Oxidative phosphorylation and its coupling to mitochondrial creatine and adenylate kinases in human gastric mucosa

Marju Gruno, Nadezhda Peet, Evelin Seppet, Lumme Kadaja, Kalju Paju, Margus Eimre, Ehte Orlova, Margot Peetsalu, Andres Tein, Jaan Sopleppmann, Uwe Schllattner, Ants Peetsalu, and Enn K. Seppet

1Department of Pathophysiology, Centre of Molecular and Clinical Medicine, Faculty of Medicine, Department of Surgery, University of Tartu, Tartu, Estonia; 2Institute of Cell Biology, ETH Zürich, Zürich, Switzerland

Submitted 8 March 2006; accepted in final form 17 May 2006

Am J Physiol Regul Integr Comp Physiol 291: R936–R946, 2006. First published June 1, 2006; doi:10.1152/ajpregu.00162.2006.—Energy metabolism in gastrobiopsy specimens of the antral and corpus mucosa, treated with saponin to permeabilize the cells, was studied in patients with gastric diseases. The results show twice lower oxidative capacity in the antral mucosa than in the corpus mucosa and the relative deficiency of antral mitochondria in complex I. The mucosal cells expressed mitochondrial and cytosolic isoforms of creatine kinase and adenylate kinase (AK). Creatine (20 mM) and AMP (2 mM) markedly stimulated mitochondrial respiration in the presence of submaximal ADP or ATP concentrations, and creatine reduced apparent Km for ADP in stimulation of respiration, which indicates the functional coupling of mitochondrial kinases to oxidative phosphorylation. Addition of exogenous cytochrome c increased ADP-dependent respiration, and the large-scale cytochrome c effect (~20%) was associated with suppressed stimulation of respiration by creatine and AMP in the mucosal preparations. These results point to the impaired mitochondrial outer membrane, probably attributed to the pathogenic effects of Helicobacter pylori. Compared with the corpus mucosa, the antral mucosa exhibited greater sensitivity to such type of injury as the prevalence of the large-scale cytochrome c effect was twice higher among the latter specimens. Active chronic gastritis was associated with decreased respiratory capacity of the corpus mucosa but with its increase in the antral mucosa. In conclusion, human gastric mucosal cells express the mitochondrial and cytosolic isoforms of CK and AK participating in intracellular energy transfer systems. Gastric mucosa disease is associated with the altered functions of these systems and oxidative phosphorylation.

human stomach mucosa; corpus; antrum

CHRONIC ACTIVE GASTRITIS, glandular atrophy, gastric ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer are the most frequent disorders of the gastric mucosa. Although they are clinically different, these entities are interrelated via having a common etiological factor, Helicobacter pylori (30, 37, 68). This bacterium induces inflammation of the mucosa by a number of factors, such as the vacuolating cytotoxin gene A (vacA), genes localized in a cag pathogenicity island, and lipopolysaccharide (39). Excess production of reactive oxygen species (ROS) exerting cytotoxic effects on gastric epithelial cells via lipid peroxidation, membrane damage, and reduction of cellular glutathione (GSH) content (10, 26, 39, 49, 65) is a major component of the inflammatory response of the tissue.

There exists evidence that mitochondria may play an important role in development of gastric inflammation not only as being a source and target of the ROS (43) but also as the organelles specifically attacked by H. pylori. In cultured gastric cell lines the vacA cytotoxin of H. pylori directly impairs mitochondrial function seen as decreased rate of mitochondrial respiration, diminished membrane potential and ATP production (29), mitochondrial fragmentation (5), release of cytochrome c (34), and activation of apoptosis (16). Mitochondrial contribution may vary between the corpus and antral mucosa, because the former contains more mitochondria than the latter (35, 50), and H. pylori-linked inflammation is associated with higher activation of SOD because of upregulation of its mitochondrial isoform in the antrum but not in the corpus (8, 14, 18, 19). Yet it is unclear whether these diversities affect regulation and intracellular organization of energy metabolism in the mucosa. In many cells with high-energy requirements (skeletal muscle, cardiac muscle, brain, retina, and spermatozoa), mitochondria and ATPases are linked to each other by specialized phosphotransfer systems mediated by different creatine kinase (CK) and adenylate kinase (AK) isoforms. These systems carry out three consequent processes: 1) functional coupling between mitochondrial CK (MtCK) or AK2 isoforms with adenine nucleotide translocase (ANT); 2) transfer of the energy-rich phosphoryl group via cytosolic muscle-type (MM)-, hetero- meric (MB)- or brain-type (BB)-CK (depending on the type of tissue) or AK1 isoforms; and 3) regeneration of ATP in the vicinity of ATPases because of the functional coupling of MM- or BB-CK or AK1 isoforms to ATPases (13, 47). Such an organization of intracellular energy metabolism enables precise matching between mitochondrial ATP synthesis and its consumption (13, 46, 47). Marked CK and AK activities have been detected in cultured gastric mucosal cells (21, 44, 55). In parietal cells BB-CK is strongly expressed (55, 63) and colocalizes and functionally couples to H+-K+-ATPase to effectively provide ATP for proton pumping (55). On the other hand, exchange between adenine nucleotides mediated by AK is markedly activated in the conditions of generation of ATP by

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Address for reprint requests and other correspondence: E. K. Seppet, Dept. of Pathophysiology, Faculty of Medicine, Univ. of Tartu, 19 Ravila St., 50411 Tartu, Estonia (e-mail: enn.seppet@ut.ee).
METHODS

Subjects and Gastric Mucosa Sampling

Gastric biopsies were carried out in accordance with the European Communities Council Directive 86/609/EEC and with the Declaration of Helsinki (64). Written informed consent was obtained from all patients, and the Tartu University Ethics Committee approved the study.

