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The use of weak acids as physiological tools: a study of the effects of fatty acids on intracellular pH and electrical plasmalemma properties of *Riccia fluitans* rhizoid cells

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The mode of action of fatty acids on electrical membrane properties and on cytoplasmic pH has been investigated on rhizoid cells of the aquatic liverwort, *Riccia fluitans*, by means of pH-sensitive microelectrodes and standard electrophysiology. (1) *Riccia* rhizoids have a cytoplasmic pH of 7.3 ± 0.13 and a vacuolar pH of 4.8 ± 0.23. In the undisturbed cell, the cytoplasmic buffer capacity was determined to be 60–80 mM H^+/pH unit, which is reduced by roughly 50% when the cytoplasm is acidified to 6.3, and is exhausted below pH 6. (2) Depending on concentration and on the carbon chain length of the tested acids, the cell first hyperpolarizes (short chains), but then depolarizes (longer chains). No hyperpolarizations are observed when the pump is deactivated (with CN^-). (3) All acids tested acidify the cytoplasm according to the concentration of their protonated form, HA. In the presence of acetic, propionic and butyric acids, the cytoplasmic pH partly recovers, while hexanoic and heptanoic acids rapidly deplete the electrochemical pH gradient across the plasmalemma. (4) All fatty acids show a concentration-dependent and reversible decrease of the membrane resistance, heptanoic acid having an approx. 10-times stronger effect than acetic acid. It is suggested that weak acids induce a net current across the plasmalemma of *Riccia* rhizoids, one portion of which is active (pump current) while the other, of opposite sign, is passive and unspecific. Since the protonmotive force remains essentially constant in the presence of lower concentrations of the shorter-chained acids, or even increases with acetic or propionic acids, we assume the lipid solubilities of the unprotonated acid (A^-) to be negligible. For hexanoic and heptanoic acids, the lipid solubilities of A^- seem high enough for them to be effective as uncouplers. It is further concluded that the hyperpolarizations are the result of a stimulated H^+-extrusion pump, whereas the depolarizations derive from (a) decreased membrane resistance, (b) strongly shifted internal pH, and (c) uncoupled mitochondria.

In recent years, weak acids and bases have been used more and more frequently to induce intracellular pH changes, in order to obtain information on the regulation of cytoplasmic pH or on proton-driven transport processes located at the plasmalemma [1–12]. In this context, it appears that too often, only a single effect of the weak acids on the cells, namely the internal pH shift, is taken into consideration, and that the physiological implications of one weak acid are assumed to be equal to those of any other one. Such an

Abbreviations: A^-, unprotonated acid; Mes, 4-morpholineethanesulphonic acid; HA, protonated acids; pH_c, cytoplasmic pH; pH_v, vacuolar pH.

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approach will necessarily lead to misinterpretation of results. The reason is evident: weak acids usually intrude into the lipid phase of the plasmalemma, and, because of their lipid solubilities, at least partly remain there, which leads to altered membrane properties according to the specific chemical structures involved. Quite obviously this will produce multiple and usually unpredictable effects on the properties not only of the plasmalemma properties, but also of other cellular membranes and compartments.

In this context, a good approach is afforded by the investigation of the mode of action of a homologous series of fatty acids. Especially the fact that the six weak acids tested have roughly the same pK_a (of 4.8) makes the measured effects directly comparable. Since fatty acids, on the one hand, are structurally similar to the membrane lipids, and on the other hand, are quite water-soluble, changes in measurable membrane parameters such as membrane potential, membrane resistance and membrane current are just as valuable for analysis as the pH changes induced by these compounds. While all electrical effects within the membrane are investigated most conveniently with the methods of classical electrophysiology, the pH changes within the cytoplasm or vacuole are measured best with pH-sensitive microelectrodes. The unicellular rhizoids of the aquatic liverwort *Riccia fluitans* [13–17] are the most suitable subjects for the quantitative measurements using both methods.

Material and Methods

Plants and general conditions

Green thalli of the aquatic liverwort *Riccia fluitans* were grown under sterile conditions in a 12/12 h light/dark cycle as described by Felle et al. [18] and Johannes and Felle [19]. 1–2 h before the tests, thalli with the rhizoids were mounted in a Plexiglas perfusion chamber and equilibrated with the basic artificial pond water. This medium comprised 5 mM Tris-Mes/1 mM KCl/1 mM NaCl/0.2 mM $CaCl_2$. The temperature was kept at 22 ± 1 °C and the object was illuminated with white light from a Leitz microscope lamp at roughly 1 W/m^2. The concentrations of the acids used are either given in total concentrations (mM) or as concentration of the protonated form (mM HA) at the indicated pH.

Electrophysiology and pH-sensitive microelectrodes

Standard electrophysiology has been applied throughout the measurements of membrane potential and membrane resistance, as described by Felle [15,16]. The current-voltage data were obtained by impaling the rhizoid cell at the very tip onto two electrodes opposite each other, one being the voltage- and the other the current-injecting electrode.

The pH-sensitive microelectrodes were fabricated as described before [10,11,17,20,21]. Briefly, glass capillaries with a solid filament (Hilgenberg) were pulled on a Getra vertical puller (Weilheim) to 0.4–0.5 μm tips, and were heated in an oven at 180 °C. The rear ends of these hot pipettes were twice dipped briefly into a mixture of dimethyldichlorosilane dissolved in chloroform (0.1 to 0.15%) and were returned immediately to the hot oven, being kept there for approx. 1 h. For stabilization of the tips against the cell turgor, the baked, cold tips were submerged for approx. 45 min in a mixture of poly(vinyl chloride) dissolved in tetrahydrofuran. Suction was applied to the rear end. The proton resin (Fluka-Buchs, No. 82500) was back-filled into the tip using a long glass pipette. Air bubbles, usually occurring during the filling procedure, disappear within 30 to 60 min. The remainder of the electrode was back-filled with a mixture of 0.5 M KCl and 0.1 M Mes-Tris, adjusted to the desired pH. Such electrodes have resistances of over $2 \cdot 10^{11}$ Ω and usually display slopes of 55 to 59 mV per pH unit between pH 4.3 and 9.6.

