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A nucleotide-regulated Cl^-/OH^- anion exchanger in endoplasmic reticulum-enriched pig pancreatic microsomes

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The anion conductive pathways in preparations of endoplasmic reticulum (ER)-enriched microsomes from pig pancreas were investigated. The rate of swelling induced by cation ionophores (nigericin (nig) and/or valinomycin (val)) was measured in iso-osmotic solutions by light scattering, in the presence or absence of an inward Cl^- and/or pH gradients. The rate of swelling in the presence of the inward Cl^- gradient and ionophores was faster than that of controls. Low pH did not change the swelling rate in the presence of valinomycin, but it increased it in the presence of nigericin. When the Cl^- gradient was abolished, valinomycin plus the pH gradient increased the rate of swelling, and this was further enhanced by nigericin. Anion transport inhibitors reduced the swelling rate. The nigericin-induced swelling was stimulated by ATP and GTP. The non-hydrolysable analogues, adenosine 5′-[β,γ-methylene]triphosphate, guanosine 5′-[β-thio]triphosphate and guanosine 5′-[β-thio]diphosphate, increased the rate of swelling, whereas adenosine 5′-[γ-thio]triphosphate inhibited it. ADP, CTP and UTP had no effect. These data suggest the presence of a Cl^-/OH^- exchanger and a Cl^- conductance in microsomes. They indicate that nucleotides may regulate the Cl^-/OH^- exchanger. Nucleotides do not need to be hydrolyzed but phosphorylation may occur to counter-balance the nucleotide-induced stimulation.

Introduction

The Cl^- transporters, the electroneutral anion exchanger and the Cl^- conductance, play an important role in epithelia transmembrane ion transport. They are involved in the maintenance of Cl^- homeostasis within cells and in overall transmural electrolyte transport [1–8].

The electroneutral anion exchanger was first described almost twenty years ago in human erythrocytes, and has since been found in numerous epithelial and non-epithelial cells [1–7]. Pancreatic acinar and ductal cells, both possess an anion exchanger [9–11], as do the intracellular secretory granules, the zymogen granules [12]. The fusion of zymogen granules with the plasma membrane is believed to allow the insertion of transport proteins into the plasma membrane, leading to increased ion transport [13].

Similarly, the Cl^- conductance has been found in all the epithelial and non-epithelial cells examined to date [8,14,15]. Its importance in the regulation of ion transport was demonstrated when it was found that a defect in the regulation of Cl^- conductance is responsible for the abnormal electrolyte transport across secretory epithelia that occurs in patients with cystic fibrosis [16].

The regulation of anion conductive pathways is critical for cells. A growing body of experimental evidence points to the importance of nucleotides in the regulation of chloride conductance, and the way in which this modulates the overall transmural ion transport [17–21]. An ATP-regulated Cl^- conductance has been identified in zymogen granules of pancreas [21]. The regulation by nucleotides is complex process and frequently involves several factors. For example, the activity of cystic fibrosis transmembrane regulated protein (CFTR), which has been identified as a Cl^-channel [22], is stimulated by both protein kinase A and ATP, while ADP blocks this action [23].

Regulation of the anion exchanger has been less extensively studied. Neverthless, the Cl^-/HCO_3^- exchanger has been shown to be activated by growth hormone in messangial cells [24], by Ca^{2+}-mobilizing agonists in pancreatic acini [11], and by MgATP in cardiac cells [25].

This article presents evidence for the existence of anion exchanger and Cl^- conductance in pig pancre-

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atic ER-enriched microsomes and describes how trinucleotides may regulate the anion exchanger rather than Cl^- conductance. The possible regulation of anion exchanger was assayed by measuring the rate of microsome swelling by light scattering using an electroneutral cation ionophore (nigericin and/or valinomycin) under iso-osmotic conditions, in the presence (or absence) of an inward Cl^- gradient. The regulation of Cl^- conductance by nucleotides using the same technique, is described elsewhere [26]. This technique has been used previously to show the role of secretagogue-receptor binding in the stimulation of anion and cation conductive pathways in pancreatic zymogen granules [12,13], and to demonstrate the presence of the Cl^-/OH^-exchanger in these granules [12]. The nucleotides, ATP and GTP, stimulated the exchanger, and neither needed to be hydrolyzed. Phosphorylation led to blockage of the stimulation by ATP.

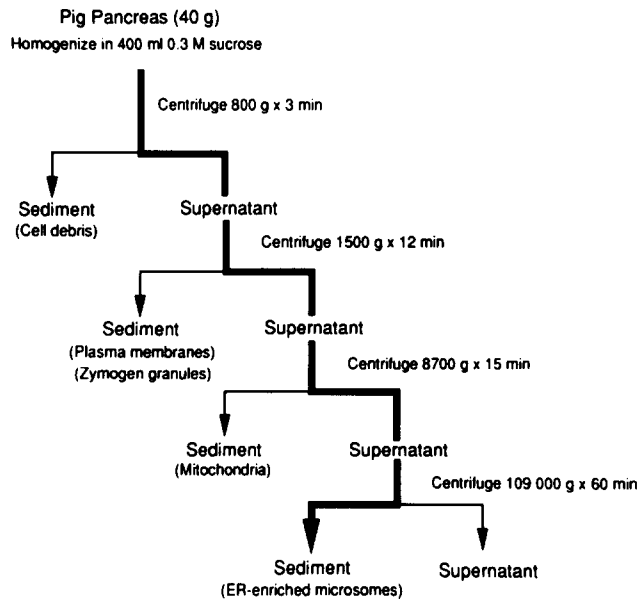

Fig. 1. Procedure for obtaining ER-enriched microsomes from pig pancreas.

