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SPECIFICITY OF NEUTRAL AMINO ACID UPTAKE AT THE BASOLATERAL SIDE OF THE EPITHELIUM IN THE CAT SALIVARY GLAND *IN SITU*

BY J. C. BUSTAMANTE*, G. E. MANN AND D. L. YUDILEVICH†

From the Department of Physiology, Queen Elizabeth College, University of London, Campden Hill Road, London, W8 7AH

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SUMMARY

1. Amino acid uptake was measured in resting cat submandibular glands with either a natural blood supply or perfused at constant flow with a Krebs-albumin solution. Following a bolus arterial injection of a ^{3}H-labelled amino acid and D-[^{14}C]mannitol (extracellular reference tracer), the venous effluent was immediately sampled sequentially. The maximal uptake, U_{max}, from the blood or perfusate was determined from the paired-tracer dilution curves using the expression: uptake % = $\{1-(^{3}H/^{14}C)\times 100\}$.

2. In glands with a natural blood supply, U_{max} values up to 46 % were measured for short-chain (serine and alanine) and long-chain (valine, methionine, leucine, isoleucine, 1-amino-cyclopentane carboxylic acid, phenylalanine, tryptophan, tyrosine, histidine and glutamine) neutral amino acids. In contrast, U_{max} was neglilible for amino acids of the imino-glycine group (proline and glycine) and the non-metabolized amino acids, 2-aminoisobutyric acid (AIB) and methylaminoisobutyric acid (MeAIB).

3. In glands with a natural blood supply addition of an unlabelled amino acid to the tracer injectate reduced U_{max} for the test amino acid by up to 80 %. The pattern of these interactions suggested the presence of two transport systems for neutral amino acids, one preferring short-chain and the other long-chain amino acids.

4. In glands perfused at constant flow rates with an amino acid-free Krebs-albumin solution high U_{max} values were measured: L-serine (66 %), L-alanine (54 %), L-leucine (43 %), L-phenylalanine (42 %) and L-tyrosine (51 %). Only a low uptake was observed for L-proline (8 %) and glycine (14 %). There was no uptake of methylaminoisobutyric acid which confirms the result obtained in glands with an intact circulation.

5. Saturation of L-phenylalanine influx was observed in perfused glands as the perfusate concentration of unlabelled L-phenylalanine was increased from 0·5 to 20 mmol.l^{-1}. A Michaelis–Menten analysis based on a single entry system indicated an apparent K_m of 6·4 ± 0·8 mmol.l^{-1} and a V_{max} of 1719 ± 94 nmol.$min^{-1}g.^{-1}$

6. Since the fenestrated capillaries in the salivary gland are readily permeable to the test amino acid and D-mannitol, it is most probable that the amino acid carriers are located in the basolateral side of the epithelium.

* World University Service Studentship.

† All correspondence and reprint requests: Professor D. L. Yudilevich, Department of Physiology, Queen Elizabeth College, University of London, Campden Hill Road, London, W8 7AH.

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7. The use of a paired-tracer dilution technique to measure uptake in a single circulatory passage has enabled a detailed characterization of neutral amino acid transport in the salivary gland and has overcome the limitation of previous studies based on solute transfer from blood to saliva.

INTRODUCTION

The permeability of the salivary gland epithelium has previously been studied by measuring the apearance of tracer in saliva after injecting labelled solutes into the carotid artery during parasympathetic nerve stimulation (see reviews of Burgen, 1967; Young & van Lennep, 1979). The latency of solute appearance in the volume of saliva secreted was used to assess whether transfer occurred across the acinar or ductal epithelium (Burgen & Terroux, 1962). When Lindsay, Catanzaro, Ueha & Hanson (1969) examined the appearance time of amino acids in dog parotid saliva using Burgen's technique, they concluded that all amino acids were transferred across the ductal epithelium. The presence of amino acids in saliva may reflect both paracellular and transcellular passage. However, the absence of a specific amino acid in saliva does not exclude the possibility that uptake has occurred at the blood-side of the epithelium. Some amino acids may simply enter the various metabolic cycles within the tissue (Martin, 1967; Lindsay, Catanzaro & Hanson, 1969).

There is little information on amino acid uptake from plasma into cells in the resting salivary gland *in vivo* and even studies with slices or isolated cells as recently reviewed by Young & van Lennep (1979) are scarce. Van Venrooij, Kuijper-Lenstra & Kramer (1973) demonstrated that the uptake of radioactive leucine from the incubation medium into fragments of rat submandibular gland was followed by rapid incorporation into cell proteins. Their results suggest that the source of amino acids to be utilized in protein synthesis is the extracellular rather than the intracellular pool of amino acids.

The present experiments were designed to measure blood-tissue uptake of amino acids at the basolateral side of the epithelium in the cat submandibular gland. We used a single-circulation, paired-tracer dilution technique which involves an intra-arterial bolus injection of a substrate and a reference extracellular marker of similar size and diffusibility. The method is similar to that used to determine the specificity and kinetics of sugar and amino acid transport in the brain (Yudilevich, de Rose & Sepúlveda, 1972; Betz, Gilboe, Yudilevich & Drewes, 1973; Betz, Gilboe & Drewes, 1975) and placenta (Yudilevich, Eaton, Short & Leichtweiss, 1979*a*; Yudilevich & Eaton, 1980).