Since the pH-sensitive electrode always records both electrical membrane potential difference (ψ_m) and the chemical activity of H^+ in the impaled compartment, a high-impedance differential amplifier (WP-Instruments, New Haven, CT, U.S.A.) measured and simultaneously subtracted the two signals. Only the trace of the voltage electrode (ψ_m) and that of the difference between the two electrodes (pH_c, pH_v) will be given throughout this paper.

The response-times of the two electrodes are very different, the pH sensitive electrode being slower ($T_{1/2} = 2$–10 s) than the voltage electrode.

With fast depolarizations, this may feign alkalinizations on the difference trace [10,17].

Localization of the electrodes

The rhizoid cells of *Riccia fluitans* allow impalements either into the vacuole (rare cases) or into the cytoplasm [10]. Since there is a substantial difference in pH between cytoplasm (7.3 ± 0.13 S.E., $n = 58$, this paper), and vacuole (4.8 ± 0.23 S.E., $n = 9$), it is easy to tell from the signal where the electrode tip was actually located.

Presentation of data and reversibility

All figures are copies or were photographed from representative originals of 3–10 equivalent tests. Since they are all continuous recordings, statistics are not given for curves. In principle, reversibility depends critically on (a) the time of incubation with the respective acids and (b) on their lipophilic nature. Reversibility of the presented effects has been obtained with all tested acids, but is demonstrated for the most lipophilic (heptanoic) acid only.

Determination of the cytoplasmic buffer capacity (β)

The cytoplasmic buffer capacity (β) has been estimated according to the weak acid method described by Roos and Boron in 1981 [22], in which

$$\beta = (-)\mathrm{d}A_c^-/\mathrm{dpH_c} \qquad (1)$$

where A_c^- is the quantity of the acid used within the cytoplasm after a new steady state is attained and is calculated from the Henderson-Hasselbalch equation. If the initial concentrations of A_c^- are taken to be zero before adding the acid, then $A_c^- = \mathrm{d}A_c^-$:

$$\mathrm{d}A_c^- = A/(10^{pK-\mathrm{pH_c}} + 10^{\mathrm{pH_o}-\mathrm{pH_c}}) \qquad (2)$$

where A is the total quantity of the acid added to the external medium [12].

The pH electrode as $\Delta\mu_{H^+}/F$ electrode

Under certain experimental conditions, namely, when the pH of the test medium was equal to the pH of the electrode-filling buffer, this pH electrode measures the electrochemical proton gradient directly, provided the external pH is kept constant.

Results

Membrane potential

The effect of the tested fatty acids (C_2 to C_7) on the electrical potential difference across the plasmalemma of *R. fluitans* cells is quite complex. In Fig. 1, a choice of data on the effects of different concentrations of acetic, pentanoic and hepatanoic acids is given. Apart from some not too reproducible wiggles, principally two major effects superimpose, namely, an earlier hyperpolarization and a later depolarization, the magnitudes of which depend on the concentration and on the carbon chain length of the tested acid. The higher the concentration and the longer the carbon chain, the more the initial hyperpolarization is suppressed in favour of a strong depolariza-

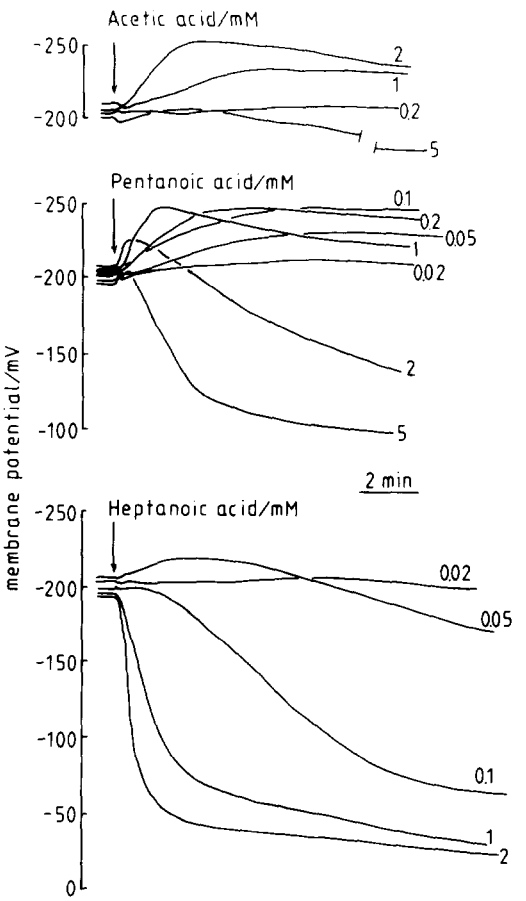

Fig. 1. Action of a selection of three fatty acids, added at the indicated concentrations (arrow), on the electrical membrane (plasmalemma) potential of *R. fluitans*. External pH = 5.0.

tion. These two overlapping effects can be observed quite distinctly by comparing the 2-mM curves in Fig. 1.