Forty consecutive patients (23 male and 17 female, aged 60 ± 2.3 and 64 ± 3.0 years, respectively) from southern Estonia who underwent upper gastrointestinal endoscopy due to epigastric complaints were included in this study. At endoscopy, different lesions were detected, such as esophagitis, hiatus hernia, gastric mucosal erosions, gastric or duodenal ulcer, duodenitis, and gastric cancer. Most of the patients (71%) were histologically H. pylori positive, which corresponds to the serological finding that 80% of the inhabitants of Estonia are carriers of that bacterium. Its virulence is highly expressed as 87% of patients with the different gastric diseases presented the cagA gene that correlated strongly with the vacA signal sequence type s1a (3). None of the subjects had received nonsteroidal anti-inflammatory drugs, H1-pump inhibitors, or antibiotics to cure the illness. Mucosal biopsies were taken from the antrum (2 cm above the pylorus from the anterior and posterior walls of the stomach) and from the anterior wall and posterior wall of the medial part of the corpus. In the patients with gastric cancer, the specimens were taken from the apparently normal mucosal region apart from the tumor tissue. One part of each biopsy specimen was used to determine the histology of the gastric mucosa and the presence of H. pylori, for which these specimens were fixed overnight in neutral buffered formalin and embedded in paraffin. Tissue sections were stained for morphological and H. pylori examination by the hematoxylin and eosin and modified Giemsa methods. The presence and severity of chronic gastritis, activity of gastritis, atrophy, and intestinal metaplasia were graded according to the Sydney system from 0 (no changes) through 1 (mild) and 2 (moderate) to 3 (severe changes) (12). Infiltration of lymphocytes was taken to indicate the chronic status of inflammation, and abundant presence of mononuclear cells was taken to mark an active chronic process. The amount of H. pylori in the mucosa was estimated semiquantitatively by microscopic counting as described earlier (42). Another part of the antral and corpus mucosa specimens was transferred into the RNA Later reagent (Qiagen) for RNA extraction, and the third part was placed in the ice-cold solution A containing (in mM): 2.77 CaK2EGTA, 7.23 K2EGTA, 6.56 MgCl2, 0.5 DTT, 50 K-Mes, 20 imidazole, 20 taurine, 5.3 Na2ATP, 15 phosphocreatine, 7.1 pH, and was used for permeabilization of the cells. Because of the limited sample availability, the number of specimens studied in different experiments varied as shown in the figure legends.

Preparation of Permeabilized Mucosal Tissue

The mucosal tissue samples were cut into 1 × 1.5-mm pieces in the ice-cold solution A. With the use of thin needles, the tissue pieces were gently stretched in all directions to mechanically separate the cells from each other. The tissue was then transferred into vessels with ice-cold solution A containing 50 µg/ml saponin and incubated at mild stirring for 30 min for permeabilization of the plasmalemma due to removal of cholesterol from the cell membrane by saponin. The indicated conditions were found to be optimal for maintaining mitochondrial function inside the permeabilized cells. The permeabilized mucosal tissues were then washed three times in solution B containing (in mM): 2.77 CaK2EGTA, 7.23 K2EGTA, 1.38 MgCl2, 0.5 DTT, 100 K-Mes, 20 imidazole, 20 taurine, 3 K2HPO4, 10 glutamate and 2 malate, and 5 mg/ml bovine serum albumin, pH 7.1 at 25°C to remove all metabolites. The extent of permeabilization was monitored by the leakage of lactate dehydrogenase (LDH), a 145-kDa cytosolic protein. The cells lost about 60-70% of LDH during saponin treatment, which corresponds to the data obtained from the study of digoxin-permeabilized rabbit gastric glands (1).

Analysis of Oxidative Phosphorylation and Its Coupling to MtCK

The saponin-treated tissues were incubated in solution B containing (nM): 2.77 CaK2EGTA, 7.23 K2EGTA, 1.38 MgCl2, 0.5 DTT, 100 K-Mes, 20 imidazole, 20 taurine, 3 K2HPO4, 10 glutamate, and 2 malate, and 5 mg/ml bovine serum albumin, pH 7.1 at 25°C in a chamber (volume 1.5-2.5 ml) of oxygraph (Rank Brothers, England) or Oroboros, Paar KG, Austria), equipped with the Clark electrode, assuming the solubility of oxygen in the medium to be 215 mM O2/ml (32). After the registration of basal respiration rate (Vo) in nonphosphorylating conditions in the presence of 10 mM glutamate and 2 mM malate, 2 mM ADP was added to monitor the maximum rate of NADH-linked ADP-dependent respiration (VGlut), followed by successive additions of 10 µM rotenone to inhibit the complex I, 10 mM succinate to activate FADH2-linked ADP-dependent respiration (VSucc), 0.1 mM atracyloside to assess respiratory control by ANT, 10 µM antimycin A to inhibit the electron flow from the complex II to cytochrome c, 0.5 mM N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) with 2 mM ascorbate to activate cytochrome c oxidase (COX), and 5 mM Na3V to quantify COX activity (VCox) as the NaF-sensitive portion of respiration. Antimycin-sensitive respiration in the presence of atracyloside was considered to measure the respiration related to proton leak. The coupling between oxidative phosphorylation and MtCK was estimated using two protocols.

Protocol 1. After the registration of Vo in nonphosphorylating mitochondria in solution B with glutamate plus malate, 50 µM ATP was added to produce a minimum amount of endogenous ADP to stimulate mitochondria. AMP (2 mM) was then added to activate the coupled reaction of AK2 with ANT followed by 0.2 mM diadenosine pentaphosphate (AP5-A) to inhibit AK (52). To assess the strength of the functional coupling independently of mitochondrial content in individual mucosal preparations, activation of respiration by AMP was normalized for the respiratory rate registered before the addition of AMP, thus producing the relative index (IAK = ...
(V_{AMP} - V_{ATP})/V_{ATP}). After AP5-A, 20 mM creatine (Cr) was added from solid to induce the coupling between MtCK and ANT, the efficiency of which was measured as CK index (ICK): \[ ICK = \frac{V_{CK}}{V_{AMP}} \]. Thereafter, 2 mM ADP was added for maximum activation of respiration (V_{AMP}) and 0.1 mM actrycloside was added to monitor control by ANT over oxidative phosphorylation. The maximum capacity of the respiratory chain was estimated in the presence of 2 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) (V_{OCCP}). In the same protocol, the intactness of mitochondrial outer membrane (MOM) was controlled by the addition of 8 μM cyt cromochrome c (48, 52).

