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Amino Acid Exchange Mechanism in the Basolateral Membrane of the Midgut Epithelium from the Larva of *Hyalophora cecropia*

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Summary. It is concluded that the amino acid, α-aminoisobutyric acid, is taken up from the blood into the midgut epithelium by an exchange mechanism for the following reasons: 1. The amino acid uptake becomes saturated with increasing amino acid concentration on the blood side; 2. The amino acid in the tissue cannot be washed out again to the blood side unless an amino acid is present in the blood side bathing solution; 3. The uptake mechanism is independent of metabolism. This amino acid exchange mechanism is independent of the active transport of potassium from blood side to lumen side, the exchange of potassium between blood and midgut tissue, and the transport of the amino acid, α-aminoisobutyric acid, across the midgut epithelium from lumen to blood.

The isolated midgut, from the larva of the American silkworm *Hyalophora cecropia*, transports α-aminoisobutyric acid (AIB) from the lumen side to the blood side (Nedergaard, 1972). This is an active transport with a flux ratio usually between 10 and 60, but it becomes close to 1 when the metabolism is inhibited by lack of oxygen. The isolated midgut has an electrical potential difference across the tissue from 100 to 150 mV with the lumen side positive. The potential difference is created by an active transport of potassium from blood to lumen, i.e. in the opposite direction of the amino acid transport. This active potassium transport is independent of sodium (Harvey & Nedergaard, 1964). The amino acid transport is independent of the active potassium transport *per se*, but depends upon the potential difference across the tissue (Nedergaard, 1973).

The present paper describes some properties of the uptake of the amino acid, AIB, from the blood into the intestinal cells. This uptake is found to be mediated by an amino acid exchange mechanism. A preliminary report has been presented at the 1977 FEBS meeting (*see* Nedergaard, 1977*a*), and the subject is mentioned in a chapter on amino acid transport in: Transport of Ions and Water in Animals (*see* Nedergaard, 1977*b*).

Materials and Methods

The moth *Hyalophora cecropia* was reared in the laboratory on either fresh willow leaves, or an artificial diet (Riddiford, 1968). About 1 cm from the middle part of the midgut was dissected out of the 5th instar larvae after they had been anaesthetized by cooling on ice. As described earlier (Harvey & Nedergaard, 1964) the gut was mounted as a cylinder in an open chamber, which was provided with short-circuiting arrangements.

The bathing solutions used were all based on a standard solution (S-1) containing (in mM): 30 KCl, 2 $KHCO_3$, 5 $CaCl_2$, 5 $MgCl_2$, and 166 sucrose. To this solution was added the amino acid α-aminoisobutyric acid (AIB) in varying amounts. In the experiments with 5 mM KCl the osmolarity was made up with sucrose. Carbon-14 labeled AIB was used to measure uptake of AIB. AIB was also present on the lumen side in all experiments; therefore the midgut also transported AIB activity from lumen side to blood side in the experiments of this paper.

The midgut was allowed to equilibrate for about 15 min before the experiment started; some experiments lasted 30 min, some 1 h and a few with other time lengths (*see* Tables). After the tissue has taken up AIB from the blood side the midgut was taken down from the mounted position in the chamber, blotted lightly on tissue paper before it was transferred to a vial, weighed and ground with 1 ml perchloric acid, 0.33 M. The total extracellular space was then measured as the sucrose space by the glucose oxidase method. In some experiments the extracellular space on one side of the tissue was measured with ^{14}C inulin.

The bathing solutions, 50 ml on the blood side and 5 ml on the lumen side, were continuously aerated and stirred by bubbling with oxygen, or with nitrogen in some cases, in order to inhibit the metabolism.

