J. Exp. Biol. (1972), 56, 167–172
Printed in Great Britain

ACTIVE TRANSPORT OF α-AMINOISOBUTYRIC ACID BY THE ISOLATED MIDGUT OF *HYALOPHORA CECROPIA*

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(*Received 7 June* 1971)

INTRODUCTION

In vertebrates the uptake of amino acids in the intestine is coupled to the active transport of sodium from the mucosal to the serosal side of the tissue (Crane, 1965). In insects, however, the amino acid uptake is assumed to be a passive process where the amino acids move along a concentration gradient established across the tissue by the rapid absorption of water from the gut (Treherne, 1959). Furthermore the amino acid uptake from the alimentary canal of the larva of *Bombyx mori* is independent of metabolism (Shyamala & Bath, 1966).

In the experiments presented here the transport of an amino acid, α-aminoisobutyric acid, across the isolated midgut of the Cecropia larva is investigated under conditions where the amino acid is present in the same concentrations on both sides of the gut. The amino acid fluxes in both directions are measured and an effect of anaerobic conditions on the amino acid uptake is demonstrated. The results show that α-aminoisobutyric acid is transported actively from the lumen to the blood of the Cecropia midgut.

The amino acid transport is also investigated in relation to the active potassium transport of the midgut epithelium, and it is found that there is no direct correlation between amino acid transport and active potassium transport.

MATERIALS AND METHODS

The animal used was the fifth-instar larva of the American silkmoth, *Hyalophora cecropia*. The larvae were reared on either lilac leaves or artificial food (Riddiford, 1968), and used when they weighed at least 9 g. The larvae grown on artificial food weighed less and showed smaller amino acid fluxes from lumen to blood and lower potential differences than larvae grown on leaves. Otherwise no differences have been found so far between the larvae fed on the two diets. A piece, about 100 mg, of midgut tissue ranging from in front of the first pair of prolegs to between the last two pairs of prolegs was dissected out and mounted as a tube in the experimental chamber. The arrangement has been described by Harvey & Nedergaard (1964), with later modifications of the short-circuiting arrangement as described by Harvey, Haskell & Zerahn (1967).

The bathing solution contained 62 mM-KCl, 2 mM-$KHCO_3$, 5 mM-$CaCl_2$, 5 mM-$MgCl_2$, 102 mM sucrose, and 10 mM α-aminoisobutyric acid; pH of the bathing solution was 8·0.

The volume of the bathing solution on the lumen side was 5 ml and on the blood side of the midgut 50 ml.

The amino acid fluxes were measured by using 1-^{14}C-labelled α-aminoisobutyric acid obtained from Amersham. Samples were taken with 10 or 15 min intervals and were usually 1 ml of size. The samples were replaced immediately with inactive bathing solution and were counted in a Packard liquid scintillation counter. The scintillator was prepared according to the recipe of Bray (1960).

The bathing solutions on both sides of the midgut were aerated and stirred by bubbling with oxygen. The aerobic metabolism was inhibited by bubbling with oxygen-free nitrogen instead of oxygen.

Table 1. *α-Aminoisobutyric acid fluxes and potential difference across the midgut of the Cecropia larva*

(The amino acid fluxes are measured for 2–4 consecutive periods: 10 min periods for the lumen-to-blood fluxes and 15 min periods for the blood-to-lumen fluxes. The potential differences are the average values for each period. The mean values and standard deviations from the means are listed for the fluxes.)