**Protocol 2.** The \[ V_{O2} \] vs. [ADP] relationships were examined in solution B supplemented with 10 mM glutamate, 2 mM malate, 4 U/ml hexokinase, and 11 mM glucose in the presence and absence of supplemented with 10 mM glutamate, 2 mM malate, 4 U/ml hexokinase, and 11 mM glucose in the presence and absence of

Determination of the Activity of Total ATPase, AK, and CK in Permeabilized Gastric Mucosa

The permeabilized mucosal specimens were incubated in a spectrophotometric cuvette in stirring conditions in the medium containing (in mM): 20 Tris-HCl (pH 8.0, 30°C), 15 KCl, 0.3 DTT, 0.24 NADH, 0.8 phosphoeneolpyruvate (PEP), and 6 IU/ml pyruvate kinase (PK) and 3 IU/ml LDH. To register total ATPase activity, 1 mM of ATP together with 0.8 mM MgCl2 was added, and the decrease in NADH concentration was measured at 340 nm by use of PerkinElmer Lambda 900 spectrophotometer, AMP (1.3 mM) was then added to determine AK activity followed by the addition of 20 mM creatine to measure CK activity. Care was taken that the measured reactions occurred in time and that the coupled enzyme systems were not limiting overall reaction rate.

**Determination of the LDH Activity**

Tissue samples were frozen in liquid nitrogen and homogenized in 300 μl of phosphate buffer (50 mM, pH 7.3) by Ultra-Turrax homogenizer (Janke and Kunkel, IKA Labortechnik, Germany) at a speed of 13,500 rpm/min for 30 s at +4°C. Then Triton X-100 was added (1% final concentration), and the homogenate was incubated for 1 h on ice. The LDH activity was measured spectrophotometrically at 340 nm in the medium containing aliquot, 0.265 mM NADH, and 50 mM phosphate buffer (pH 7.3 at 25°C). Reaction was initiated with 3 mM sodium pyruvate.

**RNA Preparation**

Total RNA from the mucosal biopsies was prepared at room temperature using the Qiagen RNeasy Protect Mini Kit, according to the manufacturer’s protocol. Approximately 25 mg of mucosal tissue from the antrum and corpus of the same patient was immediately homogenized in lysis buffer (supplied in the kit) by an Ultra-Turrax homogenizer (Janke and Kunkel, IKA Labortechnik) at a speed of 8,000 rpm/min for 30 s. Ethanol (70%) was then added to lyse, and the samples were applied to the RNeasy column (Qiagen). The contaminants (e.g., lysis buffer components) were removed by one wash spin at 8,000 g with the buffer RW1 and by two wash spins at 8,000 g with the washing buffer RPE (both supplied in the kit). Finally, membrane-bound total RNA was eluted in 30 μl RNase-free water. RNA concentration in elute was determined by measuring the absorbance in 10 mM Tris-HCl, pH 7.5 at 260 nm. The RNA isolated appeared to be pure since its 260/280 absorbance ratio varied between 1.9 and 2.0. The integrity of RNA was examined by electrophoresis in an ethidiun bromide stained 8% agarose gel (38), which revealed two characteristic ribosomal 28S and 18S bands, without any evidence of RNA degradation (Fig. 2).

**Reverse Transcription**

One microgram of total RNA was used for reverse transcription into single-strand cDNA using SuperScript II reverse transcriptase (Invitrogen). One microliter oligo(dT) at a concentration of 500 μg/ml and 1 μl 10 mM dNTP mix was added to the aforementioned amount of RNA and heated to 65°C for 5 min; thereafter, the mix was quickly chilled on ice. Final reaction volume contained (in mM) 0.5 dNTP, 10 DTT, 50 Tris-HCl (pH 8.3), 75 KCl, and 3 MgCl2, and 2 U/μl RNase inhibitor (Fermentas). Reverse transcription was performed at 42°C for 50 min. After that the reaction was inactivated by heating at 70°C for 15 min. The obtained transcripts were used for polymerase chain reaction (PCR).

**PCR Reactions**

The forward and reverse oligonucleotide primer pairs (Table 1), to match the sequences of human AK1, AK2, brain-type CK (B-CK), and uMtCK, were designed by the Primer Express software (Applied Biosystems). PCR amplification was performed using the QuantiTect SYBR Green PCR Kit (Qiagen) with the ABI PRISM 7000 Sequence Detection Systems (Applied Biosystems). The thermal profile for RT-PCR comprised initially 15 min at 94°C to activate HotStarTag DNA. This was followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30-s steps. After the 35th cycle, the amplified cDNAs were separated in a 1.7% agarose gel to verify amplicons by size.