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Results

Extracellular Spaces

To measure the concentration of a radioactive amino acid (AIB) taken up from the blood into the midgut tissue it is necessary to know both the total extracellular space and the extracellular space on the blood side of the tissue. The total extracellular space, blood side plus lumen side, is measured as the sucrose space (Zerahn, 1975). This can be done because sucrose is always present in great amounts in the bathing solutions for this preparation; otherwise the tissue does not survive *in vitro*. From the experiments shown in Table 1 the extracellular space on either side of the tissue is measured with ^{14}C labeled inulin. The total extracellular space is seen to average around 35% of the tissue wet weight. The Table also shows that the extracellular space on the blood side is on average about twice the extracellular space on the lumen side.

Table 1. Blood side, lumen side, and total extracellular space in percent of the wet weight

Experiment	Total sucrose space	Blood side		Lumen side	
		Measured	Calculated	Measured	Calculated
3/4-A	37.9	–	25.3	12.6	–
3/4-B	39.5	–	29.9	9.6	–
16/4-A	35.3	24.2	–	–	11.1
16/4-B	31.6	20.4	–	–	11.2
16/4-C	40.4	30.7	–	–	9.7
17/4-A	31.9	22.1	–	–	9.8
17/4-B	33.2	–	15.7	17.5	–
18/4-A	30.3	–	18.7	11.6	–
Average	35.0	24.35	22.4	12.8	10.45
±SD	±3.6	±3.9	±5.5	±2.9	±0.7

The isolated midgut was bathed on both sides with ordinary Ringer's (S-1), which contains 166 mM sucrose. Carbon-14 labeled inulin was added either to the blood side or to the lumen side to measure the extracellular space. The calculated extracellular space on the other side of the midgut was found by subtraction from the total extracellular space, the sucrose space, which was measured chemically in all experiments. All experiments were run for 17 min in the usual experimental set-up

Cellular Tissue Uptake of AIB from the Blood Side

Fig. 1 shows the concentration of AIB in the cells as a function of incubation time with AIB on the blood side. The bathing solutions are identical on the two sides of the isolated midgut tissue containing 10 mM AIB added to the normal Ringer's solution, S-1. Carbon-14 labeled AIB is added only to the blood side bathing solution. The total extracellular space is measured as the sucrose space. Two-thirds of this value is the blood side extracellular space (Table 1), which is contaminated with the high radioactivity (^{14}C) on the blood side. The blood side extracellular space is used to correct the tissue AIB concentration (^{14}C labeled) for the amount of ^{14}C in the extracellular space to get the true AIB concentration in the cells. The cellular volume for calculation of the AIB concentration is the total tissue volume minus the total extracellular space. The cellular concentration of AIB is seen to increase with time to reach steady state in about 1 hr with a concentration of about 30 mM.

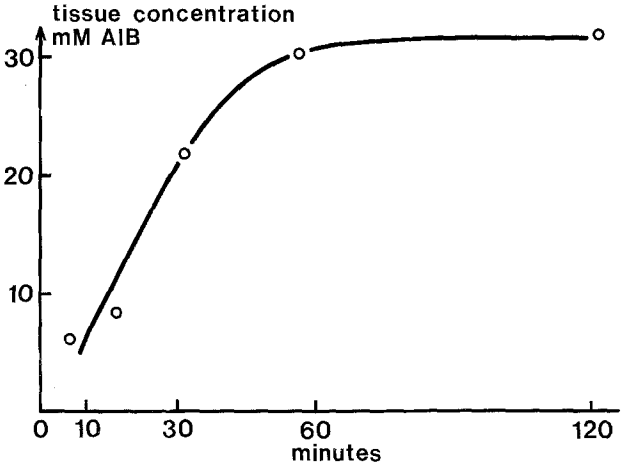

Fig. 1. Steady-state cell concentration of AIB is obtained in about 1 hr. Bathing solutions contained 10 mM AIB; the blood side was labeled with ^{14}C AIB. The cellular AIB concentration was measured as the amount of labeled AIB in the midgut corrected for extracellular space and calculated from tissue wet weight. Each point is the average of 5 to 10 experiments, one animal one experiment

The results from experiments where the concentration of AIB in the bathing solutions was varied is shown in Fig. 2. The duration of the single experiment was 1 h and the AIB concentrations were the same on the two sides of the tissue. The concentration in the cells is seen to reach saturation with an AIB concentration of about 10 mM in the bathing solutions.

