The amiloride sensitive Na^+/H^+ antiport in guinea pig pancreatic acini

Characterization and stimulation by caerulein

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Amiloride and analogs decrease the initial rate of $^{22}Na^+$ uptake by dispersed acini from guineapig pancreas in a dose-dependent manner. The initial rate of amiloride-sensitive $^{22}Na^+$ uptake depends on external Na^+ and H^+ concentrations and on internal pH. These results provide evidence for the existence of a Na^+/H^+ antiport in pancreatic acinar cells. Caerulein, a cholecystokinin analog, stimulates the activity of the Na^+/H^+ antiport.

Pancreatic acini *Amiloride* *Na^+/H^+ antiport* *Na^+ influx* *Intracellular pH* *Caerulein*

1. INTRODUCTION

The mechanism by which cellular calcium mobilizing agents such as cholecystokinin and structural analogs stimulate the growth of the exocrine pancreas [1,2] is largely unknown. The electroneutral and amiloride-sensitive Na^+/H^+ exchange has been suggested to play a central role in the initiation of cell proliferation induced by growth factors [3–6]. Here, we provide direct evidence for such an ion-exchange system in dispersed pancreatic acini and its stimulation by caerulein, a cholecystokinin (CCK) analog.

2. MATERIALS AND METHODS

Isolated pancreatic acini were prepared from guinea pig pancreas as described in [7]. Prior to $^{22}Na^+$ uptake, acini were equilibrated for 20 min in a Na^+ free medium (140 mM choline chloride, 5 mM glucose, 5 mM KCl, 0.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM Hepes-Tris, pH 7.4). This procedure was used to reduce the intracellular Na^+ concentration to levels less than 1 mM, and is known to amplify the amiloride-sensitive influx, thus allowing a more accurate analysis of its stimulation [4,6,8–10]. In some experiments, caerulein or 1 μg/ml of nigericin was added in the presence or in the absence of amiloride or analogs. Uptake experiments were performed in a 3 mM Na^+ medium containing 2.6 μCi/ml of $^{22}NaCl$, 0.5 mM ouabain and the same concentration of amiloride or analogs as in the equilibration medium. After 3 min of uptake at 37°C, acini were quickly rinsed by centrifugation (15 s at 0°C) with a 140 mM choline chloride, 1.2 mM $MgSO_4$, 25 mM Tris-HCl (pH 7.4) medium. Experiments in the pH range 6.0–6.5 were performed using a 25 mM Mes-Tris buffer, those between pH 8.0 and 9 using a 25 mM Tris-HCl buffer. When the KCl concentration was increased, the concentration of the choline chloride was decreased accordingly to maintain osmolarity. The amiloride-sensitive rate of $^{22}Na^+$ uptake is defined as the difference be-

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tween the initial rate of $^{22}Na^+$ uptake measured in the absence and in the presence of 0.1 mM amiloride.

High resolution ^{31}P-NMR has been used to measure cellular internal pH (pH_i). pH_i was determined from the pH dependent chemical shifts of the internal orthophosphate peaks [11]. NMR spectra were obtained at a frequency of 101.27 MHz with a Bruker WM 250 spectrometer operating in the Fourier transform mode. In these experiments acini were suspended in a Hepes medium (pH 7.4) [7] containing 115 mM NaCl without added phosphate.

$^{22}NaCl$ was purchased from Amersham (England). Amiloride, dimethylamiloride (DMA), benzamil were kindly provided by M.D. Paoli (Merck, Sharp and Dohme), caerulein by Dr Castiglione (Farmitalia). Other chemicals were obtained from standard commercial sources.

3. RESULTS

Amiloride (0.1 mM) decreased both the initial rate of $^{22}Na^+$ uptake and the steady state level of $^{22}Na^+$ accumulation (fig.1). When nigericin was present during the equilibration period, amiloride-sensitive $^{22}Na^+$ uptake was unchanged. Nigericin is an ionophore that exchanges K^+ for H^+, producing an intracellular acidification leading to activation of the Na^+/H^+ antiport [4,8]. In nigericin-treated cells it has been demonstrated that increasing the external K^+ concentration caused a decrease in internal H^+ concentration by reducing K^+/H^+ exchange [4]. In nigericin-treated acinar cells the rate of amiloride sensitive $^{22}Na^+$ uptake was progressively decreased by increasing the external K^+ concentration (fig.2), indicating that the rate of amiloride sensitive $^{22}Na^+$ uptake was dependent on pH_i. Nigericin was used in all ex-

