Low-Affinity Na$^+$ Uptake in the Halophyte *Suaeda maritima*[1][C][W][OA]

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Na$^+$ uptake by plant roots has largely been explored using species that accumulate little Na$^+$ into their shoots. By way of contrast, the halophyte *Suaeda maritima* accumulates, without injury, concentrations of the order of 400 mM NaCl in its leaves. Here we report that cAMP and Ca^{2+} (blockers of nonselective cation channels) and Li$^+$ (a competitive inhibitor of Na$^+$ uptake) did not have any significant effect on the uptake of Na$^+$ by the halophyte *S. maritima* when plants were in 25 or 150 mM NaCl (150 mM NaCl is near optimal for growth). However, the inhibitors of K$^+$ channels, TEA$^+$ (10 mM), Cs$^+$ (3 mM), and Ba^{2+} (5 mM), significantly reduced the net uptake of Na$^+$ from 150 mM NaCl over 48 h, by 54%, 24%, and 29%, respectively. TEA$^+$ (10 mM), Cs$^+$ (3 mM), and Ba^{2+} (1 mM) also significantly reduced ^{22}Na$^+$ influx (measured over 2 min in 150 mM external NaCl) by 47%, 30%, and 31%, respectively. In contrast to the situation in 150 mM NaCl, neither TEA$^+$ (1–10 mM) nor Cs$^+$ (0.5–10 mM) significantly reduced net Na$^+$ uptake or ^{22}Na$^+$ influx in 25 mM NaCl. Ba^{2+} (at 5 mM) did significantly decrease net Na$^+$ uptake (by 47%) and ^{22}Na$^+$ influx (by 36% with 1 mM Ba^{2+}) in 25 mM NaCl. K$^+$ (10 or 50 mM) had no effect on ^{22}Na$^+$ influx at concentrations below 75 mM NaCl, but the influx of ^{22}Na$^+$ was inhibited by 50 mM K$^+$ when the external concentration of NaCl was above 75 mM. The data suggest that neither nonselective cation channels nor a low-affinity cation transporter are major pathways for Na$^+$ entry into root cells. We propose that two distinct low-affinity Na$^+$ uptake pathways exist in *S. maritima*: Pathway 1 is insensitive to TEA$^+$ or Cs$^+$, but sensitive to Ba^{2+} and mediates Na$^+$ uptake under low salinities (25 mM NaCl); pathway 2 is sensitive to TEA$^+$, Cs$^+$, and Ba^{2+} and mediates Na$^+$ uptake under higher external salt concentrations (150 mM NaCl). Pathway 1 might be mediated by a high-affinity K transporter-type transporter and pathway 2 by an AKT1-type channel.

Various mechanisms have evolved in plants to allow them to cope with growing in saline soils; all are based on limiting the concentration of sodium and chloride ions accumulating in the cytosol, since cytosolic enzymes are sensitive to these ions in both glycophytes and halophytes (Flowers et al., 1977, 1986; Greenway and Munns, 1980). The cytosolic concentrations of sodium and chloride are determined by the net fluxes across the plasma membrane and the tonoplast. Of the tonoplast proteins, those involved in the transport of Na$^+$ are a Na$^+$/H$^+$ antiporter (Apse et al., 1999; Fukuda et al., 1999; Hamada et al., 2001; Ma et al., 2004), which is energized by the proton gradient generated by the vacuolar H$^+$-ATPase and the H$^+$-PP$_i$ase (Gaxiola et al., 1999, 2002; Sze et al., 1999, 2002). Compartmentalization of Na$^+$ in vacuoles is a particularly important aspect of the way plant cells deal with external salt, because the vacuolar ions contribute to osmotic adjustment. The extrusion of cytoplasmic Na$^+$ out of the cell, effected by SALT OVERLY SENSITIVE1 (SOS1; Shi et al., 2002, 2003; Martínez-Atienza et al., 2007), a H$^+$ exchanger present in the plasma membrane and a member of the NhaP group of proteins (Pardo et al., 2006), is another putative route to avoid Na$^+$ accumulation in the cytosol (Blumwald et al., 2000) and to transfer Na$^+$ to xylem vessels (Shi et al., 2002; see Davenport et al., 2007). Reducing Na$^+$ influx must, though, be the key step for controlling Na$^+$ accumulation compared with vacuolar Na$^+$ compartmentalization and Na$^+$ extrusion by SOS1, neither of which would be sufficient alone. However, the pathways by which plants take up Na$^+$ are uncertain.

Electrophysiological studies suggest that Na$^+$ influx across the plasma membrane occurs via voltage-independent channels or nonselective cation channels (NSCCs), but their precise molecular identities are not known (Tyerman and Skerrett, 1999; Demidchik et al., 2002). A further candidate for mediating sodium transport across the plasma membrane is a low-affinity cation transporter (LCT) from wheat (*Triticum aestivum*), although its physiological role remains to be established in planta (Schachtman et al., 1997; Amtmann et al., 2001).

