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Letters to the Editor

Mechanism of Cl^- Efflux Bursts in *Acetabularia*: Vesicle Release *versus* Permeability Transients

Recent investigations by Wendler, Zimmermann and Bentrup [4] confirm the phenomenon of Cl^- efflux bursts in *Acetabularia*, which was first described by Gradmann, Wagner and Gläsel [1] and analyzed in more detail by Mummert [2]. There is agreement upon the following characteristics of these Cl^- bursts: (a) duration (ca. 30 sec), (b) rates (ca. 60 μmol m^{-2} sec^{-1}), (c) release of ca. 4% of the cells Cl^- content in one burst, and (d) possibility of occurring without change in the transmembrane potential difference (V_m).

From these results it has been concluded [2, 3] that the large amount of Cl^- (plus counter cation) released must originate from the vacuole (the cytoplasm occupies only about 5–10% of the entire cell volume) and bypasses the cytoplasm (in which V_m is recorded) in isolated vesicles until release from the cell. This interpretation is strongly confirmed by the finding that these Cl^- bursts do not occur in freshly centrifuged preparations (without vacuole and vesicles – in contrast to normal cells – according to electron micrographs) but recover when new vacuoles and vesicles are formed [2].

Wendler et al. [4] have now presented recordings of turgor pressure (P) concerning these Cl^- bursts. Based on the slow time course in the recordings of the decrease in P ($T_{1/2}$: ca. 160 sec) after a Cl^- burst, Wendler et al. [4] repeatedly emphasize that release of aqueous Cl^- (plus counter cation) vesicles cannot take place within 30 sec of a burst. These authors suggest instead a release of sole (water-free) Cl^- salt which is followed secondarily only by a (slower) osmotic efflux of water. Wendler et al. [4], therefore, suggest that this "salt extrusion" is due to "a reversible change in the membrane permeability," i.e., temporal increase of the passive pathways in the plasmalemma for Cl^- and for the counter cation.

If this mechanism were responsible for the large efflux of Cl^- (and counter cation), dramatic changes in V_m (transient depolarization from the resting V_m of ca. −170 mV) must be expected during a Cl^- burst. However, Wendler et al. [4] particularly confirm that these Cl^- bursts can take place without changes in V_m. In addition, a loss of 4% of the entire Cl^- of the cell across the plasmalemma during one Cl^- burst would mean, according to Wendler et al. [4], that the cytoplasmic Cl^- salt concentration (normal ca. 500 mM in cytoplasm and vacuole [2]) would drop to about 250 mM within ca. 30 sec. Such dramatic and fast decreases of ion concentrations in the cytoplasm are physiologically unrealistic. Furthermore, the mentioned dependence of the Cl^- bursts on the presence of vacuole and vesicles (which has been ignored by Wendler et al. [4]), clearly favors the vesicle model and is hard to explain by the model of permeability changes in the plasmalemma.

All these reasons, in particular the insensitivity of V_m on the Cl^- bursts, render the permeability model of Wendler et al. [4] invalid.

Therefore, the question arises how to interpret the P data of Wendler et al. [4] correctly. Our suggestion is that the temporal resolution of the pressure probes used is too poor to trace the P changes as expected from the vesicle mechanism (Fig. 7*A* in [4]). This suggestion is based on the following reasons: Fig. 8 in [4] shows a single pair of ΔP recordings upon (i) a Cl^- burst and (ii) an osmolarity change of the external solution. Wendler et al. [4] base their calculations on the claimed similarity of these two recordings. Despite the statistical insignificance of this singular observation, Fig. 8 in [4] may be regarded as representative for the moment. Inspection of the temporal response ($T_{1/2}$) of the two ΔP relaxations clearly shows that $T_{1/2}$ upon an osmotic change of the external solution is *not* equivalent but much (ca. threefold) larger than $T_{1/2}$ upon a Cl^- burst. If this $T_{1/2}$ (ca. 160 sec) represents the temporal resolution (low-pass characteristics) of the turgor pressure sensing apparatus, all available results on the Cl^- bursts can consistently be explained by the vesicle release mechanism.

Unfortunately, Wendler et al. [4] do not present data on the temporal resolution of the turgor sensing device, neither by appropriate reference [5] nor directly, which would be of crucial importance in this context. It is known [5] that the response of the pressure transducer within the measuring device is fast enough, e.g., upon a position change of the piston. Required is, however, $T_{1/2}$ by which the P recording system responds to a P step in the compartment in which the tip of the microcapillary is located. In analogy to voltage recording microelectrodes, this $T_{1/2}$ is expected to be much larger, especially when plugging of the inserted microcapillaries has to be taken into account. As long as no controls of the temporal resolution of the impaled pressure probe are presented, the biological significance of the reported ΔP relaxations (and the subsequent calculations and conclusions) must be doubted.

This is not a comprehensive critique on the paper by Wendler et al. [4]. Only the part most challenging to us is dealt with by the above comments.