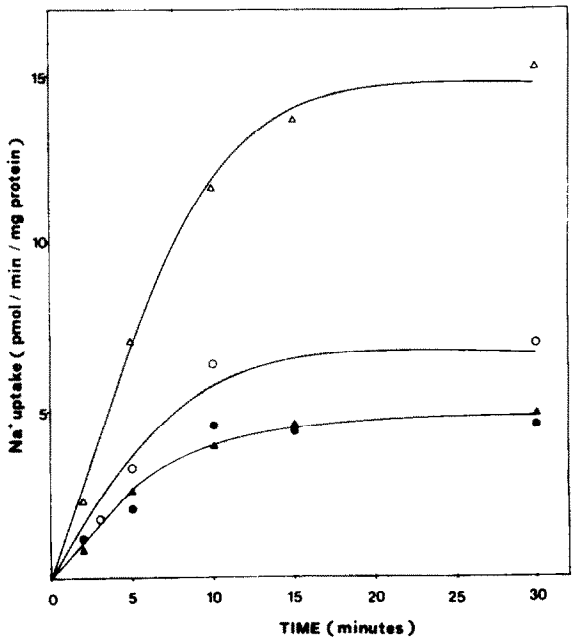

Fig.1. Time course of $^{22}Na^+$ accumulation in pancreatic acini. Acini were preincubated for 20 min under Na^+-depleted conditions in the presence (●, △) or absence (▲, ○) of 1 μg/ml of nigericin, and in the presence (▲, ●) or absence (△, ○) of 0.1 mM amiloride. $^{22}Na^+$ uptake was then followed using a 3 mM Na^+ medium supplemented with the same concentration of amiloride as in the equilibration medium. Values are means from 4 separate experiments.

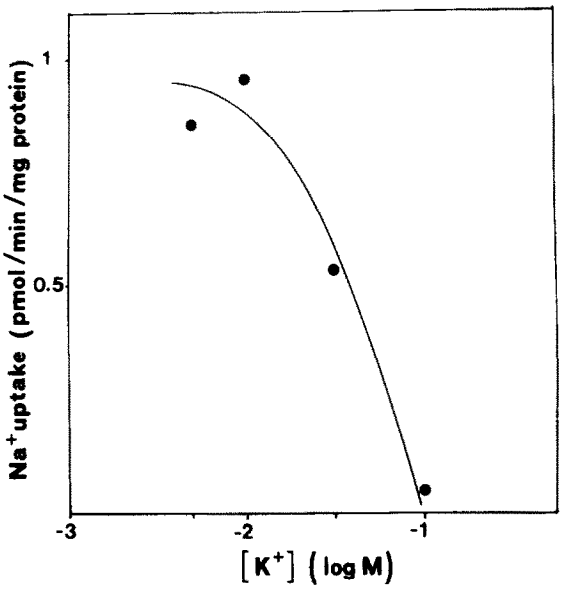

Fig.2. Influence of external K^+ concentration on the initial rate of amiloride-sensitive $^{22}Na^+$ uptake. pH gradients of varying amplitude were imposed by equilibrating acinar cells for 20 min in Na^+-free medium at pH 7.5, supplemented with 1 μg/ml of nigericin and containing various K^+ concentrations. $^{22}Na^+$ uptake was then determined as described in section 2. Amiloride-insensitive $^{22}Na^+$ uptake was not modified and was subtracted from the data. Values are means from 2 experiments.

periments, except in those with caerulein. Amiloride inhibition of initial rate of $^{22}Na^+$ uptake was dose-dependent (fig.3). The half-maximum effect of amiloride inhibition was observed at 4.9 ± 1.1 μM ($K_{0.5}$). Fig.3 also shows the dose-response curves for benzamil and DMA inhibition. $K_{0.5}$ values were 112 ± 25 and 0.25 ± 0.05 μM, respectively. The dependence on external pH (range 6.5–8.5) of the amiloride-sensitive rate of $^{22}Na^+$ uptake was characterized by a titration curve with an apparent pK of 7.57 ± 0.05 (not shown). The rate of amiloride-insensitive Na^+ uptake was not modified by external pH. The initial rate of amiloride-sensitive $^{22}Na^+$ uptake was dependent on the external Na^+ concentration at pH 7.5 and 8.5 (fig.4a). The Lineweaver-Burk representation of the data (fig.4b) indicates that the K_m value for Na^+ (24 ± 1 mM) was not dependent on pH and that the V_{max} value at pH 7.5 was increased by