In the plasma membrane, the HKT proteins (high affinity K transporters), which have also been shown to transport Na^+ (see Rodriguez-Navarro and Rubio, 2006), fall into two subfamilies, one of which (subfamily 2) has only been found in the grasses (Platten et al., 2006). Wheat TaHKT2;1 functions as a K^+-Na^+ symporter (Schachtman and Schroeder, 1994; Rubio et al., 1995; Gassmann et al., 1996) and homologs have since been cloned from many species of plants. The HKT proteins can be divided into two distinct types according to their properties of Na^+ and K^+ transport in heterologous expression systems: The Arabidopsis (*Arabidopsis thaliana*) AtHKT1;1 transported only Na^+ (Uozumi et al., 2000); rice (*Oryza sativa*) OsHKT2;1 showed an AtHKT1;1-like Na^+-specific transport activity and mediates Na^+ uptake in the absence of external K^+ (Horie et al., 2007), whereas OsHKT2;2 showed a TaHKT2;1-like K^+-Na^+ transport activity (Horie et al., 2001); both eucalyptus EcHKT1;1 and EcHKT1;2 mediated Na^+ and K^+ uptake (Fairbairn et al., 2000). Based on the capacity of Arabidopsis *hkt1* mutations to suppress Na^+ accumulation and hypersensitivity of the *sos3-1* mutant to Na^+, Rus et al. (2001) proposed that AtHKT1;1 is a determinant of Na^+ entry into plant roots. The evidence from wheat also supports this viewpoint: Transgenic wheat plants in which native *TaHK2;1* expression is significantly reduced through the introduction of antisense showed decreased Na^+ uptake into roots (Laurie et al., 2002). Recent evidence shows, however, that AtHKT1;1 is a determinant of accumulation of Na^+ in the root and retrieval of Na^+ from the xylem (Davenport et al., 2007). The analysis by Rodriguez-Navarro and Rubio (2006) suggests that HKT transporters mediate high-affinity Na^+ uptake but also function in low-affinity Na^+ transport.

At present, two obvious approaches to the functional identification of the genes encoding K^+ and Na^+ transporters, gene knock out and expression in heterologous systems, present problems that do not generally occur with other genes (Rodriguez-Navarro and Rubio, 2006). Rodriguez-Navarro and Rubio (2006) note "The problem of gene knock out is the pleiotropic effects caused by mutations that affect K^+ transporters. In fungi it is known that the disruption of the *TRK* (Madrid et al., 1998) but not *HAK* genes (Bañuelos et al., 2000) produces hyperpolarization and a consequent enhancement of K^+ uptake through non-K^+ transporters (Madrid et al., 1998)....these problems have not been reported in plants but possibly only because they have not been investigated" (p. 1156). Even if a gene of a putative transporter were cloned, Na^+ uptake tests cannot be carried out in yeast *trk1 trk2* mutants at high external Na^+ concentrations since they have intrinsic low-affinity transporters (Santa-María et al., 1997). Moreover, heterologous expression system may not reproduce kinetic characteristics of transporters in planta (Garciadeblas et al., 2003; Haro et al., 2005). There is no perfect system currently available to investigate K^+ and Na^+ uptake in plants.

To date, Na^+ uptake by plant roots has been explored using glycophytes (e.g. Arabidopsis, rice, or wheat) and a few halophytes (e.g. *Thellungiella halophila*, *Puccinellia tenuiflora*, and *Mesembryanthemum crystallinum*). Most glycophytes (Läuchli, 1984; Huang et al., 2006; James et al., 2006) and some halophytes, such as *T. halophila* (Volkov et al., 2003; Inan et al., 2004; Taji et al., 2004) and *P. tenuiflora* (Wang et al., 2002, 2004) are relatively salt-excluding plants and have a strong selectivity for K^+ over Na^+ that limits the uptake of Na^+ while maintaining the uptake of K^+. *M. crystallinum* is a salt-secreting plant and possesses epidermal bladder cells in its aerial parts, which store Na^+ (Adams et al., 1998; Su et al., 2002): Understanding ion transport in such plants may be confounded with the complex processes of salt secretion and the development of salt bladders.

Although high-affinity Na^+ uptake in plant roots can be tested by applying the depletion method (Garciadeblas et al., 2003; Haro et al., 2005), the lack of a suitable system has restricted the identification of low-affinity Na^+ uptake pathways, such as operate in plants growing under salinity. Species from within the Chenopodiaceae, the family with the highest proportion of halophytes, offer potential physiological models. Species in the genus of *Suaeda* are salt-accumulating plants (Yeo and Flowers, 1980; Wang et al., 2002), which accumulate substantial amounts of Na^+ in their shoots with apparent weak selectivity for K^+ over Na^+ (Reimann, 1992; Reimann and Breckle, 1993; Wang et al., 2002); the selectivity of roots of *Suaeda salsas* for K^+ over Na^+ ($S_{K:Na}$) was between 2% and 14% of that of *Hordeum vulgare*, *Echinochloa frumentacea*, *Calamagrostis pseudophragmites*, *Sophora alopecuroides*, and *P. tenuiflora* and the selectivity of transport from root to stem between 5% and 33% of that in the same species (Wang et al., 2002). We have used *S. maritima*, a species where net uptake of Na^+ in 50 mM NaCl is an order of magnitude greater than that of durum wheat (*Triticum durum*) growing in the same salinity (0.13 μmol/g fresh weight root/min, interpolated from the data in Yeo and Flowers, 1986; net uptake of Na^+ by durum wheat in 50 mM NaCl is 0.01–0.02 μmol/g fresh weight root/min; Davenport et al., 2005) to examine Na^+ influx and found two distinct low-affinity Na^+ uptake pathways: Pathway 1 is insensitive to TEA^+ or Cs^+, but sensitive to Ba^{2+} and mediates Na^+ uptake under low salinities such as 25 mM NaCl; pathway 2 is sensitive to TEA^+, Cs^+, or Ba^{2+} and mediates Na^+ uptake under higher external salt concentrations, such as 150 mM NaCl. We also present physiological evidence that NSCCs and LCT are not the major pathways for Na^+ entry into root cells of *S. maritima*.