Expt.		Period 1	2	3	4	Av. flux
		Lumen-to-blood flux (μmole/h; PD in mV)				
29/7	Flux	18·9	22·1	22·4	25·2	22·2
	PD	112	111	109	104	
30/7-I	Flux	15·1	16·6	19·4	19·6	17·7
	PD	120	117	113	108	
30/7-II	Flux	12·6	11·2	—	—	11·9
	PD	115	105			
31/7	Flux	20·2	25·5	—	—	22·9
	PD	114	112			
6/8	Flux	11·3	12·2	—	—	11·8
	PD	103	101			
7/8	Flux	20·9	23·4	—	—	22·2
	PD	94	96			
23/1*	Flux	8·9	9·5	8·8	8·8	9·0
	PD	89	89	85	81	
						Mean 16·8 ± 5·9
		Blood-to-lumen flux (μmole/h; PD in mV)				
1/8	Flux	0·17	0·16	0·23	0·25	0·20
	PD	109	107	104	101	
4/4*	Flux	0·22	0·24	0·23	—	0·23
	PD	108	106	101		
11/4*	Flux	0·48	0·56	0·47	—	0·50
	PD	77	73	70		
13/4*	Flux	0·29	0·30	—	—	0·30
	PD	79	77			
14/4*	Flux	0·15	0·15	0·17	—	0·16
	PD	103	101	98		
						Mean 0·28 ± 0·13

* Larvae grown on artificial food.

RESULTS

In the first series of experiments the flux of α-aminoisobutyric acid is measured in the two directions, from lumen to blood and from blood to lumen, and the potential difference across the membrane is recorded concurrently. The results are shown in Table 1. The lumen-to-blood flux is seen to be on average about 17 μmole/h, so the flux ratio of the experiments of Table 1 is around 60. The average potential difference for each experimental period is shown in the table. Whereas there is a tendency for the potential difference to decrease slowly with time, the amino acid transport tends to increase slightly.

Table 2. *Effect of anaerobic conditions on the fluxes of α-aminoisobutyric acid across the Cecropia midgut*

(The fluxes and potential differences are first measured under aerobic conditions in two or three consecutive periods for which the average values are given (control period). Then aeration with nitrogen is started and the measurements are continued for another three periods, after which aeration with oxygen is restored in some experiments (after control). The periods are 10 min for the lumen-to-blood experiments and 15 min for the blood-to-lumen experiments.)

Expt.		O_2, control	N_2, period 1	period 2	period 3	O_2 after control
		Lumen-to-blood flux (μmole/h; PD in mV)				
5/8-I	Flux	14·0	16·2	13·0	8·3	—
	PD	90	80	60	50	
5/8-II	Flux	11·8	11·9	6·1	3·8	—
	PD	87	74	18	3	
5/8-III	Flux	16·7	20·2	6·8	4·1	—
	PD	110	75	23	14	
6/8	Flux	9·3	8·7	4·0	1·5	8·3
	PD	108	32	14	9	85
24/1*	Flux	11·1	12·4	9·4	4·6	7·5
	PD	88	78	50	12	74
25/1*	Flux	10·4	10·0	3·4	2·7	6·9
	PD	84	50	6	4	73
		Blood-to-lumen flux (μmole/h; PD in mV)				
26/1-I*	Flux	0·14	0·61	1·70	1·45	0·31
	PD	95	54	8	7	67
26/1-II*	Flux	0·15	0·41	0·78	0·67	0·10
	PD	90	40	10	8	77
6/7-II	Flux	0·30	0·73	1·18	1·17	—
	PD	55	11	0	0	
7/7-I	Flux	0·29	0·92	1·50	—	0·52
	PD	83	32	6		49
8/7	Flux	0·13	0·57	0·50	—	0·16
	PD	109	19	4		57

* Larvae grown on artificial food.

When the metabolism is inhibited by lack of oxygen, the amino acid flux from lumen to blood decreases considerably with time (Table 2). The degree of inhibition of the midgut metabolism can be seen in the second line of each experiment in the table, which shows the average potential in the same periods of time as the amino acid fluxes.

The decrease of the amino acid flux is most pronounced in the experiments where the potential is inhibited the most. The flux in the opposite direction is somewhat increased during anaerobic inhibition, and reaches the same order of magnitude as the lumen-to-blood flux, the flux ratio approaching one. When oxygen is restored to the tissue the amino acid fluxes and the potential return almost to normal (last column in Table 2).