Table 2 shows the effect of decreased potassium concentration on the uptake of AIB (^{14}C AIB) from the blood side. A reduction of potassium from 32 to 5 mM has no effect on the AIB uptake from the blood side. Table 2 also shows that reduction in potassium concentration in the bathing solution from 32 to 5 mM has little effect on the potassium concentration in the tissue.

Table 3 shows that an increase in the active potassium transport across the tissue from the blood side

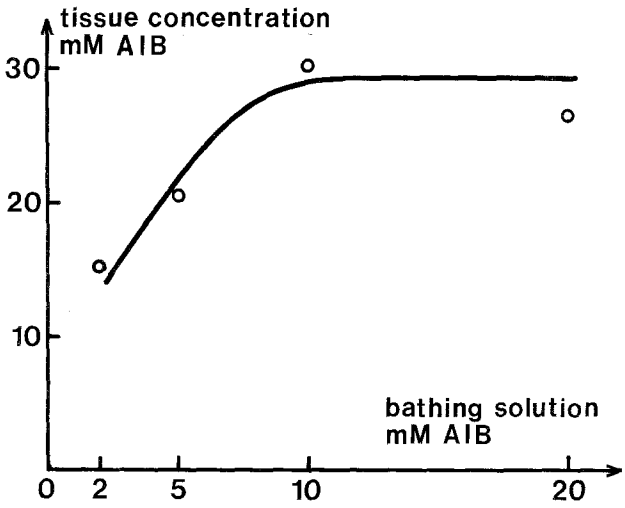

Fig. 2. AIB concentration in the midgut versus AIB concentration in the bathing solutions. The bathing solutions contained varying amounts of amino acid, AIB. The AIB in the blood side bathing solution was labeled with ^{14}C. Each point is the average value of 5 to 10 experiments, each of which represents one animal. Cellular AIB concentration calculated as in Fig. 1

Table 2. Effect of potassium ion concentration on AIB uptake from the blood into the midgut cells (Experimental period: 30 min)

Experiment	Experimental condition	AIB concentration in tissue (mM)	Potassium concentration in tissue (mM)
Control	32 mM K^+	21.8±5.9 average of 13 experiments (range 13.4 to 29.2)	127±10 average of 13 experiments (range 114 to 155)
29/4-75	5 mM K^+	26.8	137
29/4C-75	5 mM K^+	28.4	116
13/6-75	5 mM K^+	14.8	105
13/6B-75	5 mM K^+	18.7	115
16/6-75	5 mM K^+	25.8	112
16/6B-75	5 mM K^+	15.3	76
Average±SD		21.6±5.6	100±18

Table 3. Effect of active potassium transport on AIB uptake from the blood into the midgut cells (Experimental period: 30 min)

Experiment	Experimental condition	AIB concentration in tissue (mM)	SCC in μAMP (Average)
Control	not short-circuited	21.8±5.9 average of 13 experiments (range 13.4 to 29.7)	0
22/8-74	SCC	10.0	1195
23/8-74	SCC	16.3	860
18/4II-75	SCC	18.2	1800
23/4-75	SCC	19.8	1640

Table 4. Washing-out to the blood side of ^{14}C AIB labeled midgut tissue with and without AIB in the washing solution (Uptake period: 30 min; Washing period: 30 min)

Experiment	Experimental condition	AIB concentration in tissue (mM)
22/8-74	washed with AIB	4.8
23/8A-74	washed with AIB	4.5
18/4B-75	washed with AIB	9.1
18/4C-75	washed with AIB	8.0
12/4-76	washed with AIB	2.1
13/4-76	washed with AIB	5.3
13/4B-76	washed with AIB	5.1
Average±SD		5.6±2.2
23/8B-74	washed without AIB	11.1
23/8C-74	washed without AIB	13.6
21/4C-75	washed without AIB	16.2
21/4D-75	washed without AIB	20.5
23/4C-75	washed without AIB	17.7
23/1B-76	washed without AIB	16.0
26/1-76	washed without AIB	15.4
Average±SD		15.8±2.8