Acidification of cytoplasmic pH

Fatty acids are lipid-soluble, the protonated form (HA) usually more so than the anion (A^-) [23]. If no carrier exists for either species, then it can be taken for granted that HA first passively permeates the plasmalemma and then dissociates within the cytoplasm according to its pK_a. This will acidify pH_c and is shown in Fig. 2 (0.07 mM HA) and Fig. 3 (0.7 mM HA). With the exceptions of heptanoic acid in Fig. 2 and hexanoic and heptanoic acids in Fig. 3, these pH_c changes are long transients, i.e., the initial acidification is partly reversed. Please note that the changes in membrane potential do not always correspond with the simultaneously measured pH_c. When the acids are removed from the test medium, the cytoplasmic pH returns to the control level with a damped oscillation (Fig. 2), in some cases first shooting over into the alkaline region (acetic through to pentanoic acid).

The electrochemical proton gradient across the plasmalemma

Since it is widely accepted that proton cotransport, namely H^+/hexose or H^+/amino acid symport [16,19] critically depends on the electrochemical proton gradient ($\Delta\mu_{H^+}/F$) across the respective membrane, it is of great interest for transport studies to know the kinetics of $\Delta\mu_{H^+}/F$ during acid treatment. Provided the time of the acid being present was short (approx. 5 min), and the concentrations low (Fig. 4), $\Delta\mu_{H^+}/F$ can be quite stable. In some cases $\Delta\mu_{H^+}/F$ may even increase (acetic acid, propionic acid, Fig. 4), although pH_c clearly falls. On the other hand $\Delta\mu_{H^+}/F$ is massively decreased by hexanoic and heptanoic acids, eventually reaching zero (Fig. 5). Heptanoic acid (5.35 mM, pH_0 5.62) within 10 min shifts pH_c from 7.3 to 6.1, while ψ_m rapidly approaches zero. Lowering pH_0 to 4.98 further acidifies the cytoplasm to pH 5, while ψ_m, after a transient shift to positive values, reaches zero again. After removal of the acid from the medium, the pH_c readily and completely regains its control value, while the plasmalemma repolarizes considerably more slowly, within 25 min.

The effect of weak acids on internal pH in the presence of cyanide

Fig. 6 compares cytoplasmic and vacuolar pH changes caused by butyric acid and cyanide. While cyanide alone rapidly depolarizes the cell to the

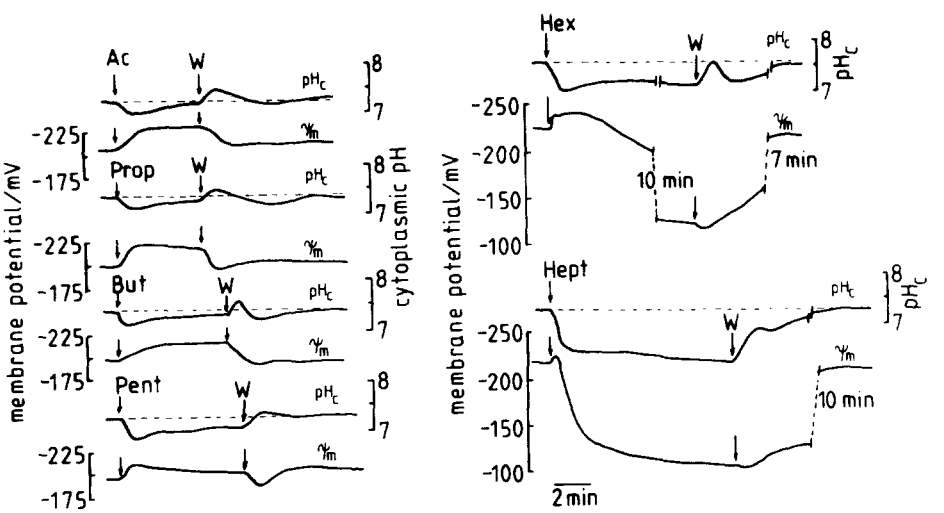

Fig. 2. Cytoplasmic pH (pH_c) and membrane potential (ψ_m) of *R. fluitans* rhizoid cells, measured simultaneously after addition and removal (W) of various fatty acids (as indicated) to the external medium. The concentration of the protonated acids [HA] was 0.07 mM and the external pH was 5.6. The dashed lines indicate the control pH. Ac, acetic acid; Prop, propionic acid; But, butyric acid; Pent, pentanoic acid; Hex, hexanoic acid; Hept, heptanoic acid.

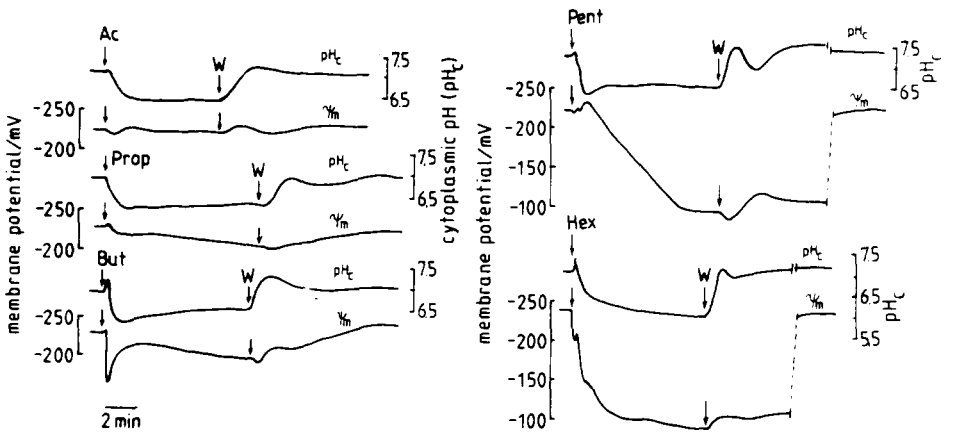

Fig. 3. Cytoplasmic pH (pH_c) and membrane potential (ψ_m) of *R. fluitans* rhizoid cells, measured simultaneously after adding and removal (W) of various fatty acids to the external medium (see Fig. 2). [HA] was 0.7 mM, and pH_o was 5.6. The conspicuous initial alkalinizations (But, Pent, Hex) are caused by the different response times of the pH-sensitive and voltage electrodes, the latter being faster [10]. Abbreviations are as in the legend to Fig. 2.

so-called diffusion potential (due to deactivation of the pump [16]), it alkalizes the vacuole. In the presence of CN^-, butyric acid, chosen as representative acid, causes substantial acidification in the cytoplasm as well as in the vacuole.

