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Amino acid specificity of the Na^+/alanine cotransporter in pancreatic acinar cells

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The method of tight-seal whole-cell recording was used to study the amino-acid specificity of the Na^+/alanine cotransporter in pancreatic acinar cells. Single cells or small clusters of electrically coupled cells were obtained by enzymatic dissociation of mouse pancreas. Inward currents were measured under 'zero-trans' conditions, i.e., at finite concentrations of Na^+ and amino acid at the extracellular side and vanishing concentrations at the cytoplasmic side. The cotransporter, which corresponds to 'system A', as previously defined in the literature, was found to exhibit a wide tolerance to neutral amino acids (L-cysteine, L-serine, L-alanine, glycine, L-phenylalanine). Competition experiments with 2-methylaminoisobutyric acid (MeAIB) indicate that for glycine a second electrogenic transport system exists in pancreatic acinar cells.

The plasma membrane of pancreatic acinar cells contains a highly active sodium-coupled cotransport system for L-alanine [1–5]. Recent electrophysiological studies using the method of tight-seal whole-cell recording have given evidence that the Na^+/alanine cotransporter, which corresponds to system A in the usual classification, operates with a stoichiometry of 1 Na^+: 1 L-alanine and is capable of sustaining inward and outward currents of up to 30–40 mA/m^2 at 37°C in pancreatic acinar cells [6–8]. In this communication we describe whole-cell recording experiments in which the amino-acid specificity of the cotransporter was studied.

Single cells or small cell clusters were obtained by collagenase treatment of mouse pancreatic tissue, as described previously [6,9]. For the whole-cell recording experiments, the medium was replaced by a solution containing 100 mM (Tris + sodium) sulfate (pH 7.4 at 20°C), 3 mM calcium sulfate (concentration of free Ca^{2+} about 0.6 mM) and various concentrations of amino acids. The medium surrounding the cell at the tip of the glas micropipette could be exchanged by superfusion of the cell within a few seconds. The interior of the pipette was filled with a solution containing 100 mM Tris sulfate (pH 7.4), 10 mM ethylene glycol bis (β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) and 0.91 mM calcium sulfate. Whole-cell recordings were carried out at 37°C with single cells or clusters consisting of 2–5 electrically coupled cells [6].

Short-circuit inward currents were measured under 'zero-trans' conditions, i.e., with finite concentrations of Na^+ and amino acid on the extracellular side and vanishing concentrations on the cytoplasmic side (Fig. 1). An example is shown in Fig. 2. Under symmetrical conditions at the start of the experiment (vanishing concentrations of Na^+ and amino acid on both sides) the transmembrane current I is zero. When the external medium is replaced by a solution containing 100 mM Na^+, a small inward current is observed which is likely to result from leakage pathways. A large inward current is recorded in the presence of both Na^+ and amino acid in the extracellular medium. The inward current I'' corresponding to the coupled transport of Na^+ and amino acid S is taken as the difference of the current amplitudes in the presence and in the absence of S (Fig. 2). When the external medium is exchanged for an amino acid and Na^+-free solution, the current returns to nearly zero.

At the end of each experiment, the inward current in the presence of 100 mM Na^+ and 20 mM L-alanine at the extracellular side was measured as a reference value. In this way experiments carried out with cells or cell clusters of different membrane areas could be compared to each other. The absolute value of the current density I_{st} in the standard experiment with L-alanine ($c'_N = c'_S$

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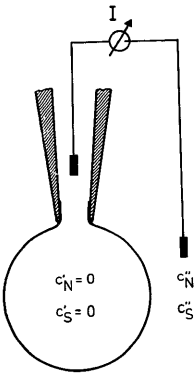

Fig. 1. Tight-seal whole-cell recording under 'zero-trans' conditions. c'_N and c'_S are the cytoplasmic concentrations of Na^+ and amino acid, respectively. c''_N and c''_S refer to the extracellular side. The transmembrane current I induced by gradients of Na^+ and amino acid was measured at vanishing membrane potential.