Table 5. Effect of metabolic inhibitors on AIB uptake from the blood into the midgut tissue (Experimental period: 30 min)

Experiment	Experimental condition	AIB concentration in tissue (mM)
Control	No inhibitor	21.8±5.9 average of 13 experiments (range 13.4 to 29.7)
20/11-75	Inhibitor: oxygen-lack	28.5
21/11-75		28.0
9/1C-76		19.7
14/1B-76		18.5
24/11-75	Inhibitor: Iodoacetic acid	21.8
25/11-75		13.5
26/11-75		26.9
27/11-75		25.6
15/1-76	Inhibitor: N_2+iodoacetic acid	21.1
15/1B-76		23.1

to the lumen has no significant influence on the AIB uptake (^{14}C AIB) from the blood side.

Table 4 shows that the loss of AIB from the cells depends upon the presence of an amino acid in the bathing medium, suggesting that the tissue uptake of amino acids is due to an exchange with some amino acids in the tissue. The tissue was first loaded with ^{14}C AIB from the blood side for half an hour then for the following half hour the blood side bathing solution was changed to a solution without AIB in the first 7 experiments, whereas in the next 7 experiments 10 mM nonlabeled AIB was present in the bathing solution. It is seen that AIB is lost from the

tissue only when AIB is present in the bathing solution.

Table 5 shows that metabolic poisons do not inhibit tissue uptake of AIB from the blood side. It was assured that the poisons were administered in adequate amounts by measuring the effects on the electrical potential difference across the tissue. In all cases the potential difference was diminished at least 75%; in some cases it was almost abolished.

Discussion

The average total extracellular space of 35% found here (Table 1) is smaller than the values found by Zerahn (1975) of 45–48% and by Giordana and Sacchi (1977) for *Philosamia cynthia* and *Bombyx mori* of 42 and 45%. Most likely the discrepancies are due to variations in the effectiveness of the blotting of the midgut tissue.

The total extracellular space, as determined by the sucrose method, is only little influenced by the fact that sucrose can be utilized by the metabolically highly active midgut tissue. Attempts to measure extracellular space by means of radioactively labeled sucrose were unsuccessful: the values were always significantly higher than those calculated from the distribution of sucrose as determined chemically, and the discrepancy increased with increasing exposure time to the radioactive sucrose.

The amount of sucrose consumed by the cells will not influence the sucrose concentration in the bathing solutions during the experimental time due to the high sucrose concentration of 166 mM. The volumes of the bathing solutions are 50 ml on the blood side and 5 ml on the lumen side, so the amount of sucrose consumed will be negligible. The sum of the extracellular space available from the blood side and that available from the lumen side agrees well with the total extracellular space in the tissue (Table 1) indicating that the two extracellular spaces are not interconnected. The extracellular space on the blood side is about twice as large as that on the lumen side, in agreement with ultrastructural studies of the midgut tissue, which show large basal infoldings and interspaces (Andersen & Harvey, 1966).

For practical reasons it has not been possible to determine the extracellular space available for inulin from the blood side in the same experiments where the distribution of labeled AIB was determined. The total extracellular space available for sucrose was determined and the blood side extracellular space was then calculated as two-thirds of the total.

From the results presented here it can be concluded that an amino acid exchange mechanism exists between the midgut cells and the blood of the silkworm *Hyalophora cecropia*. The amino acid, AIB, is taken up into the cells from the blood side and cannot be washed out again from the cells to the blood side without an amino acid being present in the blood side bathing solution (Table 4). Unpublished experiments show that the amino acid exchange mechanism is not very specific with respect to which amino acid it is exchanged for. The amino acid used here (AIB) does not normally occur in living tissues. Still it is exchanged for naturally occurring amino acids in the tissue.