Cytoplasmic buffer capacity

The buffer capacity (β) of a cell is studied best by using an acid with little or no lipid solubility for the anion (e.g., acetic acid). In doing so, we find a buffer capacity between 40 and 80 mM

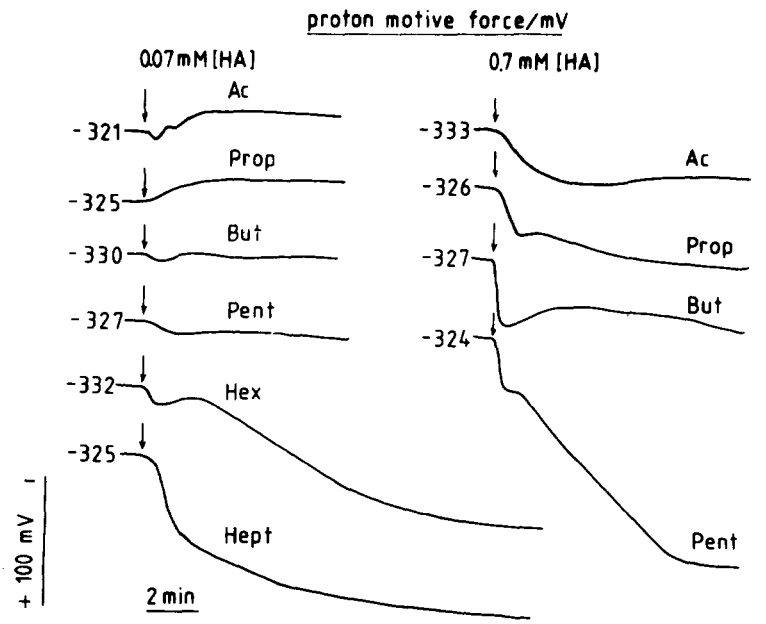

Fig. 4. Electrochemical proton gradient (proton motive force) across the plasmalemma of *R. fluitans* rhizoid cells, measured directly with the pH-electrode before adding and in the presence of various fatty acids, as indicated. Abbreviations as in the legend to Fig. 2.

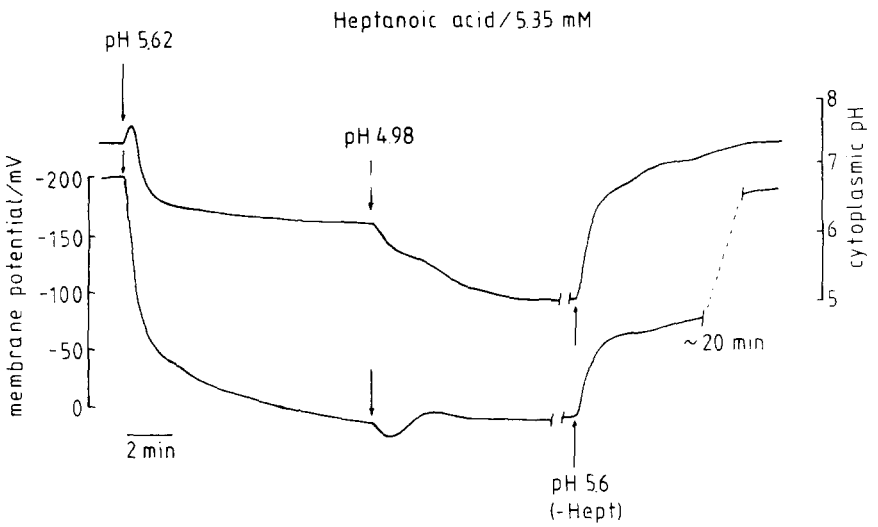

Fig. 5. The effect of heptanoic acid on cytoplasmic pH (pH_c) and membrane potential (ψ_m) of *R. fluitans* rhizoid cells at the indicated external pH values. For details of initial alkalinization at pH 5.62, see legend to Fig. 3.

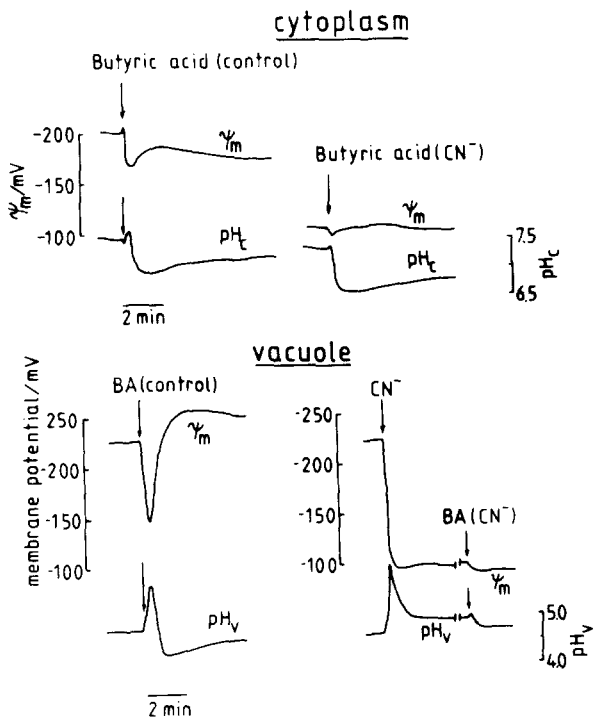

Fig. 6. Action of butyric acid ([HA] = 0.7 mM) and 1 mM NaCN on cytoplasmic pH (pH_c), vacuolar pH (pH_v), and membrane potential (ψ_m) of *R. fluitans* rhizoid cells. pH_o = 5.62. For details of initial alkalinization, see legend to Fig. 3. BA, butyric acid.

