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AMINE TRANSPORT AT THE PLASMALEMMA OF *RICCIA FLUITANS*

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Summary

Green thallus cells of the aquatic liverwort, *Riccia fluitans*, are rapidly depolarized in the presence of 1—20 μM NH_4Cl and 5—100 μM CH_3NH_3Cl, respectively. Simultaneously, the membrane conductance is increased from 0.41 to 1.2 $S \cdot m^{-2}$. Uptake of [^{14}C]methylamine is stimulated by increasing $[K^+]_o$ and inhibited by increasing $[Na^+]_o$ or $[H^+]_o$, is highly voltage sensitive, and saturates at low amine concentrations.

Double-reciprocal plots of (a) maximal membrane depolarization and (b) methylamine uptake vs. external amine concentration give apparent K_m values of 2 ± 1 μM ammonia and 25—50 μM methylamine; K_m values for changes in conductance and membrane current are greater and voltage dependent. Whereas the amine transport into the cell is strongly inhibited by CN^-, the amine efflux is stimulated.

The current-voltage characteristics of the ammonia transport are represented by a sigmoid curve with an equilibrium potential of —60 mV, and this is understood as a typical carrier curve with a saturation current of about 70 $mA \cdot m^{-2}$. It is further concluded that the evidently carrier-mediated transport is competitive for the two amines tested, and that ammonia and methylamine are transported in the protonated form as NH_4^+ and $CH_3NH_3^+$ into the cytoplasm.

Introduction

Plant cells build nitrogen-containing compounds, such as amino acids and proteins, by adding an amino group to the carbon compounds produced in

Ammonia and methylamine refer to either base without defining its state of protonation. Amine refers to both ammonia and methylamine. E_m = membrane potential (mV); E_D = diffusion potential (mV); g_m ($1/r_m$) = membrane (slope) conductance ($S \cdot m^{-2}$); P_j = permeability coefficient of ion j ($m \cdot s^{-1}$).

photosynthesis. The nitrogen of nitrate or ammonia obtained from soil or water is used for this purpose. It is only logical that water plants should be equipped with some sort of uptake system for the usually very small concentrations of ammonia in natural pond-water.

In the last two decades, amine transport has been investigated repeatedly [1—9]. In this paper an attempt is made to elucidate and characterize the amine transport system in the aquatic liverwort, *Riccia fluitans*. The general electrogenic transport properties of cell membranes have been summarized recently by Bentrup [10], and have been worked out for this plant by Felle and Bentrup [11]; also a stereo specific hexose transporter [12], and a probably less specific amino acid transport system have been demonstrated [13].

Material and Methods

Plant material and general conditions. Thalli of *R. fluitans* from a greenhouse were transferred 24 h before the experiment into a test solution which contained 1 mM K^+, 2 mM Na^+, 1 mM Ca^{2+}, 2 mM Cl^-, and 2 mM of secondary and tertiary phosphate, in order to adjust the pH from 4.9 to 7.0. If not otherwise noted, this solution was used for the experiments. NH_4Cl, CH_3NH_3Cl, L-serine, D-glucose and NaCN were added directly to this medium.

All experiments were carried out under white light of about 1 $W \cdot m^{-2}$ at 23 ± 1°C.

Electrophysiological experiments. A standard electrophysiological apparatus was used throughout the experiments [11]. Micropipettes were pulled on a David Kopf instrument (vertical) from fibre-filled borosilicate tubing (Hilgenberg), and filled by capillary displacement with 0.5 M KCl. Tip diameters were 0.3—0.5 μM. Membrane potentials were recorded from green thallus cells in a chamber which was continuously perfused by the test solution and allowed horizontal approach by the microelectrode.

Conductance measurements and *I-V* curves were obtained by inserting two or three pipettes into a rhizoid cell [11], injecting square current pulses (I_o) of 0.2—15 $\mu A \cdot cm^{-2}$ through the current electrode at the midpoint of the rhizoid. Voltage displacements were either recorded at the point of current injection or at different distances (x_1, V_1; x_2, V_2) thereof, in order to measure the conductance of the cell interior. This is given by the space constant:

$$\lambda = (x_2 - x_1)/(\ln V_1 - \ln V_2) \quad (1)$$

Because of nonlinearities of the *I-V* curves, the recorded input data had to be subjected to Cole's theorem [14] which applies to infinite cables. This condition has been considered fulfilled because the space constant of 370 μm never exceeded 30% of the length of a given rhizoid. According to Cole, the current density ($A \cdot m^{-2}$) is given by

$$i_m = (dI_o/dV_o) \cdot V_o/4c\lambda \quad (2)$$

where dI_o/V_o is the slope of the input *I-V* curve at V_o and V_o denotes the voltage displacement according to the injected current. c represents the cell circumference of usually 6—8 · 10^{-5} m.

Voltage-clamp experiments were carried out according to the method of

Gradmann [15,16] by point-clamping the cells at either their resting potential or at a chosen potential, while measuring the membrane current across a known resistor in the presence of each amine concentration. The *I-V* curves were usually taken: (a) shortly before and shortly after the amine addition; (b) 30 min after amine addition.

