CCK-related peptides stimulation of the Na^+/H^+ antiport in pancreatic acinar cells leads to cytoplasmic alkalinisation

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SUMMARY Caerulein, gastrin and pentagastrin stimulation of the Na^+/H^+ exchange leads to intracellular alkalinisation in pancreatic acinar cells. Activation of protein kinase-C is the more probable mechanism involved in the effect of caerulein.

The mechanism by which cellular calcium mobilising agents such as cholecystokinin (CCK) and gastrin stimulate the exocrine pancreatic growth¹ is largely unknown. The Na^+/H^+ exchange has been suggested to play a central role in the initiation of proliferation induced by growth factors.²⁻⁵ A Na^+/H^+ exchange has been characterised in isolated pancreatic acini.^{6,7} Amiloride sensitive (AS) ^{22}Na uptake studies and measurement of intracellular pH (pH_i) showed that caerulein, a CCK analogue activates this antiport.⁶

In the present study, we compared the action of caerulein with that of pentagastrin (PG) and non-sulphated gastrin 17 (NSG₁₇) on the activation of Na^+/H^+ exchange in pancreatic acinar cells from guinea pig.

Methods

Isolated pancreatic acini were prepared from guinea pig pancreas as described elsewhere.⁸

AMILORIDE SENSITIVE ^{22}Na UPTAKE

Before $^{22}\text{Na}^+$ uptake, acini were equilibrated for 20 minutes in a Na^+ free medium (140 mmol choline chloride, 5 mmol glucose, 5 mmol KCl, 0.5 mmol CaCl_2 , 1 mmol Mg SO_4 , 25 mmol Hepes-Tris pH = 7.4) containing varying concentrations of caerulein, PG or NSG₁₇ in the presence or in the absence of 0.1 mmol amiloride. Uptake experiments were performed in the same medium except that 3 mmol NaCl, 2.6 $\mu\text{Ci/ml}$ $^{22}\text{NaCl}$, and 0.5 mmol ouabain were added. After three minutes of uptake at 37 °C, acini were quickly washed by centrifugation (15 sec at 0 °C) with a 140 mmol choline chloride, 1.2 mmol

MgSO_4 , 25 mmol Tris medium (pH = 7.4). The AS rate of $^{22}\text{Na}^+$ uptake was defined as the difference between the basal rate of $^{22}\text{Na}^+$ uptake measured in the absence and in the presence of 0.1 mmol amiloride.

[^{14}C] BENZOIC ACID UPTAKE

Pancreatic acini were first equilibrated for 30 min at 37 °C, pH = 7.4 in a HCO_3^- free saline medium (130 mmol NaCl, 5 mmol KCl, 1 mmol CaCl_2 , 1 mmol MgCl_2 , 5 mmol glucose, 25 mmol Hepes). For pH_i measurements, [^{14}C] benzoic acid was added (1 $\mu\text{Ci/ml}$). After incubation, pancreatic acini were rapidly washed (four times) by filtration through Whatman GF/B glass fibre filters with 5 ml cold medium, pH = 7.4, (the four washes lasted 25 sec). After drying, filters were placed in scintillation vials containing 10 ml Picofluor 15 and radioactivity was determined with a beta counter (Packard).

EXTRACELLULAR SPACE

To estimate the radioactivity trapped in the extracellular space, the weak acid was replaced in parallel samples by an impermeant marker [^3H] mannitol (1 $\mu\text{Ci/ml}$). This value was routinely subtracted from the experimental points.

INTRACELLULAR WATER SPACE

The intracellular space was calculated from the equilibrium uptake of 3-O-methyl-D-[1- ^3H]glucose (5 $\mu\text{Ci/ml}$). The intracellular volume was determined to be $0.22 \pm 0.02 \mu\text{l}/\mu\text{g DNA}$.

CALCULATION OF pH_i

The counts measured after [^{14}C] benzoic acid uptake were corrected for radioactivity trapped in the extracellular space and loss of intracellular radioactivity during the washing procedure. Intracellular

Fig. 1 Na^+ -dependence of the intracellular pH_i recovery after an acid load in the guinea pig pancreatic acini. Each point is the average of triplicates in one experiment representative of three others.

pH was calculated with the simplified equation $\text{pH}_i = \text{pH}_o + \log C_i/C_o$, where pH_o = external pH , C_i = intracellular concentration of benzoic acid in $\text{cpm}/\mu\text{l}$, and C_o = $\text{cpm}/\mu\text{l}$ in the external medium.

MATERIALS

Amiloride (AMI) and dimethylamiloride (DMA) were kindly provided by Dr Paoli (Merck, Sharp, and Dohme France). $^{22}\text{NaCl}$ was purchased from Amersham (GB) [$7\text{-}^{14}\text{C}$] benzoic acid, [^3H] mannitol 3-O-methyl-D-[$1\text{-}^3\text{H}$] glucose from New England Nuclear (RFA), PG from ICI Ltd (GB) NS G_{17} from CRB Laboratories (GB) and caerulein was a generous gift of Dr Castiglione (Farmitalia). Other chemicals were obtained from standard commercial sources.

Results

DETERMINATION OF pH_i WITH [^{14}C] BENZOIC ACID AND REGULATION OF pH_i BY EXTRACELLULAR Na^+

The accumulation of [^{14}C] benzoic acid in pancreatic acinar cells reached a plateau value within 30 seconds. The steady state accumulation of benzoic acid was stable up to 30 min (Fig. 1). Experiments were done to measure the shift of benzoic acid during washing procedure. A loss of about 30% of the intracellular radioactivity was determined (not shown). Therefore all experimental values were corrected for this leakage. In these conditions the accumulation of [^{14}C] benzoic acid indicates pH_i of 7.01 ± 0.03 ($n = 11$).