Cellular uptake of amino acids in glands with a natural blood supply was compared with measurements made in glands perfused at constant flow with an amino acid-free Krebs solution. Unidirectional uptake of short- and long-chain neutral amino acids was observed in both preparations and cross-inhibition tests of uptake suggested the presence of two transport systems with differing degrees of overlap. Furthermore, successive perfusion of glands *in situ* with different concentrations of unlabelled L-phenylalanine resulted in saturation kinetics from which Michaelis–Menten parameters could be estimated.

Preliminary communications of this work have been published (Bustamante, Mann & Yudilevich, 1978, 1979; Yudilevich, Sepúlveda, Bustamante & Mann, 1979*b*; Mann, Rudolphy-Bravo & Yudilevich, 1980).

METHODS

Cats of either sex weighing between 2 and 5 kg were fasted for 12–24 h before being anaesthetized with I.P. sodium pentobarbitone (35 mg/kg, Sagatal). Supplementary anaesthetic was given via an I.V. cannula. The animal's temperature was maintained at 37–38 °C using a heating blanket. The right femoral artery was cannulated to monitor blood pressure which was displayed on a Devices M19 four channel pen recorder together with the glandular venous outflow and saliva flow.

Isolation of the submandibular gland

All branches of the carotid artery were ligated except the submandibular and lingual arteries. The latter was cannulated with a short length of 1·02 mm o.d. nylon tubing for the isotope injections. All veins draining into the external jugular vein were also tied except for the branch draining the gland. The jugular vein was cannulated after injecting heparin-saline (Pularin 1000 i.u./kg) and the outflow led by PVC tubing to a photo-electric drop recorder (see Mann, Smaje & Yudilevich, 1979*b*). In experiments performed *in vivo* the natural blood supply to the gland was maintained via the submandibular artery. In other experiments glands were perfused *in situ* at constant flow with a Krebs-albumin solution through a 'red' nylon Portex cannula (1·65 mm o.d.) in the peripheral end of the common carotid artery (Mann *et al.* 1979*b*).

The right submandibular duct was cannulated retrogradely for the collection of saliva during stimulation of the parasympathetic (chorda lingual) nerve. This nerve was dissected and the distal end stimulated using a shielded electrode with square waves of 2 msec duration and supramaximal voltage (5–10 V) at 8 Hz. The salivation rate was used as an index of the gland's physiological state between different experimental runs which were performed in glands at rest.

Perfusate

The perfusate had the following composition (mmol/l): NaCl, 118; KCl, 4·8; KH_2PO_4, 1·2; $MgSO_4.7H_2O$, 1·2; $NaHCO_3$, 25; $CaCl_2$, 2·5; and D-glucose, 11·1. Bovine serum albumin (Cohn Fraction V, Sigma Chemical Company, Poole, U.K.) was added to make a final concentration of 10 g/l. The perfusate was oxygenated at 38 °C with 95% O_2/5% CO_2 to a pH between 7·3–7·4. The osmolality ranged between 290 and 293 m-osmol.

Paired-tracer dilution experiment

A 100 μl bolus of a given isotope mixture was injected in 1–3 s retrogradely into the lingual artery. Two drops of blood or perfusate (approximately 100 μl) were immediately collected successively in each of fifteen to thirty tubes over a period of 1–5 min. The injectates were normally 0·9% saline solutions containing 2·8 μCi D-[^{14}C]mannitol (extracellular reference tracer) and 14 μCi of a given ^{3}H-labelled amino acid. When the amino acid was labelled with [^{14}C], D-[^{3}H]mannitol was used.

Radioactively labelled substances

The labelled amino acids used in the present study are listed in Table 1. D-[1-^{3}H]mannitol, 2-aminoisobutyric[Me-^{3}H] acid and [1-^{14}C]methylaminoisobutyric acid were obtained from New England Nuclear Chemicals GmBH, D6072 Dreieich, West Germany. All other labelled amino acids and D-[1-^{14}C]mannitol (mol. wt. 182) were purchased from the Radiochemical Centre, Amersham, U.K.

Addition of an inhibitor amino acid to injectates and perfusates

When inhibition of tracer uptake was tested in glands with a natural blood supply, the isotope injectates were prepared with solutions containing a specific unlabelled amino acid at a final concentration of 78 mmol.l^{-1}. L-tryptophan could only be dissolved at 39 mmol.l^{-1}. When the kinetics of phenylalanine influx were measured in perfused glands an equal concentration of unlabelled L-phenylalanine was present in the injectates and perfusates.

Unlabelled amino acids

All inhibitors added to the isotope injectate were L-amino acids, except for D-alanine. Alanine, serine, norvaline, α-amino-*n*-butyric acid and 2,4-diamino-*n*-butyric acid were purchased from Sigma Chemical Company, Poole, U.K.; lysine monohydrochloride, arginine, leucine, methionine and cysteine hydrochloride from BDH Chemicals Ltd, Poole, U.K.; tryptophan from Hopkin and Williams, Romford, U.K.; D-mannitol from Fisons, Loughborough, U.K.

Preparation of samples for liquid scintillation counting

All of the collected effluent samples, injectate mixture standards and ^{3}H and ^{14}C channel standards were prepared for liquid scintillation counting by addition of 4 ml ethanol and 10 ml toluene containing 4 g.l^{-1} PPO and 100 mg.l^{-1} POPOP (Packard). When whole blood samples were collected the tubes were centrifuged after adding ethanol and only the supernatant was counted. Negligible counts were recovered in the precipitate. Samples and standards were counted concurrently in a Packard 2425 Liquid Scintillation Spectrometer using external standardization. As little variation in the external standard ratio occurred, individual quench corrections were not made. Appropriate corrections were made for background and the overlap of ^{14}C into the ^{3}H channel. Calculations were performed using a BASIC program in a ICL Modular 1 digital computer.

