Plant Physiol. (1971) 47, 731–734

Effect of Sodium on Potassium Fluxes at the Cell Membrane and Vacuole Membrane of Red Beet[1]

Received for publication September 23, 1970

RONALD J. POOLE
Department of Biology, McGill University, Montreal, Quebec, Canada

ABSTRACT

Slices of red beet (*Beta vulgaris*) washed for 5 to 6 days are known to accumulate Na^+ in preference to K^+ from solutions containing both ions. The present work, using ion concentrations of 1.0 mM or less, with Ca^{2+} added in some cases, shows that Na^+ strongly inhibits K^+ influx at the cell membrane (plasmalemma) while K^+ efflux is increased to a lesser extent. This result from compartmental analysis is confirmed by short (15-minute) influx experiments, which indicate an immediate inhibitory effect of Na^+ on K^+ influx at the cell membrane. It is concluded that cation selectivity, even when Na^+ is favored for uptake, is primarily determined at the cell membrane. Nevertheless, a high level of K^+ in the cytoplasm is maintained during Na^+ influx, by an inhibition of K^+ transfer to the vacuole.

Recent studies of salt uptake in plant cells have had some success in discriminating between events at the cell membrane (plasmalemma) and events at the vacuole membrane (tonoplast). Thus, the work of Spanswick and Williams (11) has shown that each of these membranes has a role in Na^+-K^+ selectivity in *Nitella*. Spanswick and Williams made direct measurements of K^+ and Na^+ concentrations and electrical potentials in the cytoplasm and vacuole of *Nitella* and concluded that there is an active influx of K^+ and active efflux of Na^+ at the cell membrane, as in typical animal cells, and that in addition there is an active transport of Na^+ from cytoplasm to vacuole.

Pitman *et al.* (7) have shown that Na^+-K^+ selectivity in higher plants may be very dependent on the "salt status" of the material. Low-salt barley roots which are rapidly accumulating ions readily absorb Na^+ as well as K^+. However, as the ion content of the roots increases, they show a striking increase in selectivity for K^+ (7). Studies on higher plant cells which have reached salt saturation, *i.e.*, flux equilibrium with the external solution, have shown (3, 4, 8, 10) that the cell membrane typically has the ability to pump out Na^+, as in *Nitella*. An active influx of K^+ is not so readily detected but has been observed in several cases (4, 8, 10).

The present investigation was undertaken to learn more about the roles of the cell membrane and vacuole membrane during rapid salt accumulation. Storage tissue of red beet, washed for several days, appeared to be an appropriate material, since it not only takes up Na^+, as do low-salt barley roots (7), but shows such a preference for Na^+ accumulation that K^+ uptake is strongly inhibited when Na^+ is added to the external solution (12). It was of interest to determine whether the influx of K^+ at the cell membrane of red beet proceeds independently of Na^+ influx, leading to competition only at the vacuole membrane, or whether the preference for Na^+ uptake in this material is a property of the cell membrane. The present results show that Na^+ influx across the cell membrane strongly inhibits K^+ influx across the same membrane. They thus support the view that Na^+-K^+ selectivity, even when Na^+ is favored for uptake, is primarily determined at the cell membrane.

METHODS

Disks 10 mm in diameter and about 0.7 mm thick were cut from roots of red beet (*Beta vulgaris* L.) and kept in aerated distilled water for 5 to 6 days before use. The temperature during this pretreatment was, for the experiment of Table IA, 25 C throughout; for Table IB, 20 C for 4 days followed by 10 C for 1 day; and for Table II, 10 C throughout. Although washing at a lower temperature increases the selectivity of the tissue for Na^+ over K^+ (9), in all of the experiments reported here the tissue showed a marked preference for Na^+ uptake from solutions containing both ions.