Materials and Methods

Materials

All chemicals were obtained from commercial sources and were of the highest grade available. EDTA, nigericin, valinomycin, Hepes, pepstatin A, phenylmethylsulfonyl fluoride (PMSF), leupeptin, ATP, CTP, UTP, GTP, ADP, adenosine 5′-[γ-thio]triphosphate (ATPγS), adenosine 5′-[β,γ-methylene]triphosphate ($AppCH_2p$), guanosine 5′-[γ-thio]diphosphate (GDPβS), guanosine 5′-[γ-thio]triphosphate (GTPγS), and bovine serum albumin fraction V were from Sigma. 4-Acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) were from Fluka, Indanyloxyacetic acid, IAA 94-95, was a gift from D. Landry, Columbia University, New York, USA. All other chemicals were from Merck.

Methods

Tissue preparation. Adult pig pancreases were transported on ice from the slaughter house and all subsequent manipulations were performed at 4°C.

Endoplasmic reticulum (ER)-enriched microsomes were prepared essentially according to Jamieson et al. [27], as outlined in Fig. 1. Briefly, fat and connective tissue was removed and the pancreas was cut into cubes. A 40 g sample was mixed (1:10, w/vol) with homogenizing buffer (0.3 M sucrose containing a cocktail of proteinase inhibitors: 1 mM EDTA, 0.1 mM PMSF, 2 μM leupeptin and 10 μM pepstatin A) and homogenized twice at 500 r.p.m. using a low shear continuous tissue homogenizer (Yamato, model LH-21, Roucaire, France). The first three centrifugation steps were run in a Sigma 4K 10 centrifuge, Rotor No. 12166, and the fourth in a Beckman Optima XL-70, rotor 70 Ti. The final pellet was resuspended at 25 mg protein/ml in the same buffer using a Dounce homogeneizer or by 10 passages through a 22-gauge needle. The resulting vesicles were stored at −80°C; when needed they were rapidly thawed and the enzymes assayed.

Enzyme assays, protein determination. Protein was determined using the BCA protein assay reagent and bovine serum albumin fraction V as standard (Pierce, Touzard et Matignon, Paris, France). The enrichment of microsomes with endoplasmic reticulum was determined by measuring total RNA [28]. Mitochondrial contamination was assessed by measuring cytochrome-*c* oxidase activity [29]. Prior to the assay, the cytochrome *c* (40 μM in phosphate buffer pH 7.4) was fully oxi-

TABLE I

Composition of buffers used for transport assays

	K^+ (mM)	Gluconate$^-$ (mM)	Cl^- (mM)	Hepes (mM)	pH
Buffer A	150	150	–	20	7.0
Buffer B	150	–	150	20	7.0
Buffer C	150	–	150	20	5.5

dized with ferricyanide and subsequently reduced using Na-dithionite to obtain a fully reduced form of cytochrome *c* which served for determination of cytochrome-*c* oxidase activity. Plasma membrane contamination was determined by measuring Na/K-ATPase activity [30]. Na/K-ATPase activity was calculated as the difference between the activities obtained in the presence and absence of 3 mM ouabain.

Measurement of microsomal Cl^- permeability. Chloride permeability was assayed by measuring the iso-osmotic swelling of microsomes using cation probes and light scattering [12,13,21]. For transport assays, the vesicles were resuspended in an appropriate buffer at a protein concentration of 10 mg/ml (buffer A or buffer B, Table I). The microsomes (~ 5 μl vesicles ~ 50 μg protein) were then added to 1 ml iso-osmotic assay buffer (buffers A, B or C, Table I) with or without the electroneutral K^+/H^+ ionophore, nigericin (5 μg/ml), and/or the electrogenic K^+ ionophore, valinomycin (25 μg/ml). The kinetics of swelling were followed by measuring the time-dependent change in the absorbance of microsomes at 540 nm. The Cl^- conductive pathways were identified by permeabilizing the vesicles for cations using nigericin or valinomycin. Using such conditions for light-scattering experiments implies that the endogenous Cl^- conductive pathways become rate-limiting for the influx of salt and water, resulting in microsomal swelling. Stock solutions of nigericin or valinomycin in 95% ethanol were added to assay buffer to obtain a given concentration of ionophore (see above) and 0.01% ethanol. This concentration of ethanol did not influence vesicle swelling. The absorbance was continuously measured for 1 min at room temperature, in a Hitachi U-2000 spectrophotometer (Braun Sci, Paris, France).