**SDS-PAGE and Immunoblotting**

Thirty-five micrograms of total protein in homogenates was separated by standard 12% SDS-PAGE and electrotransferred by semidy blotting (Hoefel Pharmacia Biotech, San Francisco, CA) on a nitrocellulose membrane (Shleicher and Schuell, Dassel, Germany), according to the manufacturer’s instructions. The membranes were blocked with 4% fat-free milk powder in T-TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) overnight at 4°C, incubated for 15 min at room temperature and washed for 4×5 min with T-TBS. The membranes were then incubated for 1 h with uMtCK rabbit immunesera (1:2,000 dilution in a blocking buffer) or with affinity-purified chicken anti-B-CK IgY (1:500 dilution in a blocking buffer) at room temperature (51). For detecting AKs, the membranes were incubated for 1 h at room temperature with rabbit polyclonal antibodies against AK1 (H-90; Santa Cruz Biotechnology, Santa Cruz, CA) or AK2 (H-65; Santa Cruz Biotechnology) (dilution 1:500 in a blocking buffer), washed for 4×5 min in T-TBS and incubated for 1 h with the peroxidase-coupled secondary antibody, either goat anti-rabbit IgG (Nordic, Lausanne, Switzerland) (1:1,000 dilution in a blocking buffer) or rabbit anti-chicken IgY (Jackson ImmunoResearch, West Grove, PA) (1:3,000 dilution in a blocking buffer), and finally washed for 4×5 min with T-TBS. The blots were developed

**Table 1. Characteristics of the oligonucleotide primers used in PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Orientation</th>
<th>Primer Sequence</th>
<th>Base Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AK1</td>
<td>Forward</td>
<td>5′-AAGGTTTGACGCACGATTG-3′</td>
<td>82</td>
</tr>
<tr>
<td>AK2</td>
<td>Reverse</td>
<td>5′-GTCTTGACTGTTGCTGACAC-3′</td>
<td>82</td>
</tr>
<tr>
<td>B-CK</td>
<td>Reverse</td>
<td>5′-CCCAGTTGCTGAAAATCTTCT-3′</td>
<td>82</td>
</tr>
<tr>
<td>uMtCK</td>
<td>Forward</td>
<td>5′-CGGTATCTGGACAATGACAA-3′</td>
<td>99</td>
</tr>
<tr>
<td>uMtCK</td>
<td>Reverse</td>
<td>5′-GGTATCTGGACAATGACAA-3′</td>
<td>99</td>
</tr>
</tbody>
</table>

The length of amplicons is expressed as base pairs. AK, adenylate kinases; B-CK, brain-type creatine kinase; uMtCK, ubiquitous mitochondrial creatine kinase.

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with the enhanced chemiluminescence substrate (Amersham, Buckinghamshire, UK) and exposed to an X-ray film.

Statistical Analysis

The means ± SE are presented. Differences between the groups were evaluated using Student’s t-test.

RESULTS

Function of Oxidative Phosphorylation in Gastric Mucosa

Table 2 summarizes the analysis of the function of the respiratory chain in the permeabilized mucosal specimens from the antrum and corpus. For the antrum mucosa, the mean values of all respiratory rate indices (V0, VGlut, VSucc, VAir, VCOX, and VFCCP) were lower than for their corpus counterparts (by a factor of 1.6, 2.3, 1.7, 2.1, 2.1, and 1.8, respectively), which refers to less tissue content of mitochondria in antral mucosa. When the ratios of respiration rates with different substrates, independent of individual variation of mitochondrial content, were compared in the same mucosal specimen, different functional properties of mitochondria in antrum and corpus became evident. In corpus, the VGlut-to-VSucc ratio was 1.38, comparable to that in other tissues, such as muscle or heart in the healthy state (17, 52), and the VGlut-to-VCOX ratio exceeded the VSucc-to-VCOX ratio. Thus complex I-dependent respiration was higher than complex II-dependent respiration, which implies limitation of the electron flow at the level of complex II. Compared with the VGlut-to-VCOX ratio in the corpus, this parameter was reduced in the antral mucosa. Presumably, this change resulted from a combination of decreased complex I-dependent respiration and increased complex II-dependent respiration, as suggested by the tendencies to reciprocal alterations in VGlut-to-VCOX and VSucc-to-VCOX ratios. Compared with the corpus mucosa, the antral mucosa exhibited lower respiration control index (RCI)Glut but higher RCI succ, this difference being attributable to a larger deficit in VGlut than in VSucc, to a smaller deficit in V0 than in VAir, and to a smaller proton leak.

Function of Energy Transfer Systems

The activities of total tissue ATPase and CK in the corpus mucosa exceeded those in the antrum mucosa, while no difference between these tissues was observed in total AK activity (Fig. 1). Figure 2 shows that the antral and corpus mucosa expressed mRNA encoding mitochondrial (AK2 and ubiquitons CKMT1) and cytosolic (AK1 and CKB) isoforms of AK and CK, respectively. The expression of KCM originating from the microvessels and the muscularis mucosae layer was negligible (not shown), which suggests that the expression signals for AK and CK emerged chiefly from the epithelial cells. A Western blot analysis of the tissue homogenates confirmed the presence of the B-CK, uMtCK, AK1, and mitochondrial AK2 proteins (Fig. 3). The presence of mitochondrial and cytosolic isoforms of CK and AK points to the existence of intracellular energy transfer systems linking mitochondria to ATPase. The presence of the functional coupling between mitochondrial kinases and ANT, a key stage in these systems, was tested by means of two previously validated protocols (52). In one of them (Fig. 4) the permeabilized mucosal cells were incubated with 50 μM ATP, which led to a minimum stimulation of respiration because of the limited amounts of ADP that the ATPases could produce. Addition of 2 mM AMP strongly stimulated respiration as it triggered the functional coupling of mitochondrial AK2 to ANT, thereby increasing the local [ADP] near ANT. Participation of AK in this process was confirmed by the effect of the AK inhibitor AP5A: suppression of respiration down to the levels before AMP addition. Inclusion of 20 mM creatine reactivated respiration, because now...
MtCK, coupled to ANT, provided a local activator, ADP. Stimulation of respiration with AMP was stronger than stimulation with creatine because each cycle of AK2 reaction coupled to ANT produced two times more ADP compared with MtCK reaction (52). The IAK, an index independent of the tissue mitochondrial content, was smaller for the antrum (0.517 ± 0.038, P < 0.05, n = 27) than for the corpus (0.651 ± 0.050, n = 27). This change may indicate a less tight coupling between AK2 and ANT and/or less active AK2 in the antrum mucosa. Regarding ICK, a similar tendency was observed, although without statistical significance (0.372 ± 0.056, n = 26, and 0.504 ± 0.090, n = 26, in the antrum and corpus, respectively).