Incubation of acini with nigericin (an ionophore that exchanges K^+ for H^+), led to an intracellular acidification (Fig. 1). Thereafter when cells were

Fig. 2 Effect of caerulein, NS G_{17} and PG on AS ^{22}Na uptake in guinea pig pancreatic acini. Results are expressed as stimulated ^{22}Na uptake divided by control value in the absence of peptides. Values are means $\pm$ SEM from 8 separate experiments.

placed in a 130 mM Na^+ nigericin free medium, pH_i nearly returned to the control level within 10 min (Fig. 1). Acidification caused by nigericin was markedly increased when Na^+ was omitted from the medium, as pH_i fell abruptly to 6.3 and recovered only slowly (Fig. 1). The Na^+ dependence for pH_i recovery could show that extrusion of protons is regulated by Na^+ and suggests that Na^+/H^+ antiport regulates cytoplasmic pH .

EFFECT OF CAERULEIN, PENTAGASTRIN AND GASTRIN ON AMILORIDE SENSITIVE ^{22}Na UPTAKE AND pH_i

We have previously shown that caerulein increased the AS ^{22}Na uptake in pancreatic acini.⁶

The effect of the CCK analogues PG and NSG17 was studied and the stimulation of the AS ^{22}Na uptake by these peptides was concentration dependent. NS G_{17} ($\text{EC}_{50} = 1.2 \text{ pM}$) and PG ($\text{EC}_{50} = 0.02 \text{ nM}$) were more potent than caerulein ($\text{EC}_{50} = 0.2 \text{ nM}$) (Fig. 2). NS G_{17} was much less efficient than PG and caerulein.

Concomitantly, a significant alkalinisation of the cytoplasm appeared. The time course of caerulein induced alkalinisation is shown in Fig. 3A. Intracellular pH increased, about 0.15 pH units after a few minutes and reached a stable ΔpH_i of 0.3 pH units after 10–15 min. Alkalinisation of the cytoplasm by CCK analogues was concentration dependent. Rela-

Fig. 3 Effect of caerulein, NS G_{17} and PG on ΔpH_i in guinea pig pancreatic acini. (a): Time-course for pH_i in the presence of 1 mM caerulein. Each point is the average of triplicates. (b) Peptide-concentration dependence. Values are means $\pm$ SEM from 4 separate experiments.

tive potencies were: NS G_{17} ($\text{EC}_{50} < 10 \text{ pm}$) < PG ($\text{EC}_{50} = 0.1 \text{ nM}$) < caerulein ($\text{EC}_{50} = 0.3 \text{ nM}$). Caerulein activation of the Na^+/H^+ exchange was directly responsible for this alkalinisation, as caerulein-induced pH_i rise observed in a 25 mmol external Na^+ concentration ($\Delta \text{pH}_i = 0.22$) was abolished in the presence of 0.1 mmol AMI or DMA ($\Delta \text{pH}_i = 0.01$).

SECOND MESSENGERS INVOLVED IN CCK PEPTIDES INDUCED CYTOPLASMIC ALKALINISATION

To test the possible involvement of second messengers such as diacylglycerol and inositol triphosphate in CCK-peptides induced cytoplasmic alkalinisation, we tested the effect of the tumour promoter tetradecanoyl phorbol acetate (TPA) and that of the Ca^{2+} ionophore A 23187 on pH_i . TPA triggered a significant increase in pH_i (Fig. 4); that was concentration dependent and maximal at $1 \mu\text{M}$ ($\text{EC}_{50} = 30 \text{ nM}$). By contrast the Ca^{2+} ionophore caused a significant decrease in pH_i (Fig. 5) that was maximal within three minutes and was reversed within 10 minutes.

Discussion

Na^+/H^+ antiport has been characterised in pancreatic acinar cells.⁶⁷ Several sources of evidence indicate

Fig. 4 Effect of phorbol 12-myristate, 13-acetate (TPA) on pH_i in guinea pig pancreatic acini. Values are means $\pm$ SEM from 3 separate experiments.

Fig. 5 Effect of ionophore A23187 on pH_i in guinea pig pancreatic acini. Points are average of triplicates.

that Na^+/H^+ antiport is activated by caerulein, NS G_{17} and PG. A very similar concentration dependence has been observed between initial rates of AS. ^{22}Na uptake and the extent of the pH_i rise caused either by caerulein, NS G_{17} or PG. Furthermore AMI and DMA inhibited the increase in pH_i caused

by caerulein. A link between activation of Na^+/H^+ exchange and initiation of growth has been suggested by a number of studies.⁸ Growth factors activate this exchange in quiescent cells so that their cytoplasm becomes more alkaline.² Activation of a quiescent Na^+/H^+ exchange in pancreatic acinar cells by caerulein, NS G17 and PG might play a role in the initiation of pancreatic growth. A question arises as to whether the growth factor/receptor interaction activates the Na^+/H^+ exchange. Calcium does not seem to be involved: (1) Ca^{2+} ionophore caused a decrease in pH_i rather than an increase; (2) TPA, NS G17 and PG do not mobilise intracellular calcium at concentrations effective on the Na^+/H^+ exchange. Activation of protein-kinase C is the more probable mechanism involved in the effect of caerulein. Two models could account for the effect of PG and NS G17. These agents might act *via* the CCK-receptor. In that case postreceptor signalling pathways must determine a degree of coupling with the antiport specific for each peptide. Another hypothesis is that PG and NS G17 might act *via* a specific gastrin receptor utilising additional pathways to activate Na^+/H^+ .

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