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Reply to: Mechanism of Cl^- Efflux Bursts in *Acetabularia*: Vesicle Release *versus* Permeability Transients

The critique of Gradmann and Mummert is mainly based on the assumption that the time resolution of the pressure probe is not sufficient to detect the hypothetical vesicle-mediated turgor-pressure behavior described in our paper (ref. [6], Fig. 7*A*). They think that the water resistance of the capillary tip of the pressure probe was the limiting factor of time resolution in our experiments. This would mean that the tips were clogged in all our measurements by cytoplasmatic and/or cell wall material, although tip diameters of several μm have been used. This inferred experimental failure is in sharp contrast to the built-in control mechanism of the probe.

First of all we refer to several examples in the literature where half times of pressure relaxations were measured in the range of a few seconds [3, 4] or even less [1]. As the time scale of the interesting processes in *A. mediterranea* is about two orders of magnitude larger than what can be resolved with the pressure probe, the critique of Gradmann and Mummert really is surprising.

Although the pressure probe technique has been published in detail (cited in ref. [7]), we think it necessary to point out some details pertinent to the raised objections. After insertion of the pressure probe into a cell and continuously during the whole measurement, the meniscus between oil and cell sap in the tip of the microcapillary is controlled under the microscope. A free tip is indicated by the meniscus easily shifting within the glass capillary when volume changes are induced by means of the metal rod in the probe. Besides, there is additional evidence in the pressure response to distinguish a free tip from a clogged one. When the tip is free, the pressure response is mainly due to the elasticity of the cell wall. With a blocked tip, the same volume change results in a much higher pressure response, as the compressibility of the oil and the rubber seals in the probe is lower than the extensibility of a cell wall. This is demonstrated in Fig. 1. The graph shows the pressure track recorded on an *A. mediterranea* cell. At a turgor pressure of 2.7 bar, the cell exhibited a typical pressure regulation (Fig. 1*A*). After the pressure had attained steady state, definite volume changes were applied to the cell and the corresponding pressure changes measured (Fig. 1*B*, note that the time scale is changed). In Fig. 1*C*, the same procedure is shown with a significant difference: the capillary tip was clogged in this experiment! While the same sequence of volume changes was applied, the pressure response was about sevenfold. A volume change of 10 nl produced a pressure change of 2.3 bar instead of 0.33 bar with the capillary tip open towards the cell. Evidently, this is a strong criterium to detect clogging of the pressure probe tip. As the measurement of the volumetric elastic modulus in Fig. 1*B* directly followed the pressure regulation, there can be no doubt that the time course of the pressure response to a Cl^- burst was measured correctly. No artificial slowdown was involved.

A straightforward demonstration of the sufficiency of time resolution in our experiments is given in Fig. 2. After the insertion of the pressure probe into a cell of *A. mediterranea* (Fig. 2*B*) there was a short loss of pressure due to a leakage (probably around the tip). By penetrating the tip a bit deeper into the cell the leakage vanished and a stable turgor pressure was achieved. In the moment when the microelectrode was inserted (Fig. 2*A*), the pressure dropped immediately (Fig. 2*B*) due to the compressibility of the KCl-Agar and the seals in the pressure-tight potential-measuring electrode. Note that the event causing the pressure change occurred outside the pressure probe. Therefore, the pressure signal had to pass the capillary tip of the probe before it could be detected by the pressure transducer in the probe chamber. A comparison of the sudden drop of pressure with the time course of a typical turgor regulation (dashed line in Fig. 2*B*) illustrates that the response of the pressure probe certainly is sufficient to resolve easily the genuine pressure regulation.

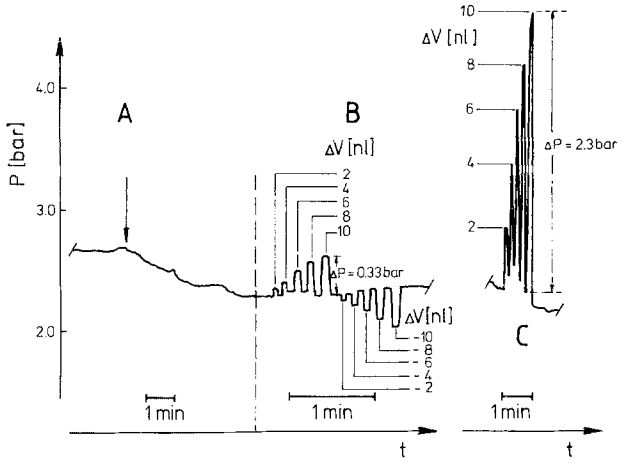

Fig. 1. Turgor pressure *P* recorded on an *Acetabularia mediterranea* cell. (*A*): At a turgor pressure of 2.7 bar, the cell exhibited a typical pressure regulation as a response to a Cl^- burst (not shown). Salt extrusion occured at the time marked by an arrow. The subsequent water flow was responsible for the turgor pressure decrease. (*B*): Immediately after the pressure relaxation, definite volume changes were applied to the cell and the corresponding pressure changes were measured. Note the different time scale in *B*. (*C*): The same sequence of volume changes were applied, but in this experiment the capillary tip of the pressure probe had been clogged. As the pressure response was about seven fold in *C*, clogging of the capillary tip of the pressure probe would have been easily detected in a regular experiment