ATP was used as the Mg salt, other nucleotides were Na salts and were used in the presence of 5 mM $Mg(CH_2COOH)_2$ (i.e., Mg(acetate)$_2$). The pH of the solutions containing nucleotides was adjusted to pH 7.0. All experiments were performed at room temperature.

Analysis of light scattering data. The swelling of microsomes corresponding to the Cl^- conductive pathways were determined by measuring absorbance for 1 min in the presence of nigericin and/or valinomycin. The results were quantified by measuring the initial relative rate constant (IRC) which corresponds to the decrease in optical density (OD) measured within one second after adding vesicles to the assay buffer (ΔOD/s). It was estimated from the data (absorbance vs. time) recorded by a spectrophotometer. The changes in IRC measured under different experimental conditions are expressed as a percent of their respective controls. Because the values of IRC varied from one day to another, each set of appropriate controls were taken as 100%. The test IRC values for the conditions on a given day were compared to the controls for the same day.

All experiments were repeated at least three times. 4–10 determinations of ΔOD/s for control and test conditions were performed for each experiment. The results are expressed as means ± S.E., and analysed by Student's unpaired *t*-test.

Results

Enzymatic properties of the isolated pig pancreatic microsomes

Table II shows the marker enzymes measured in the pancreatic microsomes preparation. The ER-enriched fraction contained low levels of the enzyme markers specific for mitochondria (cytochrome-*c* oxidase) and plasma membrane (Na/K-ATPase). It was 4-fold enriched in a marker for endoplasmic reticulum (RNA).

Tests for anion conductive pathways in the ER-enriched pancreatic microsomes

Addition of ER-enriched pancreatic microsomes suspended in the buffer A to the iso-osmotic KCl solution (buffer B) to impose an inward Cl^- gradient on the vesicles, caused only a slight decrease in absorbance (Fig. 2), indicating that cationic and/or an-

TABLE II

Distribution of enzyme markers in microsomal fractions obtained by differential centrifugation

Specific activity is expressed as means ± S.D. Enrichment is expressed as the specific activity divided by the specific activity of the total homogenate. *n*, number of independent experiments.

	Na/K-ATPase		Cytochrome-*c* oxidase		RNA	
	μmol/mg per min ($n = 3$)	enrichment	nmol/mg per min ($n = 4$)	enrichment	μg/mg ($n = 3$)	enrichment
Homogenate	21.1 ± 13.9	1	12.2 ± 4.2	1	365.9 ± 65.1	1
ER-enriched microsomal fraction	2.0 ± 1.1	0.09	6.6 ± 0.9	0.54	1562.1 ± 394.1	4.27
Mitochondrial-enriched microsomal fraction	23.9 ± 11.5	1.13	27.1 ± 6.8	2.22	593.3 ± 29.1	1.62

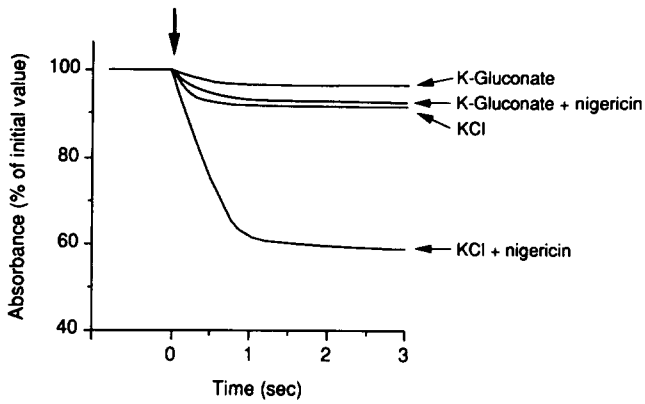

Fig. 2. Swelling of ER-enriched pancreatic microsomes in the presence and absence of nigericin. Microsomes were suspended in buffer A. The arrow indicates the introduction of microsomes in buffer A or B. There was a significant drop in absorbance in KCl-buffered solutions containing 5 μg/ml nigericin, the experiment shown corresponds to IRC = 17 ΔOD/s.

ionic permeabilities were low at steady state. Nigericin significantly increased the rate of decrease in absorbance (Fig. 2), indicating that the cation permeability was rate-limiting for microsomal swelling. The swelling resulted from intravesicular accumulation of KCl, and the resulting in flow of water. This result also suggests the presence of the Cl^- conductive pathways, a Cl^- conductance and an electroneutral anion transporter (Cl^-/OH^- exchanger, or Cl^-/cation cotransporter), with the efflux of OH^- ions preserving electroneutrality. The absorbance dropped only slightly when microsomes were added to buffer A in the absence of nigericin (no inward gradients in the absence of Cl^- ions), indicating that Cl^- ions were necessary for iso-osmotic swelling. There was a slightly more pronounced decrease in absorbance under the same conditions plus nigericin (5 μg/ml), probably due to non-specific swelling, but this effect was not further investigated. The initial rate constant (IRC) measured in the presence of inward Cl^-gradient (buffer A inside vesicles, buffer B outside) varied from one experiment to another. But despite these variations, nigericin was always stimulatory. The mean IRC values in the presence and absence of nigericin are shown in Table III (columns 'ctrl' and '+nig', line 1).

