Na_2HPO_4 (pH 7.2). Chemicals were obtained from Merck (Darmstadt, Germany). Blocking solution contained 10 g/L bovine serum albumin from Sigma (Budapest, Hungary) and 1 mL/L scintillation grade Triton X-100 (BDH Chemicals, Poole, UK).

Statistical analysis

Results were expressed as mean±SE (n = 5 ducts). Statistical analyses were performed using ANOVA. $P<0.05$ was accepted as statistically significant.

RESULTS

Efficacy of viral infection

Two GFP-expressing cassettes were inserted into the PRV genome[15] enabling virus infection to be visualized in both living and fixed cells. Monitoring of GFP expression of BDG was utilized to assess the extent of viral infection of ductal epithelial cells. We observed that BDG virus practically infected all accessible cells of the duct when used at a titer of 10^7 PFU/mL after 24 h (Figure 1A). We used a higher concentration (10^{10} PFU/mL) of KEG virus and found a similarly high efficiency of infection (Figure 1C).

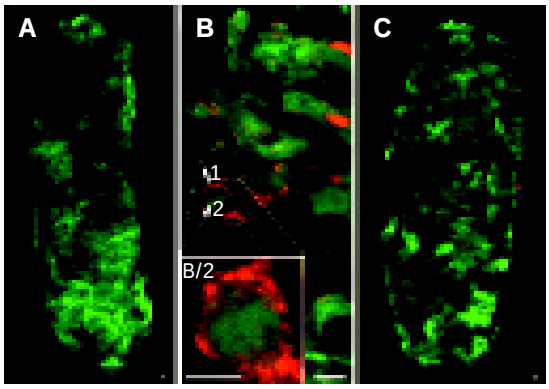

Figure 1 Infection of guinea pig pancreatic ducts with BDG and Ka-EP0lacgfp (KEG). A: Infection of the pancreatic ductal epithelial cells with BDG; B: appearance of viral antigens indicating the productive BDG infection and GFP fluorescence; C: non-virulent KEG strain-produced high efficiency of infection. Scale bars represent 10 μm.

Cytotoxic effect of PRV infection

BDG is a virulent strain; therefore, virus uptake by cells results in the immediate initiation of the lytic viral cycle and the unavoidable death of the infected cells. Productive virus infection was detected in the BDG group by labeling the structural viral proteins by means of immunohistochemistry. The GFP gene was placed under the control of the constitutive human cytomegalovirus immediate early promoter, which conferred a very early expression of the reporter gene. In contrast, viral structural proteins, which are recognized by the anti-PRV antibodies, appeared at a later stage of viral infection[15]; therefore, a shift between the appearance of GFP and immunofluorescence could be observed (Figure 1B). In contrast, in the KEG group, we did not observe any cytopathic effects and could not detect viral antigens. However, GFP expression indicated the presence of the virus within the infected cells (Figure 1C).

Pancreatic ductal bicarbonate secretion

Exposing the ducts to 0.5 mmol/L H_2DIDS and 0.2 mmol/L amiloride caused an acidification of pH_i (Figure 2A), due to inhibition of the basolateral Na^+/HCO_3^- co-transporters and Na^+/H^+ exchangers. The effect of H_2DIDS and amiloride was reversible, removal of the inhibitors caused pH_i to return to the control value. In these series of experiments, we tested whether virus infection affected the net HCO_3^- secretion of pancreatic duct cells. Base efflux was significantly elevated

in the BDG group compared to the non-infected ducts, 24 h after the infection (7.84±1.24 mmol/L B^-/min *vs* 2.2±0.18 mmol/L B^-/min, respectively; $n = 5$). However, no change was observed in the KEG group (2.4±0.32 mmol/L B^-/min; $n = 5$) compared to the non-infected ducts. These data are summarized in Figure 2B.