TABLE 1. The position of radioactive label within the molecule, specific activity, molecular weight and abbreviated nomenclature for each amino acid studied

Amino acid	Specific activity (Ci/mmol)	Mol. wt.	Abbreviated nomenclature
L-(2,3-^{3}H)alanine	37	89	L-Ala
L-(3-^{3}H)serine	19	105	L-Ser
L-(2(n)-^{3}H)methionine	2·9	149	L-Met
L-(3,4(n)-^{3}H)valine	31	119	L-Val
L-(4,5-^{3}H)leucine	53	135	L-Leu
L-(4,5-^{3}H)isoleucine	98	131	L-Ile
1-aminocyclopentane-(^{14}C) -carboxylic acid	59×10^{-3}	131	CycloLew
L-(4-^{3}H)phenylalanine	27	165	L-Phe
L-(G-^{3}H)tryptophan	8·8	204	L-Trp
L-(3,5-^{3}H)tyrosine	45	184	L-Tyr
L-(2,5-^{3}H)histidine	43	155	L-His
(2-^{3}H)glycine	21	75	L-Gly
L-(3,4-^{3}H)proline	60	115	L-Pro
L-(G-^{3}H)glutamine	20	146	L-Gln
2-aminoisobutyric (methyl-^{3}H) acid	10	103	AIB
(1-^{14}C)methylamino-isobutyric acid	52×10^{-3}	117	MEAIB

Determination of the free amino acid concentrations in plasma and homogenates of the submandibular gland

Plasma samples were obtained from the femoral artery at the beginning of an experiment. One ml plasma was mixed with 0·1 ml 5 mmol.l^{-1} norleucine (internal standard) and 30 mg sulphosalicylic acid were added for deproteinization. The mixture was shaken gently, left standing in ice for 10–15 min and then centrifuged at 40,000 *g* for 1 h at −2 °C. The supernatant was decanted and stored frozen at −20 °C up to the time of amino acid analysis.

The free amino acid concentrations in submandibular tissue were estimated in non-experimental glands flushed free of blood, *in situ*, with 0·9% LiCl. The glands were excised and 1·0–1·2 g tissue were homogenized with 3 ml ice cold 0·9% LiCl and 0·4 ml 5 mmol.l^{-1} norleucine (internal standard). The homogenates were centrifuged at 40,000 *g* for 1 h at −2 °C and the supernatant was added to 120 mg of sulphosalicylic acid. This mixture was shaken gently and left standing in ice for 10–15 min for complete deproteinization. The samples were recentrifuged and stored frozen for analysis which was identical to that for the plasma supernatant samples.

Aliquots of the supernatant (100 μl) were run on a Technicon NCIIP Amino Acid Analyser, using a 6 h physiological fluids programme (single column, stepwise elution with Li citrate buffers; column 400 cm × 0·9 cm; flow rate = 0·45 ml.min^{-1}; temperature = 47 °C; chromobeads type C_3 resin).

RESULTS

Measurement of cellular uptake from paired-tracer dilution curves

Fig. 1 *A* shows typical venous tracer dilution profiles obtained for D-[^{14}C]mannitol and L-[^{3}H]phenylalanine in a gland perfused at constant flow with a Krebs-albumin solution containing 0·05 mmol . l^{-1} unlabelled phenylalanine. The activities of the two tracers are expressed as a percentage of the injected dose and plotted against the venous sample number and collection time. The curve for the test amino acid lies below that of the extracellular reference tracer, D-[^{14}C]mannitol, suggesting uptake of the amino acid at the interstitial side of the salivary epithelium. It is assumed that