To see whether a change in active potassium transport gave a corresponding change in amino acid flux, the active potassium transport, after a control period, was increased by short-circuiting the midgut potential. The active potassium flux against the normal

Table 3. *Effect on the amino acid transport of short-circuiting the midgut potential*

(Each column is the average of at least three 10 min periods in the lumen-to-blood experiments and three 15 min periods in the blood-to-lumen experiments. The experimental period, where the potential is short-circuited, is placed between two sets of control periods, where the potential is not short-circuited. SCC: short-circuit current. Each value in the table is the average of two or three consecutive periods. Mean values and standard deviations from the means are shown for the fluxes.)

	Potential		SCC		Potential	
Expt.	PD (mV)	Flux (μmole/h)	SCC (μA)	Flux (μmole/h)	PD (mV)	Flux (μmole/h)
			Lumen-to-blood flux			
1/2*	101	7·1	2000	2·7	—	—
2/2-I*	94	9·1	2310	4·1	92	9·6
2/2-II*	71	13·9	1700	6·1	—	—
14/4-II*	86	6·5	1330	2·1	55	3·9
15/4-I*	63	5·8	1470	3·6	—	—
17/4-I*	98	6·6	1965	2·0	—	—
17/4-II*	109	8·5	2240	2·9	—	—
17/4-III*	82	10·2	1840	3·9	—	—
Mean flux		8·5 ± 2·5		3·4 ± 1·3		6·4
			Blood-to-lumen flux			
3/2-I*	71	0·37	1060	1·02	54	0·42
5/2*	76	0·37	1100	1·02	50	0·31
6/2*	104	0·23	1510	0·58	89	0·24
4/4*	105	0·23	1820	1·19	66	0·23
11/4*	73	0·50	1440	1·77	53	0·39
13/4*	78	0·29	1690	0·76	68	0·27
14/4-I*	101	0·16	1820	0·52	76	0·12
Mean flux		0·31 ± 0·11		0·98 ± 0·40		0·28 ± 0·11

* Larvae reared on artificial food.

potential is only half the flux measured in the short-circuited condition (Harvey *et al.* 1967). The effect of the increase in active potassium transport on the amino acid flux is shown in Table 3. The results show that there is no straightforward relation between amino acid flux and active potassium transport; the amino acid transport appears to be inhibited when the midgut potential is short-circuited. Table 3 shows a large decrease in amino acid flux from lumen to blood when the potential is short-circuited and an increase in the blood-to-lumen flux. The flux ratio falls from 30 in the control periods to around 3 in the short circuited periods. It should be mentioned that the difference between the control period of Table 3 and the corresponding last column of Table 1

is assumed to be due to a difference in the experimental animals. The larvae used for the experiments given in Table 3 were all grown on artificial food, whereas the larvae used for Table 1 were grown on leaves.

DISCUSSION

The bathing solutions are identical on both sides of the midgut and there is therefore no concentration gradient of any ion or solute across the membrane, and no osmotic gradient that could result in a net water flux from one side of the midgut to the other. The only difference between the two sides of the midgut is the potential difference, the lumen being 60–160 mV more positive than the blood side. The potential difference is measured throughout the experiment to assure that the midgut preparation is alive. Usually the potential difference decreases with time, but so slowly that it is possible to run the experiment over several hours. Only midguts with reasonably steady potentials are used. The potential is dependent on the metabolism of the midgut and is abolished when the tissue is depleted of oxygen.

α-Aminoisobutyric acid is used as test amino acid because it is not metabolized.

The amino acid fluxes in the two directions are found to be different, the flux from lumen to blood is about 60 times the flux in the opposite direction, although the amino acid concentration is the same on both sides of the tissue.

The pH of the bathing solution is 8·0, the isoelectric point of α-aminoisobutyric acid is 6·3, and pK_2 is 10·2, so most of the amino acid is electrically neutral and only a minor part is negatively charged. The net amino acid transport is from lumen to blood, which is opposite to the direction expected from the charge. This shows that the amino acid transport from the lumen of the Cecropia midgut is an active process.