RESULTS

S. maritima grows optimally in salt concentrations of about 150 mM sodium chloride (Yeo and Flowers, 1980) and in 200 mM NaCl accumulates sodium ions in its leaves to concentrations of around 5.5 mmol per g dry weight or about 430 mM based on the tissue water

content (plants grown for 35 d: Clipson, 1984; see also Flowers et al., 1986). To throw light on the way in which sodium ions enter the root of *S. maritima*, we have examined the effects of a range of inhibitors on growth and ion uptake.

Effects of cAMP, Ca^{2+}, and Li$^+$ on Growth and Ion Accumulation of *S. maritima* Growing at Near-Optimal Salinity (150 mM NaCl)

We evaluated the effects of cAMP and Ca^{2+}, which inhibit Na$^+$ influx through NSCCs (Maathuis and Sanders, 2001; Demidchik and Tester, 2002), and Li$^+$, which is considered an analog of Na$^+$ and is a competitive inhibitor for Na$^+$ uptake and transport (Serrano et al., 1999; Rubio et al., 2004) on growth and ion accumulation of *S. maritima*. Compared to control plants (plus NaCl, but no inhibitor), cAMP (500 μM), Li$^+$(10 mM), and Ca^{2+}(10 mM) did not have any significant effect on dry weight or tissue water content of leaf, stem, or root (Fig. 1); neither did these compounds have any significant effect on Na$^+$ or K$^+$ concentrations in different plant parts (Fig. 2). Prolonging the treatment time from 48 to 144 h did not induce any significant effect of cAMP, Li$^+$, or Ca^{2+} on whole plant Na$^+$ content of *S. maritima* (data not presented). Since these data suggest that NSCCs do not mediate Na$^+$ uptake in *S. maritima*, we investigated the potential roles of other ion channels.

Effects of Various K$^+$ Channel Blockers on Growth and Ion Accumulation of *S. maritima* Growing at Near-Optimal Salinity (150 mM NaCl)

TEA$^+$ and Cs$^+$ inhibit K$^+$ uptake through most K$^+$ channels and some other transporters (Hedrich and Schroeder, 1989; Tester, 1990). In preliminary experiments (data not presented), we found that plants whose roots were treated with 10 mM TEA$^+$ and 3 mM Cs$^+$ wilted, an effect not seen with other inhibitors that we used. So, in the experiments that we report involving TEA$^+$ and Cs$^+$ treatments, we reduced transpiration by keeping the plants in a chamber with 100% relative humidity (compare with Nublat et al., 2001; Shi et al., 2002). In these experiments, we added the inhibitors at the same time as the salt; *S. maritima* is a salt-accumulating plant and has a strong capacity for Na$^+$ accumulation, so it adjusts very rapidly to increase in external Na$^+$ concentration (Clipson, 1987). Using non-Na$^+$-adapted plants allowed us to investigate the properties of those proteins involved in initial Na$^+$ uptake and avoid any possible consequences of a high internal Na$^+$ concentration interfering with the uptake of the inhibitors. Under these conditions, TEA$^+$ and Cs$^+$ significantly decreased leaf fresh weight (by 46% and 42% compared with the control, respectively; data not presented) of plants growing in 150 mM NaCl. TEA$^+$ also decreased leaf dry weight (by 29%; Fig. 1); the effect of Cs$^+$ was not statistically significant. Leaf tissue water content was significantly reduced by both

TEA$^+$ (by 40%) and Cs$^+$ (by 31%) treatments compared with the control (Fig. 1).

The exposure of plants to external Na$^+$ invariably leads to an increase in the concentration of that ion in the plant. TEA$^+$, however, significantly decreased Na$^+$ concentrations in leaf, stem, and root by 48%, 45%, and 21%, respectively, compared with corresponding organs of control plants, which were treated with NaCl (150 mM) but not the inhibitor (Fig. 2A). Although Cs$^+$ treatment also reduced Na$^+$ concentrations in leaves, the concentrations in stems and roots increased (Fig. 2A). Further analysis of the data showed that while TEA$^+$ altered the quantity of Na$^+$ in different plant parts, it had little effect on its distribution within the plant (Table I). However, Cs$^+$ reduced the quantity of Na$^+$ in leaf tissue and increased the proportion in the stem (Table I).