Compartmental Analysis. The fluxes of K^+ in each direction across the cell and vacuole membranes were determined by the method of Pitman (6). Samples of tissue (2 g) were pretreated for 3 hr in unlabeled solution and were then placed in solution containing ^{42}K for a period long enough to allow the isotope to reach a steady level in the cytoplasm. (The isotope uptake periods for the experiments of Tables IA and IB were 4 hr and 5.5 hr, respectively, while the calculated times for half-exchange of K^+ in the cytoplasm were 45–55 min and 70–90 min, respectively.) The tissue was then transferred to unlabeled solutions of similar composition, which were counted and renewed at intervals for a further period of 9–10 hr to determine the change in the amount of isotope in the tissue with time. Net uptake of K^+ was determined by flame photometry of the solutions and of extracts of the tissue at the end of the experiment. For the experiment of Table IA, which was done in the absence of Ca^{2+}, exchangeable Ca^{2+} was removed by treatment with 50 mM KCl at 2 C for 1.5 hr (6) on the day before the experiment.

As shown in Figure 1, the over-all loss of isotope from the tissue may be resolved graphically into two first order processes, apparently representing exchange of ions from the vacuole and from the cytoplasm, respectively, plus a third component attributable to the cell wall. The method of calculating individual fluxes has been described by Pitman (6) and Cram (1) and may be summarized as follows.

[1] This work was supported by a grant from the National Research Council of Canada.

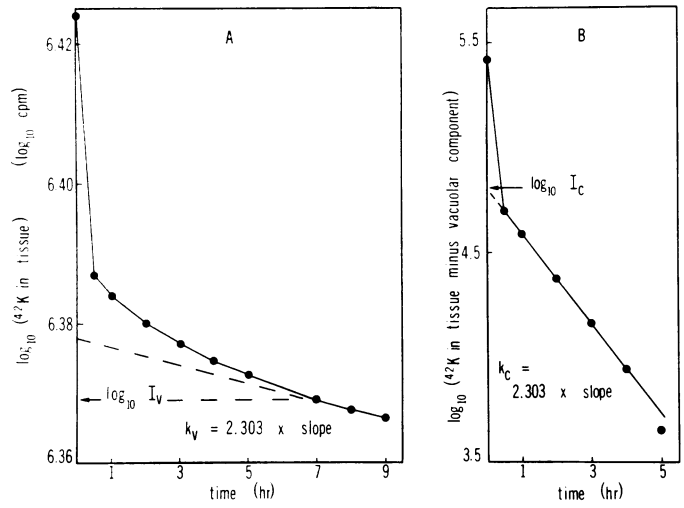

FIG. 1. A: Loss of isotope from beet tissue placed in unlabeled solution after exposure to a solution containing ^{42}K. B: Data from part A replotted after subtraction of the slowly exchanging (vacuolar) component. (Time for half-exchange of vacuolar component: 9.4 days; cytoplasmic component: 1.4 hr.) The data are taken from one of the replicates of the experiment of Table IB. External solution: 1.0 mM KCl + 1.0 mM $CaCl_2$ + 0.5 mM NaCl. Temperature: 10 C. I_v, I_c, k_v, k_c are defined in text.

Quantities to be Determined.

ϕ_{oc}, flux from external solution to cytoplasm
ϕ_{co}, flux from cytoplasm to external solution
ϕ_{cv}, flux from cytoplasm to vacuole
ϕ_{vc}, flux from vacuole to cytoplasm
Q_c, content of cytoplasmic compartment

Measurable Parameters.

ϕ, net (chemical) flux into cell ($= \phi_{oc} - \phi_{co}$)
Q_v, content of vacuole (approximately 90% of content of tissue at end of expt.)
k_c, rate constant for exchange of cytoplasmic compartment (see Fig. 1)
k_v, rate constant for exchange of vacuolar compartment (see Fig. 1)
I_c, apparent isotope content of the cytoplasm (see Fig. 1)
I_v, isotope content of vacuole after exchange of isotope in cytoplasm (see Fig. 1)
s_o, specific activity of external solution during isotope uptake
s_v, specific activity of vacuole content at end of experiment ($= I_v/Q_v$)
t, duration of exposure of tissue to isotope solution.

