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 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 (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.

AMILORIDE SENSITIVE Na+ UPTAKE
Before 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 CaCl2, 1 mmol MgSO4, 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 μCi/ml Na22Cl, 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 Na22 uptake was defined as the difference between the basal rate of Na22 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-[1-3H]glucose (5 μCi/ml). The intracellular volume was determined to be 0.22±0.02 μl/μg DNA.

CALCULATION OF pHi
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
\[ \text{pH}_i = \text{pH}_o + \log \frac{C_i}{C_o}, \]
where \( \text{pH}_o \) = external pH, \( C_i \) = intracellular concentration of benzoic acid in cpm/\( \mu l \), and \( C_o \) = cpm/\( \mu l \) in the external medium.

**Materials**
Amiloride (AMI) and dimethylamiloride (DMA) were kindly provided by Dr Paoli (Merck, Sharp, and Dohme France). 22NaCl was purchased from Amersham (GB) [7-14C] benzoic acid, [3H] mannitol 3-O-methyl-D-[1-3H] glucose from New England Nuclear (RFA), PG from ICI Ltd (GB) NS G17 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 PHi WITH [14C] BENZOIC ACID AND REGULATION OF PHi BY EXTRACELLULAR Na**
The accumulation of [14C] 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 [14C] benzoic acid indicates pH 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, pHi 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 pHi fell abruptly to 6.3 and recovered only slowly (Fig. 1). The Na+ dependence for pHi 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 22NA UPTAKE AND PHi**
We have previously shown that caerulein increased the AS 22Na uptake in pancreatic acini.6

The effect of the CCK analogues PG and NSG17 was studied and the stimulation of the AS 22Na uptake by these peptides was concentration dependent. NS G17 (EC50 = 1.2 pM) and PG (EC50 = 0.02 nM) were more potent than caerulein (EC50 = 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 Δ pHi of 0.3 pH units after 10–15 min. Alkalisation of the cytoplasm by CCK analogues was concentration dependent. Rela-
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**Fig. 3**  Effect of caerulein, NS G17, and PG on \(\Delta pHi\) in guinea pig pancreatic acini. (a): Time-course for \(pHi\) in the presence of 1 nM caerulein. Each point is the average of triplicates. (b) Peptide-concentration dependence. Values are means ± SEM from 4 separate experiments.

Effective potencies were: NS G17 (EC\(_{50}\) < 10 pm) < PG (EC\(_{50}\) = 0.1 nM) < caerulein (EC\(_{50}\) = 0.3 nM). Caerulein activation of the Na\(^+\)/H\(^+\) exchange was directly responsible for this alkalinisation, as caerulein-induced \(pHi\) rise observed in a 25 mmol external Na\(^+\) concentration (\(\Delta pHi\) = 0.22) was abolished in the presence of 0.1 mmol AMI or DMA (\(\Delta pHi\) = 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 Ca\(^{2+}\) ionophore A23187 on \(pHi\). TPA triggered a significant increase in \(pHi\) (Fig. 4); that was concentration dependent and maximal at 1 \(\mu\)M (EC\(_{50}\) = 30 nM). By contrast the Ca\(^{2+}\) ionophore caused a significant decrease in \(pHi\) (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.\(^{7}\) 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. \(^{22}\)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 \(pHi\) 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.\(^8\) 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 pHi, 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