K$^+$ concentrations in salinized plants fell when compared with the situation before salinization (Fig. 2B). However, neither TEA$^+$ nor Cs$^+$ (nor any of the other inhibitors used) had any effect on the K$^+$ concentration in leaf, stem, or root (Fig. 2B). After a NaCl treatment of 48 h, TEA$^+$ and Cs$^+$ did not significantly reduce K$^+$
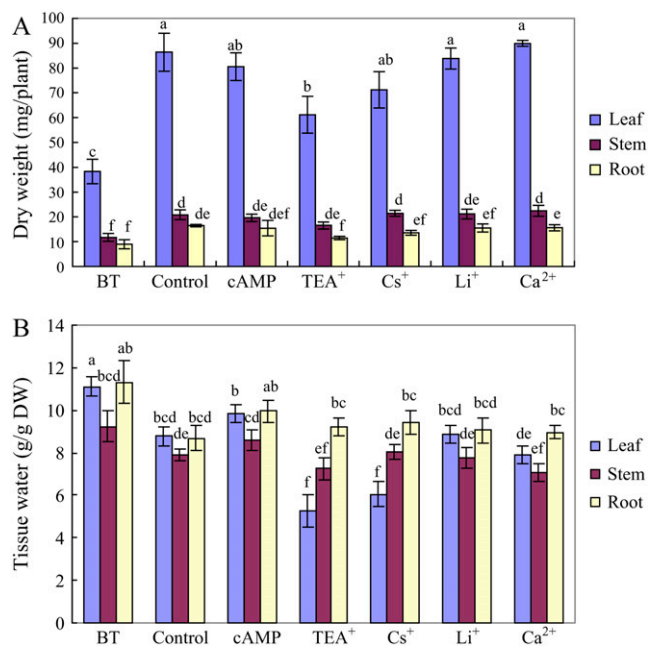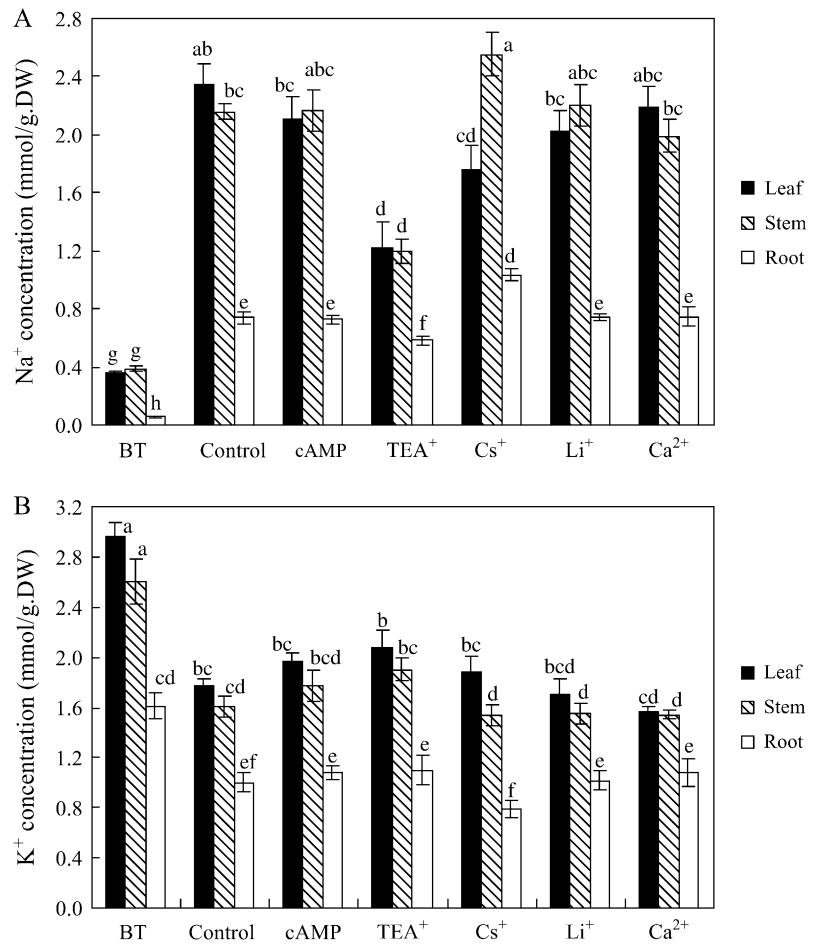

Figure 1. Growth and tissue water content of *S. maritima* seedlings in response to treatment with NaCl (150 mM) together with cAMP (500 μM), TEA$^+$ (10 mM), Cs$^+$ (3 mM), Li$^+$ (10 mM), or Ca^{2+} (10 mM). Three-week-old seedlings were transferred to Hoagland solution supplemented with 75 mM NaCl and 24 h later the concentration was increased to 150 mM NaCl for another 24 h (Control); for the inhibitor treatments, the plants were subjects to the same salinization procedure but in the presence of the inhibitor (i.e. from the time of salinization with 75 mM NaCl). Plants were grown in 100% relative humidity. BT represents before treatment with NaCl and inhibitors. Values are means ± SD ($n = 24$) and bars indicate SD. Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test). [See online article for color version of this figure.]