Cl^- conductance was detected in the microsomes by measuring the iso-osmotic swelling in the presence and absence of valinomycin with an inward Cl^- gradient (buffer A inside vesicles, buffer B outside). Valinomycin significantly increased the rate of swelling, suggesting the existence of the Cl^- conductance (compare columns 'ctrl' and '+val', line 1, Table III).

Two series of experiments were performed to detect Cl^-/OH^- exchanger in the microsomes. First, the microsomes were suspended in buffer A and placed in buffer C (inward Cl^- and pH gradients were imposed on vesicles). Under such conditions IRC increased 2-fold, as compare to the controls without a pH gradient (column 'ctrl', lines 1 and 2, Table III). This suggests the existence of Cl^-/OH^- exchanger. Nigericin, in the presence of inward Cl^- and pH gradients, further increased IRC (columns 'ctrl' and '+nig', line 2, Table III). This effect may occur because, following the influx of Cl^- ions exchanged for OH^-, nigericin facilitates the entry of K^+ ions (and the exit of H^+ ions) into the microsomes, thus accelerating water flow and provoking swelling.

The nigericin-induced swelling in the presence of inward $Cl^-$and pH gradients may also be due to stimulation of Cl^- conductance by the low pH, which would allow the influx of Cl^- ions followed by K^+ ions and water. This hypothesis was tested by measuring the effect of low pH, in the presence of inward Cl^- gradient, on valinomycin-induced swelling, i.e., on Cl^- conductance. The microsomes were suspended in buffer A and placed in buffer C plus valinomycin (inward Cl^- and pH gradients were imposed on the vesicles), and the results were compared to those obtained in the absence of a pH gradient (buffer A inside vesicles, buffer B outside). The IRC in the presence and absence of the pH gradient were not significantly different (compare column '+val' lines 1 and 2, Table III). Thus the low pH does not stimulate the Cl^- conductance, so that the nigericin-induced swelling in the

TABLE III

IRC values for ER-enriched pancreatic microsomes measured under different experimental conditions

Lines 1 and 2: Microsomes suspended in buffer A were added to external solutions containing buffer B or buffer C, in the absence (ctrl) or presence of nigericin (+nig) or valinomycin (+val). Lines 3 and 4: as above except that the microsomes were suspended in buffer B. [(a)] Lines 3 and 4, +nig, the microsomes were placed in buffer containing valinomycin before the addition of nigericin. * $P < 0.05$, ** $P < 0.01$ and otherwise = non significant, using Student's t-test for unpaired values. n, number of independent experiments.

	Buffer		pH		ΔOD/s					
	internal	external	internal	external	ctrl	n	+nig	n	+val	n
1	K-Glu.	KCl	7	7	10.5±4.6 **	19	17.1±7.9 **	24	20.0±10.4 **	22
2	K-Glu.	KCl	7	5.5	19.9±9.8 **	9	26.5±9.6 **	6	19.3± 8.7 **	12
3	KCl	KCl	7	7			2.5±1.5 [(a)]	12	1.5± 1.0	11
4	KCl	KCl	7	5.5			4.6±2.3 *[(a)]	8	2.9± 1.4 *	11

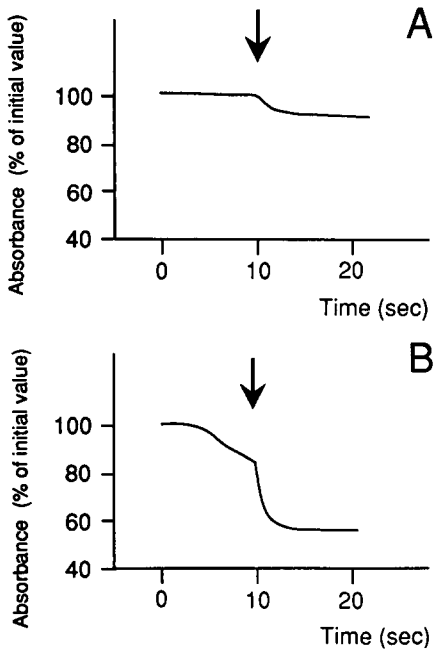

Fig. 3. Swelling of ER-enriched pancreatic microsomes in the absence of inward Cl^- gradient and in the presence or absence of pH gradient. (A) Microsomes were suspended in 150 mM KCl, pH 7 and placed in the same solution plus valinomycin. The arrow indicates the addition of nigericin, the corresponding IRC is 4 ΔOD/s. (B) Microsomes suspended as above were added to the same solution buffered to pH 5.5. The arrow indicates the addition of nigericin, the corresponding IRC is 9 ΔOD/s.

presence of inward $Cl^-$and pH gradients is at least partly due to the Cl^-/OH^-exchanger.