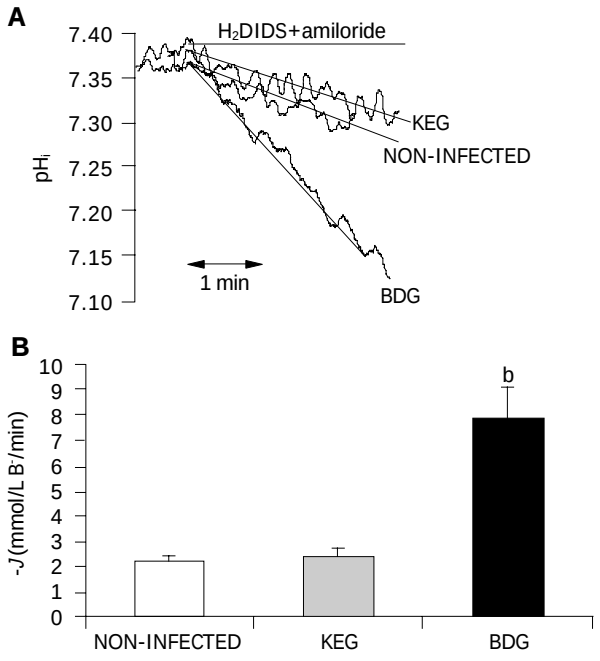

Figure 2 Inhibitor stop method for determining bicarbonate secretion. A: Representative pH_i traces; B: summary of the results obtained using the inhibitory stop method. $^bP<0.001$ *vs* the control (ANOVA).

Exposure of duct cells to 20 mmol/L NH_4Cl induced an immediate rise in pH_i due to the rapid entry of NH_3 into the duct cells (Figure 3A). In this series of experiments, base efflux was significantly elevated in the BDG group compared to the control group 24 h after the infection (160.04±19.16 mmol/L B^-/min *vs* 36.37±1.08 mmol/L B^-/min, respectively; $n = 5$). However, as we found with the inhibitor stop method, no change was observed in the KEG group (39.34±3.49 mmol/L B^-/min, $n = 5$) compared to the non-infected ducts (Figure 3B).

Using the ammonium pulse technique, we also tested whether PRV affected the ability of duct cells to recover from an acid load following removal of NH_4Cl from the superfusate. The transporters most likely to be involved in this process are the Na^+/HCO_3^- co-transporter, the Na^+/H^+ exchanger and the H^+ pump located on the basolateral membrane of the duct cells. No significant change was observed between the PRV infected and non-infected groups (Figure 3A, $n = 5$).

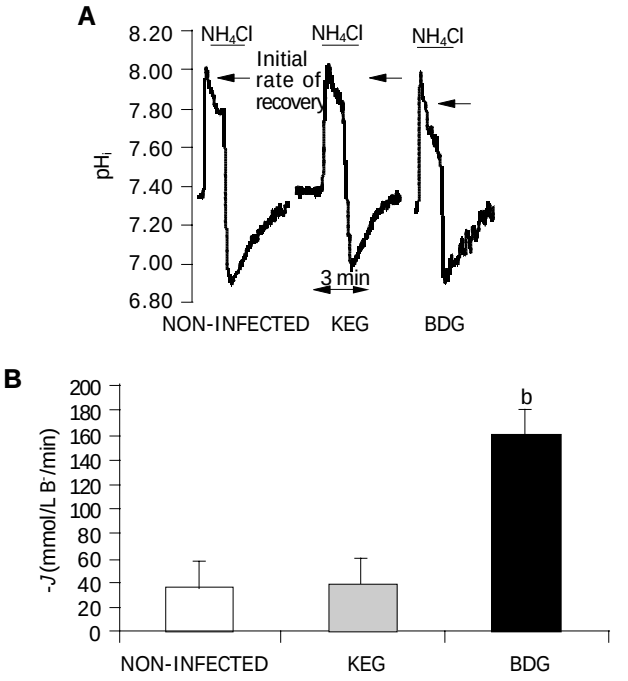

Figure 3 Alkali load method for determining bicarbonate secretion. A: Representative pH_i traces; B: summary of the results obtained using the alkali load method. $^bP<0.001$ *vs* the control (ANOVA).

