Short Communication

Proton Efflux from Corn Roots Induced by Tripropyltin\(^\dagger\)

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ABSTRACT

Tripropyltin restores medium acidification by washed corn root tissue in which electrogenic \(H^+\) efflux has been blocked by ATPase inhibitors or injury. However, the restored \(H^+\) efflux is not electrogenic and will not drive \(K^+\) influx, and, by itself, tripropyltin is inhibitory to \(K^+\) influx. Tripropyltin elicits a 5-fold increase in endogenous chloride efflux, and \(Cl^-/OH^-\) exchange can, thus, account for the acidification of the medium. This explanation cannot be applied equally to the acidification produced by the \(K^+\)/\(H^+\) exchanging ionophore nigericin.

In connection with a study of the block in net \(H^+\) efflux from washed corn root tissue caused by protein synthesis inhibitors, it was found that nigericin, a \(K^+\)/\(H^+\) exchanging ionophore, would partially restore apparent \(H^+\) efflux, somewhat in the manner of fusicoccin (2). However, unlike FC, nigericin did not even partially restore \(K^+\)\(^{(\text{\textsuperscript{86}}\text{Rb})}\) influx or growth, and, by itself, it was inhibitory to these processes. Oddly enough, nigericin did restore the small depolarization of membrane electrical potential in cells, a result not expected of an ionophore carrying out neutral exchange.

We wondered if another ionophore, which can carry out a neutral exchange at the expense of a pH gradient, would also produce acidification of the medium. We report, here, an investigation with tripropyltin, which is known to facilitate rapid \(Cl^-/OH^-\) exchange across membranes (5). We also reinvestigated the action of nigericin but failed to confirm the previously reported repolarization.

MATERIALS AND METHODS

Corn seedlings (\textit{Zea mays} L.) were raised, as described, on paper toweling wetted with 0.1 mM CaCl\(_2\), and 0.5 to 2.5-cm root segments were washed for 4 h in the standard medium of 0.2 mM CaSO\(_4\) + 0.2 mM KH\(_2\)PO\(_4\) (pH 6.0) at 30 C (2). Lots of 40 segments (about 0.7 g) were transferred to 50 ml aerated fresh medium at 30 C, and net acidification was recorded as described (2).

Additions were made as indicated with the data. TPT (Pfaltz and Bauer, Stamford, CT), in the range of 2.5 to 10 \(\mu\)M, was determined to give near maximal \(H^+\) efflux. Oligomycin was added with sufficient ethanol to give 1% final ethanol, which prevented oligomycin from coming out of solution. By itself, 1% ethanol did not affect \(H^+\) efflux or \(K^+\) influx.

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For cold shock, the segments were transferred for 3 min to ice cold (2-4 C) medium. For osmotic shock, they were transferred for 3 min to medium + 0.75 mM mannitol at 30 C. For handling, the segments were gently rubbed between the fingers for 2 min; surgical gloves were used.

Uptake of \(K^+\)\(^{(\text{\textsuperscript{86}}\text{Rb})}\) was measured as described (2). Prelabeling the tissue \(Cl^-\) was done over the 4-h wash period by adding 0.23 \(\mu\)Ci of \(\text{\textsuperscript{38}}\text{Cl}^-\) to 100 ml medium. The labeled tissue was rinsed, and free space \(\text{\textsuperscript{38}}\text{Cl}^-\) was desorbed for 10 min in unlabeled medium, after which it was transferred to 25 ml fresh medium \(\pm 5 \mu\)M TPT, and \(\text{\textsuperscript{38}}\text{Cl}^-\) was released to the medium determined at intervals.

RESULTS

Figure 1 shows that TPT restores apparent net \(H^+\) efflux from washed root segments that have had \(H^+\) pumping blocked by ATPase inhibitors. (Depending on penetration, some inhibition of mitochondrial ATPase also may be affected.) This result differs in two ways from the release of \(H^+\) efflux by FC; the response to TPT occurs immediately rather than after a lag of 2 to 3 min, and TPT can restore \(H^+\) efflux in the presence of DES (cf. Ref. 2). Nigericin does not restore \(H^+\) efflux blocked by ATPase inhibitors (2).

