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Reply to: Comments on "The Role of Electrogenic Pump in *Chara corallina*"

Walker raises several questions about the validity of our assumptions in the analysis of electrogenicity in the plasma membrane of *Chara* cells.

What seems important for us in our experiments is that the conductance, G, of the *Chara* membrane, which is the sum of the conductance of passive channel, g_d, and that of electrogenic channel, g_p, decreases asymptotically to a smaller value with the progress of TPC-poisoning; and that the electromotive force (emf) of the *Chara* membrane, which is a weight average of the electromotive forces of these two channels, E_d and E_p, depolarize also to a less negative level asymptotically. If an inhibitor is really an ideal one, attacking only the pump mechanism, then such asymptotic values of conductance and emf should correspond to conductance, g_d, and emf, E_d, of the passive channel and that g_p should decrease to a very small value in the parallel circuit model which we adopted in our analysis (Kishimoto, Kami-ike, Takeuchi, 1980). Thus, adequacy of our analysis is to be judged by how close TPC is to an ideal one or not in the plasma membrane of *Chara* cells.

Walker's comments on our report seem to come from his strong confidence that the passive channel of *Chara* membrane should be modified during a process of metabolic poisoning. He shows in his comments an equation describing a possible change in E_d based on his own model. If this is proved by some direct measurements, we need, indeed, to correct our estimations of g's and E's.

We are not sure that g_p will become strictly zero by TPC. However, we know that the final value of g_p is a very small quantity, which corresponds to an inactivation of the pumping mechanism. During our experiments we did not find a definite evidence to show an increase of g_d like what Walker claims. This is why we assumed that g_d was unchanged during TPC-poisoning, which we mentioned in the text.

In the internally perfused *Chara* cell with very low ATP solution the electrogenic pump is expected to be blocked. However, we are not sure that only the pump channel was blocked in such a treated cell. Actually we noticed that the behavior of the passive channel including the excitatory mechanism was also evidently modified. Therefore, in such a case we need a careful correction.

The depolarized level attained during TPC-poisoning can be expressed either with a Goldman equation or with a parallel combination of Nernst potentials by knowing ionic concentrations of K^+, Na^+, Cl^-, etc., in the cytoplasm and in the external solution. However, we are aware that this is a simulation and is not necessarily definite evidence in support of our assumption.

Walker points out that TPC causes Cl^-/OH^- antiport in the mitochondrial membrane, citing Aldridge, Street and Skilleter's report (1977). This may be true for the mitochondrial and chloroplast membranes in the *Chara* cell. However, again we have so far no definite evidence to indicate such an effect of TPC on the plasma membrane. If the exchange mechanism is of an electroneutral one, it may cause no change in g_d. If we need to assume TPC causes such a Cl^-/OH^- antiport system in the plasma membrane, we can expect a change of the internal pH, which is to be proved with a direct measurement. Anyway, we found by another experiment that g_d was practically unchanged against the change of external pH (Kishimoto, Kami-ike & Takeuchi, 1981). What changed with pH was g_p.

We also measured the change in the internal ATP level during the process of TPC-poisoning. The time course of its decrease was almost an exponential type (1981). We could also find a close correlation of g_p with the level of internal ATP (U. Kishimoto, N. Kami-ike, and Y. Takeuchi, *unpublished*). A similar result was also found by Spanswick (1980). It seems very likely that the main cause for the decrease of g_p in the TPC experiments is in the decrease of internal ATP concentration. Thus, we believe that the main effect of TPC is on chloroplast and mitochondria in the cytoplasm and that its effect on the plasma membrane is minor.

As Walker noted, if there is an over- or under-estimation in the value of g_d and/or E_d, value of E_p also changes. For instance, if g_d is overestimated, the level of the transient hyperpolarization may become more negative than -525 mV, which is certainly ridiculous. It is our experience that if the conductance can be determined accurately by correcting an error which arises from a contribution of change in emf during the test pulse, which we emphasized in our report (1980), the estimated values of g_d and E_d become very reasonable ones.

The transient hyperpolarization of E_p had been puzzling to us for some time. Keifer and Spanswick (1978) also showed a transient hyperpolarization of the membrane potential of *Chara* during poisoning with 5 μM CCCP in the light. Recently we determined values of g_d, E_d, g_p and E_p at different pH solutions. According to our analysis, g_d remains almost unchanged for the change in external pH from 5.4 to 8.3 (U. Kishimoto, N. Kami-ike, and Y. Takeuchi, *in preparation*). A matter of importance is what type of pumping mechanism one assumes in the plasma membrane of *Chara* cells. We assume that the electrogenic pump works as a linear combination of two chemical reactions, one being ATP hydrolysis another proton driving reaction. We could determine the weight of contribution of these two reactions at different pH solutions. We also found evidence to suggest a close coupling of these

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two reactions. This model seems to explain the reason for the transient hyperpolarization and can anticipate the final level of E_p. The results will be published in some detail elesewhere.

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