Biochimica et Biophysica Acta, 692 (1982) 157–160
Elsevier Biomedical Press

BBA Report

BBA 70064

UNIDIRECTIONAL UPTAKE OF L-ALANINE AND L-SERINE BY SYSTEM ASC IN THE BASOLATERAL MEMBRANE OF CAT SALIVARY EPITHELIUM

SELECTIVE INHIBITION OF RAPID ALANINE METABOLISM BY AMINOOXYACETATE

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(Received May 29th, 1982)

Key words: Amino acid uptake; Alanine transport; Serine transport; Aminooxyacetate; (Cat salivary gland)

Unidirectional influx of L-alanine and L-serine across the basolateral membrane of cat salivary epithelium was investigated in perfused submandibular glands using a rapid (<30 s) paired-tracer dilution technique. (Bustamante, J.C., Mann, G.E. and Yudilevich, D.L. (1981) J. Physiol. 313, 65–79). High uptakes were measured for both neutral amino acids, however, only the uptake of L-alanine was followed by an immediate tracer efflux into the circulation. Aminooxyacetate, an inhibitor of alanine aminotransferase, completely inhibited this efflux and glandular pyruvate production. These results demonstrate that in assessing unidirectional amino acid uptake, the influence of metabolism should be considered even during short intervals of measurement.

Detailed studies of amino acid transport and accumulation in exocrine organs have been performed in the pancreas in vitro [2], however, little such information is available for the salivary epithelium [3]. It has recently been possible to characterize the specificity and kinetics of parallel transport systems for neutral amino acids at the basolateral membrane of the epithelium in the perfused salivary gland [4] and pancreas [5]. Cross-inhibition experiments of tracer amino acid uptake in the pancreas [5] have revealed a much lesser degree of system specificity for short- and long-chain neutral amino acids than that observed in the salivary gland [1,4]. We have previously shown that in the salivary epithelium short-chain neutral amino acids are transported by an Na^+-dependent, stereospecific and high-affinity ASC-type [6] system, whereas long-chain neutral amino acids such as leucine, phenylalanine and tryptophan are transported by an Na^+-independent system for which alanine exhibited a low affinity [1,4].

Among the limitations of studies on amino acid transport in vitro has been the difficulty of distinguishing transport phenomena at either the basolateral or luminal poles of the cell and separating unidirectional transport from intracellular metabolic conversions. The use of plasma membrane vesicles or parenchymal cells isolated from tissues such as the pancreas [7] and liver [8–11] has permitted some transport processes to be studied under more well-defined conditions. However, it is now well documented that even isolated hepatocytes readily metabolize alanine and that this process can be blocked by aminooxyacetate [8–11], an inhibitor of pyridoxal phosphate-dependent enzymes such as alanine aminotransferase [12–14].

In view of these reports and the fact that alanine is rapidly metabolized during its passage from plasma to saliva [15], we have re-examined the

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unidirectional uptake of L-alanine and L-serine by the epithelium of the perfused salivary gland under conditions where alanine aminotransferase was inhibited by aminooxyacetate. Ten cats, of either sex, were fasted 12–24 h before being anaesthetized with i.p. sodium pentobarbitone (35 mg/kg). After the arterial supply and the venous drainage of the submandibular gland was isolated, the gland was perfused in situ at a constant flow rate (about 1 $ml \cdot min^{-1} \cdot g^{-1}$) via the carotid artery with an amino acid-free Krebs-Ringer solution containing 10 $g \cdot l^{-1}$ bovine albumin [1,16]. The perfusate was oxygenated at 38°C with 95% O_2/5% CO_2 to a pH between 7.3 and 7.4. Each gland was perfused successively with a 'control' solution and one containing 2.5 $mmol \cdot l^{-1}$ aminooxyacetate (Sigma Chemical Co., U.K.). The radioactive substances, L-[2,3-^{3}H]alanine (37 Ci/mmol), L-[3-^{3}H]alanine (82.7 Ci/mmol), L-[3-^{3}H]serine (28 Ci/mmol) and D-[1-^{14}C]mannitol (45 mCi/mmol), were obtained from either New England Nuclear Chemicals, Dreieich, F.R.G., or Amersham International, U.K.

Epithelial uptake of these two neutral amino acids was measured using a single circulation, paired-tracer dilution technique which has previously been described in detail [1]. Essentially a rapid bolus arterial injection of a tritiated amino acid and D-[1-^{14}C]mannitol, an extracellular tracer in the salivary gland [1,4], was immediately followed by sequential sampling of the glandular venous effluent. Fig. 1A illustrates simultaneous dilution profiles obtained for L-[3-^{3}H]alanine and D-[^{14}C]mannitol in an isolated gland perfused with a 'control' amino acid-free Ringer solution. It is apparent that the concentration-time curve for L-[^{3}H]alanine lies below that of the extracellular tracer, D-[^{14}C]mannitol. Since the fenestrated salivary capillaries are highly permeable to small solutes [16] and D-mannitol has previously been used as an index of passive diffusion [1], the initial difference in these normalized tracer profiles re-