Figure 2 shows the block in \(H^+\) efflux, caused by cold shock, osmotic shock, and handling, and the apparent reversal by TPT. FCCP stops the TPT-mediated efflux. FC gives partial release after a lag period of about 3 min (a more extensive investigation of the FC response will be published separately). Nigericin is also effective on shocked tissue.

These inhibitions of \(H^+\) efflux are associated with strong inhibitions of \(K^+\)\(^{(\text{\textsuperscript{86}}\text{Rb})}\) influx which can be partially released by FC (2)(unpublished data). However, as with nigericin (2), TPT proved to be completely ineffective in restoring \(K^+\) influx in inhibited roots and was, itself, inhibitory to \(K^+\) influx (e.g. \(K^+\)\(^{(\text{\textsuperscript{86}}\text{Rb})}\) influx of 4.38 \(\mu\)mol/g fresh weight \(\cdot\) h in control, 0.36 with 5 \(\mu\)g/ml oligomycin, 0.38 with 2.5 \(\mu\)M TPT, and 0.21 with oligomycin + TPT).

Attempts to determine if TPT could restore the amount of decrease in cell potential accompanying MDMP inhibition of \(H^+\) pumping (2) gave erratic results, ranging from small repolarizations to small depolarizations. Reexamination of the response to nigericin, which initially gave repolarization (2), also gave erratic results, and we must withdraw the previous conclusions, since it is not consistently reproducible.

If an exchange were set up between cellular \(Cl^-\) and external \(OH^-\) by TPT, the result would be acidification of the medium. Figure 3 shows that this is undoubtedly what is occurring, since the rate of \(\text{\textsuperscript{38}}\text{Cl}^-\) efflux is increased 5-fold by TPT. This result makes understandable the apparent restoration of \(H^+\) efflux in DES-blocked tissue (Fig. 1); the acidification reflects passive \(Cl^-/OH^-\) exchange.

TPT does not affect tissue respiration (data not shown).
Fig. 1. Inhibition of net H⁺ efflux from washed corn root segments by ATPase inhibitors and resumption of apparent H⁺ efflux on addition of TPT. Oligomycin, 10 μg/ml; DCCD, 50 μM; DES, 50 μM; TPT, 2.5 μM; FC, 5 μM. (---), Abrupt acidification.

Fig. 2. Restoration of net H⁺ efflux in washed root segments subjected to shock treatment (see "Materials and Methods"). Break in the pH recording results from removal of tissue for treatment and subsequent reequilibration. TPT, 2.5 μM; FCCP, 15 μM; nigericin, 10 μM; FC, 5 μM. Inhibition due to cold shock can last up to 40 min.

Fig. 3. Loss of tissue ³⁵Cl⁻ to external medium ± 5 μM TPT (see "Materials and Methods").

**CONCLUSIONS**

It is evident that the action of FC in partially releasing the electrogenic H⁺ efflux blocked by proton channel inhibitors (2), protein synthesis inhibitors (2, 3), physical injury or shock (4) (Fig. 2), and nonpenetrating sulphydryl reagents such as mersalyl (Chastain and Hanson, unpublished data; mersalyl depolarizes corn root cells and FC repolarizes [11]) is quite distinct from the release produced by ionophores. In the case of TPT, the result can be explained by exchange of Cl⁻ accumulated during growth for external OH⁻. However, simple exchange will not account for the action of nigericin. Exchange of internal K⁺ for external H⁺ should alkalize the medium, and it is acidified, while the reverse exchange should give (⁶⁷Rb)K⁺ influx, which was not found (2). As we suggested previously, nigericin may eventually prove useful in studies of the control of the H⁺ pump. Nigericin has been used to stimulate plasmalemma vesicle ATPase (6), and there is a possibility that this could occur in vivo.

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**LITERATURE CITED**