[^{14}C]methylamine/ammonia uptake experiments. Uptake of [^{14}C]methylamine hydrochloride (Amersham, U.K.) was measured with thallus samples of 5—10 mg dry wt., and was carried out at 23 ± 1°C in 20 ml of constantly stirred test solution. Uptake was stopped by transferring the sample into a non-radioactive, but otherwise identical medium, and washed for 1 min. Various tests at different rinse times up to 5 min and efflux experiments proved that after a 1 min rinse, the radioactivity in the free space was less than 2% of the total activity found in the tissue. After killing with CO_2 at −79°C, samples were lyophilized for 48 h, weighed, partly dissolved in TS-1 (Zinsser), and tested for activity in a Berthold-Frieseke scintillation counter (BF-5000). Corrections for quenching due to green colour and tissue residues were made by using [^{14}C]-methylamine as internal standard. Uptake of NH_4^+ was carried out in a bioassay by using the plants themselves as an ammonia-electrode. Thalli of *R. fluitans* were transferred into solutions of different ammonia concentrations and of known volume for a given time, during which the plants took up some of the ammonia. The solution was filtered off, and the plants were freeze-dried for 48 h and weighed. The remaining ammonia content of the filtrate was measured by means of membrane potential measurements. Less ammonia in the solution produces a smaller depolarization (see Results), and therefore gives an estimate of how much ammonia the plants took from the medium. Comparison of this difference with the dry weight data gives a measure for the ammonia uptake. The error due to the concentration change during uptake decreases with increasing external ammonia concentration because a concentration change of 0.5 μM could easily be measured. This means that in the presence of, say, 5 μM external ammonia, the maximum error never exceeded 10%.

Velocity of perfusion. The influence of the velocity of perfusion on amine uptake and the electrical behaviour, respectively, was tested. A significant difference could only be found by comparing the stagnant medium and very low flow velocities. The responses saturated at 0.2 ml/s which caused a total exchange of the chamber volume within 4 s. A velocity of perfusion of 0.4 ml/s was then chosen for the experiments.

Estimation of the area. Thalli of *R. fluitans* are tissues of six to ten cell layers: therefore, the area is difficult to calculate. Consequently, uptake data will in general be given in mols/g dry wt. Nevertheless, an attempt has been made to estimate the 'effective area' in order to bring electrical and flux data into some relation. This was carried out by equilibration experiments with [^{14}C]methylamine at different external concentrations and different membrane potentials. If methylamine is transported in the protonated form (see Discussion), then the uptake of methylamine is a function of the membrane potential. Methylamine uptake is measured at different membrane potentials up to saturation. A relationship can be found between this membrane potential and the ratios of internally accumulated methylamine. The amount of internal methylamine is in relation to a volume (and area) pertinent to these experi-

ments. From this an area of approx. 0.1 m^2/g dry wt. was calculated.

All data given are from representative single experiments or mean values ±S.E. from at least triplicates.

Results

Electrical phenomena under different conditions

Ammonia concentrations of 20 μM or more rapidly depolarize the membrane potential, E_m, of *R. fluitans* to a level close to the membrane potential, E_D, which is usually obtained by inhibiting the ATP-dependent electrogenic pump or ATP synthesis. Lower ammonia concentrations result in smaller, but for each concentration specific, depolarizations (Fig. 1). Even after a 30 min incubation in NH_4Cl, repolarization occurs readily upon washout. Fig. 1 further shows that, although methylamine is less effective by a factor of about 10, saturation occurs at the same membrane potential as measured in the presence of ammonia. This is demonstrated as well in Fig. 2 where the changes in membrane potential (ΔE_m) in the presence of methylamine and ammonia (C) are subject to Michaelis-Menten kinetics according to:

$$1/\Delta E_m = (K_m/\Delta E_m max)/C + 1/\Delta E_m max \quad (3)$$

From this a double-reciprocal plot gives apparent K_m values of about 2 μM ammonia and 25 μM methylamine.

Fig. 3 shows that changes in external potassium under standard conditions result in small but significant changes in E_m. In the presence of 20 μM or more ammonia, however, the membrane responds much more strongly to changes in external K^+. But once the membrane was depolarized to E_D, no or only small changes in potential were measured even in the presence of ammonia concentrations of 0.1—1.0 mM.

Of all the other ions tested (Na^+, H^+, Mg^{2+}, Ca^{2+}), only protons had a significant effect on the extent of membrane depolarizations. An increase in the

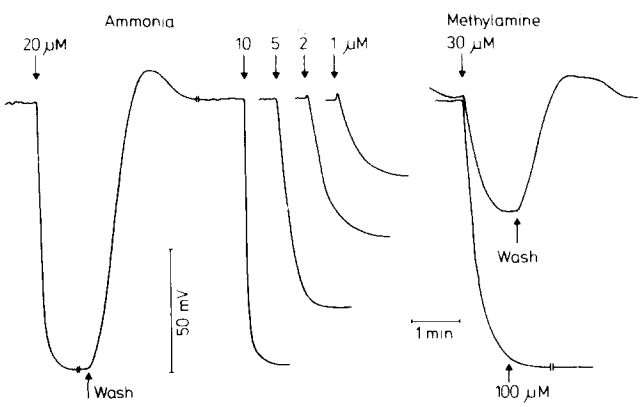

Fig. 1. Recordings of changes in membrane potential (ΔE_m) from green thallus cells of *R. fluitans* after adding different NH_4Cl concentrations (μM) to the external medium (pH_o = 5.6). Recovery of E_m takes place almost immediately after washout. For comparison, ΔE_m in the presence of 30 and 100 μM methylamine is shown.