Theory. The main assumptions are as follows. The cytoplasm and vacuole behave as well mixed compartments in series. The tissue need not be in flux equilibrium but is assumed to be in a steady state, so that the content of the cytoplasm and the fluxes are constant, and

$$\phi_{oc} - \phi_{co} = \phi_{cv} - \phi_{vc} = \phi \quad (1)$$

Movement of isotope is assumed to be random, so that exchange of the contents of the cytoplasm is described by

$$\phi_{co} + \phi_{cv} = k_c \cdot Q_c \quad (2)$$

By the end of the isotope uptake period, and again by the end of the washout period, the isotope in the cytoplasm is assumed to reach a steady state in which the amount of isotope leaving the compartment equals the amount entering. Of the isotope leaving the cytoplasmic compartment, the proportion which goes to the vacuole is $\phi_{cv}/(\phi_{co} + \phi_{cv})$, while the proportion going to the external solution is $\phi_{co}/(\phi_{co} + \phi_{cv})$. On this basis, expressions may be written for each of three steady state isotope fluxes distinguishable in the experiments, namely, the isotope flux from external solution to vacuole at the end of the isotope uptake period, from cytoplasm to solution at the moment of transfer to the washout solution, and from vacuole to external solution at the end of the washout period. These are, respectively

$$(\phi_{oc} \cdot s_o)\phi_{cv}/(\phi_{co} + \phi_{cv})$$
$$(\phi_{oc} \cdot s_o)\phi_{co}/(\phi_{co} + \phi_{cv})$$
$$(\phi_{vc} \cdot s_v)\phi_{co}/(\phi_{co} + \phi_{cv})$$

These expressions may be identified with the observable parameters as follows. The total isotope influx into the cytoplasm during the labeling period ($\phi_{oc} \cdot s_o \cdot t$) will, by the end of the washout period, have been lost to the vacuole and to the external solution in the proportions $\phi_{cv}/(\phi_{co} + \phi_{cv})$ and $\phi_{co}/(\phi_{co} + \phi_{cv})$. Therefore, the isotope content of the vacuole at the end of the experiment is

$$I_v = (\phi_{oc} \cdot s_o \cdot t)\phi_{cv}/(\phi_{co} + \phi_{cv})$$
$$\therefore \quad (\phi_{oc} \cdot s_o)\phi_{cv}/(\phi_{co} + \phi_{cv}) = I_v/t \quad (3)$$

The steady state isotope content of the cytoplasm during isotope uptake is $\phi_{oc} \cdot s_o/k_c$. On transfer to the washout solution, the *apparent* isotope content of the cytoplasm will be

$$I_c = (\phi_{oc} \cdot s_o/k_c)\phi_{co}/(\phi_{co} + \phi_{cv})$$
$$\therefore \quad (\phi_{oc} \cdot s_o)\phi_{co}/(\phi_{co} + \phi_{cv}) = I_c \cdot k_c \quad (4)$$

And for exchange of the vacuole contents at the end of the washout period

$$(\phi_{vc} \cdot s_v)\phi_{co}/(\phi_{co} + \phi_{cv}) = k_v \cdot Q_v \cdot s_v \quad (5)$$

Then, from equations 1 to 5

$$\phi_{oc} = (I_v/t + I_c \cdot k_c)/s_o$$
$$\phi_{co} = \phi_{oc} - \phi$$

or, alternatively,

$$\phi_{co} = I_c \cdot k_c/s_o + k_v \cdot Q_v$$
$$\phi_{cv} = \phi_{co}(I_v/t)/(I_c \cdot k_c)$$
$$\phi_{vc} = \phi_{cv} - (\phi_{oc} - \phi_{co})$$
$$Q_c = (\phi_{co} + \phi_{cv})/k_c$$

Short Term Influx Experiments. These were designed to check the plasmalemma fluxes by a different method. In order to ensure that uptake was not limited by extracellular diffusion, the tissue was placed in isotope solution for 15 min at 2 C before the uptake period of 15 min at 30 C. The small influx during the 2 C exposure was measured in a parallel experiment and subtracted from the total influx to give the influx during the 30 C uptake period. This influx was taken as a measure of the plasmalemma influx, ϕ_{oc}.

RESULTS

Tables I and II show the effect of sodium on the influx and efflux of K^+ at the cell membrane and at the vacuole membrane of red beet cells. In all cases, Na^+ inhibits the influx of K^+ at the cell membrane, and this inhibition is large enough to account for most of the effect of Na^+ on the over-all accumulation of K^+. In Table IA, for instance, the net uptake of K^+ falls from 1.19 μeq/g·hr to 0.11 μeq/g·hr in the presence of Na^+, a change of 1.08 μeq/g·hr. The cell membrane fluxes show that this change is due to a decrease of 0.87 μeq/g·hr in the influx plus an increase of 0.21 μeq/g·hr in the efflux.