The second series of experiments designed to unmask the Cl^-/OH^- exchanger in the ER-enriched pancreatic microsomes were performed using such conditions in which there was no driving force for the influx of Cl^- ions through Cl^- conductance. The vesicles were suspended in buffer B and placed in the same buffer plus valinomycin, in the presence or absence of a pH gradient (buffer B or C outside vesicles). Valinomycin clamped the transvesicular potential to 0 mV, so pH gradient unmasking any electroneutral anion exchanger (or H^+/anion co-transporter) that may be present. Fig. 3A shows that in the presence of valinomycin and in the absence of any ion gradient (buffer B inside and outside vesicles), there was no change in absorbance (column '+val', line 3, Table III). Subsequent additional nigericin induced a slight but non-significant change in absorbance (column '+nig', line 3, Table III). A pH gradient across the vesicles in the presence of valinomycin (buffer C outside and buffer B inside vesicles) caused a significant decrease in IRC (column '+val', line 4, Table III) suggesting that the Cl^-/OH^- exchanger is responsible for the swelling. Nigericin caused a significant decrease in absorbance in vesicles pretreated with valinomycin, suggesting that nigericin-induced swelling is at least partially due to the Cl^-/OH^-exchanger (Fig. 3B, and column '+nig', line 4, Table III).

Effects of anion transport inhibitors on nigericin-induced swelling

The effects of three anion transport inhibitors were tested on nigericin-induced microsomal swelling in the presence of an inward Cl^- gradient (buffer A inside, buffer B outside vesicles), using 0.1 mM DIDS, 0.1 mM SITS and 0.01 mM IAA 94–95. Preincubation of the vesicles for 10 min with DIDS and SITS significantly reduced the rate of swelling. A longer incubation with IAA 94–95 was needed (1 h) to inhibit the nigericin-induced swelling. DIDS caused the IRC to decrease to $20.4 \pm 4.7\%$ ($n = 4$) of controls, SITS caused a decrease to $35.9 \pm 5.4\%$ ($n = 4$) while IAA 94-95 caused a decrease to $31.5 \pm 5.9\%$, $n = 4$. (n is the number of independent experiments). Thus, the Cl^- conductive pathway in the ER-enriched pancreatic microsomes is sensitive to the classical anion transport inhibitors. The same drugs inhibited the valinomycin-induced swelling (not shown, see Ref. 26).

The following experiments were performed to study the regulation of the nigericin-induced swelling by nucleotides. All of them were done in the presence of nigericin. The results are expressed as % change in IRC in the presence of nucleotides.

Effect of ATP

ATP (0.1 μM–2 mM) was tested in the presence of an inward Cl^- gradient (buffer A inside, buffer B outside vesicles) (Fig. 4). Maximal stimulation occurred at 100 μM and the concentration giving half-maximum stimulation (IC_{50}) was 80 μM. The ATP stimulation of nigericin-induced swelling decreased at concentrations

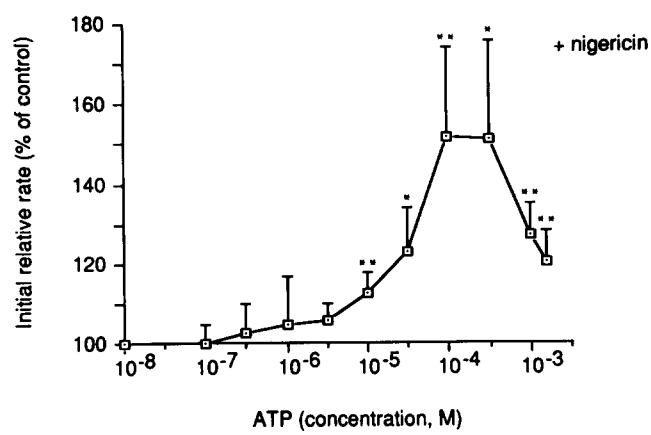

Fig. 4. Effect of ATP on the nigericin-induced swelling of ER-enriched pancreatic microsomes. Initial relative rates (IRC) of nigericin-induced microsomal swelling are expressed as percentages of control values without ATP. The control IRC in the presence of nigericin was 10.7–29 ΔOD/s. All experiments were performed three times ($n = 3$). * $P < 0.05$, ** $P < 0.03$ and otherwise = non significant, using Student's t-test for unpaired values.

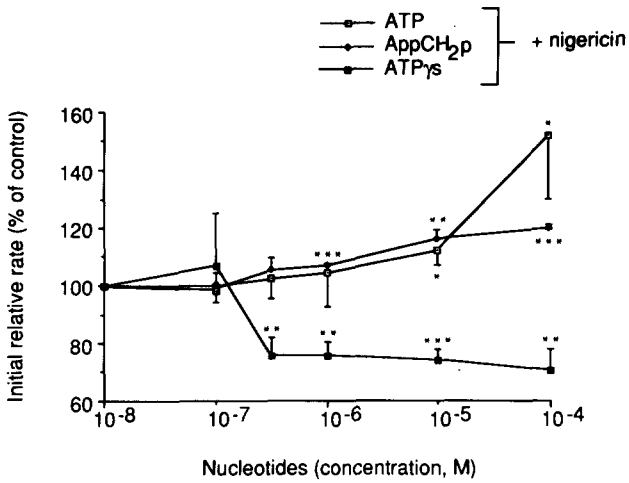

Fig. 5. Effects of ATP and non-hydrolysable analogues on the nigericin-induced swelling of ER-enriched pancreatic microsomes. The control IRC in the presence of nigericin was 9.2–30 ΔOD/s. All experiments were performed three times ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ and otherwise = non significant, using Student's t-test for unpaired values.