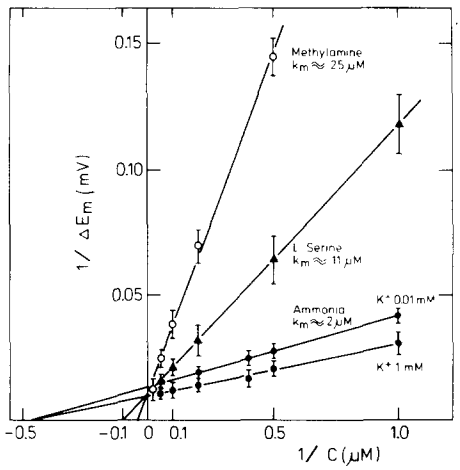

Fig. 2. Double-reciprocal plot of depolarization vs. the concentration of ammonia, methylamine and L-serine, respectively. $[K^+]_o$ = 1 mM, otherwise as indicated; pH_o = 5.6 (see text).

external pH from 5.0 to 6.5 enhanced ΔE_m by about 40% after addition of amines.

Parallel to the membrane depolarizations, a significant change in membrane conductance (Δg_m) from 0.41 to 0.93 S · m^{-2} at 50 μM methylamine takes place. Fig. 4A shows that this change in g_m also saturates with concentration. Evaluation of these data according to Eqn. 3 yields:

$$1/\Delta g_m = (K_m/\Delta g_m max/C + 1/\Delta g_m max \quad (3a)$$

The K_m values of 12 μM ammonia and 50 μM methylamine differ considerably from the data derived above for ΔE_m. A common $\Delta g_m max$ of about 0.8 S · m^{-2} can be obtained from Fig. 4.

[^{14}C]Methylamine uptake

The concentration-dependent influx of [^{14}C]methylamine and ammonia is

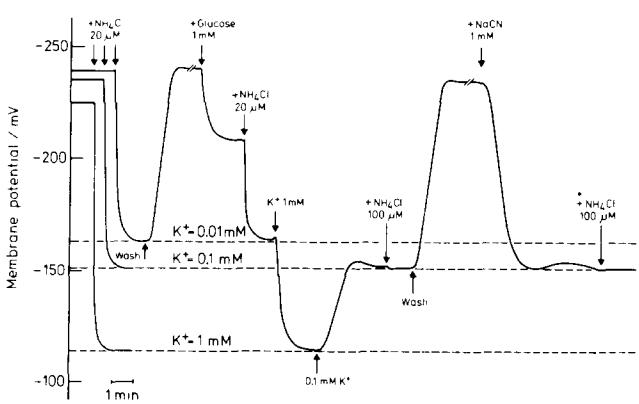

Fig. 3. Representative recordings of E_m from thallus cells of *R. fluitans*. The dotted lines indicate the Nernst potentials at different potassium concentrations (see text).

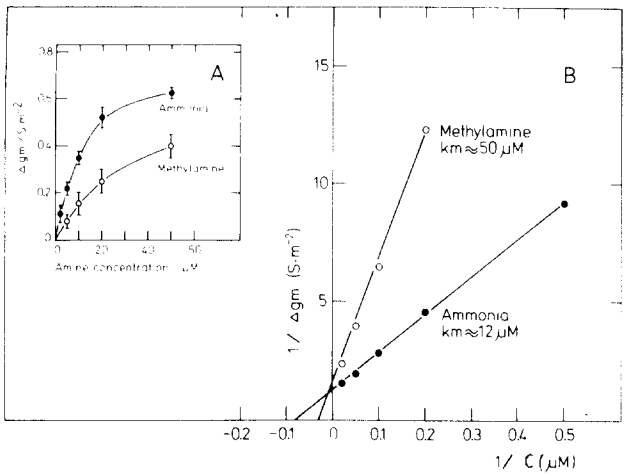

Fig. 4. (A) Change in membrane conductance (Δg_m) in the presence of ammonia and methylamine as indicated. (B) Same values plotted double reciprocally according to Eqn. 3a. *C* represents amine concentration.

shown in Fig. 5A. The data display saturation-like curves, levelling off at 50—70 μM. It is important to realize that in the presence of (a) 0.1 mM methylamine, (b) 1 mM CN^- or (c) 0.1 mM NH_4Cl, the membrane potential is depolarized to the same diffusion potential E_D (see Discussion), and that the methylamine uptake is identical in all three cases. The rate of [^{14}C]methylamine uptake into thallus cells of *R. fluitans* is constant for approx. 30 min