H^+/pH unit in the resting cell (calculated from experiments with low acid concentrations). When the acid is added at different concentrations to each different cell, (Fig. 7), the maximal cytoplasmic pH changes indicate a decreasing buffer capacity with falling pH_c (Fig. 7C). However, if calculated from the recovered niveaus of pH_c (same experiment), the buffer capacity apparently increases to unusually high values of around 200 mM H^+/pH (not plotted). No recovery of pH_c is observed when the acid loads are superimposed to one cell without prior washing out, and the drifting pH trace is indicative of an exhausted cytoplasmic buffer capacity beyond pH 6.

Membrane resistance

The entry of the fatty acids into the cell is accompanied by changes in electrical resistance (R_m) of the plasmalemma, depending on the concentration and the lipid solubility of the acids tested. In the presence of 0.58 mM (0.07 mM HA) heptanoic acid, the membrane resistance gradually decreases within 10 min to roughly a quarter of the original value, whereas the 10-fold concentration increase causes a rapid decrease of R_m to about 10% of the control (Fig. 8). The reduction of R_m caused by the same concentrations of the shorter fatty acids is considerably smaller, but

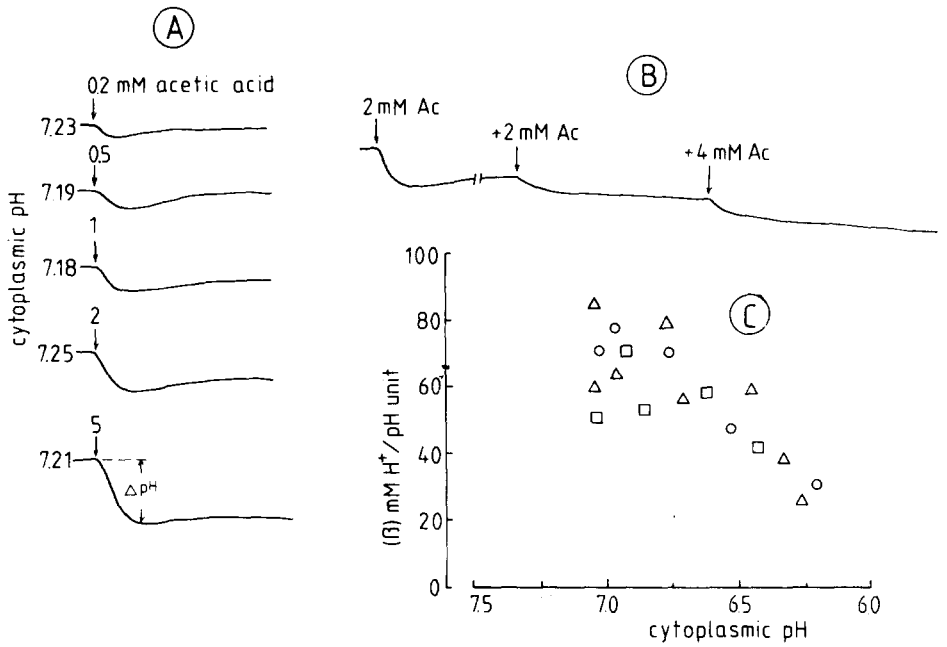

Fig. 7. Estimation of cytoplasmic buffer capacity (β) of *Riccia* rhizoid cells according to Eqns. 1 and 2: (A) action of different acetic acid concentrations on cytoplasmic pH ($pH_o = 5.2$). Each curve represents a different cell; (B) action of acetic acid on pH_c of one cell without washing out (final concentrations, 8 mM acetic acid); (C) cytoplasmic buffer capacity as a function of pH_c; (○) data from (A); △, □ data from other, similar experiments (same conditions).

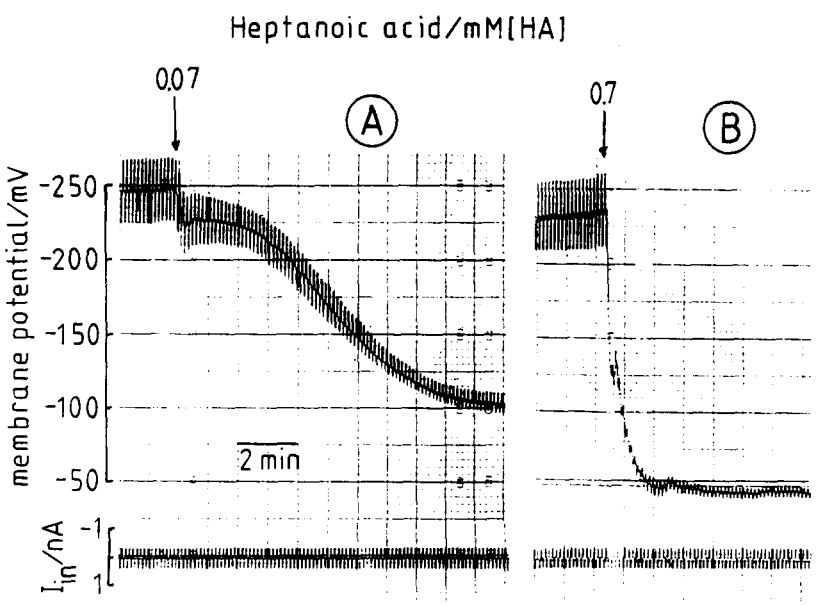

Fig. 8. Decrease of plasmalemma resistance of *R. fluitans* rhizoid cells after the addition of heptanoic acid, photographed from the original recording; (A) [HA], 0.07 mM; (B) [HA], 0.7 mM; $pH_o = 5.6$. The deflections from the membrane potential (dark line) denote the reaction of the cell to the injected current (I_{in}).