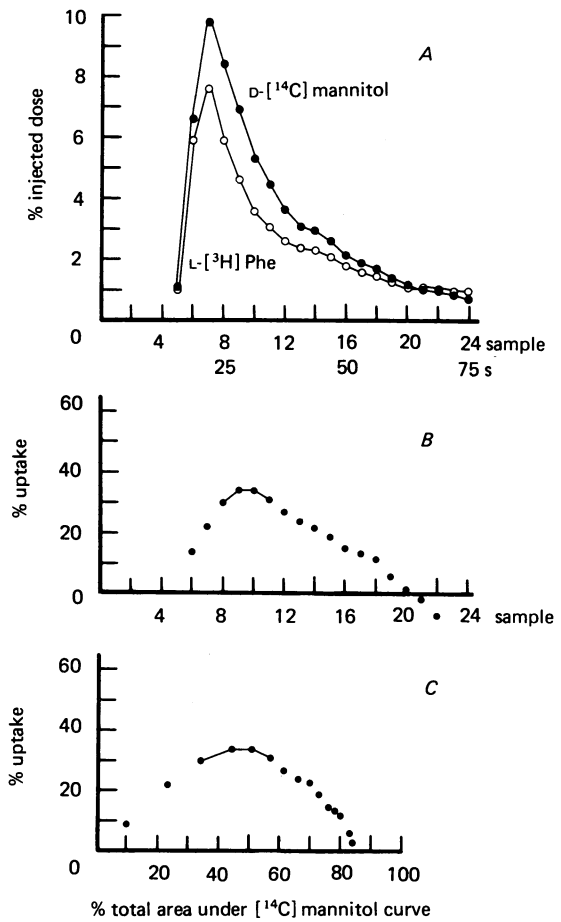

Fig. 1. Analysis of single-circulation paired-tracer dilution curves in a perfused gland. *A*, simultaneous venous concentration–time curves for D-[^{14}C]mannitol (extracellular reference) and L-[^{3}H]phenylalanine (test amino acid). The tracer concentrations are expressed as a percentage of the respective radioactive dose injected into the lingual artery. *B*, uptake of L-[^{3}H]phenylalanine relative to D-[^{14}C]mannitol (uptake % = $\{1-(^{3}H/^{14}C)\} \times 100$) in successive venous effluent samples. Maximal uptake (U_{max}) is indicated by the joined data points in panels *B* and *C*. *C*, the individual uptake measurements in panel *B* may be weighted for the recovered reference tracer by plotting them as a function of the per cent accumulated area under the D-[^{14}C]mannitol curve in panel *A*.

mannitol remains extracellular and that small molecules of similar size, such as mannitol (mol. wt. 182) and phenylalanine (mol. wt. 165), readily diffuse across the fenestrated capillaries of the submandibular gland in equal proportions (Mann *et al.* 1979*b*). An inference may thus be made about the facilitated transport of substrate into the epithelium based on the difference in the venous tracer profiles of D-[^{14}C]mannitol and L-[^{3}H]phenylalanine.

The percentage uptake of an amino acid relative to mannitol may be determined from uptake % = $\{1-(^{3}H/^{14}C)\} \times 100$ in each successive venous sample. The calculated uptakes are shown graphically in Fig. 1*B*. The relative importance of individual uptake values is assessed from the fraction of the injected extracellular reference dose recovered in each sample as described previously by Yudilevich *et al.* (1972). This weighting is illustrated in Fig. 1*C* by plotting uptake versus the per cent accumulated area under the D-[^{14}C]mannitol dilution curve in Fig. 1*A*. The maximal uptake (U_{max}) obtained by averaging the joined points in Fig. 1*C* is followed by a rapid decline indicating tracer backflux from the epithelium into the circulation. The early rising portion of the uptake curve reflects low uptake in short transit time pathways.

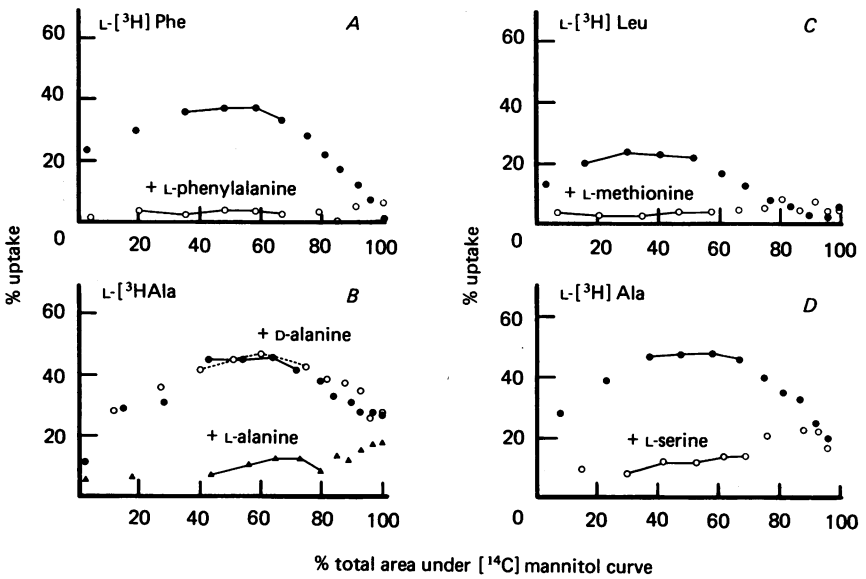

Fig. 2. Inhibition of ^{3}H-labelled L-amino acid uptake in glands with a natural blood supply. Self- (graphs *A*, *B*) and cross-inhibition (graphs *C*, *D*) tests of uptake were performed by adding a specific unlabelled amino acid to an isotope injectate at a concentration of 78 mmol.l^{-1}. Control patterns of uptake, indicated by the filled circles, were obtained in the same gland using an injectate concentration of 78 mmol.l^{-1} unlabelled D-mannitol to maintain isotonicity. Maximal unidirectional uptake, U_{max}, in control runs (●—●) is compared with uptake measured in the presence of an inhibitor amino acid (○—○ and ▲—▲).

Patterns of amino acid uptake in the presence of injectate inhibitor amino acids

The uptake of phenylalanine in glands with a natural blood supply (Fig. 2*A*) was similar to that observed in glands perfused with a Krebs–albumin solution (Fig. 1*C*). In Fig. 2 the joined filled circles in each graph were used to estimate control U_{max}

values for the specified ^{3}H-labelled amino acid. When an unlabelled amino acid was added to the tracer injectate (78 mmol.l^{-1}), U_{max} for the labelled amino acid could be markedly reduced. Both self-inhibition (Fig. 2*A* and *B*) and cross-inhibition (Fig. 2*C* and *D*) tests of uptake were demonstrated. In the case of L-[^{3}H]ALA only the L-enantiomer inhibited tracer uptake.

Assuming that the unlabelled amino acid is diluted in a similar manner to D-[^{14}C]mannitol (Fig. 1*A*) the peak concentration of the amino acid would approximate 8 mmol.l^{-1}. This concentration is still 2·6 times higher than the total neutral amino acid concentration in cat plasma (see Table 2).