> 1 mM. The ATP effects were inhibited by 0.1 mM DIDS (70% inhibition of IRC, data not shown).

Effects of non-hydrolysable ATP analogues

Two non-hydrolysable ATP analogues, $AppCH_2p$ and ATPγS, were tested (0.1–100 μM) on nigericin-induced swelling of ER-enriched pancreatic microsomes. $AppCH_2p$ increased the IRC, while ATPγS (which is a substrate for kinases [31]), slightly but significantly inhibited the swelling (Fig. 5). This indicates that ATP does not need to be hydolyzed to exert its action, and that the spatial conformation of ATP is important for its action.

Effects of ADP and trinucleotides

The possible involvement of purinergic/pyrimidinergic receptors, was tested using GTP, CTP and UTP (0.1–100 μM). Only GTP stimulated swelling, with a maximum at 10 μM (Fig. 6). The presence of a purinergic receptor was checked using ADP. If the purinergic receptor were involved in the stimulation of nigericin-induced swelling, ADP should also stimulate swelling. ADP did not affect swelling (means (% of control): $103.8 \pm 6.8\%$, $106.2 \pm 7.2\%$, $106.4 \pm 13.4\%$ and $107 \pm 8.7\%$ for 0.1 μM, 1 μM, 10 μM and 100 μM of ADP, respectively ($n = 3$)). Thus purinergic receptors are not involved in the stimulation of nigericin-induced swelling.

Effects of non-hydrolysable GTP analogues

Since GTP stimulated the nigericin-induced swelling, G-proteins may be implicated. The effects of the non-hydrolysable GTP analogues, GTPγS and GDPβS

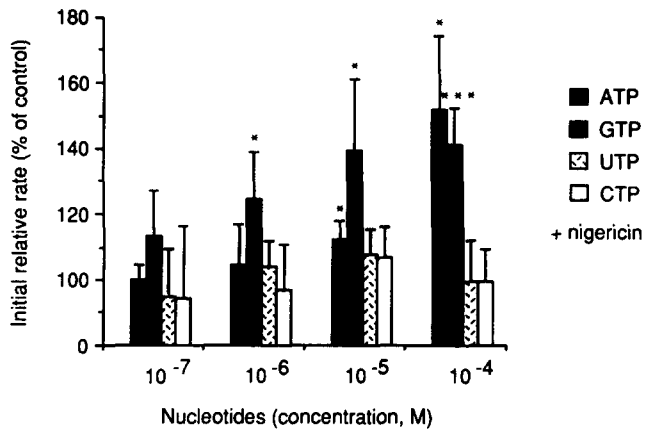

Fig. 6. Effects of trinucleotides on the nigericin-induced swelling of ER-enriched pancreatic microsomes. The control IRC in the presence of nigericin was 9.2–39.7 ΔOD/s. All experiments were performed three times ($n = 3$). * $P < 0.05$, ** $P < 0.005$ and otherwise = non significant, using Student's t-test for unpaired values.

(0.1–100 μM), were investigated. If G-proteins were involved in the stimulation of the anion exchanger, the effects of GTPγS and GDPβS should be opposite; if G_s were involved, GTPγS should stimulate the exchanger, but GDPβS should inhibit it; and if G_i was involved, GDPβS should stimulate it whereas GTPγS should inhibit it. Fig. 7 summarizes the results. Both GTP analogues stimulated microsomal swelling albeit less than GTP itself, suggesting that the G-proteins do not mediate the stimulation of nigericin-induced swelling by nucleotides, and suggesting that GTP does not need to be hydrolyzed to exert its action.

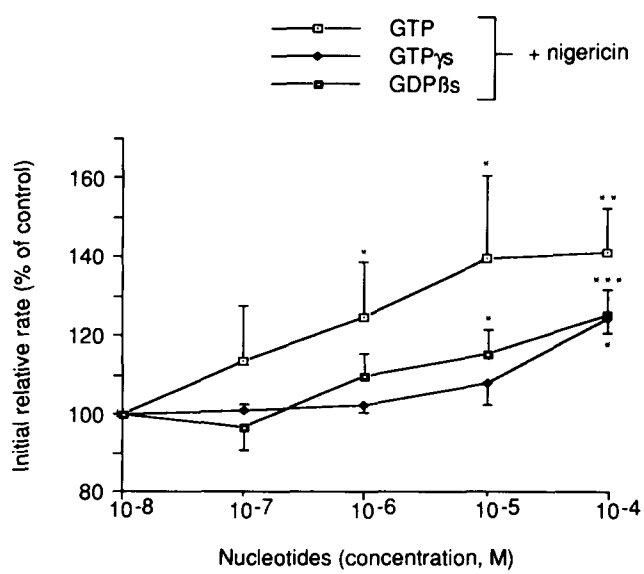

Fig. 7. Effects of GTP and non-hydrolysable analogues on the nigericin-induced swelling of ER-enriched pancreatic microsomes. The control IRC in the presence of nigericin was 9.2–39.5 ΔOD/s. All experiments were performed three times ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ and otherwise = non significant, using Student's t-test for unpaired values.