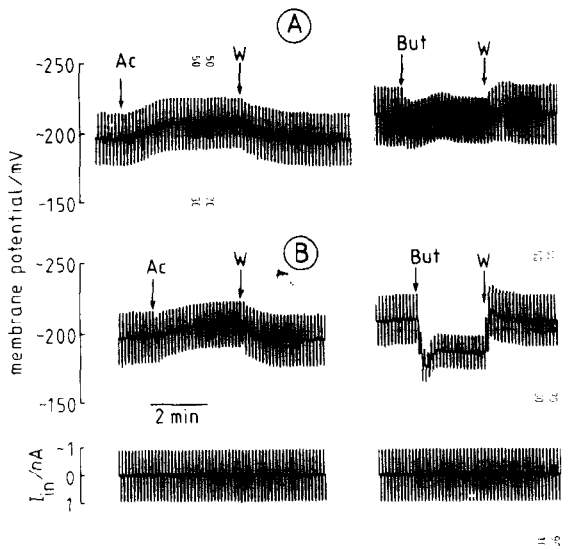

Fig. 9. Plasmalemma resistance of *R. fluitans* rhizoid cells before and after the addition of acetic and butyric acids (Ac and But, respectively). (A) 0.07 mM [HA]; (B) 0.7 mM [HA]; pH_o 5.6; I_{in} = input current. Photographed from original recordings.

increases with lipid solubility (Fig. 9). In the presence of 1 mM NaCN, where mainly passive processes determine the electrical behaviour of the membrane [15], butyric acid yields only a small depolarization, but a significant decrease in R_m (Fig. 10A) which leads to an electrical current either inwardly directed and of positive value or vice versa (Fig. 10B). However, when the electrogenic proton pump is shortcircuited by rapid import of cationic methylamine [20,24], acetic and butyric acids almost completely repolarize the cell, although the membrane resistance is markedly reduced (Fig. 11A, B).

Discussion

The data presented prove that ΔpH and $\Delta\psi_m$ do not have a simple causal relationship, either after adding the acids, or after washing them out. While the cytoplasm is always acidified, either a hyperpolarization (Figs. 1 and 2), a depolarization

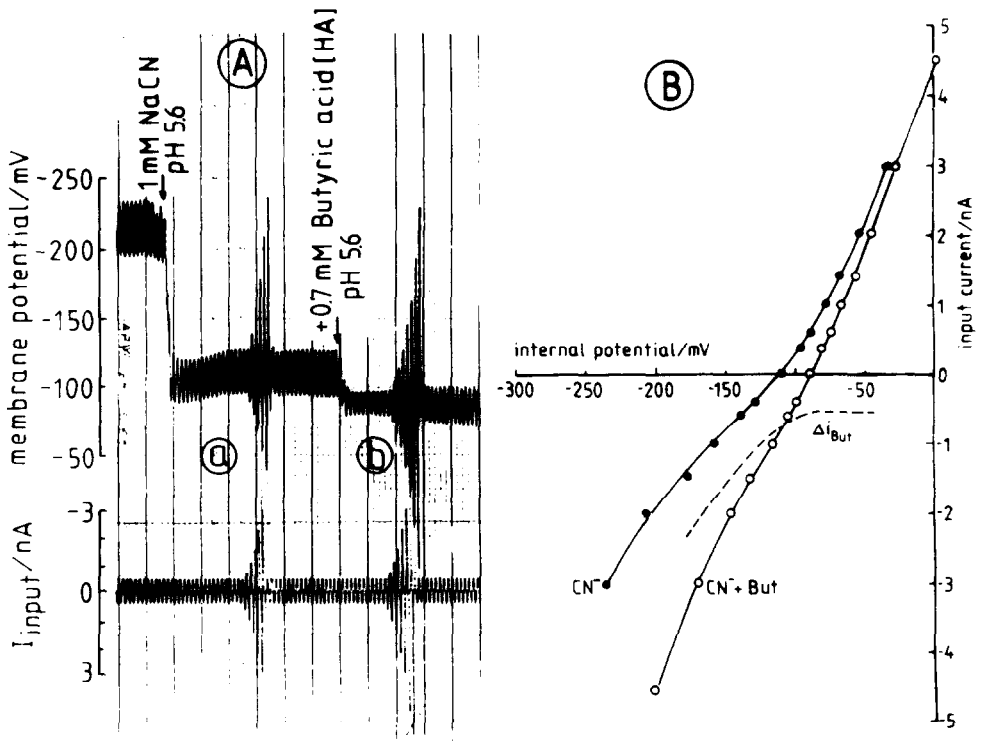

Fig. 10. (A) Action of butyric acid (But) on membrane resistance of *R. fluitans* rhizoid cells measured in the presence of 1 mM NaCN. (B) Current-voltage curves plotted from A. The dashed curve (Δi_{But}) denotes the electrical membrane current induced by the addition of 0.7 mM butyric acid (HA). The current was extrapolated by subtracting the cyanide control curve (CN^-) from the curve measured in the presence of butyric acid (CN^- + But).