DISCUSSION

Though the broad spectrum of etiological factors is involved in acute pancreatitis, the pathophysiology of the disease is less understood. Most investigators believe that acute pancreatitis results from an early intra-acinar cell activation of zymogens[26]. Following this early activation, a trypsin cascade occurs in the gland leading to the auto-digestion of acinar cells[26]. However, a possible pathophysiological role of the ductal epithelium has not been investigated. The permeability of the pancreatic ductal epithelium to HCO_3^- and Cl^- is increased by exposure to various bile salts at concentrations within the range normally found in the duodenum[27]. Moreover, *E. coli*-infected bile causes further increases of the permeability of the ductal epithelium to HCO_3^- and Cl^-[27]. Ethanol could induce fluid hypersecretion from guinea pig pancreatic duct cells. Low concentrations of ethanol directly augment pancreatic ductal fluid secretion stimulated by physiological and pharmacological concentrations of secretin (cAMP pathway) and via Ca^{2+} mobilization[28]. The effects of other etiologic factors for acute pancreatitis on pancreatic ductal HCO_3^- secretion have not been characterized as yet.

Viruses can alter ion transport by epithelial cells. Kunzelmann *et al*.[29], reported that parainfluenza virus I (Sendai virus) produces rapid changes in ion transport across tracheal epithelium. The Sendai virus, at concentrations observed during respiratory infections, activates Cl^- secretion and inhibits Na^+ absorption[29] by triggering the release of ATP, which then acts on apical P2Y receptors to produce changes in ion transport[29]. Bacterial infection (e.g., *Pseudomonas aeruginosa* or *Staphylococcus aureus*) triggers mucus and interleukin production[30,31]. Mucus clearance is a primary innate defense mechanism for mammalian airways[32].

In this study, we developed a model to investigate the effect of acute infection with a herpes virus (PRV) on the pancreatic ductal epithelium. Incubating BDG at a dose of 10^7 PFU/mL for 6 h resulted in the infection of the majority

of accessible epithelial cells within the duct. As expected, the virulent PRV strain resulted in a productive infection of epithelial cells, indicated by the appearance of viral antigens. We used two different measures of HCO_3^- secretion (inhibitor stop and alkali load methods) to study the effect of acute PRV infection on pancreatic ducts. Both methods showed that BDG infection stimulated HCO_3^- secretion in guinea pig pancreatic duct by about four or fivefold. However, BDG had no effect on the pH_i recovery after an acid load, suggesting that neither the basolateral Na^+/HCO_3^- co-transporter nor the basolateral Na^+/H^+ exchanger is involved in the hypersecretory effect. We have previously reported that pancreatic hypersecretion is observed during the early phase of acute necrotizing pancreatitis[33]. Furthermore, the early phase hypersecretion is accompanied with a simultaneous decrease in protein output[34], suggesting that the pancreatic ducts are at least in part involved in the change of secretory pattern. In our study, the genetically engineered control virus (KEG) did not evoke hypersecretion in the ductal cells, indicating that the presence of the virus in the cell is not enough to trigger hypersecretion. Hypersecretion was only induced by the BDG virus, which is able to initiate a lytic viral cycle.

Our finding that BDG stimulates HCO_3^- secretion from pancreatic ducts may represent a defense mechanism against invasive pathogens in order to avoid pancreatic injury. We speculate that the stimulated secretion can wash out activated enzymes, viruses and other toxic factors from the pancreas. Moreover, the high efficiency of PRV infection in the pancreatic ductal epithelium may open the possibility for gene transfer and gene therapy of the duct cells using non-replicating or conditionally replicating PRV variants (KEG)[2,19].

ACKNOWLEDGMENTS

We would like to thank Dr. János Szabad for his helpful discussions.