Discussion

The results demonstrate Cl^- conductive pathways, a Cl^- conductance and a Cl^-/OH^- exchanger (or a Cl^-/cation co-transporter), in ER-enriched pig pancreatic microsomes. The origin (acinar or ductal cells) of the microsomes cannot be precisely defined, since the preparation was obtained from whole pancreas. However, acini represent 99% of the total tissue, so that most of the ER-enriched microsomes were derived from acinar cells.

The presence of Cl^- conductance is demonstrated by valinomycin-induced microsomal swelling in the presence of an inward Cl^- gradient. The swelling which occurs under the same experimental conditions, but in the presence of nigericin rather than valinomycin, may be provoked by both the Cl^-/OH^- exchanger and the Cl^- conductance. In this study both nigericin- and valinomycin-induced swellings were inhibited by stilbene and indanyloxyacetic acid derivatives, but did not differ in their sensitivities to these drugs. It is therefore not possible to distinguish between the transporters on the basis of their sensitivity to inhibitors.

The existence of the Cl^-/OH^- exchanger in the ER-enriched pig pancreatic microsomes is suggested by swelling of microsomes in the presence of a pH gradient, while the electrochemical potentials for K^+ and Cl^- and the transvesicular potential were equal to 0 mV. The involvement of the Cl^-/OH^- exchanger in the nigericin-induced swelling is further supported by the enhanced swelling of microsomes induced by nigericin under the same experimental conditions. The Cl^-/OH^- exchanger may also contribute to the nigericin-induced swelling in the presence of an inward Cl^- gradient, as suggested by the enhanced swelling recorded in the presence of inward Cl^- and pH gradients. This is supported by absence of any effect of acid pH on Cl^- conductance, implying that the Cl^- conductance in the nigericin-induced swelling does not change in the presence or absence of a pH gradient. Therefore an increase of the rate of swelling in the presence of a pH gradient plus nigericin may be due to the Cl^-/OH^- exchanger alone.

Another argument suggesting that the Cl^-/OH^- exchanger contributes to the nigericin effects is the regulation by nucletotides of nigericin- and valinomycin-induced microsomal swelling. Another article [26] analyzes the effects of nucleotides on Cl^- conductance of the ER-enriched pancreatic microsomes. The action of nucleotides on nigericin- and valinomycin-induced microsomal swelling differs in three main ways. First, the IC_{50} (half-maximal stimulation by ATP) for the stimulation of nigericin-induced swelling is about 10-fold higher (80 μM) than that for Cl^- conductance (8 μM). Second, GTP activates the nigericin-induced swelling, whereas it is without effect on Cl^- conductance. Lastly, ATPγS inhibits the nigericin-induced swelling but has no effect on Cl^- conductance.

This suggests that the Cl^-/OH^- exchanger is present in ER-enriched pig pancreatic microsomes and is partly responsible for nigericin-induced swelling in the presence of inward Cl^- gradient. Nucleotides probably regulate the exchanger through the binding of ATP or GTP to the Cl^-/OH^- exchanger or to a regulatory protein. Pyrimidinergic, P_2-purinergic receptors, G-proteins and phosphorylation are not involved in this process. Pyrimidinergic receptors can be eliminated because CTP and UTP did not stimulate the anion exchanger. Similarly, the involvement of P_2-purinergic receptor seems unlikely, since ADP was without effect. The G-proteins do not appear to transduce the nucleotide signal, since the non-hydrolysable GTP analogues, GDPβS and GTPγS, stimulated the exchanger, whereas they should have had opposite actions if they activated the G-proteins. Lastly, the hydrolysis of ATP (or GTP) was unnecessary to stimulate the Cl^-/OH^- exchanger, since the non-hydrolysable ATP analogue, $AppCH_2p$, and those of GTP, GDPβS and GTPγS, all stimulated the exchanger.

The data in Figs. 4 and 5 show that the ATP-induced stimulation of anion exchanger decreased at ATP concentrations above 500 μM. This may be due to phosphorylation of the exchanger or a regulatory protein, since ATPγS, the non-hydrolysable analogue of ATP, which is a substrate for the kinases [31], inhibited the nigericin-induced microsomal swelling at concentrations from 1 to 100 μM. Alternatively, there may be a second, inhibitory binding site for ATP. This binding site would be only for ATP, since GTP and its non-hydrolysable analogues were stimulatory. We attempted to distinguish between the two mechanisms by measuring the nigericin-induced microsomal swelling in the presence of protein kinase A. Preincubation of microsomes with protein kinase A abolished the ATP-induced swelling, suggesting that phosphorylation is responsible for the lack of ATP stimulation at higher concentrations. These experiments also suggest that the inhibition of ATPγS may be due to phosphorylation. This process may mask the stimulatory action of ATP at concentration above 0.1 μM, since ATPγS is active at all concentrations above 1 μM.