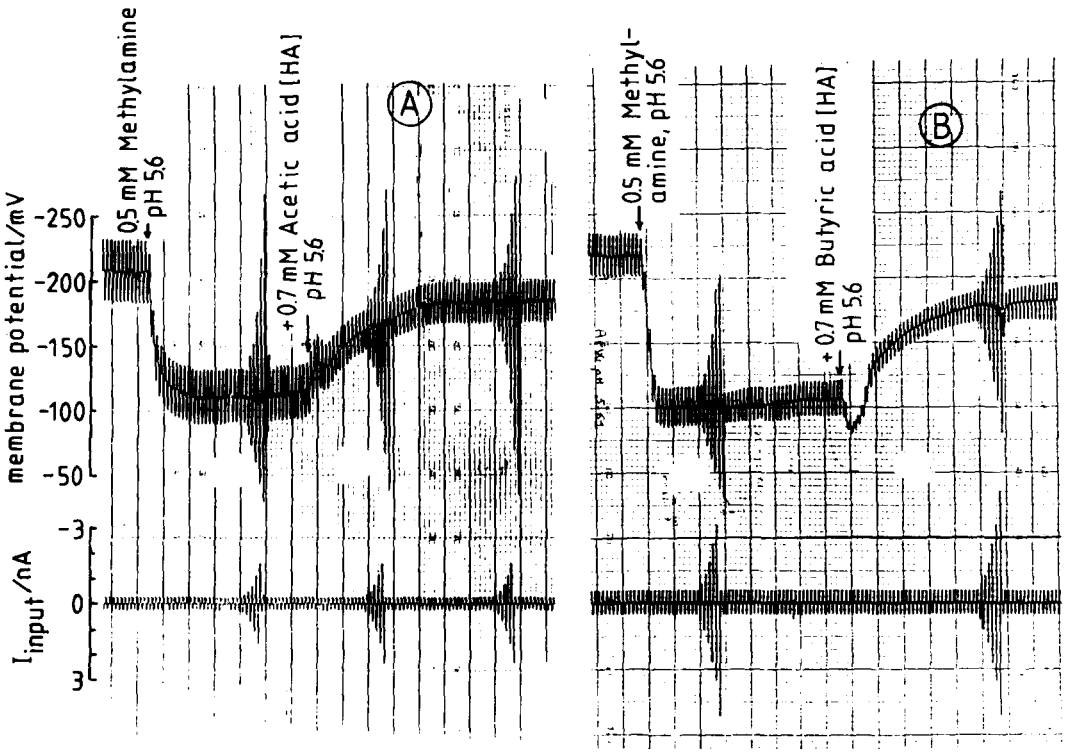

Fig. 11. Effect of 0.7 mM acetic acid (HA) (A) and butyric acid (B) on membrane resistance of *R. fluitans* rhizoid cells measured in the presence of 0.5 mM methylamine at pH 5.6. The deflections from the membrane potential (dark line) are caused by the injection of different test currents (I_{input}) in order to obtain current voltage data, as plotted out in Fig. 10. Photographed from the original recordings.

(Figs. 1–3) or a decrease in R_m (Figs. 8–11) is the first measurable reaction of the cells to the weak acids. Relatively stable hyperpolarizations are generally observed with low concentrations of the C_2–C_4 acids, while the depolarization usually follows in the presence of hexanoic and heptanoic acids, Depolarization and cytoplasmic acidification enhance with increasing acid concentration, and may suppress the hyperpolarization completely. It is evident that the mode of action of these fatty acids is multiple and overlapping, and becomes rather complicated as the lipid solubilities change. In the following sections we attempt to separate and analyse some of the observed effects.

Entry of HA into the plasmalemma

Since we assume that the translocation of these acids is not facilitated by a specific porter, the first interaction will be with the plasmalemma components, leading to a change in membrane resistance (Figs. 8–11). It can be assumed quite safely that the intrusion of the uncharged HA between the lipid molecules and the remaining of some fraction thereof will disturb the structure and hence the function of some membrane elements, including transport proteins. Since the changes in R_m are likewise observed with a deactivated pump (CN^-, Fig. 10) the initially decreased electrical membrane resistance may be looked upon as intrinsically unspecific, indicating that the enhanced membrane current (Δi_{But}, Fig. 10B) consists of several as yet unidentified components. This is confirmed by the finding that the decrease in R_m clearly depends on the lipid solubilities of the acids tested (Fig. 9).

Dissociation of HA within the cytoplasm

According to its pK_a and the cytoplasmic pH, the permeated HA dissociates and acidifies the

cytoplasm (Figs. 2–7). This will necessarily have consequences for membrane transport and for pH_c regulation, including all pH-dependent reactions within that compartment. Drastic effects on amino-acid and sugar transport due to changes in cytoplasmic pH have been reported recently [25]. But also the primary H^+-export pump reacts to acid loads for different reasons: (a) H^+ is a substrate; (b) as a protein, it has a pH-optimum; (c) H^+ will have catalytic effects on the pump. The hyperpolarizations, as observed in the presence of mainly low acid concentrations, therefore point to such a reaction of the electrogenic proton pump. This is supported by the observation that cyanide prevents hyperpolarization in the presence of these acids. However, at first sight it is puzzling that the hyperpolarizations remain, while the acidifications caused by acetic, propionic and butyric acids are partly reversed (Fig. 2). But this is by no means contradictory: The H^+-pump reacts first to the acid load with increased H^+-extrusion and thereby partly restores pH_c, but it also prevents full equilibration of HA across the plasmalemma. Because of this nonequilibration ($HA_o > HA_i$), the pump remains stimulated and the cell hyperpolarized. In the instant of reducing the load on the pump, namely, by removal of the acid(s) from the medium (Fig. 2), the cell responds immediately with a temporary overshoot, indicative of a stimulated active proton extrusion.