REFERENCES

1 **Boldogkoi Z**, Bratincsak A, Fodor I. Evaluation of pseudorabies virus as a gene transfer vector and an oncolytic agent for human tumor cells. *Anticancer Res* 2002**;** **22**: 2153-2159
2 **Boldogkoi Z**, Nogradi A. Gene and Cancer Therapy - Pseudorabies Virus: A Novel Research and Therapeutic Tool? *Curr Gene Ther* 2003; **3**: 155-182
3 **Steer ML**. Etiology and pathophysiology of acute pancreatitis. In: The Exocrine *Pancreas: Biology, Pathobiology and Diseases*. Edited by Go VLW, Dimagno EP, Gardner JD, Lebenthal E, Reber HA, Scheele GA. *Raven Press New York* 1993: 581-593
4 **Vuorinen T**, Kallajoki M, Hyypia T, Vainionpaa R. Coxsackievirus B3-induced acute pancreatitis: analysis of histopathological and viral parameters in a mouse model. *Br J Exp Pathol* 1989; **70**: 395-403
5 **Vella C**, Brown CL, McCarthy DA. Coxsackievirus B4 infection of the mouse pancreas: acute and persistent infection. *J Gen Virol* 1992; **73**: 1387-1394
6 **Imrie CW**, Ferguson JC, Sommerville RG. Coxsackie and mumpsvirus infection in a prospective study of acute pancreatitis. *Gut* 1977; **18**: 53-56
7 **Kirschner S**, Raufman JP. Varicella pancreatitis complicated by pancreatic pseudocyst and duodenal obstruction. *Dig Dis Sci* 1988; **33**: 1192-1195
8 **Khanna S**, Vij JC. Severe acute pancreatitis due to hepatitis A virus infection in a patient of acute viral hepatitis. *Trop Gastroenterol* 2003; **24**: 25-26
9 **de Oliveira LC**, Rezende PB, Ferreira AL, de Freitas AA, de Carvalho AM, Guedes CA, Costa WO. Concurrent acute hepatitis and pancreatitis associated with hepatitis B virus: case report. *Pancreas* 1988; **16**: 559-561
10 **Wilcox CM**, Forsmark CE, Grendell JH, Darragh TM, Cello JP. Cytomegalovirus-associated acute pancreatic disease in patients with acquired immunodeficiency syndrome. Report of two patients. *Gastroenterology* 1990; **99**: 263-267
11 **Pulik M**, Teillet F, Teillet-Thiebaud F, Lionnet F, Genet P, Petitdidier C. Varicella-zoster virus pancreatitis in hematologic diseases (in French). *Ann Med Interne* 1995; **146**: 292-294
12 **Shintaku M**, Umehara Y, Iwaisako K, Tahara M, Adachi Y. Herpes simplex pancreatitis. *Arch Pathol Lab Med* 2003; **127**: 231-234
13 **Hegyi P**, Czako L, Takacs T, Szilvassy Z, Lonovics J. Pancreatic secretory responses in L-arginine-induced pancreatitis: comparison of diabetic and nondiabetic rats. *Pancreas* 1999; **19**: 167-174
14 **Bartha A**. Experimental reduction of virulence of Aujeszky's disease (in Hungarian). *Magy Allatorv Lapja* 1961; **16**: 42-45
15 **Boldogkoi Z**, Reichart A, Toth IE, Sik A, Erdelyi F, Medveczky I, Llorens-Cortes C, Palkovits M, Lenkei Z. Construction of recombinant pseudorabies viruses optimized for labeling and neurochemical characterization of neural circuitry. *Brain Res Mol Brain Res* 2002; **109**: 105-118
16 **Boldogkoi Z**, Sik A, Denes A, Reichart A, Toldi J, Gerendai I, Kovacs KJ, Palkovits M. Novel tracing paradigms-genetically engineered herpesviruses as tools for mapping functional circuits within the CNS: present status and future prospects. *Prog Neurobiol* 2004; **72**: 417-445
17 **Boldogkoi Z**, Medveczky I, Glavits R, Braun A, Fodor I. *In vivo* studies on Aujeszky's disease virus mutants. *Acta Microbiol Immunol Hung* 1996; **43**: 307-318
18 **Boldogkoi Z**, Braun A, Fodor I. Replication and virulence of early protein 0 and long latency transcript deficient mutants of the Aujeszky's disease (pseudorabies) virus. *Microbes Infect* 2000; **2**: 1321-1328
19 **Boldogkoi Z**, Szabo A, Vrbova G, Nogradi A. Pseudorabies virus-based gene delivery to rat embryonic spinal cord grafts. *Hum Gene Ther* 2002; **13**: 719-729
20 **Argent BE**, Arkle S, Cullen MJ, Green R. Morphological, biochemical and secretory studies on rat pancreatic ducts maintained in tissue culture. *Q J Exp Physiol* 1986; **71**: 633-648
21 **Thomas JA**, Buchsbaum RN, Zimniak A, Racker E. Intracellular pH-measurements in Ehrlich ascites tumor cells utilizing spectroscopic probes generated *in situ*. *Biochemistry* 1979; **18**: 2210-2218
22 **Hegyi P**, Rakonczay Z Jr, Gray MA, Argent BE. Measurement of intracellular pH in pancreatic duct cells: A new method for calibrating the fluorescence data. *Pancreas* 2004; **28**: 427-434
23 **Weintraub WH**, Machen TE. pH regulation in hepatoma cells: roles for Na-H exchange, Cl-HCO_3 exchange, and Na-HCO_3 cotransport. *Am J Physiol* 1989; **257**: G317-G327
24 **Hegyi P**, Gray MA, Argent BE. Substance P inhibits bicarbonate secretion from guinea-pig pancreatic ducts by modulating an anion exchanger. *Am J Physiol* 2003; **285**: C268-C276
25 **Szalmay G**, Varga G, Kajiyama F, Yang XS, Lang TF, Case RM, Steward MC. Bicarbonate and fluid secretion by cholecystokinin, bombesin and acetylcholine in isolated guinea-pig pancreatic ducts. *J Physiol* 2001; **535**: 795-807
26 **Lerch MM**, Albrecht E, Ruthenburger M, Mayerle J, Halangk W, Kruger B. Pathophysiology of alcohol-induced pancreatitis. *Pancreas* 2003; **27**: 291-296
27 **Reber HA**, Mosley JG. The effect of bile salts on the pancreatic