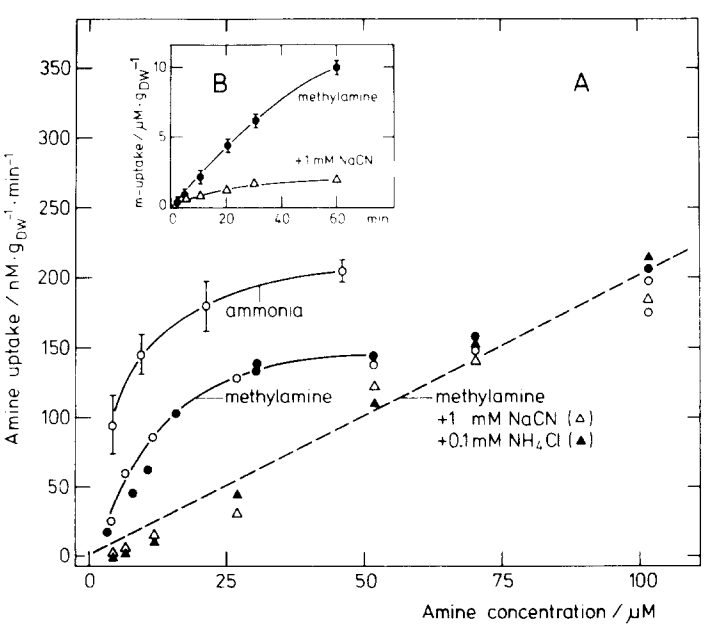

Fig. 5. Uptake (5 min) of ^{14}C-labelled methylamine by thalli of *R. fluitans* (control) at pH 6.1 and $[K^+]_o$ = 1 mM. (▲) With addition of 0.1 mM NH_4Cl, and (△) in the presence of 1 mM CN^- (see text). Ammonia uptake values are from the bioassay described in Material and Methods. Inset: [^{14}C]methylamine uptake with or without 1 mM CN^- as a function of time. g_{DW}, g dry wt.

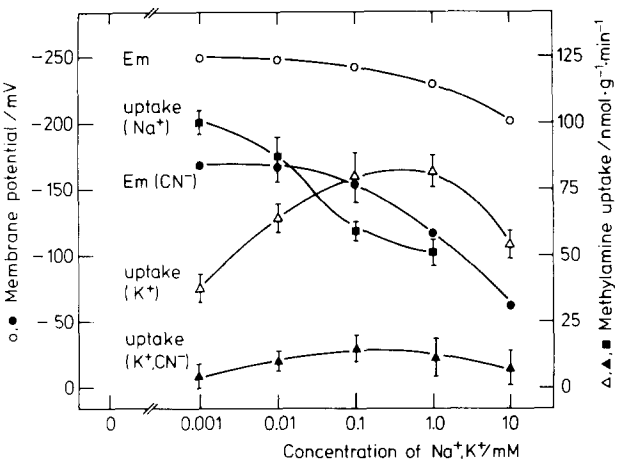

Fig. 6. Uptake of [^{14}C]methylamine by thalli of *R. fluitans* at different potential levels: (○) membrane potential and (△) [^{14}C]methylamine uptake, both as functions of external potassium concentration. (●) Membrane potential and (▲) [^{14}C]methylamine uptake, both as functions of external potassium concentration, and in the presence of 1 mM CN^-, respectively. (■) [^{14}C]methylamine uptake at different external sodium concentrations.

after which slower uptake was observed (Fig. 5B). However, compartmental analysis shows that net uptake continues until a ratio of methylamine content of 60 : 1 in the vacuole and cytoplasm, respectively, is reached (efflux data not shown). In contrast, the volume ratio of these compartments is only 5 : 1. While 0.1—1 mM CN^- stimulates the efflux of [^{14}C]methylamine, the influx is strongly inhibited and is only 20% of the control after 1 h of uptake (Fig. 5B).

Whereas K^+ concentrations from 1 μM to 1 mM stimulate [^{14}C]methylamine uptake by a factor of 2 to 3 in the presence or absence of CN^-, Na^+ concentrations in the same range inhibit methylamine uptake to 50% of its original value

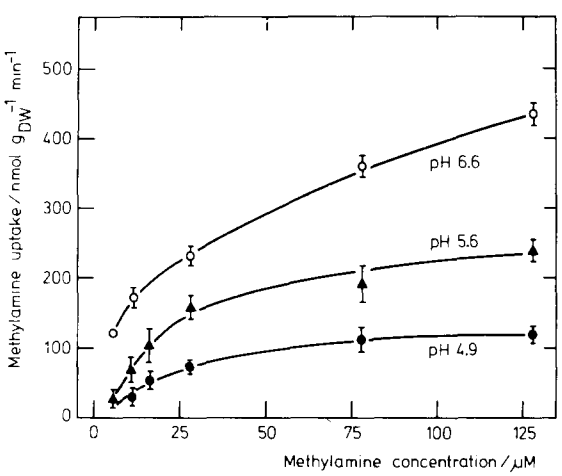

Fig. 7. [^{14}C]methylamine uptake by thalli of *R. fluitans* as a function of external methylamine concentration at three different pH_o values as indicated, and external potassium = 1 mM, after 5 min of incubation in methylamine. g_{DW}, g dry wt.