Figure 2. Na$^+$ and K$^+$ concentrations in *S. maritima* seedlings treated with NaCl (150 mM) together with cAMP (500 μM), TEA$^+$ (10 mM), Cs$^+$ (3 mM), Li$^+$ (10 mM), or Ca^{2+} (10 mM). Three-week-old seedlings were transferred to Hoagland solution supplemented with 75 mM NaCl and 24 h later the concentration was increased to 150 mM NaCl for another 24 h (Control); for the inhibitor treatments, the plants were subjects to the same salinization procedure but in the presence of the inhibitor (i.e. from the time of salinization with 75 mM NaCl). Plants were grown in 100% relative humidity. BT represents before treatment with NaCl and inhibitors. Values are means ± SD ($n = 8$) and bars indicate SD. Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

content of the whole plant compared with Na$^+$ content (data not presented). These plants had been grown in 6 mM K$^+$ external solution and we did not add Na$^+$ to the solution until the treatment, so the K$^+$ content was high (159 ± 21.2 μmol/plant) and Na$^+$ content was low (18.9 ± 2.51 μmol/plant). The high K$^+$ background is the most likely reason why TEA$^+$ and Cs$^+$, the inhibitors of K$^+$ channels and transporters, did not significantly affect whole plant K$^+$ content in our experiments.

Effects of Inhibitors on Growth, Tissue Water, and Ion Accumulation of *S. maritima* Growing in 25 mM NaCl

Although the optimal salinity for the growth of *S. maritima* is around 150 mM NaCl, about 85% of growth measured as organic dry weight is achieved in 25 mM NaCl (Yeo and Flowers, 1980), allowing separate assessment of the response that stimulates growth from that which requires osmotic adjustment for survival. Treating plants with 25 mM NaCl and Li$^+$ or cAMP or Ca^{2+} for 144 h did not have any significant effect on whole plant Na$^+$ and K$^+$ contents (Supplemental Fig. S1), even though cAMP and Ca^{2+} improved water status and Na$^+$ or K$^+$ concentrations in some parts of *S. maritima* (Supplemental Fig. S2).

We also examined the effects of TEA$^+$ (10 mM) and Cs$^+$ (3 mM) on *S. maritima* growing at the lower concentration of 25 mM NaCl in a chamber with 100% relative humidity. TEA$^+$ severely reduced leaf fresh weight and tissue water (Fig. 3); Cs$^+$ significantly decreased leaf fresh weight and tissue water by 26% and 23% compared with the control, respectively. Neither TEA$^+$ nor Cs$^+$ had any effect on the dry weight of leaves or roots: TEA$^+$ reduced the dry weight of stem by 32%; Cs$^+$ did not significantly affect stem dry weight (data not presented). Although TEA$^+$

Table I. *Effect of TEA$^+$ and Cs$^+$ on Na$^+$ relative distribution in different plant parts*

The quantity of sodium in each plant part was calculated from the data in Figures 1 and 2 and used to calculate the proportions of the total quantity in each part. Different letters within the second column indicate significant differences at $P < 0.05$ (Duncan test).

Treatments	Total Quantity	Na$^+$ Proportion		
		Leaf	Stem	Root
	μmol/plant	%		
Control	259 ± 23 a	78	17	5
10 mM TEA$^+$	101 ± 11 c	74	20	6
3 mM Cs$^+$	194 ± 17 b	65	28	7

and Cs$^+$ significantly affected Na$^+$ or K$^+$ concentrations in some parts of *S. maritima* (Fig. 4), they did not significantly affect whole plant Na$^+$ and K$^+$ contents (data not presented).

Effects of TEA$^+$ and Cs$^+$ on Net Uptake of Na$^+$ in 25 mM and 150 mM NaCl

TEA$^+$ significantly reduced the net uptake of Na$^+$, measured over a period of 48 h, from 0.56 ± 0.04 μmol g^{-1} fresh weight min^{-1} in the untreated plants growing in 150 mM NaCl to 0.26 ± 0.03 μmol g^{-1} fresh weight min^{-1} (Table II). Net uptake of Na$^+$ in the presence of Cs$^+$ (0.43 ± 0.03 μmol g fresh weight^{-1} min^{-1}) was also significantly less than that of the control plants (plus 150 mM NaCl, but without inhibitor; Table II). These results suggest that TEA$^+$ strongly inhibits Na$^+$ absorption by the root, but did not affect Na$^+$ transport within the plant; Cs$^+$ not only inhibited Na$^+$ uptake, but also blocked Na$^+$ transport to the leaves, causing a greater portion of Na$^+$ to be retained in the stem and root in comparison with control (Table I).

In contrast to the situation in 150 mM NaCl, 10 mM TEA$^+$ or 3 mM Cs$^+$ did not significantly reduce net Na$^+$ flux (the net quantity of Na$^+$ absorbed by the plant per unit of root and per unit of time) of *S. maritima* growing in 25 mM NaCl (Table II).