TABLE 2. Blood to tissue maximal uptake of sixteen labelled neutral amino acids in the cat submandibular gland. The calculated injectate concentration for each amino acid is listed beside the U_{max} values measured in thirty cats. The plasma concentrations of free amino acids were determined in four animals and intracellular concentrations in three glands. Values reflect mean ± S.E. of means and the numbers in parentheses denote the number of observations

Amino acid	Injectate (mmol.l^{-1})	Maximal uptake (U_{max} %)	Plasma (mmol.l^{-1})	Gland (μmol/g cells*)
L-Ala	0·003	31 ± 2 (45)	0·32 ± 0·04	8·5 ± 2·6
L-Ser	0·006	45 ± 6 (8)	0·22 ± 0·03	3·3 ± 1·1
L-Met	0·038	28 ± 2 (21)	0·06 ± 0·01	0·9 ± 0·3
L-Val	0·003	14 ± 4 (8)	0·16 ± 0·01	1·8 ± 0·9
L-Leu	0·002	33 ± 3 (17)	0·14 ± 0·02	2·2 ± 0·5
L-Ile	0·001	31 ± 9 (3)	0·07 ± 0·003	0·5 ± 0·2
CycloLeu	0·370	12 ± 3 (4)	—	—
L-Trp	0·004	25 ± 2 (12)	0·1†	—
L-Tyr	0·012	28 ± 3 (3)	0·06 ± 0·01	1·6 ± 0·1
L-Phe	0·004	46 ± 2 (29)	0·07 ± 0·01	1·8 ± 0·3
L-His	0·002	25 ± 3 (7)	0·25 ± 0·10	1·1 ± 0·3
L-Gln	0·005	37 ± 5 (3)	1·21 ± 0·25	16·4 ± 0·9
Gly	0·005	−3 ± 4 (8)	0·31 ± 0·06	5·5 ± 0·8
L-Pro	0·002	−2 ± 2 (8)	0·10 ± 0·02	2·1 ± 0·5
AIB	0·011	0 ± 2 (3)	—	—
MeAIB	0·420	2 ± 5 (3)	—	—

* Glandular concentrations have been corrected for an interstitial volume of 0·15 ml.g^{-1} (Mann, Smaje & Yudilevich, 1979*b*) and reflect intracellular concentrations.

† Tallen, Moore & Stein (1954).

Comparison of U_{max} values obtained in vivo *and in glands perfused* in situ

Table 2 summarizes U_{max} values measured for sixteen labelled neutral amino acids against the background of the endogenous amino acids present in plasma. The amino acid concentration in each tracer injectate (see Table 2) was related to the dose of radioactivity required to achieve significant counts in the venous effluent of the gland and the specific activity of the tracer. With the exception of the synthetic amino acids, 1-aminocyclopentane-[^{14}C]carboxylic acid and [1-^{14}C]methylaminoisobutyric acid, the injectate concentration was much lower than the corresponding plasma amino acid concentration. The last two columns in Table 2 summarize our measurements of the free amino acid concentrations in cat plasma and their corresponding intracellular concentrations in submandibular gland homogenates. Among the naturally occurring amino acids all except L-proline and glycine had significant uptakes ranging from 14% for L-valine to 46% for L-phenylalanine. Cycloleucine had an

uptake of 12%, whereas the uptake for two other synthetic amino acids was negligible.

Since the variable amino acid concentrations in the plasma could modify the U_{max} measurements obtained *in vivo*, experiments were performed in glands perfused at constant flow with an amino acid-free Krebs-albumin solution. The U_{max} values determined for some neutral amino acids in the perfused preparation (Table 3) should be compared with those obtained *in vivo* (Table 2). The absence of uptake for MeAIB, a model substrate for characterizing an A-system (Christensen, 1979), in perfused glands confirms the results obtained *in vivo*. A low uptake of L-proline and glycine from amino acid-free perfusates was observed.

TABLE 3. Maximal cellular uptake (U_{max}) in glands perfused at constant flow rates between 0·6–1·2 ml.min^{-1}.g^{-1} with an amino acid-free Krebs solution. Values are given as mean ± S.E. of mean, n = number of observations

Amino acid	Maximal uptake (U_{max} %)
L-(2,3-^{3}H)Ala	54 ± 2 (10)
L-(3-^{3}H)Ser	66 ± 3 (8)
L-(4,5-^{3}H)Leu	43 ± 1 (4)
L-(3,5-^{3}H)Tyr	51 ± 2 (3)
L-(4-^{3}H)Phe	42 ± 5 (7)
(2-^{3}H)Gly	14 ± 2 (3)
L-(3,4-^{3}H)Pro	8 ± 3 (3)
(1-^{14}C)methylamino isobutyric acid	1 ± 1 (3)

Characterization of neutral amino acid transport systems in vivo

The experiments described in Fig. 2 were performed by injecting a ^{3}H-labelled amino acid and different unlabelled inhibitor amino acids. Frequently twenty-five such single passage runs were made successively over a 5 h period in one gland with a natural blood supply. Two possible carrier systems for neutral amino acid transport were studied using L-[^{3}H]Leu, L-[^{3}H]Met and L-[^{3}H]Phe as probes for a long-chain system and L-[^{3}H]Ala as a test substrate for a short-chain system.