Three more arguments by Gradmann and Mummert must be discussed in detail. Firstly, the authors expect a dramatic change in the electrical resting potential when transport of Cl^- is due to a permeability change of the plasma membrane. This is not the case, however, because the Cl^- bursts are electroneutral as was emphasized in our paper [6]. Our argument is that the permeability for a Cl^- salt (Cl^- and its counter ion) is increased. Thus, the osmotic pressure inside the cell can be reduced without an electrical current through the membrane. Since we did not try to identify the counter ion, we did not speculate about the molecular mechanism of the permeability change in the membrane. Our results suffice, however, to exclude exocytosis by isosmotic vesicles to account for the Cl^- efflux bursts.

Secondly, Gradmann and Mummert think that a permeability change of the plasmalemma would result in an enormous decrease of Cl^- in the cytoplasm of the cell because of the small volume of this compartment, which they estimate to be about 5–10% of the cell volume. Although our estimation of the cytoplasmatic volume is somewhat higher (about 27% [6]), chloride depletion in the cytoplasm would be less if the permeability of the tonoplast to Cl^-(salt) was not negligible, because then a loss of Cl^-(salt) in the cytoplasm would soon draw Cl^-(salt) out of the vacuole. In fact, as there is no essential difference in the Cl^- concentration of the vacuole and the cytoplasm, there is no hint for a vanishing permeability of the tonoplast.

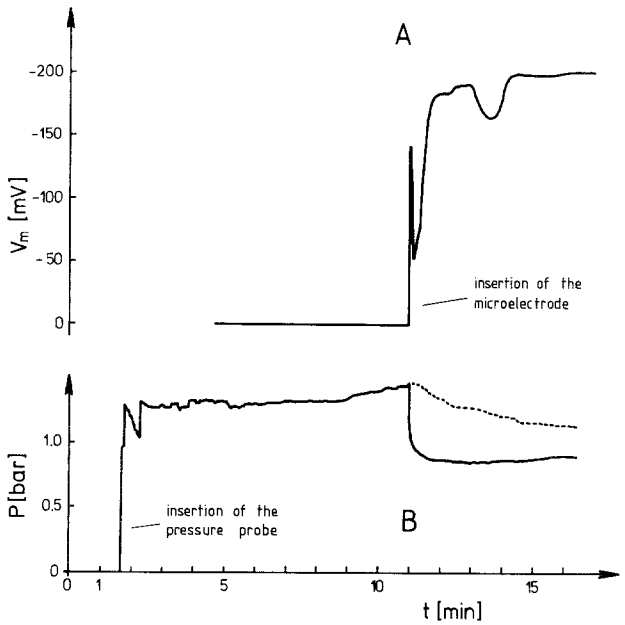

Fig. 2. Straightforward demonstration of the time resolution of the pressure probe during measurements on *Acetabularia* cells. (*A*): Recording of the membrane potential V_m. (*B*): Simultaneous recording of the turgor pressure P. After insertion of the pressure probe (*B*), there was a short loss of pressure due to a leakage. By penetrating the tip a bit deeper into the cell, the leakage vanished and a steady-state turgor pressure was achieved. At the moment where the microelectrode was inserted into the cell (*A*), the pressure dropped immediately due to a small loss of cell volume (*B*). Though pressure-tight microelectrodes have been used, the compressibility of the KCl-Agar and of the seals in the electrode was large enough to account for the observed pressure decrease. As this pressure drop was induced outside the pressure probe, the pressure signal had to pass the capillary tip of the probe before it could be detected by the pressure transducer in the probe chamber. Compare this rapid change of turgor pressure with the time course of a typical turgor pressure regulation (dashed line). It is obvious that the genuine pressure regulation process can easily be resolved by the pressure probe

The third argument of Gradmann and Mummert is based on Mummert's centrifugation experiments yielding cell segments free of vacuoles and vesicles [2]. From the observation that the ligatured nonvacuolated cell segments did not show Cl^- bursts during the first days after centrifugation, the authors concluded that vesicles are necessary for the process of Cl^- extrusion. We also have examined such presumably vesicle-free cell segments using the preparation described by Mummert [2]. With the pressure probe, we were also able to test the cell turgor of these segments. Our results show that a major fraction of the centrifuged cells had no measurable turgor pressure after centrifugation [5], and the turgor pressure established 12–18 hr after centrifugation and ligature was generally less than 2 bar (*unpublished*). As we have pointed out in our paper [6], Cl^- bursts are triggered by turgor pressure values between 2.0 and 2.8 bar. Our explanation of Mummert's observation would be that the cell segments must have had such a low turgor pressure that no change of the permeability of the plasmalemma and therefore no Cl^- bursts could be expected. These experimental findings therefore are not in contradiction to our hypothesis, but a particular confirmation of it.

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