Ba^{2+} Inhibits Na$^+$ Uptake of *S. maritima* Both in 25 mM and 150 mM External NaCl

Ba^{2+} is known to be another K$^+$ channel blocker in plants (Tester, 1988; Kelly et al., 1995), dramatically reducing K$^+$ uptake mediated by K$^+$ transporters AtKUP1, EcHKT1;1, or EcHKT1;2 (Fu and Luan, 1998; Fairbairn et al., 2000). Ba^{2+} also inhibited Na$^+$ uptake mediated by OsHKT2;1 or OsHKT1;1 (formerly OsHKT4) and completely inhibited Na$^+$ uptake
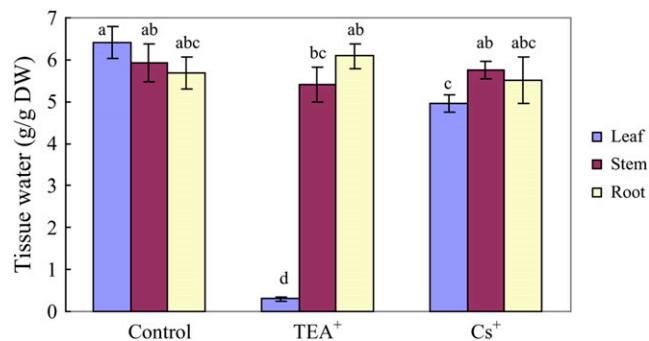

Figure 3. Tissue water content of *S. maritima* seedlings in response to treatment with NaCl (25 mM), TEA$^+$ (10 mM), or Cs$^+$ (3 mM). Three-week-old seedlings were transferred to Hoagland solution supplemented with 25 mM NaCl (Control), or 25 mM NaCl with different inhibitors for 48 h (to change each 24 h) in 100% relative humidity. Values are means ± SD (*n* = 24) and bars indicate SD. Columns with different letters indicate significant difference at *P* < 0.05 (Duncan test). [See online article for color version of this figure.]
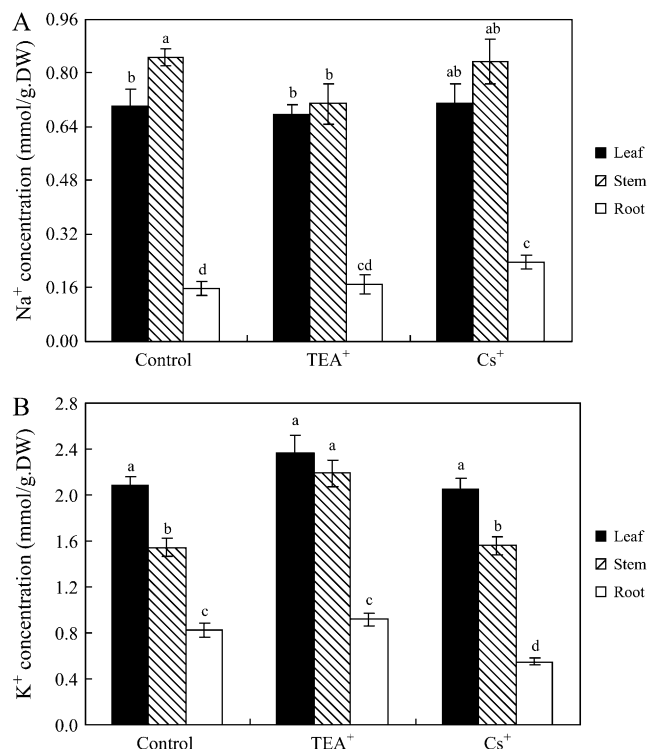

Figure 4. Na$^+$ and K$^+$ concentrations in *S. maritima* seedlings treated with NaCl (25 mM), TEA$^+$ (10 mM), or Cs$^+$ (3 mM). Three-week-old seedlings were transferred to Hoagland solution supplemented with 25 mM NaCl (Control), or 25 mM NaCl with different inhibitors for 48 h (to change each 24 h) in 100% relative humidity. Values are means ± SD (*n* = 8) and bars indicate SD. Columns with different letters indicate significant difference at *P* < 0.05 (Duncan test).

in rice roots (Garciadeblas et al., 2003). Thus, we tested the effects of Ba^{2+} on Na$^+$ and K$^+$ uptake in *S. maritima* under different external NaCl concentrations.

Plant dry weight was not affected significantly over the period of treatment (48 h) by the different concentrations of NaCl with or without 5 mM Ba^{2+} (data not presented). However, Ba^{2+} had a remarkable effect on water content. In the absence of added NaCl to the culture solution, Ba^{2+} severely decreased tissue water both in leaf and stem of *S. maritima* (Fig. 5). For plants growing in 25 mM NaCl, although Ba^{2+} still significantly reduced fresh weight (data not presented) and tissue water, leaf water status was much better in the presence (25NaBa treatment) than in the absence of salt (0NaBa treatment). In 150 mM NaCl, Ba^{2+} again significantly reduced tissue water in leaf, stem, and root, but again the water content was higher than in the absence of salt or in 25 mM NaCl (Fig. 5).