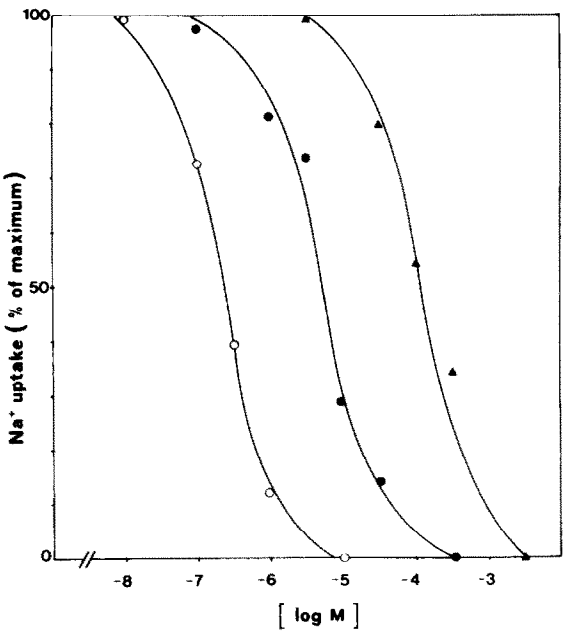

Fig.3. Dose-response curves for benzamil (▲), amiloride (●) and DMA (○) inhibition of the initial rate of $^{22}Na^+$ uptake by acini. Acini were Na^+-depleted in the presence of 1 μg/ml of nigericin. The rate of $^{22}Na^+$ uptake that cannot be inhibited by a saturating concentration of analog (37%) was subtracted from the experimental values. Values are means from 3 separate experiments.

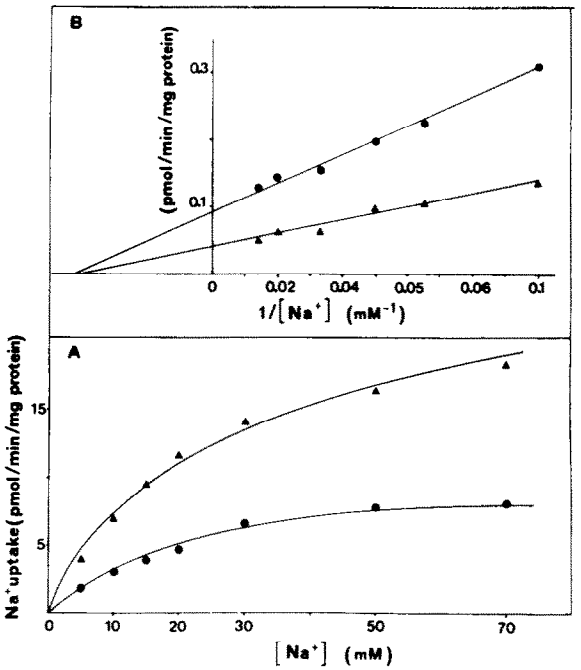

Fig.4. Influence of increasing external Na^+ concentration on the rate of $^{22}Na^+$ uptake. Acini were Na^+-depleted in the presence of 1 μg/ml of nigericin. (A) Dose-response curves for Na^+ activation of the amiloride-sensitive rate of $^{22}Na^+$ uptake determined at external pH of 8.5 (▲) and pH 7.5 (●). The basal rate of $^{22}Na^+$ uptake measured in the presence of 0.1 mM amiloride was subtracted. (B) Lineweaver-Burk plots of the data of panel A. Symbols are the same as for A. Values are means from 3 separate experiments.

2-fold at pH 8.5. Caerulein, a CCK analog, has been shown to increase $^{22}Na^+$ uptake in pancreatic acini [12–14]. Fig.5 shows that caerulein stimulated in a dose-dependent manner the initial rate of amiloride-sensitive $^{22}Na^+$ uptake. The maximal effect was obtained at 1 nM caerulein. The same stimulation was found when caerulein was omitted from the equilibration medium indicating that its effect was not due to a decrease in pH_i during the 20 min preincubation in Na^+-free medium. Finally, we studied the effect of caerulein on pH_i in physiological Na^+ conditions. Caerulein caused pH_i to increase by about 0.2 pH units after 15 min (table 1). In the continuous presence of the peptide pH_i remained elevated for at least 30 min.