In another set of experiments the interaction between MtCK and ANT was estimated from the effects of creatine on the V˙O₂ vs. [ADP] relationships. Linearization of these dependencies in the double-reciprocal coordinates (Fig. 5A) provides the apparent Km values for ADP to be 107.7 ± 6.6 µM and 93.5 ± 11.2 µM (Fig. 5B) in the antral and corpus mucosa, respectively. In the presence of creatine, the apparent Km decreased markedly (Fig. 5B). Like the results described above (Fig. 4), this finding confirms an effective functional coupling of oxidative phosphorylation to MtCK because the latter provides the activator ADP for mitochondria and thus reduces apparent Km for exogenous nucleotide. Figure 5 also demonstrates that creatine exerted no effect on Vmax because with increasing exogenous [ADP] a direct ADP flux into the intermembrane space through MOM becomes sufficient to maximally stimulate respiration independently of MtCK. The mean creatine index calculated from the data shown in Fig. 5 tended to be lower for the antrum compared with the corpus but, similar to changes in ICK, this difference was statistically insignificant. Concerning the function of mitochondrial AK2, it would have been desirable to...
analyze the effects of AMP on [ADP] vs. $V_{\text{O}_2}$ relationships. However, registration of these relationships is impossible in the presence of AMP because stationary ADP concentrations cannot then be established (52). Therefore, the coupling of AK2 to ANT was estimated on the basis of IAK only.

Monitoring the effect of excess cytochrome $c$ added to the uncoupled mitochondria (Fig. 4) enabled an assessment of the intactness of MOM. In the case of impaired (leaky) MOM, the mitochondria would have lost cytochrome $c$, and its replenishment would have increased the respiration rate (48). In fact, practically all gastrobiopsy specimens exhibited a smaller or larger stimulation of respiration by cytochrome $c$, i.e., leakiness of MOM, and for a whole group of specimens studied ($n = 54$ from 27 patients) the mean cytochrome $c$ effect was 17 ± 2%, with minimum and maximum individual value of 0 and 42%, respectively. Increased permeability of the MOM may be associated with impaired function of mitochondrial kinases, as is shown for cardiac cells (27, 28, 47, 48). To test this association, the gastric specimens were split into two groups: one group exhibiting <20% of cytochrome $c$ effects (here the cutoff level was set arbitrarily at 19%, a sum of mean ± SE values for 54 specimens) and the another group with ≥20% effects; and I$_{\text{CK}}$ and I$_{\text{AK}}$, measured as shown in Fig. 4, were compared for these groups (Fig. 6). The specimens with a larger cytochrome $c$ effect (≥20%, i.e., with a larger defect in MOM) were characterized by smaller values of I$_{\text{CK}}$ and I$_{\text{AK}}$ than those showing less sensitivity to cytochrome $c$ (<20%). Thus disintegration of the MOM was indeed combined with the suppressed coupling of mitochondrial kinases to oxidative phosphorylation. It was also found that the prevalence of the cytochrome $c$ effect >20% was twice larger for the preparations of the antral mucosa (14 of 27 patients, 52%) than for the preparations of the corpus mucosa (7 of 27 patients, 26%).

The data obtained within the protocol shown in Fig. 4 were also analyzed in relation to the activity of $H.\text{pylori}$-induced inflammation among the patients participating in this set of experiments. We found that the parameters of oxidative phosphorylation for the mucosal preparations exhibiting signs of chronic gastritis (lymphocyte infiltration in the presence of $H.\text{pylori}$) did not differ from the corresponding parameters for the preparations without chronic inflammation (not shown). Therefore, the corresponding data were pooled to create a group assigned as control. The rest of the specimens formed a group with active chronic gastritis as confirmed on the basis of the prominent mononuclear infiltration. Compared with control, this group was characterized by significant decreases in the basal, ADP-stimulated, and FCCP-stimulated respiration rates in the corpus mucosa (Fig. 7) but not in the antrum mucosa, where opposite changes took place. Regarding the other parameters of oxidative phosphorylation (e.g., RCI), its coupling to kinases (I$_{\text{AK}}$, I$_{\text{CK}}$), and the cytochrome $c$ effects, no changes related to active inflammation were observed, probably because the low number of specimens available.

![Fig. 6](image_url)  
**Fig. 6.** Relationships between the extent of cytochrome $c$ effect on ADP-stimulated respiration (%) and coupling of mitochondrial kinases expressed as CK index (I$_{\text{CK}}$; A) and AK index (I$_{\text{AK}}$; B) in permeabilized gastric mucosa. Open bars, $n = 33$, A and B; filled bars, $n = 19$ (A) and $n = 21$ (B). *P < 0.05 vs. preparations with the cytochrome $c$ effect <20%.