Fig. 3 graphically summarizes all the inhibition experiments performed with the long-chain neutral amino acids. The control U_{max} value for the specified labelled amino acid is indicated in the upper left-hand corner of each graph. U_{max} measured in the presence of a given inhibitor amino acid was always compared with an averaged U_{max} estimate for the preceding and succeeding control runs. Fig. 3 shows that self-inhibition was always observed as was cross-inhibition by the two other long-chain neutrals and L-tryptophan. L-alanine and L-lysine had no inhibitory effect on U_{max} for the long-chain test amino acids. It is interesting that 39 mmol.l^{-1} L-phenylalanine and L-tryptophan each inhibited L-[^{3}H]phenylalanine uptake by about 40%, whereas 78 mmol.l^{-1} L-phenylalanine caused a 73% self-inhibition (Fig. 3). This finding demonstrates the sensitivity of the U_{max} measurement to the diluted unlabelled amino acid concentration at the exchange site.

The specificity of a short-chain neutral amino acid carrier is summarized in Fig. 4. In addition to showing marked stereospecificity, the system was inhibited significantly by four other short-chain neutral amino acids (L-serine, L-cysteine, L-amino-*n*-butyric acid and L-norvaline). As shown in Fig. 4 increasing the length of

the neutral amino acid side chain (L-methionine), the branching of the side chain (L-leucine) or the introduction of an aromatic ring (L-phenylalanine) reduced the degree of inhibition of L-[^{3}H]Ala uptake. The basic amino acids, L-lysine and L-arginine, enhanced rather than inhibited the uptake of L-[^{3}H]Ala while the dibasic amino acid, L-2,4-diamino-*n*-butyric acid, had a variable but over-all slightly inhibitory effect. As all these injectates were buffered in the plasma, it seems improbable that the stimulatory effects of L-lysine and L-arginine could be the result of a high pH.

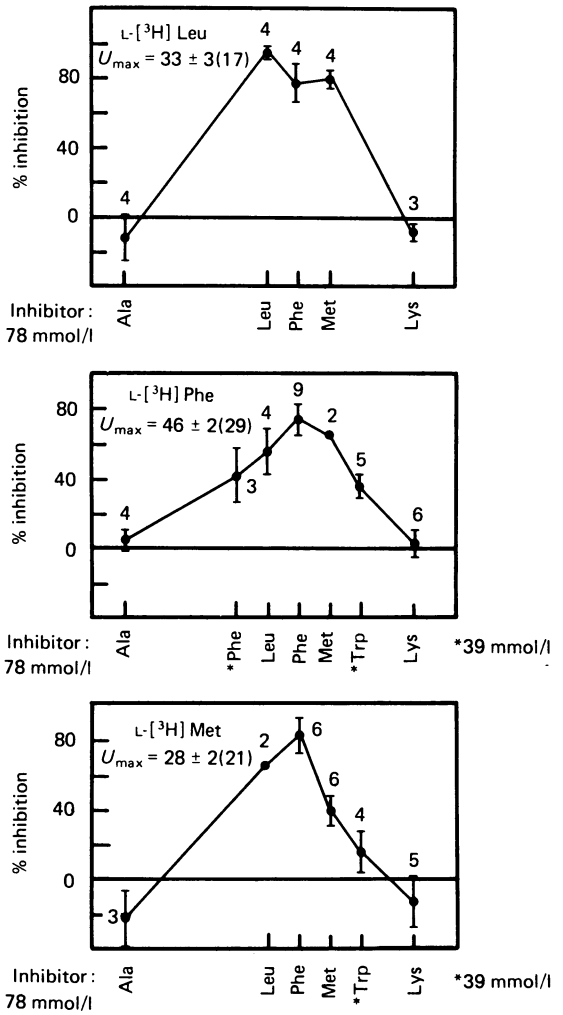

Fig. 3. Characterization of a long-chain neutral amino acid transport system. Inhibition of U_{max} for L-[^{3}H]leucine, L-[^{3}H]phenylalanine and L-[^{3}H]methionine was studied using the experimental procedures described in Fig. 2. % inhibition was calculated from

$$\frac{U_{max}(\text{control}) - U_{max}(\text{inhibitor})}{U_{max}(\text{control})} \times 100.$$

Control U_{max} values are listed in the upper left hand corner of each graph (mean ± S.E. of mean, n = number of observations in a total of fourteen cats). Points are joined to suggest a relationship between the different effects of the inhibitor L-amino acids.

Kinetics of L-*phenylalanine unidirectional influx*

To estimate kinetic parameters for amino acid influx across the basolateral membrane of the epithelium experiments were performed in perfused glands. Glands were perfused successively, in random order, with different unlabelled L-phenylalanine concentrations ranging between 0·5 and 20 mmol . l^{-1}. Each tracer injectate contained D-[^{14}C]mannitol, L-[^{3}H]phenylalanine and unlabelled phenylalanine at a final concentration equal to that of the specific perfusate. Each mixture was injected after 3 min of perfusion with a given solution. In between experimental runs, 8–10 per animal, the gland was perfused with a low phenylalanine concentration.