duct mucosal barrier. *Br J Surg* 1980; **67**: 59-62

28 **Yamamoto A**, Ishiguro H, Ko SB, Suzuki A, Wang Y, Hamada H, Mizuno N, Kitagawa M, Hayakawa T, Naruse S. Ethanol induces fluid hypersecretion from guinea-pig pancreatic duct cells. *J Physiol* 2003; **551**: 917-926

29 **Kunzelmann K**, Konig J, Sun J, Markovich D, King NJ, Karupiah G, Young JA, Cook DI. Acute effects of parainfluenza virus on epithelial electrolyte transport. *J Biol Chem* 2004; **279**: 48760-48766

30 **McNamara N**, Khong A, McKemy D, Caterina M, Boyer J, Julius D, Basbaum C. ATP transduces signals from ASGM1, a glycolipid that functions as a bacterial receptor. *Proc Natl Acad Sci USA* 2001; **98**: 9086-9091

31 **Ratner AJ**, Bryan R, Weber A, Nguyen S, Barnes D, Pitt A, Gelber S, Cheung A, Prince A. Cystic fibrosis pathogens activate Ca2+-dependent mitogen-activated protein kinase signaling pathways in airway epithelial cells. *J Biol Chem* 2001; **276**: 19267-19275

32 **Knowles MR**, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest* 2002; **109**: 571-577

33 **Hegyi P**, Rakonczay Z Jr, Sari R, Gog C, Lonovics J, Takacs T, Czako L. L-arginine induced experimental pancreatitis. *World J Gastroenterol* 2004; **10**: 2003-2009

34 **Czako L**, Yamamoto M, Otsuki M. Exocrine pancreatic function in rats after acute pancreatitis. *Pancreas* 1997; **15**: 83-90

Science Editor Wang XL and Guo SY **Language Editor** Elsevier HK