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

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SUMMARY Caerulein, gastrin and pentagastrin stimulation of the Na⁺/H⁺ exchange leads to intracellular alkalisation 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 growth is largely unknown. The Na⁺/H⁺ exchange has been suggested to play a central role in the initiation of proliferation induced by growth factors. A Na⁺/H⁺ exchange has been characterised in isolated pancreatic acini. Amiloride sensitive (AS) Na⁺ uptake studies and measurement of intracellular pH (pHi) showed that caerulein, a CCK analogue activates this antiport.

In the present study, we compared the action of caerulein with that of pentagastrin (PG) and non-sulphated gastrin 17 (NSG,17) 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.

AMILORIDE SENSITIVE ²²NA UPTAKE

Before ²²Na⁺ 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 CaCl₂, 1 mmol Mg SO₄, 25 mmol Hepes-Tris pH = 7-4) containing varying concentrations of caerulein, PG or NSG,17 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 μCi/ml ²²NaCl, 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 MgSO₄, 25 mmol Tris medium (pH = 7-4). The AS rate of ²²Na⁺ uptake was defined as the difference between the basal rate of ²²Na⁺ uptake measured in the absence and in the presence of 0-1 mmol amiloride.

[¹⁴C] BENZOIC ACID UPTAKE

Pancreatic acini were first equilibrated for 30 min at 37 °C, pH = 7-4 in a HCO₃⁻ free saline medium (130 mmol NaCl, 5 mmol KCl, 1 mmol CaCl₂, 1 mmol MgCl₂, 5 mmol glucose, 25 mmol Hepes). For pH, measurements, [7-¹⁴C] benzoic acid was added (1 μCi/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 μCi/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-[¹³C]glucose (5 μCi/ml). The intracellular volume was determined to be 0.22±0.02 μl/μg DNA.

CALCULATION OF pH

The counts measured after [¹⁴C] benzoic acid uptake were corrected for radioactivity trapped in the extracellular space and loss of intracellular radioactivity during the washing procedure. Intracellular
pH was calculated with the simplified equation

\[ \text{pH}_i = \text{pH}_o + \log \frac{\text{Ci}}{\text{Co}}, \]

where \( \text{pH}_o \) = external pH, \( \text{Ci} \) = intracellular concentration of benzoic acid in cpm/\( \mu l \), and \( \text{Co} \) = cpm/\( \mu l \) 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-\( ^{14}\)C] benzoic acid, [\( ^{3}\)H] mannitol 3-O-methyl-D-[\( ^{1}\)H] glucose from New England Nuclear (RFA), PG from ICI Ltd (GB) NS G\(_{12}\) 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 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 G17 (EC\(_{50}\) = 1.2 pM) and PG (EC\(_{50}\) = 0.02 nM) were more potent than caerulein (EC\(_{50}\) = 0.2 nM) (Fig. 2). NS G17 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 \( \Delta \) pH\(_i\) of 0.3 pH units after 10–15 min. Alkalination of the cytoplasm by CCK analogues was concentration dependent. Rela-
**CCK-related peptides stimulation of the Na⁺/H⁺ antiport in pancreatic acinar cells**

Na⁺/H⁺ antiport has been characterised in pancreatic acinar cells. Several sources of evidence indicate that Na⁺/H⁺ antiport is activated by caerulein, NS G17 and PG. A very similar concentration dependence has been observed between initial rates of AS Na⁺ uptake and the extent of the pH rise caused either by caerulein, NS G17 or PG. Furthermore AMI and DMA inhibited the increase in pH caused by caerulein activation of the Na⁺/H⁺ exchange was directly responsible for this alkalisation, as caerulein-induced pH rise observed in a 25 mmol external Na⁺ concentration (△pH = 0.22) was abolished in the presence of 0.1 mmol AMI or DMA (△pH = 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 alkalisation, we tested the effect of the tumour promoter tetradecanoyl phorbol acetate (TPA) and that of the Ca²⁺ ionophore A 23187 on pH. TPA triggered a significant increase in pH (Fig. 4); that was concentration dependent and maximal at 1 μM (EC₅₀ = 30 nM). By contrast the Ca²⁺ ionophore caused a significant decrease in pH (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. Several sources of evidence indicate...
by caerulein. A link between activation of Na+/H+ exchange and initiation of growth has been suggested by a number of studies. Growth factors activate this exchange in quiescent cells so that their cytoplasm becomes more alkaline. 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) Ca²⁺ ionophore caused a decrease in pH, 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⁺.

References

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