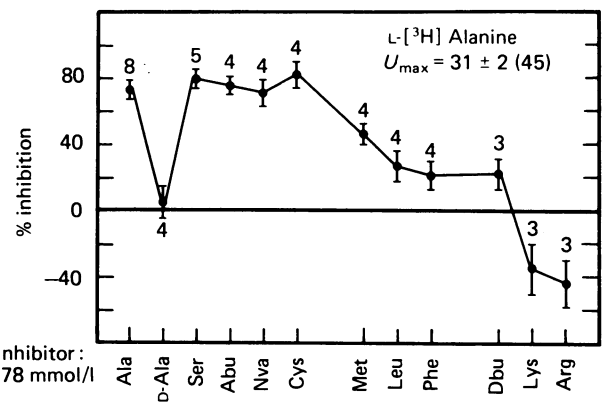

Fig. 4. Characterization of a short-chain neutral amino acid transport system. Inhibition of U_{max} for L-[^{3}H]alanine was studied using the procedures outlined in Figs. 2 and 3. Unless specified all inhibitors are L-amino acids. Values reflect mean ± S.E. of mean (n = number of observations in ten cats). Abu, L-amino-n-butyric acid; Nva, L-norvaline; Cys, L-cysteine; Dbu, L-2,4-diamino-n-butyric acid and remaining details are given in Table 1 and legends to Figs. 2 and 3.

The insert in Fig. 5 shows that the measured U_{max} for L-[4-^{3}H]Phe decreases as the perfusate concentration of unlabelled phenylalanine is increased. Assuming that uptake into the tissue is proportional to the substrate concentration, an integration along the length of the capillary (Renkin, 1959; Crone, 1963) may be used to calculate the initial influx, v,

$$v = C_a . \{-F \ln(1 - U_{max})\} \quad (1)$$

C_a is the perfusate concentration of unlabelled phenylalanine and F is the outflow rate in ml . min^{-1} per gram of gland wet weight. A small fraction of the inflow was lost through small arteries before the glandular exchange site, and hence the outflow rate was used to estimate influx.

When saturation is approached in carrier-mediated transport, influx becomes independent of the substrate concentration and a linear transformation should be applied:

$$v = C_a . F . U_{max} \quad (2)$$

Pappenheimer & Setchell (1973) have discussed this problem in detail and eqn. (1) has previously been used to estimate unidirectional solute influx across the blood-brain barrier (Pardridge, Connor & Crawford, 1975; Cunningham & Sarna, 1979). In our

experiments saturation results in low U_{max} measurements (Fig. 5) so that the difference between the logarithmic and linear transformations becomes insignificant. We have used the logarithmic transformation (eqn. (1)) to assess phenylalanine influx kinetics.

A single rectangular hyperbola has been fitted directly to the data points (Fig. 5) as the simplest and commonest solution, though more complicated models could also fit the experimental points (Atkins & Gardner, 1977). An iterative, least-squares method was used to fit the Michaelis–Menten equation and the computer program provided estimates of K_m, V_{max} and their respective standard errors (Cleland, 1967).

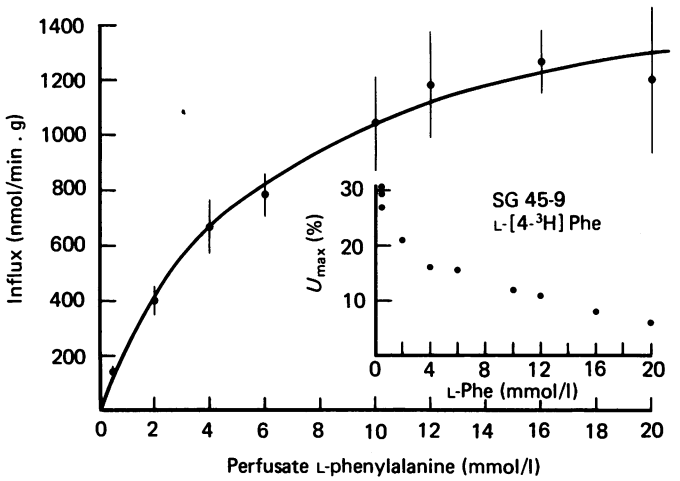

Fig. 5. The rate of unidirectional L-phenylalanine influx across the basolateral membrane of salivary epithelium. Influx of phenylalanine was measured during successive perfusion, in random order, with eight different unlabelled L-phenylalanine concentrations. The curve is a rectangular hyperbola obtained by a direct fit to the mean influx values weighted for the reciprocal of the standard error at each perfusate concentration. The vertical lines denote the standard error for each mean (n = five cats). The inset illustrates the effect of increasing phenylalanine perfusate concentrations on the U_{max} for L-[4-^{3}H]Phe in one of these experiments.

An apparent K_m of $6{\cdot}4 \pm 0{\cdot}8$ mmol . l^{-1} and V_{max} of 1719 ± 94 nmol . min^{-1} . g^{-1} was calculated. These results do not differ markedly from our preliminary values ($K_m = 4{\cdot}7 \pm 0{\cdot}5$ mmol . l^{-1}, $V_{max} = 1340 \pm 196$ nmol . min^{-1} . g^{-1}) calculated using eqn. (2) (Mann *et al.* 1980).

DISCUSSION

In a recent review, Young & van Lennep (1979) referred to the lack of information on amino acid uptake by salivary glandular cells. The present studies were directed to the identification of neutral amino acid transport systems in the salivary gland, *in vivo*, which may be compared with carriers widely represented in different animal cells (for reviews see Christensen, 1975; Lerner, 1978).

Extension of a paired-tracer dilution technique used in the brain (Yudilevich *et al.* 1979*b*) has demonstrated that in the salivary gland during a single capillary transit a large fraction of a specific amino acid in plasma could be transferred into a cellular compartment by carrier-mediated processes. The relative specificity of these trans-

port systems was characterized using self- and cross-inhibition experiments which formed the basis for measuring unidirectional influx kinetics for L-phenylalanine.