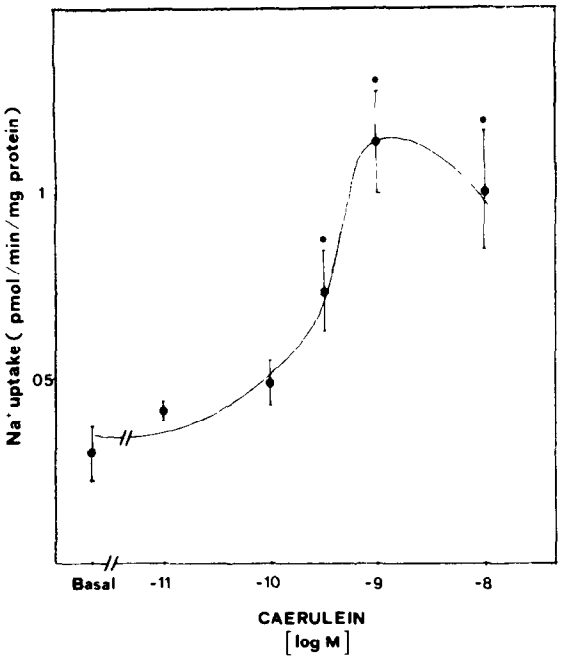

Fig.5. Dose-response relationship for the stimulation of $^{22}Na^+$ uptake by caerulein. Acini were preincubated under Na^+-depleted conditions with increasing concentrations of caerulein, in the presence or absence of 0.1 mM amiloride and without nigericin. Uptake experiments were performed in the presence of the same concentrations of caerulein and amiloride. Amiloride-insensitive $^{22}Na^+$ uptake was not modified and was then subtracted from the data. Values are means ± SE from 3 separate experiments. * Significantly higher ($p < 0.05$) than control values.

Table 1

Effect of caerulein on pH_i

Experimental conditions	pH_i
Basal	7.11 ± 0.06
Caerulein (1 nM)	7.35 ± 0.08

pH_i was determined at 37°C as described in section 2, under basal conditions (without caerulein) and 15 min after caerulein addition. Data are means ± SE from 3 separate experiments

4. DISCUSSION

Our data are the first demonstration for the presence of an amiloride-sensitive Na^+ transporting system in guinea-pig pancreatic acini. This ion-exchanger shares the following properties: (1) Amiloride-sensitive Na^+ uptake is a saturable function of external Na^+ concentration. The K_m value for Na^+ (24 mM) is invariant between pH 7.5 and 8.5 whereas V_{max} increases, indicating that Na^+ recognition by the entry system remains unchanged at this pH range, and that H^+ is a non-competitive inhibitor for Na^+ binding. (2) Amiloride-sensitive Na^+ uptake is pH dependent and an ionizable group with a pK 7.6 is essential for the Na^+ pumping activity. (3) Amiloride-sensitive Na^+ uptake is favored by a decrease in pH_i. (4) The relative potency of amiloride analogs in inhibiting Na^+ uptake at 3 mM Na^+ concentration is DMA ($K_{0.5}$ = 0.25 μM) > amiloride ($K_{0.5}$ = 4.9 μM) > benzamil ($K_{0.5}$ = 112 μM). These results indicate that the amiloride-sensitive Na^+ transporting system we observed in pancreatic acinar cells can be described as a Na^+/H^+ exchange [4,8–10] and confirms the ubiquity of this transport system. Na^+/H^+ exchange is involved in broad areas of cell function including regulation of intracellular pH for initiation of specific cellular events and transepithelial ion transport [15]. In pancreatic acinar cells the Na^+/H^+ antiport which exhibits a slight activity without nigericin or caerulein is significantly activated by these agents. In physiological Na^+ conditions caerulein increased pH_i. Thus, the activation of the Na^+/H^+ exchange by an increase of intracellular H^+ production as a consequence of metabolic activation caused by caerulein might be excluded. There are specific receptors for CCK and analogs in acinar cells from guinea pig pancreas [16]. The dose-response curve for the action of caerulein on amiloride-sensitive Na^+ uptake is closely correlated with those for the action of caerulein on amylase release, Ca^{2+} outflux and inhibition of iodinated CCK binding [16]. This indicates that the action of caerulein on amiloride-sensitive Na^+ uptake is probably receptor mediated. Activation of the Na^+/H^+ antiport by growth factors triggers increase in Na^+ influx and subsequently activation of the Na^+, K^+-ATPase [17,18]. These intracellular events are also involved in the caerulein-stimulated acinar secretions [19]. Finally, our finding that caerulein caused a cytoplasmic alkalinization under physiological Na^+ conditions promised further investigation to assess whether the activation of a Na^+/H^+

antiport might be involved in acinar cell proliferation and/or acinar secretions induced by cacrulein.

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