$= 0$, $c''_N = 100$ mM, $c''_S = 20$ mM) was obtained by determining the membrane area from the electrical capacitance of the membrane, as described previously [6]. I_{st} was found to vary by less than 20% in a series of experiments with 10 different cells and cell clusters. Assuming a specific membrane capacitance of 1 $\mu F/cm^2$, the average value of I_{st} was determined to be 24.1 mA/m^2.

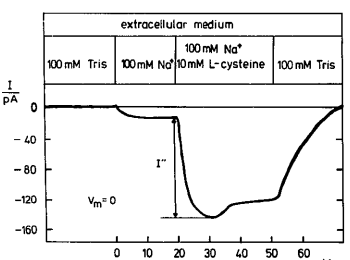

Fig. 2. Inward current I at vanishing cytoplasmic concentrations of Na^+ and amino acid and vanishing transmembrane voltage V_m. The current I'' associated with coupled transport of Na^+ and L-cysteine is taken as the difference of the current amplitudes in the presence and in the absence of S. $T = 37°C$. The current trace has been redrawn from the original record.

TABLE I

Inward currents I'' under 'zero-trans' conditions ($c'_N = c'_S = 0$, $c''_N = 100$ mM, $c''_S = 20$ mM) at vanishing transmembrane voltage

$T = 37°C$. The values represent averages from 2–10 separate experiments. MeAIB, 2-methylaminoisobutyric acid.

Amino acid	I'' (mA/m^2)
L-Cysteine	30
L-Serine	26
L-Alanine	24
Glycine	17
L-Histidine	13
MeAIB	10
L-Phenylalanine	6
L-Glutamate	4
L-Lysine	≤1
β-Alanine	1–2

Table I summarizes inward currents I'' observed with different amino acids under 'zero-trans' conditions with $c''_N = 100$ mM and $c''_S = 20$ mM. I'' was obtained from the ratio of the currents measured with the given amino acid and with L-alanine, together with $I_{st} = 24.1$ mA/m^2. Large inward currents were observed for L-cysteine, L-serine, L-alanine, glycine and L-histidine. L-Phenylalanine which has a bulky side chain exhibited a 4-times smaller transport rate than L-alanine. With the charged amino acids L-glutamate and L-lysine the currents were close to or below the detection limit. In these two cases it is possible that the zwitterionic form of the amino acid which is present in low concentration in the solution at pH 7.3 is responsible for the (marginal) current.

Mammalian cells are known to contain several different transport systems for amino acids [1,2]. The electrogenic Na^+/L-alanine cotransport system in pancreatic acinar cells was previously shown to correspond to 'system A' [2,10] by its ability to accept N-methylated amino acids such as 2-methylaminoisobutyric acid (MeAIB). In order to test for competition between MeAIB and the amino acid under study, we have carried out the following experiment. First, a nearly saturating concentration of MeAIB (20 mM) was added together with 100 mM Na^+ to the extracellular medium under 'zero-trans' conditions. This resulted in a large inward current. Then the amino acid under study was added to the medium (in addition to MeAIB) in a concentration which normally leads to an easily detectable current. When MeAIB and the second amino acid compete for the electrogenic cotransporter, addition of the second amino acid should not give rise to a further current increase. Except for glycine, competition was observed for all amino acids tested, i.e., for L-cysteine, L-serine, L-alanine, and L-phenylalanine. This indicates

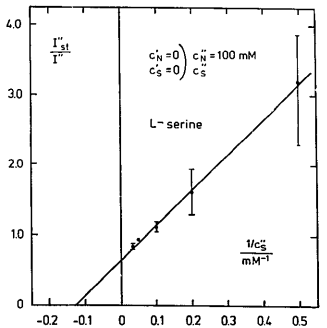

Fig. 3. Inward current I'' as a function of extracellular concentration c''_S of L-serine under 'zero-trans' conditions. The values of I'' are referred to the current I''_{st} observed in the standard experiment with L-alanine, as described in the text.

that these amino acids are transported by system A. On the other hand, the current increase which was normally observed after addition of 2 mM glycine could be inhibited by only about 50% by 20 mM MeAIB. It is therefore likely that a second electrogenic transport system for glycine exists in pancreatic acinar cells.