An active amino acid transport in the insect midgut was suggested by Ussing earlier in 1946, since he found that the concentration of all examined amino acids was higher in the blood than in the gut of *Melolontha*.

Shyamala and Bath (1966) found no inhibition of the amino acid transport by metabolic inhibitors, such as dinitrophenol and KCN, in the intestine of the larva of *Bombyx mori*, and they concluded that the amino acid transport is not an active process. The results reported in this paper show, however, that the amino acid transport from the larval midgut of the American silkmoth is inhibited by lack of oxygen. This indicates that the transport is dependent on an active aerobic metabolism in the tissue and confirms the suggestion that the amino acid transport is active.

The amino acid transport has been found to be passive in the locust gut in consequence of a rapid uptake of water from the lumen of the midgut caeca, whereby a concentration gradient of the amino acids is established across the gut (Treherne, 1959). Across the isolated Cecropia midgut there is no concentration gradient of the amino acid at the start of an experiment. The net water flux across the Cecropia midgut was found by measuring the [^{14}C]sucrose concentration in the bathing solution of the lumen. In three experiments the net water flux from lumen to blood was 9 μl/h with a variation of ±70 μl/h. This small water flux cannot create a concentration gradient of the right magnitude across the tissue. Neither can the amino acid transport be explained by assuming that the net water flux is a solute flow, because a bulk flow of more than 1 ml/h from lumen to blood is needed in most experiments to account for the amino acid transport.

It is obvious that the mechanism for amino acid transport in the Cecropia midgut cannot be similar to that in the vertebrate intestine. In vertebrates the active sodium transport, which is responsible for the amino acid uptake, is in the same direction as the amino acid flux. The active potassium transport of the Cecropia midgut is in the direction from blood to lumen, i.e. in the opposite direction of the amino acid transport. The results from the experiments designed to show the influence of the active potassium transport on the amino acid flux show clearly that there is no direct relation between the two, the amino acid transport being inhibited when the potassium transport is increased by short-circuiting the midgut potential.

It may be reasonable to suggest that the amino acid transport of the Cecropia midgut is not dependent on the active potassium transport *per se*, but dependent on some result of the active potassium transport, such as the potential difference, or may be an effect of the potential difference.

SUMMARY

1. The α-aminoisobutyric acid flux from lumen to blood of the isolated Cecropia midgut is around 17 μmole/h, while the amino acid flux in the opposite direction is on average 0·3 μmole/h.

2. The amino acid uptake is inhibited by lack of oxygen. It is suggested that the amino acid transport from lumen to blood is an active process.

3. The amino acid uptake is inhibited by short-circuiting the midgut potential, indicating that there is no direct correlation between the active transport of potassium and the uptake of the amino acid by the midgut.

REFERENCES

BRAY, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a scintillation counter. *Anal. Biochem.* **1**, 279.

CRANE, R. K. (1965). Na^+-dependent transport in the intestine and other animal tissues. *Fedn Proc.* **24**, 1000–6.

HARVEY, W. R., HASKELL, J. A. & ZERAHN, K. (1967). Active transport of potassium and oxygen consumption in the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **46**, 235–48.

HARVEY, W. R. & NEDERGAARD, S. (1964). Sodium-independent active transport of potassium in the isolated midgut of the Cecropia silkworm. *Proc. natn. Acad. Sci. U.S.A.* **51**, 757–65.

RIDDIFORD, L. M. (1968). Artificial diet for Cecropia and other saturniid silkworms. *Science, N.Y.* **160**, 1461–2.

SHYAMALA, M. B. & BATH, J. V. (1966). Intestinal transport in the silkworm, *Bombyx mori* L., of amino acids from mixtures. *J. Insect Physiol.* **12**, 129–35.

TREHERNE, J. E. (1959). Amino acid absorption in the locust (*Schistocerca gregaria* Forsk.). *J. exp. Biol.* **36**, 533–45.

USSING, H. H. (1946). Amino acids and related compounds in the haemolymph of *Oryctes nasicornis* and *Melolontha vulgaris*. *Acta physiol. Scand.* **11**, 61–84.