(Fig. 6). Change in external pH from 4.9 to 6.6 stimulates methylamine uptake by a factor of 3 to 4 (Fig. 7). No significant effect on methylamine uptake by Ca^{2+} or Mg^{2+} in the range from 0.01 to 1 mM was found.

Current-voltage relationships

Whereas small voltage displacements from the resting potential by injected current pulses give information about the total membrane conductance due to all electrical pathways involved, a train of different voltage displacements (i.e., *I-V* curves) may display nonlinearities due to the different voltage dependence of the single pathways. This information is indispensable for calculating charged fluxes across the membrane, such as amine transport.

In Fig. 8, the control curve (no ammonia) is fairly linear from −300 to about −130 mV; it curves upwards to much higher conductance (i.e., membrane current/E_m) at −120 mV which is close to the diffusion potential (E_D) of −110 mV in the presence of 1 mM potassium. In the presence of different ammonia concentrations, the *I-V* curves have a similar shape but different slopes (i.e., conductance). The membrane conductance increases for negative currents (i.e., ammonia influx) according to the external concentrations, but there is ob-

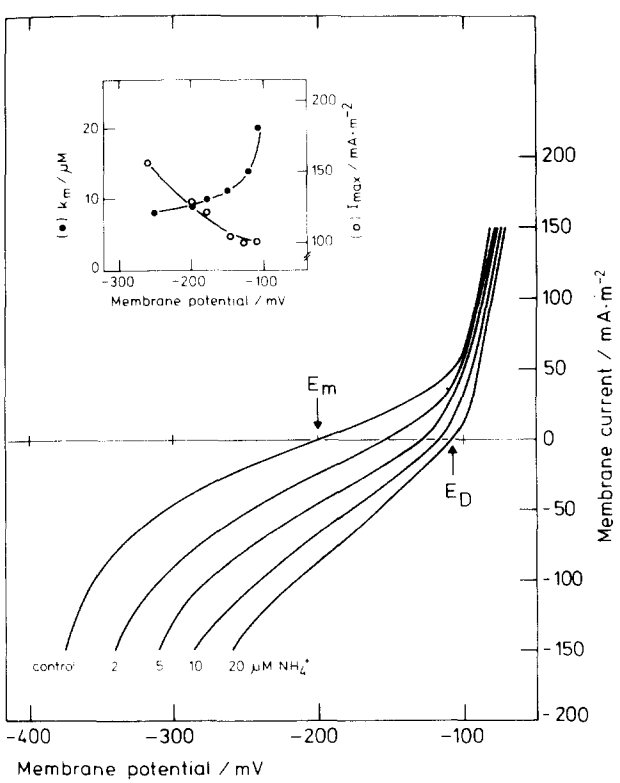

Fig. 8. *I-V* characteristics of the *Riccia* plasmalemma in the presence of different NH_4Cl concentrations (µM) as indicated. The control curve was taken just before addition of NH_4Cl. $[K^+]_o$ = 1 mM, pH_o = 5.6. Inset: (•) voltage dependence of the apparent K_m values. Each point represents electrical current data measured in the presence of different NH_4Cl concentrations while clamped at the indicated voltage. (○) Maximal positive inward current from the same measurements.

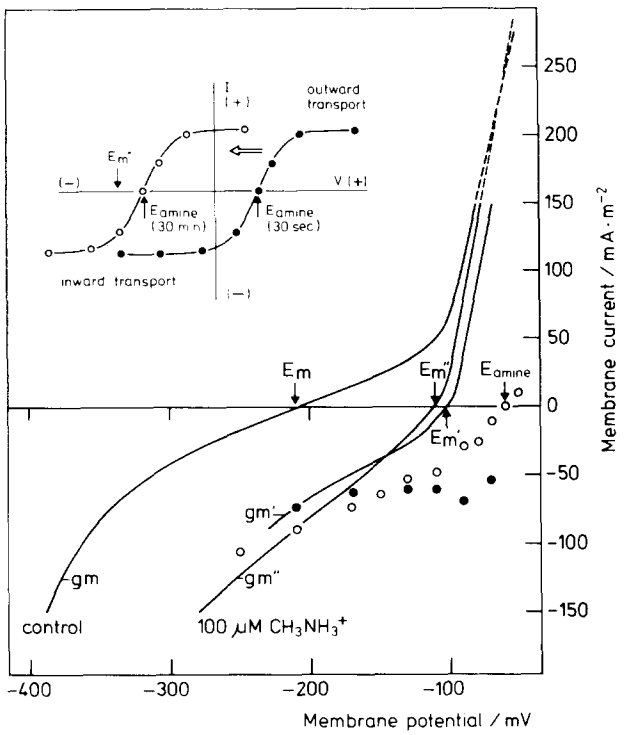

Fig. 9. *I-V* characteristics from rhizoid cells of *R. fluitans*. g_m denotes the standard control curve. g_m' denotes the curve obtained about 30 s after addition of methylamine, g_m'' denotes the curve from the same cell 30 min after addition of methylamine. Subtraction of g_m from g_m' yields the solid-circled curve (•), from (g_m''—g_m) the open-circled curve (○) is obtained (see text). Inset: hypothetical sigmoid carrier *I-V* curves and their shift along the voltage axis with time of incubation in the substrate (see text).