In the absence of added NaCl (the culture solution contains a trace quantity of NaCl), Ba^{2+} did not significantly affect Na$^+$ concentrations in different plant parts of *S. maritima* although it decreased leaf and root K$^+$ concentrations by 12% and 18%, respectively, compared to control plants. In 25 mM NaCl, Ba^{2+} significantly decreased leaf and root Na$^+$ concentrations by

Table II. *Effect of TEA^+ and Cs^+ on net Na^+ flux (μmol g^{-1} fresh weight root min^{-1}) of S. maritima growing in different salt concentrations for 48 h*

Na^+ fluxes for *S. maritima* treated with 150 mM NaCl were calculated from the data of Figures 1 and 2; Na^+ fluxes of *S. maritima* treated with 25 mM NaCl were calculated from the data of Figures 3 and 4. Net uptake was calculated, using the control data as an example, as (Na^+ content in whole plant of control − Na^+ content in whole plant before NaCl and inhibitor treatments)/(fresh weight of control root × 48 × 60). Different letters within the same row indicate significant differences at $P < 0.05$ (Duncan test).

NaCl Concentration	Inhibitor		
	None (Control)	TEA^+	Cs^+
mM		*10 mM*	*3 mM*
150	0.56 ± 0.04 a	0.26 ± 0.03 c	0.43 ± 0.03 b
25	0.20 ± 0.02 a	0.15 ± 0.01 a	0.18 ± 0.03 a

58% and 30%, respectively; Ba^{2+} also significantly decreased stem and root K^+ concentrations by 19% and 26%, respectively. In 150 mM NaCl, Ba^{2+} again significantly decreased leaf and root Na^+ concentrations (by 51% and 36%, respectively) and root K^+ concentrations (by 29%; Fig. 6). However, Ba^{2+} did not significantly affect whole plant K^+ contents under either 25 mM NaCl or 150 mM NaCl (data not presented).

These changes in Na^+ contents and concentrations resulted from the effects of Ba^{2+} in significantly lowering net Na^+ fluxes (calculated from uptake data obtained over 48 h) by 47% and 29%, in 25 mM and 150 mM NaCl, respectively (Table III). Ba^{2+} decreased whole plant Na^+ content by 56% in 25 mM NaCl and by 49% in 150 mM NaCl. In contrast, Na^+ uptake was not inhibited under low 25 mM external NaCl by TEA^+ and Cs^+ (Table II). Since net fluxes confound influx and retranslocation, we investigated the effects of various inhibitors on $^{22}Na^+$ influx into the roots.

Effects of K^+ and Inhibitors on $^{22}Na^+$ Influx

To investigate the effects of inhibitors on $^{22}Na^+$ influx, rather than net accumulation, we used whole root systems of *S. maritima* attached to about 4 cm of stem. We confirmed that influx of $^{22}Na^+$ to the roots was linear over the first 2 min of exposure to $^{22}Na^+$, as for Arabidopsis (Essah et al., 2003); after that, root $^{22}Na^+$ content increased gradually and over the same time $^{22}Na^+$ began to accumulate in the remnant of the stem. The influx of $^{22}Na^+$ into the roots showed evidence of a plateau (of some 0.5 μmol g^{-1} fresh weight root min^{-1}) as the external salt concentration increased between 50 and 75 mM NaCl; above this concentration, the influx more than doubled as the external concentration doubled (Fig. 7). Double reciprocal plots (Lineweaver-Burk) were linear over the concentration ranges 2.5 to 75 mM Na^+ (1/influx = 58.6[1/{NaCl}] + 0.73; R^2 = 0.97) and 100 to 200 mM Na^+ (1/influx = 68.1[1/{NaCl}] + 0.27; R^2 = 0.98), allowing approximate estimates to be made of the K_m and V_{max} values, which

were 80 mM and 1.4 μmol g^{-1} fresh weight root min^{-1} and 243 mM and 3.6 μmol g^{-1} fresh weight root min^{-1} over the low and high concentration ranges, respectively.

K^+ (10 or 50 mM) had no effect on $^{22}Na^+$ influx at concentrations below 75 mM NaCl, but the influx of $^{22}Na^+$ was inhibited by 50 mM K^+ when the external concentration of NaCl was above 75 mM (Fig. 7). However, the double reciprocal plots were not characteristic of K^+ being either a competitive or noncompetitive inhibitor (Supplemental Fig. S3).

Li^+ (1 and 10 mM) and Ca^{2+} (5 and 10 mM) did not have any significant effect on $^{22}Na^+$ influx of *S. maritima* growing in either 25 mM or 150 mM external NaCl (Supplemental Fig. S4) in agreement with their lack of effect on growth and ion accumulation.