It has been suggested that the transport systems are sited in the basolateral plasma membrane of the glandular epithelium (Young & van Lennep, 1978), and that amino acids are transferred directly from the blood via these carriers to the ribosomes for protein synthesis (van Venrooij *et al.* 1973; Ekfors & Barka, 1971; Barka, 1971). Our results support the proposed site for the carriers since equal proportions of the extracellular reference marker and the test amino acid should reach the basolateral side of the epithelium. This assumption is based on the finding that the fenestrated salivary capillaries (Takada, 1970) offer little diffusional resistance to the passage of small lipid-insoluble molecules (Mann, 1978; Mann *et al.* 1979*b*). Furthermore, active transport of amino acids by endothelial cells has only been demonstrated in isolated cells of brain capillaries (Betz & Goldstein, 1978).

Specificity of neutral amino acid transport

In the salivary gland, as in many other cells, at least two transport systems appear to exist for neutral amino acids. One of these systems preferentially transports short-chain neutral amino acids and was characterized using L-[^{3}H]Ala as a test substrate (see Fig. 4). This system shows marked stereospecificity, exhibits a high affinity for four other small neutral amino acids and is inhibited between 12 and 46 % by large neutral amino acids. The long-chain basic amino acids apparently stimulated alanine uptake while diaminobutyric acid caused inhibition. This pattern of cross-inhibition is similar to that reported for sheep erythrocytes (Young & Ellory, 1977) with the exception that in those experiments basic amino acids were inhibitory. This subdivision between the effects of long- and short-chain basic amino acids is a phenomenon which has been observed in brain slices (Neame, 1968), and Christensen & Handlogten (1979) have previously described the observation of 'competitive stimulation'.

Further characteristics of the short chain neutral system were obtained from the measurement of a negligible uptake for the non-metabolized model A-system amino acid, MeAIB, both from plasma (Table 2) and amino acid-free perfusates (Table 3). Uptake from plasma for representatives of an imino-glycine system was negligible and only a low uptake for L-proline (8 %) and glycine (14 %) was measured in glands perfused with an amino acid-free Krebs solution. This uptake corresponds to an insignificant influx since the isotope injectate contained either 2 μmol . l^{-1} proline or 5 μmol . l^{-1} glycine. We believe that this minimal influx is not mediated by a specific transport system but rather reflects the low affinity of these substrates for the other transport systems.

Since there is no uptake of MeAIB at the basolateral side of the salivary epithelium, we may be dealing with one of the few tissues in which short-chain neutral amino acids are transported by an alanine-serine-cysteine (ASC) type carrier (Kilberg, Christensen & Handlogten, 1979) rather than by both alanine (A-type) and ASC-type carriers. The only other tissues that apparently lack an A-type system are rabbit reticulocytes and avian erythrocytes (Thomas & Christensen, 1971).

The long-chain neutral amino acids appear to share a common transport site for which alanine and lysine have no affinity (Fig. 3). At a similar exchange site

concentration ($\simeq$ 8 mmol.l^{-1}) alanine is ineffective in inhibiting the uptake of large neutral amino acids while methionine, leucine and phenylalanine all reduce the uptake of alanine (Fig. 4). These results suggest that two transport sites, probably with overlapping affinities, are operative in the basolateral membrane of the epithelium. Further studies in the perfused gland *in situ* should enable us to determine the relationships between them. It is interesting that preliminary experiments in the perfused gland have shown the K_m for alanine to be an order of magnitude lower than that reported for phenylalanine in the present paper. Furthermore, the influx of alanine and serine is highly Na^+-dependent while the influx of phenylalanine is relatively unaffected by the absence of Na^+ from the perfusate (G. E. Mann & D. L. Yudilevich, unpublished observations).

Kinetics of L-*phenylalanine influx*

The present estimates of unidirectional phenylalanine influx would appear to be independent of cellular metabolism, as phenylalanine is poorly metabolized by salivary glands (Lindsay *et al.* 1969). *In vitro* and steady-state investigations are limited in that only estimates of the net influx and efflux rate can be obtained. The K_m of 6·4 mmol.l^{-1} in the salivary gland may be compared with values reported for the rat heart sarcolemma (K_m = 3·7 mmol.l^{-1}, Baños, Daniel, Moorhouse, Pratt & Wilson, 1978) and rat brain endothelium (K_m = 0·12 mmol.l^{-1}, Pardridge & Oldendorf, 1977). Both of these values were obtained by measuring maximal tracer uptake at the blood–tissue interface during a single circulation. It is interesting that the K_m of 6·4 mmol.l^{-1} is similar to the total endogenous neutral amino acid concentration (3·0 mmol.l^{-1}) in cat plasma (Table 2). The value of V_{max} in the salivary gland (1719 nmol.min^{-1}.g^{-1}) is twice the estimate of 760 nmol.min^{-1}.g^{-1} reported for rat heart sarcolemma (Baños *et al.* 1978).

The use of the paired-tracer dilution technique to measure kinetic constants in the salivary gland *in situ* should provide a basis for further characterization of the identified membrane carriers for neutral amino acids. In a variety of tissues these seem to be under hormonal control (Guidotti, Borghetti & Gazzola, 1978), and it may be possible to test for an alteration in membrane carriers and/or receptors in the intact organ.

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