viously little change in conductance for positive currents (i.e., ammonia efflux). In order to avoid errors due to nonlinearities of the *I-V* curves, inwardly directed net current was also measured by holding the membrane potential constant at a given value in the presence of different ammonia concentrations (voltage clamp). These current data were plotted reciprocally vs. $1/[NH_4^+]$. The

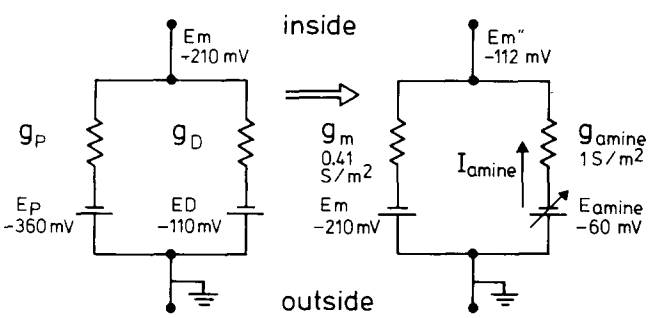

Fig. 10. Equivalent circuits for the membrane potential across the membrane of rhizoid cells of *R. fluitans* before (left) and after (right) addition of the amine. The left circuit is represented by the left branch in the right circuit.

apparent K_m values shown in the inset of Fig. 8 are voltage dependent, especially when the clamped membrane potential approaches E_D. The inwardly directed maximal current (I_{max}) decreases as the potential is shifted from −250 to −110 mV which appears logical according to the reduced driving force for ammonia.

The *I-V* curves of Fig. 9 are obtained from experiments during the first 30 s after addition of methylamine (g_m', E_m'), and from those after 30 min of incubation in methylamine (same cells; g_m'', E_m''). These curves have been subtracted from the control curve (g_m) according to the equivalent circuit for the membrane potential of Fig. 10. It is assumed that addition of methylamine induces an additional conductance which is parallel to g_m. If g_m itself is not altered by the amine, then subtraction of g_m' from g_m yields a curve almost parallel to the voltage axis (closed circles). However, from subtraction of g_m'' from g_m another curve is obtained which intercepts with the voltage axis at about −60 mV (open circles).

Discussion

As reported previously [11,17], an electrical potential difference of −220 to −250 mV (inside negative) exists across the plasmalemma of *R. fluitans*, depending on external pH. $[K^+]_o$ and overall ionic strength. Since this voltage is far too negative to be explained by mere passive transport, an electrogenic (proton) pump has been suggested for *R. fluitans*. This pump is probably driven by ATP and can be inhibited by addition of CN^-, by uncouplers [17] or low temperature (Felle, H., unpublished observations). The diffusion potential, E_D, is dominated by potassium in the range 0.1—10 mM ($P_{Na}/P_K \approx 0.1$).

Charged transport of amines?

Ammonia and methylamine probably establish a new electrical pathway across the plasmalemma* of *R. fluitans* thallus and rhizoid cells. It has been an issue of major discussions, whether NH_3 [5,18,19] or NH_4^+ [7—9] is the predominant species crossing the cell membrane. Results in favour of an NH_4^+/$CH_3NH_3^+$ transport are given in Figs. 5 and 6 where [^{14}C]methylamine uptake is shown to be correlated with changes in E_m. Comparison of Figs. 3 and 5 further shows that in the presence of 1 mM CN^-, 0.1 mM NH_4Cl or 0.2 mM methylamine, respectively, [^{14}C]methylamine uptake and membrane potential are correlated. Also, if the membrane potential is changed by increased external K^+ concentration from 1.0 to 10 mM, methylamine uptake is strongly reduced. The quantitative aspect of the flux changes is less satisfactory. As we have seen in Material and Methods, it is very difficult to calculate an exact cell surface area for the *Riccia* thallus. However, using the value of 0.1 m^2/g dry wt, it is possible to calculate the ratio of electrical flux to electrical current. Whereas at low concentrations of external methylamine, the labelled methylamine influx can almost account for the electrically measured inward current,

*Although microelectrode measurements were usually carried out across plasmalemma and tonoplast, the term 'membrane' refers to the plasmalemma only in this paper because a potential difference of only +10 to +20 mV across the tonoplast was measured.

about 5 times as much electrical charge passes through the membrane at 100 μM of amine. Explanations for this discrepancy may be found in some non-uniformity of the applied current over the cell surface, or in the stimulation of the fluxes of other ions due to the amine. The free base of methylamine (pK = 10.65) probably does not contribute significantly to the influx because of its extremely low concentration at the external pH of 5.7, although it probably is transported. Therefore, these results strongly support the idea of a charged amine transport in this pH range and give some confidence that at least part of the increase in membrane conductance and current can be attributed to influx of positive (amine) charge. Transport of the uncharged molecule is not ruled out completely, but certainly does not play a significant role at this pH.