Table I. *Effect of Na^+ on K^+ Fluxes in Red Beet*

K^+ fluxes and contents of compartments were determined by the method of Pitman (6). Na^+ was either included throughout the periods of pretreatment, isotope uptake, and isotope washout, or omitted throughout.

A. External solution containing 0.7 mM KCl + 0.3 mM $KHCO_3$ ± 0.4 mM NaCl, 25 C. Means of three replicates.

	K^+ Flux, or K^+ Content of Compartment	
	− Na^+	+ Na^+
	μeq/g·hr or μeq/g	
Net uptake (ϕ)	1.19 ± 0.09	0.11 ± 0.27
Influx to cell (ϕ_{oc})	2.63 ± 0.09	1.76 ± 0.09
Efflux from cell (ϕ_{co})	1.44 ± 0.09	1.65 ± 0.36
Cytoplasm content (Q_c)	4.7 ± 0.1	5.1 ± 0.7
Influx to vacuole (ϕ_{cv})	2.80 ± 0.04	2.27 ± 0.26
Efflux from vacuole (ϕ_{vc})	1.61 ± 0.10	2.16 ± 0.51
Vacuole content (Q_v) at end of experiment	72.7 ± 1.5	57.0 ± 3.6

B. External solution containing 1.0 mM KCl + 1.0 mM $CaCl_2$ ± 0.5 mM NaCl, 10 C. Means of four replicates.

	K^+ Flux, or K^+ Content of Compartment	
	− Na^+	+ Na^+
	μeq/g·hr or μeq/g	
Net uptake (ϕ)	1.98 ± 0.04	0.51 ± 0.03
Influx to cell (ϕ_{oc})	2.14 ± 0.04	0.74 ± 0.02
Efflux from cell (ϕ_{co})	0.16 ± 0.01	0.23 ± 0.01
Cytoplasm content (Q_c)	8.1 ± 0.7	7.1 ± 0.9
Influx to vacuole (ϕ_{cv})	4.69 ± 0.18	3.11 ± 0.27
Efflux from vacuole (ϕ_{vc})	2.71 ± 0.17	2.60 ± 0.29
Vacuole content (Q_v) at end of experiment	92.3 ± 0.9	73.0 ± 2.0

Calcium was omitted in this experiment, and bicarbonate was included to increase the uptake rates. In the experiment of Table IB, in which the presence of Ca^{2+} greatly decreased the efflux of K^+, Na^+ inhibits the influx by 1.40 μeq/g·hr and increases the efflux by only 0.07 μeq/g·hr.

The data on vacuolar fluxes of K^+ are rather variable in Table IA, but from Table IB it seems that the influx of K^+ into the vacuole, rather than the efflux from vacuole to cytoplasm, is affected by Na^+. Neither experiment showed a significant effect of Na^+ on the amount of K^+ in the cytoplasmic compartment.

The results of Table I were obtained by the method of Pitman (6), in experiments of about 14 hr duration. Because of the assumptions and approximations involved in this technique (see "Discussion"), short influx experiments were designed to confirm the above picture. Comparing the isotope influx with the net uptake of K^+ for a 15-min period (Table II), one again finds that the effect of Na^+ on the net uptake of K^+ is mainly due to an inhibition of influx at the cell membrane, although, as before, the efflux of K^+ is also increased by Na^+ to a variable extent. This is particularly noticeable in the cases where Na^+ causes a net loss of K^+ from the tissue, but even here the change in K^+ efflux is somewhat smaller than the change in influx.

The short influx experiments of Table II are particularly significant in that K^+ influx at the cell membrane is strongly inhibited by Na^+ during an uptake period of only 15 min. Figure 2 of the accompanying paper (9) shows that under some conditions the K^+ influx can be reduced almost to zero in a 15-min influx period. This is strong evidence that K^+ uptake at the cell membrane is affected directly, and not, for example, as a consequence of competition with Na^+ at the vacuole membrane.