The dependence of inward current I'' on extracellular concentration c''_S of L-serine is represented in Fig. 3. Using the notation of Ref. 7, I'' can be described by a Michaelis-Menten relation of the form

$$I'' = I''_{\infty S}\frac{c''_S}{c''_S + L''_S} \qquad (1)$$

This corresponds to a linear relationship between $1/I''$ and $1/c''_S$ in the double-reciprocal plot of Fig. 3. Values of the half-saturation concentration L''_S and of the maximal current $I''_{\infty S}$ which were determined from Fig. 3 are given in Table II. Table II also summarizes the results obtained for L-cysteine and glycine. For L-histidine, MeAIB, L-phenylalanine, L-glutamate and β-alanine (Table I), the concentration dependence of inward currents could not be measured with sufficient accuracy to allow the evaluation of L''_S and $I''_{\infty S}$. The dependence of $1/I''$ on $1/c''_S$ for glycine is shown in Fig. 4. It is seen from Fig. 4 that the experimental results tend to deviate from a linear relationship between $1/I''$ and $1/c''_S$. This again indicates that glycine is transported by a second electrogenic system. Accordingly, the values of L''_S and $I''_{\infty S}$ for glycine given in Table II should be considered as apparent values.

The results obtained in this study may be compared with previous observations. In experiments with rat

TABLE II

Half-saturation concentrations L''_S and maximal inward current $I''_{\infty S}$ measured under 'zero-trans' conditions ($c'_N = c'_S = 0$, $c''_N = 100$ mM) at 37°C

Transmembrane voltage $V_m = 0$.

Amino acid	L''_S (mM)	$I''_{\infty S}$ (mA/m²)	Notes
L-Serine	7.9	38	this study
L-Alanine	2.9	30	Ref. 5
L-Glycine	2.7	17	this study [a]
L-Cysteine	2.3	33	this study [b]

[a] Since glycine is likely to be transported by two different electrogenic systems (see text), the values of L''_S and $I''_{\infty S}$ should be considered as apparent values.

[b] Tentative values, based on incomplete data set.

pancreas, a variety of amino acids (L-serine, L-cysteine, glycine, L-phenylalanine, L-glutamine, L-lysine, L-glutamate) was found to inhibit the influx of radioactively labelled MeAIB, indicating that these amino acids are substrates of system A [10]. A similarly wide tolerance of system A has been described for hepatocytes and hepatoma cells [12,13].

Our finding that glycine seems to be transported by an additional electrogenic system different from system A in pancreatic acinar cells parallels similar conclusions derived from studies with other cell types. A sodium-de-

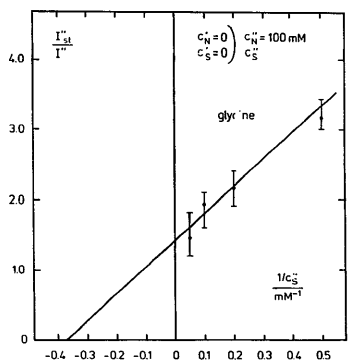

Fig. 4. Inward current I'' (referred to the current I''_{st} observed in the standard experiment with L-alanine) as a function of extracellular concentration c''_S of glycine under 'zero-trans' conditions. The other experimental conditions were the same as in Fig. 3.

pendent MeAIB-insensitive component of glycine uptake has been observed with hepatoma cells [14]. In hepatoma cells [14] as well as in erythrocytes [15], the transport rate of this system ('system Gly') was found to be proportional to the square of extracellular sodium concentration, indicating that system 'Gly' operates with a 2 Na^+: 1 amino acid stoichiometry.

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