The physiological role of the Cl^-/OH^- exchanger in ER-enriched microsomes may be two-fold. It may, together with other transport systems such as H^+-ATPase, regulate the intravesicular pH and volume of the ER, the two parameters which are fundamental for correct protein synthesis. It may also participate in the net Cl^- transport across the epithelium, and regulate intracellular pH. If this is the case, the newly Cl^-/OH^- exchanger synthesized, must be transported and inserted into the plasma membrane. A similar mechanism has been recently proposed for the ATP-regu-

lated Cl^- channels in the pancreatic ER and zymogen granules [12,26].

This is to our knowledge, the first report of the activation of Cl^-/OH^- exchanger by nucleotides in epithelial cells, althrough ATP has been reported to regulate the Cl^-/HCO_3^- exchanger in cardiac cells, and perhaps be involved in certain pathological situations leading to arrhythmia [25]. The nucleotide effect on the Cl^-/OH^- exchanger described here may be an ubiquitous phenomenon in the ER of many cell types (epithelial and non epithelial). The two an ion transport systems present in ER-enriched pancreatic microsomes appear to be stimulated by nucleotides, which may be important for the regulation of Cl^- transport in epithelia.

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References

1 Brazy, P.C. and Gunn, R.B. (1976) J. Gen. Physiol. 68, 583–599.
2 Cabantchik, T.I., Knauf, P.A. and Rothstein, A. (1978) Biochim. Biophys. Acta 515, 239–302.
3 Cabantchik, T.I. and Rothstein, A. (1974) J. Membr. Biol. 15, 226–248.
4 Alpern, R.J. (1990) Physiol. Rev. 70, 79–114.
5 Ferdows, M., Golchini, K., Adler, S.G. and Kurtz, I. (1989) Kidney Int. 35, 783–789.
6 Edelman, A., Bouthier, M. and Anagnostopoulos, T. (1981) J. Membr. Biol. 62, 7–17.
7 Hoffmann, E.K. (1986) Biochim. Biophys. Acta 864, 1–31.
8 Welsh, M.J. (1987) Physiol. Rev. 67,1143–1184.
9 Novak, I. and Greger, R. (1988) Pflügers Arch. 411, 546–553.
10 Stuenkel, E.L., Machen, T.E. and Williams, J.A. (1988) Am. J. Physiol. 254 (Gastrointest. Liver Physiol. 17), G925–G930.
11 Muallem, S. and Loessberg, P.A. (1990) J. Biol. Chem. 265, 12813–12819.
12 DeLisle, R.C. and Hopfer, U. (1986) Am. J. Physiol. 250 (Gastrointest. Liver Physiol. 13), G489–G496.
13 Gasser, K.W., DiDomenico, J. and Hopfer, U. (1988) Am. J. Physiol. 254 (Gastrointest. Liver Physiol. 17), G93–G99.
14 Pahapill, P.A. and Schlichter, L.C. (1992) J. Membr. Biol. 125, 171–183.
15 Jentsch, T.J., Steinmeyr, K. and Schwarz, G. (1990) Nature 348, 510–514.
16 Quinton, P.M. and Bijman, J. (1983) N. Engl. J. Med. 308, 1185–1187.
17 Stutts, M.J., Chinet, T., Mason, S.J., Fullton, J.M., Clarke, L.L. and Boucher, R.C. (1992) Proc. Natl. Acad. Sci. USA 89, 1621–1625.
18 Bégault, B. and Edelman, A. (1992) 6th Annu. Northern. American CF Conference, Oct, pp. 15–18.
19 Knowles, M.R., Clarke, L.L. and Boucher, R.C. (1991) New. Engl. J. Med. 325, 533–538.
20 Anderson, M.P., Berger, H.A., Rich, D.P., Gregory, R.J., Smith, A.E. and Welsh, M.J. (1991) Cell 67, 775–784.
21 Thevenod, F., Gasser, K.W. and Hopfer, U. (1990) Biochem. J. 272, 119–126.
22 Bear, C.E., Li. C., Kartner, N., Bridges R.J., Jensen, T.J., Ramjeesingh, M. and Riordan, J.R. (1992) Cell 68, 809–818.
23 Andersen, M.P. and Welsh, M.J. (1992) Science 257, 1701–1704.
24 Ganz, M.B., Boyarski, G., Sterzel, R.B. and Boron, W.F. (1989) Nature 337, 648–651.
25 Pucéat, M., Clément, O. and Vassort, G. (1991) J. Physiol. 444, 241–256.
26 Bégault, B., Anagnostopoulos, T. and Edelman, A. (1993) Biochim. Biophys. Acta, submitted.
27 Jamieson, J.D. and Palade, G.E. (1967) J. Cell. Biol. 34, 577–596.
28 Hatcher, D.W. and Goldstein, G. (1969) Anal. Biochem. 31, 42–50.
29 Cooperstein, S.J. and Lazarow, A. (1951) J. Biol. Chem. 189, 665–675.
30 Penefsky, H.S. and Bruist, M.F. (1974) Methods Enzym. Anal. 2, 324–328.
31 Eckstein, F. (1985) Annu. Rev. Biochem. 54, 367–402.