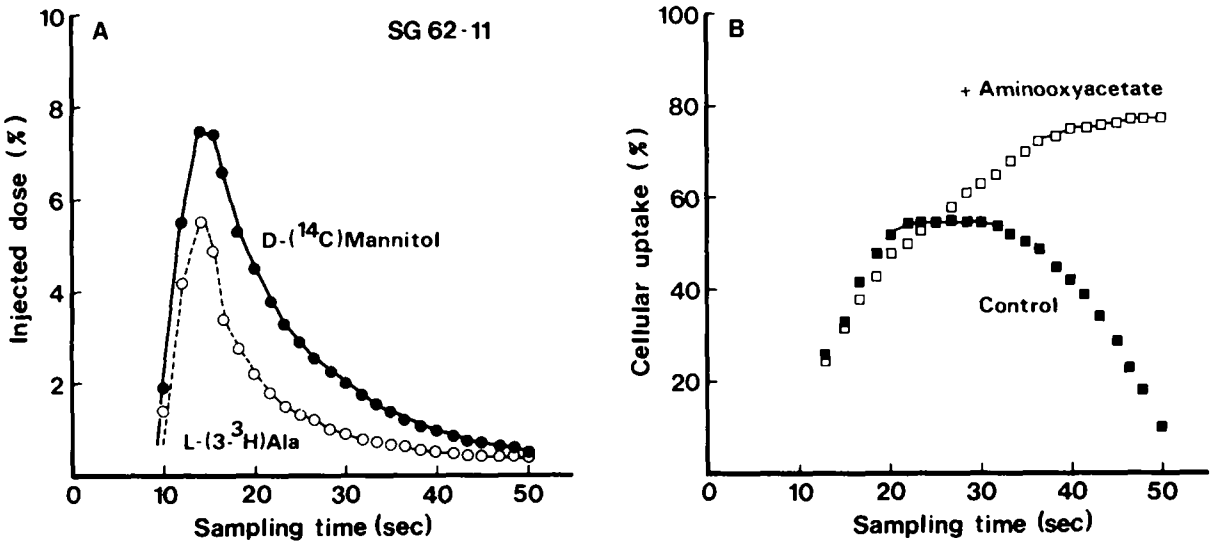

Fig. 1. Effect of aminooxyacetate on the unidirectional uptake of L-alanine at the basolateral membrane of cat salivary epithelium. A. Single circulation, paired-tracer dilution profiles for D-[1-^{14}C]mannitol (extracellular reference) and L-[3-^{3}H]alanine in a gland perfused at 1.4 $ml \cdot min^{-1} \cdot g^{-1}$. A 100 µl bolus of this tracer mixture was rapidly (1–2 s) injected via the lingual artery into the perfusate supplying the gland. Following the tracer injection, 30 successive samples (approx. 100 µl) were immediately collected from the cannulated external jugular vein. A final 4-min sample was accumulated for assessing tracer efflux and total recovery (data not shown). Tracer concentrations have been normalized with respect to the radioactive doses injected and plotted against the time of sampling. Glands were perfused successively in the absence or presence of 2.5 mmol/l aminooxyacetate with an amino acid-free Krebs-Ringer solution containing 11.1 mmol/l D-glucose. B. Cellular uptake of L-[3-^{3}H]alanine was estimated in successive samples and plotted against the time of collection. The maximal uptake (U_{max}) was calculated in each case from the average of the joined data points.

flects uptake of alanine at the basolateral side of the epithelium. Cellular uptake was calculated using the expression:

$$\text{uptake } \% = \left[1 - (\text{L-}[^3\text{H}]\text{alanine}/\text{D-}[^{14}\text{C}]\text{mannitol})\right] \times 100$$

When transport of L-[^{3}H]alanine was measured in the absence of aminooxyacetate the time course of uptake (Fig. 1B) revealed a maximal value, U_{max}, 10 s after the appearance of the tracers in the glandular venous effluent. This maximal uptake remained constant for a further 15 s and was followed by a rapid decrease due to the efflux of tracer from the epithelium into the circulation. When the same preparation was perfused with 2.5 mmol · l^{-1} aminooxyacetate only the maximal uptake for L-[^{3}H]alanine was significantly higher and tracer efflux was not detectable during the 50 s of sampling. Table I summarizes the effects of aminooxyacetate on the maximal tracer uptake of alanine and serine and the respective tracer effluxes across the basolateral membrane of the epithelium. In the case of both L-[2,3-^{3}H]alanine and L-[3-^{3}H]alanine, aminooxyacetate increased the maximal uptake by about 34% and markedly reduced the tracer efflux. In contrast, serine, which as alanine is transported by System ASC [1], was not affected by aminooxyacetate. The high maximal uptake as well as the low tracer efflux remained unchanged (Table I).