Characteristic for an exchange mechanism is that it need not consume metabolic energy, as suggested by Ussing (1947). Table 5 shows clearly that the amino acid exchange mechanism in the midgut is independent of metabolic energy, because it is neither affected by lack of oxygen nor by poisoning with iodoacetate.

Another property of an exchange mechanism is that it usually becomes saturated when the concentration of the exchanging substance is increased. This is also the case for this mechanism as shown in Fig. 2, where the process is saturated at an amino acid concentration of about 10 mM AIB in the blood side bathing solution.

The amino acid exchange mechanism of the midgut blood side is not a fast reaction; it takes about an hour before a steady state is reached (Fig. 1).

The amino acid exchange mechanism is situated at the blood side of the midgut tissue, which is at the same time actively transporting potassium from the blood side across the tissue into the lumen side, and actively transporting AIB in the opposite direction, from lumen to blood. On the blood side of the tissue there is also a potassium exchange mechanism (Harvey & Zerahn, 1969; Zerahn, 1975). This potassium exchange is very fast: in 2.2 min it has exchanged 50% of the tissue potassium and it is able to exchange 90–95% of the total amount of potassium present in the tissue.

The potassium exchange works well when no amino acids are present in the bathing media (Zerahn, 1975), and the amino acid exchange is unaffected by a reduction of the potassium concentration in the media from 32 to 5 mM, which reduces the potassium exchange considerably (Table 2). So the potassium exchange and the amino acid exchange appear to be independent of each other.

The reduction in potassium in the medium will affect the potassium concentration ratio between tissue and medium. In the control experiments the ratio $K^+_{tissue}/K^+_{medium}$ equals $127/32 = 4.0$, whereas in the experimental period the ratio equals 22. However, the amino acid exchange is unaffected.

The active transport of potassium which goes from blood side to lumen side across the midgut tissue has no effect on the amino acid exchange at the blood side. Table 3 shows that when the potential difference across the tissue is abolished, whereby the active potassium transport is increased about twofold (Harvey, Haskell & Zerahn, 1967), there is little effect on the amino acid exchange. The efflux of potassium from lumen to blood is also without effect on the amino acid exchange since the potassium efflux is decreased in the short-circuited tissue. The potassium efflux is passive and therefore influenced by the potential gradient across the epithelium.

The active transport of the amino acid, AIB, from the lumen side across the tissue to the blood side is without influence on the amino acid exchange mechanism on the blood side. The active amino acid transport is dependent on the potential difference across the midgut tissue and is therefore inhibited when the PD is short-circuited as in Table 3. The amino acid exchange should have decreased if there was a relation between the two transports. Another reason why these two mechanisms must be separate is that the active transport of amino acid goes on whether or not there is amino acid present on the blood side. Furthermore, the uptake of AIB into the tissue, arising from the active AIB transport from lumen side to blood side, is not indifferent to metabolic poisons. On the contrary, the amount of AIB taken up is heavily reduced during N_2 inhibition (*unpublished results*). It should also be pointed out that in the experiments of this paper there is also amino acid, AIB, in the solution bathing the lumen side of the midgut, which means that in all the experiments reported here concerning the AIB uptake from the blood side into the cells there is at the same time an active transport of AIB across the midgut from lumen side to blood side.

It is therefore concluded that the amino acid exchange mechanism is independent of the other transports of amino acids or potassium going on at the same time in the same tissue. The function of the amino acid exchange mechanism in the midgut tissue might be to supply amino acids for cellular activities such as enzyme production for the digestion. It seems reasonable that the system supplying amino acids for such functions is independent of the active amino acid transport across the tissue.

It would be interesting to compare this amino acid exchange mechanism to similar mechanisms in other transporting epithelia, but this is not possible at present due to the scarcity of available data.

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