![Fig. 7](image_url)  
**Fig. 7.** The effect of active chronic gastritis (filled bars, $n = 10$ and 9 for antrum and corpus group, respectively) on respiratory parameters compared with absence of active process (open bars, $n = 17$ and 18 for antrum and corpus group, respectively) in permeabilized mucosa. *P < 0.05, **P < 0.01 vs. control without active process. $V_0$, basal respiration rate.
Different Oxidative Phosphorylation in the Corpus and Antral Mucosa

Mucosal oxidative capacity was far higher in the corpus than in the antrum, in accordance with the larger content of mitochondria in the corpus mucosa (35, 50). The prominent oxidative capacity of the corpus mucosa relates to massive acid generation by parietal cells for which the mitochondria account for 25-45% of cell volume (24, 67). The tissue-specific differences were related not only to the amount of mitochondria but also to their functional properties, as indicated by decreased VGlut-to-VSucc ratio, RCIGlut, and proton leak, but also by increased RCISuc in the antrum compared with corpus. Because a reduced VGlut-to-VSucc ratio is considered to be a reliable index of respiratory chain deficiency at the level of the complex I as observed in gene-modified animals and in human muscle disease (17, 45, 62), the low VGlut-to-VSucc ratio can be taken to indicate the impaired complex I function resulting from the pathogenic effects of H. pylori. However, the diminished complex I activity is also seen in the mitochondria of healthy rabbit antral mucosa, as indicated by a twice lower RCI, because of reduced state 3 respiration with glutamate compared with the corpus mitochondria (35). Besides, the normal antral mucosa contains more GHS than its corpus counterpart, which renders the former less susceptible to free radical damage during ischemia-reperfusion (58). Thus it could be speculated that the relative complex I deficiency is a normal property of the antral mitochondria, in which the increased antioxidative capacity serves to neutralize the excess ROS generated during hampered electron flow through the inadequate complex I, similarly to the processes taking place in many types of cells in response to the failure of complex I (43).

Having considered these mechanisms, the role of variations in the complex II function as a reason for altered VGlut-to-VSucc ratio should not be overlooked. For example, fumarase deficiency is associated with suppressed succinate-dependent respiration (11), whereas atrial fibrillation is characterized by its increase (52), without changes in NADH-linked respiration. Our findings that the VSucc-to-VCOX ratio tended to increase and the VSucc was relatively less reduced than VGlut in the antrum compared with corpus imply that the electron transfer from succinate to oxygen was less limited in the mitochondria of antral mucosa. Hence, this change could also contribute to the relative deficit in complex I in that type of mucosa. However, further studies, aimed at direct assessment of the enzyme activities of different respiratory chain complexes in these two types of gastric mucosa, are required to validate that assumption.

For the first time, this study demonstrates different responses of mitochondria to active chronic inflammation in the antral and corpus mucosa, as oxidative phosphorylation (e.g., measured as VADP) was suppressed in the corpus but was upregulated in the antrum (Fig. 7). These changes are surprising because active chronic gastritis and associated oxidative stress induced by H. pylori are always more pronounced in the antral than in the corpus mucosa (6, 15, 40), which would predict a more severe injury of antral mitochondria. Yet this apparent controversy can be explained by a tissue type-specific control of the cellular bioenergetic processes by this bacterium. First, H. pylori induces expression of mitochondrial MnSOD exclusively in the antral mucosa (8, 14, 18, 19), which might enhance its capacity to eliminate ROS, in addition to that afforded by intrinsically excessive GSH. Second, suppression of mitochondrial respiration in the corpus mucosa might result from inhibition of acid production. This assumption relies upon the findings that IL-1β and the TNF-α, the two major cytokines mediating H. pylori effects, strongly inhibit acid secretion in cultured parietal cells (7) and that treatment of patients with omeprazole, a H+–K+-ATPase inhibitor, simultaneously downregulates mitochondrial activity (23). Finally, in our experiments, the H. pylori-linked inflammation was associated with concerted changes in VADP, VO2, and VFCCP, without variations in RCI. Thus it seems that H. pylori effects were not related to regulation of mitochondrial function but to changes in tissue mitochondrial content that decreased in the corpus but increased in the antrum. In principle, these inverse changes could result from the differently altered balance between the antiapoptotic and apoptotic pathways in the corpus and antral mucosa. H. pylori promotes apoptosis mainly through the mitochondrial route by triggering cytochrome c release (16, 34) via translocation of Bax (5, 34) and/or the N1H2-terminal 34-kDa fragment of H. pylori vacA cytotoxin into the mitochondria (16) in association with depolarized mitochondrial membranes, depressed cellular respiration and ATP content, and mitochondrial fragmentation (5, 29). Among major pathogenic factors inducing these changes is ammonia, a product of strong urease activity of H. pylori (31, 56, 60, 61). Therefore, suppressed respiration (Fig. 7) in the gastric corpus mucosa can be related to apoptotic changes in the mitochondria. On the other hand, H. pylori exerts a powerful antiapoptotic influence through many ways, such as activation of the cellular inhibitor of the apoptosis 2 gene (66), epithelial cell-cycle arrest (54), and upregulation of cyclooxygenase-2 (COX-2) in epithelial, mononuclear, and parietal cells (15, 36, 59). The products of COX-2 (15d-PGJ2 and PGA1) directly inhibit NF-κB-mediated apoptotic pathways via activating the peroxisome proliferator-activated receptor-γ (PPARγ) (20, 41), whereas PPARγ accelerates biosynthesis of mitochondria, thus increasing the tissue’s oxidative capacity (9, 33). It is known that H. pylori upregulates COX-2 in the antral mucosa to a greater extent than in the corpus mucosa (15). Hence, activation of PPARγ should also be more pronounced in the antral mucosa, which could account for the augmented respiration in this tissue registered by us (Fig. 7). Besides these mechanisms, host response to H. pylori involves increased gastrin production by mucosal G-cells, which in turn stimulates gastric epithelial cell proliferation (41). Thus it cannot be excluded that at least a fraction of increased oxidative capacity in the antrum stemmed from proliferation of G-cells.

Coupling of Mitochondrial Kinases to Oxidative Phosphorylation in Gastric Mucosa

Observations that short-term inhibition of proton pumps (H+–K+-ATPase) with omeprazole or ranitidine suppresses mitochondrial activity in the body of the human stomach (23), that blockade of mitochondrial processes abolishes the H+-pumping activity of the gastric glands (44), and that alterations in the secretory activity of parietal cells occur together with changes in mitochondrial morphology (25, 57), collectively
imply that gastric mucosal cells must possess intracellular mechanisms enabling exact regulation of mitochondrial ATP production in accordance with altered ATP utilization in secretory processes.