Permeability of amines

With 0.1 mM methylamine in the external medium and a driving membrane potential of −120 mV, a flux of $3–4 \cdot 10^{-14}$ M $\cdot$ m$^{-2} \cdot$ s^{-1} can be measured at pH 5.7. From this a permeability of $6.25–8.3 \cdot 10^{-8}$ m $\cdot$ s^{-1} can be calculated which is about 15 times larger than the values for potassium (1 mM) of $0.5 \cdot 10^{-8}$ m $\cdot$ s^{-1} [11]. From the much faster depolarizations and the larger increases in membrane conductance with ammonia, it can be assumed that $P_{NH_4^+}$ must be considerably bigger than $P_{CH_3NH_3^+}$. In spite of these relatively high permeabilities, a simple approximation with the Goldman voltage equation [20] shows that the effect on the diffusion potential due to the amine influx and its accumulation inside the cell can indeed only be of the order of a few millivolts; this is demonstrated in E_m'' of Fig. 9 after a 30 min incubation with methylamine.

Interaction of amines with other ions

There are some distinct effects of K^+, Na^+ and pH on electrical and flux parameters of the two amines tested (Figs. 6 and 7). Therefore, amines probably do not only establish a new pathway across the plasmalemma, but also affect properties of other ion channels, and vice versa. Whereas different external potassium concentrations cause significant shifts in amine uptake and membrane depolarization (Fig. 6), the apparent K_m does not change (Fig. 2). From Fig. 3 we know that the diffusion potential, E_D, is more K^+ sensitive than E_m;

TABLE I

INFLUENCE OF EXTERNAL POTASSIUM ON MEMBRANE DEPOLARIZATIONS ΔE_m AT pH 5.6 AND DIFFERENT NH_4Cl CONCENTRATIONS

The K_m values are identical for both potassium concentrations. The two ΔE_mmax values are due to the different levels of E_D, and are, in fact, identical as percentages ΔE_mmax.

$[NH_4Cl]_o$ (μM)	ΔE_m (mV)		% of ΔE_mmax (mV)	
	$[K^+]_o$ = 0.01 mM	$[K^+]_o$ = 1 mM	$[K^+]_o$ = 0.01 mM	$[K^+]_o$ = 1 mM
1	13	30	23.5	23.6
2	24	49	43.1	38.2
5	34	78	60.9	61.4
10	43	98	75.0	76.4
20	49	111	86.7	86.6

subsequently, both ΔE_m and E_D are in function of external $[K^+]$. This results in the K^+-dependent ordinate intercepts (ΔE_mmax) of Fig. 2. Table I shows that these effects are due to shifts in E_D, and are identical results if expressed as a percentage of ΔE_mmax.

It was suggested before that K^+ (and Na^+) and NH_4^+ could be transported via a single mechanism into the cell [21,22]. Although the potassium permeability is evidently enhanced in the presence of amines, this may not be the case in *Riccia* because of the amine uptake stimulation in the low K^+-concentration range (Fig. 6). On the other hand, it has been shown [11] that the permeability for K^+ rises with increasing $[K^+]_o$ which could explain the increase in amine influx. The inhibition of uptake at external $[K^+]$ greater than 1 mM is simply explained by the lower electrical driving force. Also, comparison of the *I-V* curves does not give satisfactory answers yet because of the uncertainty about the internal amine concentrations. The question as to whether NH_4^+ just modulates the K^+ pathway (as the changed K^+ permeability indicates) or is indeed transported by the same system is under current investigation in our laboratory. The observed inhibition of [^{14}C]methylamine uptake at lower pH may be explained by an increased protonation of the putative carrier molecules, or of the membrane per se, resulting in a changed mobililty for the carrier-substrate complex inside the membrane. Of course, the same effect could also be taken to be stimulation of the methylamine uptake at higher pH_o, because of increasing CH_3NH_2 concentration. However, the very low concentration of the free base due to its pK of 10.65 renders this stimulation very unlikely.

Common carrier for ammonia, methylamine and amino acids?

In Fig. 2, where ΔE_m is plotted double reciprocally vs. the concentrations of ammonia, methylamine and L-serine, a common ΔE_mmax for all three compounds exists. Does this suggest also a common transport system?

The K_m values from different approaches listed in Table II can be split into two groups: data obtained from *I-V* analysis show higher values than those from uptake and depolarization experiments. It is possible that the maximal membrane depolarization in the presence of amines is possibly not as large as it would be in the case of a totally linear *I-V* curve with quasi ohmic properties. Clearly, from Fig. 8 it can be concluded that, for E_m values more positive than −130 Mv, the curves are non-linear, and it would require a much higher current in order to display a depolarization proportional to the concentration.

TABLE II

APPARENT K_m VALUES (μM) FROM DIFFERENT EXPERIMENTS ON *RICCIA FLUITANS* THALLUS AND RHIZOID CELLS (SEE TEXT)

1/concentration vs.	Ammonia	Methylamine
1/depolarization	2 ± 1	25 ± 5
1/conductance	12 ± 3	50 ± 8
1/current	11 ± 3	46 ± 9
1/uptake	(6) *	23 ± 4

* See Material and Methods.