DISCUSSION

Validity of Methods. The method of compartmental analysis described above is based on the assumption of a steady state for the duration of the experiment, which in the present investigation was about 14 hr. Uptake rates determined by analysis of the solutions (Table III) are not constant, and so the calculated fluxes (Table I) can only be approximate. Nevertheless, each of the replicates gave an efflux curve which was clearly separable into two first order components in addition to the cell wall exchange, and the calculated fluxes showed good agreement among the replicates. In addition, there is an internal check in this method of compartmental analysis (see "Methods") in that there are two alternative ways to calculate the fluxes, one based

Table II. *K^+ Fluxes at Cell Membrane of Red Beet*

The effect of Na^+ was investigated in short term uptake experiments. Tissue was pretreated in solution without isotope or Na^+ for 2 hr at 30 C, then with ^{42}K ± Na^+ for 15 min at 2 C, followed by uptake for 15 min at 30 C. After the uptake period, the tissue was washed in 1.0 mM KCl + 1.0 mM $CaCl_2$ for 30 min at 2 C, and ashed at 500 C. Isotope incorporation during the pretreatment at 2 C was measured separately and subtracted from the total to give the influx during 15 min at 30 C. Net uptake was determined by flame photometry of the uptake solutions after decay of isotope. Means of three replicates.

Solution			Net K^+ Flux		K^+ Influx		K^+ Efflux (Influx minus Net Flux)	
KCl	$CaCl_2$	NaCl[1]	− Na^+	+ Na^+	− Na^+	+ Na^+	− Na^+	+ Na^+
mM			*μeq/g·hr*					
0.2	1.0	0.1	0.73	0.33	1.11	0.78	0.38	0.45
0.5	1.0	0.25	1.26	0.41	1.87	1.25	0.61	0.84
0.2	1.0	0.2	0.48	−0.23	0.92	0.44	0.44	0.67
0.5	1.0	0.5	0.91	−0.44	1.25	0.47	0.34	0.91

[1] Where present.

Table III. *Estimates of Net Uptake of K^+ during Experiment of Table IA*

K^+ uptake was determined by flame photometry of external solutions or of tissue extracts after decay of isotope. Means of three replicates.

	Net Uptake	
	− Na^+	+ Na^+
	μeq/g·hr	
K^+ absorbed from isotope solution (0–4 hr)	1.50 ± 0.08	0.15 ± 0.05
K^+ absorbed from efflux solution (2–3 hr after start of efflux)	1.07 ± 0.10	−0.51 ± 0.07
Average K^+ absorption for whole experiment (from tissue extract)	1.19 ± 0.09	0.11 ± 0.27

on a determination of the net flux, and the other on the exchange rate for the vacuolar compartment. In the experiment of Table IA, the average net flux for the duration of the experiment was used, and the calculated loss of isotope from the vacuole was found to agree with the mean observed values to within 20%, in both the presence and absence of Na^+. In the case of the experiment of Table IB, the K^+ efflux in the presence of Ca^{2+} is too small to estimate from the difference between influx and net uptake. In this case, a check on the results may be made by comparing the calculated net flux (Table IB) with the net uptake determined by flame photometry of tissue extracts. In the absence of Na^+, the calculated and observed values are 1.98 and 2.16 μeq/g·hr; and in the presence of Na^+, 0.51 and 0.76 μeq/g·hr, respectively.

Although the above methods of calculating fluxes appear to give plausible and reasonably self-consistent results, the assumption that the cytoplasm and vacuole behave strictly as compartments in series has been questioned by MacRobbie (5). If it turns out that the series model must be modified, it is unlikely to affect the main result of the present experiments, since the effect of Na^+ on K^+ influx is large.

The experiments of Table II were designed to check the results by a different method. The significance of short term influx experiments has been discussed by Cram (2), and the experiments of Table II approach Cram's requirements for a reliable determination of cell membrane influx. In particular, the uptake time of 15 min at 30 C and the final wash for 30 min at 2 C are reasonably short compared to the times for half-exchange of the cytoplasm (45–55 min at 25 C and 70–90 min at 10 C). Moreover, it was found that the results followed the same pattern after a rinse of only 30 sec as after washing for 30 min. (Subtraction of the low temperature influx corrects for the isotope remaining in the cell wall, but, as might be expected, the variability of the data is greater after a short rinse, when the wall component is very large.) The outcome of these experiments is reassuring, since the effect of Na^+ during a 15-min influx period is very similar to that calculated from the 14-hr experiments described above.