In other types of cells, e.g., cardiomyocytes, these mechanisms are well characterized, and there exists consensus on the issue that mitochondria and ATPases communicate with each other via different isoforms of CK and AK coupled to ANT in mitochondria and to ATP-consuming processes at different intracellular sites (13, 46, 47). That CK is involved in these mechanisms in gastric mucosal cells was first demonstrated by Sistermans et al. (55), who showed a functional coupling between BB-CK and H^+–K^+-ATPase in parietal cells. Here we report that human gastric mucosal cells express not only BB-CK, but also uMtCK, which is functionally coupled to oxidative phosphorylation. To confirm this, one set of experiments demonstrated stimulation of respiration with creatine in the presence of minute concentrations of ATP (50 μM) (Fig. 4). Here, two principally different mechanisms can account for the creatine effect. First, creatine added to the medium, which represents cytoplasm in permeabilized cells, in the presence of ATP may activate cytosolic CK and the CK bound to endoplasmic reticulum and mitochondria, which increases stationary [ADP] in the cytoplasm/medium. The latter stimulates oxidative phosphorylation after being diffused through MOM and transported into the matrix. This mechanism described (Model I) assumes a fast equilibrium between ADP concentrations in the intermembrane space, cytosol, and medium to take place because of simultaneous action of cytosolic CK, AK, and ATPases. Alternatively, creatine could stimulate local production of ADP near ANT by MtCK at the expense of mitochondrially produced ATP. This mechanism (Model II), known as the functional coupling between ANT and MtCK, allows establishment of higher local concentrations of ADP in the microcompartment between MtCK and ANT than in the surrounding mitochondria cytoplasm (47).

To discriminate between these two models, the kinetics of regulation of mitochondrial respiration by ADP was analyzed from [ADP] vs. VO_{2} relationships in the presence of excess amounts of hexokinase and glucose. This system completely utilizes mitochondrial ATP once it reaches the cytosolic compartment to become available for hexokinase and allows clamping of cytosolic [ADP] at desired levels in experimental settings (Fig. 5). It was found that the apparent K_{m} value for ADP in regulation of respiration was ~100 μM in both the antral and corpus mucosa, which is far higher than its value for the same process in isolated mitochondria (10-20 μM) (32). The concerns that the low apparent affinity of mitochondria to exogenous ADP in mucosal preparations is an artifact (resulting from incomplete permeabilization of cells or large diffusion distances within the bulk multicellular tissue) can be dispelled for several reasons. First, stimulation of respiration by cytochrome c (Figs. 4 and 6) directly demonstrates effective penetration of much larger molecules than ADP into the cell interior, thus indicating effective permeabilization. Second, glutamate-dependent respiration with exogenous ADP yielded a RCI value (2-3; Table 2) similar to that for mitochondria in homogenized mucosal preparations (50). Presence of intact (impermeabilized) cells in the medium would have resulted in RCI values lower than that for mucosal homogenates, due to the high rate of cellular respiration supported by endogenous ADP, which was not the case, however. Third, the straight-line double reciprocal relationships between the rates of respiration and [ADP] (Fig. 5) exclude existence of different populations of mitochondria with variable kinetic properties (e.g., mitochondria in permeabilized cells and intact cells, and mitochondria extracted from cells). Finally, high apparent K_{m} for ADP in regulation of respiration in oxidative muscle cells, permeabilized in the same manner as mucosal cells in this study, has been shown not to be proportional to cellular diffusion distances (i.e., cell dimensions), but related to organization of mitochondria in permeabilized cells and intact cells, and mitochondria extracted from cells)

<table>
<thead>
<tr>
<th>Parameter of Oxidative Phosphorylation</th>
<th>No.</th>
<th>Antrum</th>
<th>Corpus</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_{0}</td>
<td>16</td>
<td>0.164±0.014‡</td>
<td>0.300±0.019</td>
</tr>
<tr>
<td>V_{Glu}</td>
<td>16</td>
<td>0.365±0.023‡</td>
<td>0.850±0.059</td>
</tr>
<tr>
<td>RCIC_{Glu}</td>
<td>16</td>
<td>2.322±0.154‡</td>
<td>2.976±0.256</td>
</tr>
<tr>
<td>V_{Succ}</td>
<td>16</td>
<td>0.378±0.033‡</td>
<td>0.650±0.057</td>
</tr>
<tr>
<td>RCISucc</td>
<td>16</td>
<td>2.040±0.011‡</td>
<td>0.425±0.032</td>
</tr>
<tr>
<td>RCIC_{Succ}</td>
<td>16</td>
<td>1.848±0.116‡</td>
<td>1.526±0.070</td>
</tr>
<tr>
<td>V_{Glu}/V_{Succ}</td>
<td>16</td>
<td>1.027±0.067†</td>
<td>1.376±0.080</td>
</tr>
<tr>
<td>Proton leak_{Norm}</td>
<td>16</td>
<td>0.161±0.032*</td>
<td>0.275±0.044</td>
</tr>
<tr>
<td>V_{COX}</td>
<td>16</td>
<td>0.620±0.034‡</td>
<td>1.297±0.097</td>
</tr>
<tr>
<td>V_{Glu}/V_{COX}</td>
<td>16</td>
<td>0.603±0.040</td>
<td>0.675±0.033§</td>
</tr>
<tr>
<td>V_{Succ}/V_{COX}</td>
<td>16</td>
<td>0.615±0.047</td>
<td>0.515±0.037</td>
</tr>
<tr>
<td>V_{FCCP}</td>
<td>27</td>
<td>0.449±0.032‡</td>
<td>0.812±0.055</td>
</tr>
</tbody>
</table>