This results in an apparent saturation near E_D which in turn might give K_m values which are too low. The same may be true for L-serine (Fig. 2), i.e., the common K_m values would be incidental!

If, however, the K_m values are calculated from the change in current through the membrane (Fig. 8) in the presence of ammonia or methylamine, no such misleading interpretation has to be taken into account. This approach gives common saturation values for both ammonia and methylamine which makes a competitive transport of these two amines very likely.

The amine pathway

The saturating methylamine uptake curve of Fig. 5A has a sharp increase in curvature at about 70 μM, and follows the data from experiments in the presence of 1 mM CN^- and 0.1 mM NH_4^+. Two questions arise:

(1) Why does the curve increase in curvature?

(2) Why is the uptake of methylamine the same in the three experiments?

Methylamine uptake in the presence of CN^- and NH_4^+ is directly proportional to the external amine concentration and hence indicates diffusion. This again would mean that methylamine uptake without CN^- is coupled in some way to active transport. The identical uptake with or without CN^- for methylamine concentrations higher than 70 μM makes this very unlikely. For the same reason, a second uptake system is also not favoured to explain the enhanced uptake. But, if it is recalled that:

(a) the membrane is depolarized to the same E_D value in the presence of 1 mM CN^-, 0.1 mM NH_4^+ or methylamine (Figs. 1 and 3);

(b) the *I-V* curves have a sharp increase in curvature near E_D (Figs. 8 and 9) which means higher conductance and probably higher fluxes;

(c) the K_m values increase near E_D (Fig. 8) which means smaller affinity of the carrier, it can be argued in the following way: In the depolarized state of the membrane (CN^-, Figs. 5 and 6), the binding of the substrate (amine) to the carrier may be so poor (high K_m) that a possible saturation curve of the methylamine uptake in the presence of 1 mM CN^- or 0.1 mM NH_4Cl cannot be distinguished from a straight line. In fact, the changes in membrane current, plotted double reciprocally vs. the different ammonia concentrations, yield voltage-dependent K_m values (Fig. 8, inset) in such a way that the binding of the substrate drastically decreases as E_m approaches E_D. It seems possible that the binding site lies within the membrane, and thus is under control of the transmembrane voltage; or the amine binding site of the carrier is active at the membrane surface, but is allosterically controlled by that part of the carrier located within the membrane. In *Chara*, a binding site of the substrate inside the membrane rather than at the surface has been proposed by Walker et al. [9] to explain a voltage-dependent K_m.

A model of Finkelstein [23] predicts a general sigmoid form of the *I-V* relationship which would be expected between the membrane potential and current through a carrier system. This curve would intercept with the *V*-axis at the equilibrium potential for the transported substrate where no net current flows because the electrical forces just balance the chemical forces [24].

The carrier is saturated if the inwardly or outwardly directed driving forces and the substrate concentration are large enough. Therefore, if amine is added

to the outside medium, the membrane is depolarized towards the equilibrium potential, E_{amine}, which initially may even be positive because it depends upon the ratio of $[amine]_o/[amine]_i$, namely at a given pH_o.

$$E_{amine} = RT/F \cdot \ln[amine]_o/[amine]_i \quad (4)$$

E_{amine} is shifted on the V-axis as long as amine accumulation in the cytoplasm proceeds, and finally reaches the membrane potential. However, these considerations only hold strictly if the amines are indeed transported via a single pathway and, secondly, are not metabolized, such as methylamine. Therefore, according to Eqn. 4, E_{amine} and the amine carrier curve are shifted to the left along the voltage axis (inset in Fig. 9). I-V curves measured 30 s after addition of the amine indeed show little change in conductance if compared with the control at the same voltage (cf. g_m and g_m' in Fig. 9). Therefore, as a result of the subtraction of g_m from g_m' (as explained in Results), a constant current of about 70 mA $\cdot$ m^{-2} (filled circles, Fig. 9) is displayed. This is exactly what one would expect if the equilibrium potential for the amine were far off from E_m; it is in full agreement with the hypothetical sigmoid carrier curve of the inset of Fig. 9.

Whereas 30 s after addition of methylamine, $E_{CH_3NH_3^+}$ cannot be determined because membrane breakdown prevents depolarization of the membrane beyond —40 mV, this is possible after 30 min of incubation in methylamine, when $E_{CH_3NH_3^+}$ is about —60 mV according to the open circles of Fig. 9. During these 30 min of incubation, methylamine evidently has accumulated in the cytoplasm, and the equilibrium potential has been shifted to —60 mV. Both curves (open and solid circles) can therefore be understood as portions of the same I-V curve and represent one branch (amine influx) of carrier characteristics. The conductance of the transport system at the equilibrium potential is about 1 S $\cdot$ m^{-2}. This is about twice the value found for the K^+ conductance of 0.43 S $\cdot$ m^{-2} at $E_K \approx$ —110 mV.

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