In parallel experiments, we analyzed the venous effluent for the amount of ^{3}H-activity still associated with the native amino acid using thin-layer chromatography (Berthold LB 2760 Scanner). Following the arterial injection of L-[2,3-^{3}H]alanine (50 μCi in 100 μl amino acid-free perfusate), the effluent was collected in three pooled samples over a 300 s interval: 0–30 s, 30–60 s, 60–300 s. 1-ml aliquots were precipitated with 4 ml absolute ethanol, and the supernatant was evaporated to dryness and resuspended in 100 μl 96% ethanol. The samples were then eluted on 0.1 mm cellulose-coated TLC plastic sheets (Merck 5577, BDH Chemicals, U.K.) with *n*-butanol/acetic acid/water (12:3:5).

In the second sample only 60 ± 11% (mean ± S.E., $n = 3$) of the ^{3}H-activity was still associated with native alanine and in the third sample this recovery was reduced to 36 ± 11%. Addition of aminooxyacetate to the perfusate completely inhibited the production of these metabolites. Similar results were obtained with L-[3-^{3}H]alanine. Further biochemical experiments were performed to demonstrate inhibition of alanine aminotransferase. Our results indicated that 2.5 mmol · l^{-1} aminooxyacetate decreased the glandular production of pyruvate by 80%, whereas venous concentrations of lactate remained unaffected by aminooxyacetate (Table II).

The results presented here show that measurements of unidirectional L-alanine uptake via System ASC are influenced by the rapid efflux of

TABLE I

EFFECTS OF AMINOOXYACETATE ON THE UNIDIRECTIONAL UPTAKE OF SHORT-CHAIN NEUTRAL AMINO ACIDS AND EFFLUX OF THEIR LABELLED METABOLITES ACROSS THE BASOLATERAL MEMBRANE OF SALIVARY EPITHELIUM

Glands were initially perfused with an amino acid-free 'control' Ringer solution and subsequently with one containing 2.5 mmol · l^{-1} aminooxyacetate (AOA). In these experiments D-glucose (11.1 mmol · l^{-1}) was the only substrate present. Tracer efflux was estimated from $(1 - (U_T / U_{max})) \times 100$. The overall amino acid uptake (U_T) was calculated from the integrated tracer recoveries starting from the time of maximal uptake and including the final 4-min sample. Values are given as mean ± S.E., n = number of cats.

	n	Maximal uptake (U_{max})		% Efflux	
		Control	+2.5 mM AOA	Control	+2.5 mM AOA
L-[2,3-^{3}H]Alanine	3	55 ± 1	74 ± 1	108 ± 26	36 ± 10
L-[3-^{3}H]Alanine	4	51 ± 2	68 ± 3	101 ± 13	33 ± 4
L-[3-^{3}H]Serine	3	82 ± 1	81 ± 1	22 ± 1	23 ± 5

TABLE II

EFFECTS OF AMINOOXYACETATE ON THE CONCENTRATIONS OF PYRUVATE AND LACTATE IN THE VENOUS EFFLUENT OF THE CAT SUBMANDIBULAR GLAND

As specific assays for pyruvic and lactic acids have been described [17], biochemical determinations (Sigma Assays No. 726-UV, 826-UV) were performed using the effluent collected from glands perfused, successively in the absence or presence of 2.5 mmol · l^{-1} aminooxyacetate. Both the control and aminooxyacetate perfusates contained D-glucose (11.1 mmol · l^{-1}) and L-alanine (0–5 mmol · l^{-1}) as substrates. Values are given as mean ± S.E. (n) = number of measurements in a total of six animals.

	Pyruvic acid (μmol · l^{-1})	Lactic acid (μmol · l^{-1})
Control	39 ± 5 (15)	720 ± 130 (17)
Aminooxyacetate	8 ± 2 (18)	620 ± 120 (24)

labelled metabolites. Thus, at tracer concentrations or low perfusate concentrations of unlabelled alanine the prominent and rapid efflux of tritiated metabolites results in an underestimate of the maximal cellular uptake (Table I). Aminooxyacetate appeared to inhibit only the metabolism of alanine, since there was no change in the uptake or efflux measured for L-serine. This observation contrasts with findings in the liver, where serine metabolism is catalyzed by a pyridoxal phosphate-dependent enzyme sensitive to aminooxyacetate [9]. Chromatographic identification of the tritiated metabolites was not attempted; however, based on extensive studies with hepatocytes [8–14], it seems likely that in the absence of aminooxyacetate, alanine was transaminated to pyruvate. Our biochemical results provide further support for this hypothesis, since aminooxyacetate caused a 5-fold decrease in the steady-state glandular pyruvate production (Table II). It is interesting that transaminases have been suggested to be responsible for the initial metabolism of some amino acids in dog parotid and submandibular glands [15].

The present study reveals that alanine and serine, which in most other tissues share similar transport systems, may enter different metabolic pathways in the salivary epithelium. Furthermore, the measurement of unidirectional influx for different amino acids even with methods of high time resolution may selectively be influenced by metabolism.

We gratefully acknowledge research grants from the Medical Research Council, U.K. and the Cystic Fibrosis Research Trust, U.K. Mr. S.J. Peachell provided skilled technical assistance in these experiments.

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