Values are means ± SE; No. = the number of specimens studied. The rates of respiration (V) are given in nmol O_{2}·min⁻¹·mg wet weight. V_{0}, basal respiration without ADP or ATP; V_{Glu}, ADP-stimulated respiration in the presence of glutamate and malate; RCIC_{Glu}, respiration control index calculated as V_{Glu}/V_{Succ}; V_{Succ}, ADP-stimulated respiration in the presence of rotenone and succinate; V_{COX}, respiration after inhibition of succinate-stimulated respiration by atractyloside; RCISucc, V_{Succ}/V_{Atr}; Proton leak_{Norm}, proton leak normalized for V_{Succ}/V_{COX}; the respiratory equivalent of cytochrome oxidase activity calculated as [V_{COX} = V_{TMPD} – V_{TMPD} + NaN_{3}] where V_{TMPD} and V_{TMPD} + NaN_{3} are TMPD-stimulated respiration rates before and after addition of NaN_{3}; V_{FCCP}, respiration in the presence of the uncoupler assessed as in Fig. 4. *P < 0.05, †P < 0.01, ‡P < 0.001 compared with corpus mucosa. §P < 0.01 compared with V_{Succ} to V_{COX} ratio in corpus mucosa.
tion is characteristic of the functional coupling between ANT and McCK [Model II described above (47)]. These findings in combination with earlier results (55) allow us now to envisage the entire system of CK-phosphotransfer comprising all three necessary stages: 1) synthesis of PCr in mitochondria, 2) transfer of energy-rich phosphoryl groups from PCr via cytosolic BB-CK, and 3) regeneration of ATP in the vicinity of ATPases because of the coupling of BB-CK to ATPases in mucosal cells. Expression of mitochondrial (AK2) and cytosolic (AK1) isoforms and the coupling of mitochondrial ATP production to AK2 (Fig. 4) strongly suggest the presence of a functional AK-phosphotransfer network as well.

We found that mitochondria in gastric mucosa specimens exhibited leaky MOM, and the larger permeability of MOM was associated with decreased ICCK and IKM (Fig. 6). The simplest explanation for these findings might be that the mitochondria degraded and lost their cytochrome c and kinases due to mechanical sample damage imposed during skinnning procedure and the following oxygraphy. However, some lines of evidence argue against considerable sample impairment. No sign of time-dependent progression of the degradation of mitochondria was observed. For example, the VADP values registered as in Fig. 4 for the whole groups of antral and corpus specimens (0.453 ± 0.033 and 0.782 ± 0.056 nmol O2·min−1·mg−1, n = 27, respectively) were not lower than the corresponding values of analogous parameter, VGIH (Table 2), despite the markedly longer incubation period before ADP addition within the former protocol (35-40 min, Fig. 4) than within the protocol for VGIH assessment (usually 5–10 min; see METHODS). On the other hand, for the groups of probes with different cytochrome c effects (<20% and ≥20%), the mean incubation period before addition of cytochrome c into the medium was found to be similar: 51.73 ± 1.46 (Figs. 6, A and B, bars <20%), 52.84 ± 2.43 (Fig. 6A, bar ≥20%), and 51.43 ± 2.4 min (Fig. 6B, bar ≥20%); likewise, the duration of the skinnning procedure was kept constant for all mucosal probes (METHODS). In addition, as mentioned above, analysis of the 1/[ADP] vs. 1/V relationships (Fig. 5A) did not reveal the population of isolated mitochondria, characteristic of degradation of the cells. Finally, in recent studies on the function of mitochondria in human atrial cells, the positive cytochrome c effect was not observed (52) using the same protocols of cell permeabilization and oxygraphy as applied in this study. Altogether these data rule out significant nonspecific damage of the gastric mucosal specimens, at least during oxygraphy. At the same time, most of the patients had an H. pylori infection, capable of altering the processes of oxidative phosphorylation (Fig. 7) and of imposing the structural changes in mitochondria via multiple mechanisms as described above. It is therefore possible that increased permeability of MOM and impaired coupling of kinases to ANT are related to the specific effects of H. pylori and associated inflammatory reactions exerted upon mitochondrial structures. Notably, partial disintegration of cell structure, resulting from ischemia-reperfusion injury and genetic knockout of cytoskeletal proteins is also characterized by altered functions of the MOM and mitochondrial kinases (27, 28), which suggests that for these disturbances and those produced by H. pylori the impairment of the MOM represents a common stage with adverse consequences. First, it facilitates diffusion of cytoplasmic ADP into the intermembrane compartment in which excess ADP may hinder the functional coupling between mitochondrial kinases and oxidative phosphorylation (27, 47). Second, it enables mitochondrial leak of cytochrome c and kinases, these changes being causal for suppressed electron flow along the respiratory chain and for impaired intracellular energy transfer, respectively (28, 48). Thus we provide first evidence that the inflammation related to H. pylori affects the cellular energy metabolism not only by influencing the system of oxidative phosphorylation, but also by interfering with the systems of coupled reactions of kinases and ANT in the mitochondria. Because others have shown an association of gastric adenocarcinoma with suppressed expression of B-CK (21, 22), it appears that the whole system of intracellular energy transfer might be targeted by H. pylori and accompanied inflammatory factors in the pathogenesis of different gastric diseases. Recently, regulation of oxidative phosphorylation by ADP was found to be markedly affected by the changes in muscle cell contractile function and structure (4). Further research is required to elucidate whether analogous structure-function relationships exist in different cell types within the gastric mucosa.

ACKNOWLEDGMENT

The authors thank Dr. F. N. Gellerich for valuable comments, E. Gvozdkova for technical assistance, and E. Jaigma for correcting the English.

GRANTS

This work was supported by Estonian Science Foundation’s Grant 5266, and by Grants 0182549603 and 0182558603 from the Estonian Ministry of Education and Research.

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