Entry of HA into other compartments

It has to be emphasized that the HA concentration added to the external medium will likewise be present within all intracellular compartments after some time. This will also lead to pH-shifts in these compartments,, and influence the physiological state of the cell. Since this will have impacts on membrane potential, membrane resistance and cytoplasmic pH, long-term experiments were avoided throughout this study. With the methods presented here, effects of externally added acids can be measured directly within the vacuole itself (Fig. 6), and indeed, a direct pH change of vacuolar pH in the presence of butyric acid with and without cyanide can be observed. Of course, similar pH changes have to be taken into consideration for other compartments also.

Lipid solubility of A^-

With increasing carbon chain length, not only the lipid solubility of HA increases, but also that of A^-, which brings an additional aspect into the discussion [26]. As soon as A^- is lipid permeable, the negative membrane potential will drive A^- out of the cell. This is equivalent to an additional membrane current, which will lead to further depolarization (Figs. 1–3,4,5,8). The A^- thereby translocated will bind H^+ on the outside, and can now return as HA into the cell, where it can dissociate and acidify the cytoplasm even more (Fig. 3). This process will continue until electrical and chemical driving forces balance. Figs. 2,3,6 demonstrate the following: (a) Identical concentrations of the various acids lead to quite different cytoplasmic pH shifts; (b) whereas the pH_c shifts induced by acetic, propionic and butyric acids are partly reversed, hexanoic and heptanoic acids force pH_c to drift towards increased acidity; (c) heptanoic acid, as the most lipid-soluble of the acids tested, within minutes almost completely depletes both $\Delta\psi_m$ and ΔpH across the plasmalemma, thus revealing its properties as an uncoupling agent.

Impact on the electrochemical proton gradient

$\Delta\mu_{H^+}/F$ is the driving force for proton cotransport. As shown in all figures, weak acids change both ψ_m and ΔpH across the plasmalemma. All the more it is interesting that $\Delta\mu_{H^+}/F$ remains quite constant in the presence of acetic through to butyric acids, and even at a 10-fold higher concentration, acetic acid still depresses $\Delta\mu_{H^+}/F$ by only 50 mV (out of −300!). It thus seems that moderate acidification of pH_c is compensated by a hyperpolarization. On the other hand, it is not surprising that hexanoic and hepatanoic acids rapidly force $\Delta\mu_{H^+}/F$ to very low values, as one would expect from uncoupling agents. In the presence of heptanoic acid (0.7 mM HA), not only does the membrane resistance drastically drop (Fig. 8), but also intracellular ATP levels fall to about 20% (data not shown), indicating severe uncoupling of the mitochondria.

Aspects of cytoplasmic pH regulation

The neutralization of intracellular acid derived from a variety of sources (normally metabolic) is

the central problem of pH_c regulation. The cell has several possibilities to handle this: (a) Cytoplasmic buffer capacity; (b) membrane bound H^+-translocators (e.g., H^+-extrusion pump, H^+-cotransport); and (c) cellular H^+-consumption [22,27]. The massive acidification caused by the entry of the weak acids into the *Riccia* cells must trigger counteractions with a clear tendency to restore the original pH. Indeed, the pH_c first decreases, then slowly returns towards normal pH_c, without reaching it entirely. Although, at first glance, this behaviour appears intrinsically logical for pH regulation and has seemingly been found in other systems also [28–33], some findings cast doubt as to a simple interpretation. Since $[HA]_o$ is clamped, the tendency is at all times for $[HA]_i = [HA]_o$. Therefore, any attempt by pH_c regulators to neutralize protons will necessarily be countered by dissociative processes, as long as the weak acid is present externally at a constant concentration. It therefore appears that only fast H^+-consuming processes or membrane bound H^+-translocators with high proton transport rates will be able to restore pH_c. The electrogenic H^+-extrusion pump seems predestined to do this job, and there are indications for this (such as hyperpolarization and increased pump current, data not shown). But, as demonstrated in Fig. 6, a slight restoration of pH_c also occurs in the presence of CN^-, where the pump is supposed to be deactivated. A catalytically activated H^+-cotransport, as discussed for animal cells (H^+/Na^+-antiport [33]) could well be responsible for this. Seisjo's idea of a time-dependent buffer capacity [31] does not appear likely here, because according to the data of Fig. 7, it would mean an increase in buffer capacity from 50 to 200 mM H^+/pH unit. This is highly doubtful, because the data clearly state that the cytoplasmic buffer capacity is overcome when pH_c is about 6 or lower.

Concluding remarks

The data reported in this work clearly demonstrate the problems in using weak acids as a physiological tool. Although the question of metabolizing the used compounds has not been raised here, it is concluded that the shorter fatty acids, e.g., acetic and propionic acids, may, within limits, be quite useful tools for investigating problems of pH regulation or pH-dependent transport processes. This is because the effects of A^- (acetic acid, propionic acid) were found to be negligible, at least for short time intervals. The problem of the overlapping effects (such as a decrease in resistance, hyperpolarization, depolarization, transient acidification and drifting acidification) makes the use of hexanoic and heptanoic acids extremely difficult. Because of their marked anion lipid solubility and the resulting strong decrease in membrane resistance, even their use as uncouplers is quite uncomfortable and unpredictable. Therefore, such substances are useful tools only if the induced changes, under the relevant experimental conditions, are either known before or are measured simultaneously during the experiment.

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