Effect of Na^+ on K^+ Fluxes. The main result of the present investigation is that, during Na^+ uptake, K^+ influx is inhibited at the cell membrane of red beet. Even so, Table I shows that K^+ in the cytoplasm does not become replaced by Na^+ but is maintained at a constant level within the limits of experimental error, the inhibition of K^+ influx at the cell membrane being balanced by a decrease in the rate of transfer of K^+ to the vacuole. This inhibition of K^+ influx to the vacuole perhaps results from competition between Na^+ and K^+ for a transport mechanism at the vacuole membrane.

An effect of Na^+ on K^+ movement from cytoplasm to vacuole has been previously suggested by Pitman (6) on the basis of an experiment in which beet tissue was first allowed to absorb ^{42}K and then transferred to unlabeled solutions of KCl or NaCl. Between 2 hr and 11 hr after transfer, much more label was lost to the Na^+ solution, and the specific radioactivity of this K^+ diffusing out of the tissue was higher than that in the vacuoles, indicating that it came largely from the cytoplasm. It was argued that the cytoplasm exchanges ions with both the vacuole and the external solution, and that Na^+, by competing with K^+ for uptake to the vacuole, causes more K^+ to be lost to the solution.

Inhibition of K^+ influx at the cell membrane in the present investigation is not considered to result indirectly from an effect of Na^+ at the vacuole membrane, since inhibition occurs in a time much less than 15 min. This may be compared with the effect of Na^+ on ^{42}K loss from the vacuole, which, according to Pitman (6), takes about 3 hr to reach a maximal value.

Although it is concluded that Na^+ directly inhibits K^+ influx at the cell membrane, there is evidence that Na^+ and K^+ do not interact by competition for a single carrier at the cell membrane, but are taken up by distinct transport mechanisms, which compete in some way for electrically balancing transported ions. This interaction of Na^+ and K^+ transport is described in the following paper (9).

Acknowledgment—The author thanks Dr. W. J. Cram for valuable discussion.

LITERATURE CITED

1. CRAM, W. J. 1968. Compartmentation and exchange of chloride in carrot root tissue. Biochim. Biophys. Acta 163: 339–353.
2. CRAM, W. J. 1969. Short term influx as a measure of influx across the plasmalemma. Plant Physiol. 44: 1013–1015.
3. ETHERTON, B. 1963. Relationship of cell transmembrane electropotential to potassium and sodium accumulation ratios in oat and pea seedlings. Plant Physiol. 38: 581–585.
4. ETHERTON, B. 1967. Steady state sodium and rubidium effluxes in *Pisum sativum* roots. Plant Physiol. 42: 685–690.
5. MACROBBIE, E. A. C. 1969. Ion fluxes to the vacuole of *Nitella translucens*. J. Exp. Bot. 20: 236–256.
6. PITMAN, M. G. 1963. The determination of the salt relations of the cytoplasmic phase in cells of beetroot tissue. Aust. J. Biol. Sci. 16: 647–668.
7. PITMAN, M. G., A. C. COURTICE, AND B. LEE. 1968. Comparison of potassium and sodium uptake by barley roots at high and low salt status. Aust. J. Biol. Sci. 21: 871–881.
8. PITMAN, M. G. AND H. D. W. SADDLER. 1967. Active sodium and potassium transport in cells of barley roots. Proc. Nat. Acad. Sci. U.S.A. 57: 44–49.
9. POOLE, R. J. 1971. Development and characteristics of sodium-selective transport in red beet. Plant Physiol. 47: 735–739.
10. SCOTT, B. I. H., H. GULLINE, AND C. K. PALLAGHY. 1968. The electrochemical state of cells of broad bean roots. I. Investigations of elongating roots of young seedlings. Aust. J. Biol. Sci. 21: 185–200.
11. SPANSWICK, R. M. AND E. J. WILLIAMS. 1964. Electrical potentials and Na, K, and Cl concentrations in the vacuole and cytoplasm of *Nitella translucens*. J. Exp. Bot. 15: 193–200.
12. SUTCLIFFE, J. F. 1957. The selective uptake of alkali cations by red beet root tissue. J. Exp. Bot. 8: 36–49.