We found similarities between the effects of TEA^+, Cs^+, and Ba^{2+} on growth and net ion accumulation and on $^{22}Na^+$ influx. Neither TEA^+ (1, 5, 7.5, or 10 mM) nor Cs^+ (0.5, 1.5, 3, or 10 mM) had any significant effects on $^{22}Na^+$ influx of *S. maritima* growing in 25 mM external NaCl (Fig. 8, A and B). However, in 150 mM external NaCl, influx of $^{22}Na^+$ into the roots decreased significantly with the increase of TEA^+ and Cs^+ concentration (Fig. 8, A and B). $^{22}Na^+$ influx into the roots of *S. maritima* was also significantly reduced by Ba^{2+}, but in this case in both 25 mM and 150 mM external NaCl (Fig. 8C). Even 1 mM Ba^{2+} decreased $^{22}Na^+$ influx, by 36% and 31% in 25 mM and 150 mM external NaCl, respectively, compared to a control without Ba^{2+}.

So, Li^+ and Ca^{2+} have no inhibitory effects on $^{22}Na^+$ influx of *S. maritima* in either 25 mM or 150 mM external NaCl; K^+, Cs^+, and TEA^+ have no inhibitory effects on $^{22}Na^+$ influx in 25 mM NaCl, but inhibit $^{22}Na^+$ influx in 150 mM NaCl. Ba^{2+} inhibits $^{22}Na^+$ influx both in 25 mM and 150 mM external NaCl. The effects of Cs^+ and
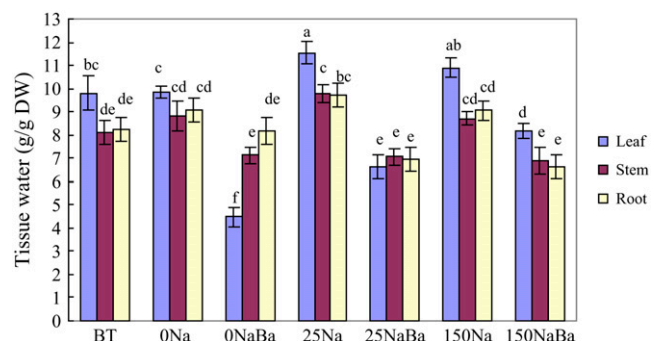

Figure 5. The effect of different external concentrations of NaCl, with or without 5 mM Ba^{2+}, on the water content of *S. maritima* seedlings. Three-week-old seedlings were transferred to Hoagland solution supplemented with different concentrations of NaCl with or without 5 mM Ba^{2+} for 48 h (150 mM NaCl was added at 75 mM NaCl/24 h) in 100% relative humidity. BT represents before treatments. The figure preceding Na is its concentration in the culture solution; Ba signifies the presence of 5 mM Ba^{2+}. Values are means ± SD ($n = 24$) and bars indicate SD. Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test). [See online article for color version of this figure.]
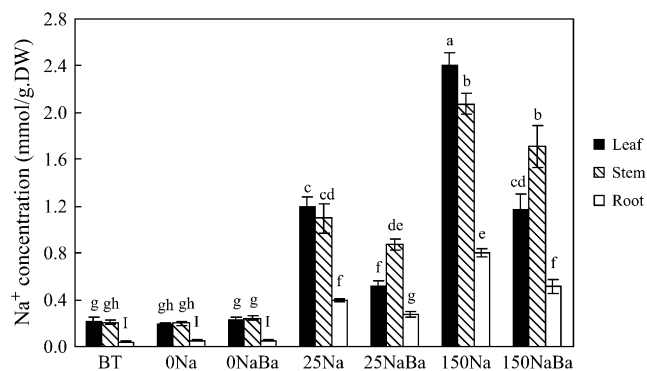
Figure 6. Na$^+$ concentrations in *S. maritima* seedlings treated under different concentrations of NaCl with or without 5 mM Ba^{2+}. Three-week-old seedlings were transferred to Hoagland solution supplemented with different concentrations of NaCl with or without 5 mM Ba^{2+} for 48 h (150 mM NaCl was added at 75 mM NaCl/24 h) in 100% relative humidity chamber. BT represents before treatments. The figure preceding Na is its concentration in the culture solution; Ba signifies the presence of 5 mM Ba^{2+}. Values are means ± SD ($n = 8$) and bars indicate SD. Columns with different letters indicate significant difference at $P <$ 0.05 (Duncan test).

TEA$^+$ on ^{22}Na$^+$influx measured over 2 min matches closely the effects on net uptake estimated over 48 h.

Interestingly, in 25 mM NaCl, the ^{22}Na$^+$ influx of 0.23 ± 0.04 μmol g^{-1} fresh weight root min^{-1} (Fig. 8) measured over 2 min was virtually the same as net flux (0.20 ± 0.02 μmol g^{-1} fresh weight root min^{-1}) determined over 48 h (Table II). ^{22}Na$^+$ influx of 0.76 ± 0.10 μmol g^{-1} fresh weight root min^{-1} in 150 mM NaCl (Fig. 8) exceeds net flux of 0.56 ± 0.04 μmol g^{-1} fresh weight root min^{-1}, but this net flux is the average of 24 h in 75 mM NaCl and 24 h in 150 mM NaCl (Table II).

Na$^+$ Alleviation of Wilting of *S. maritima* Induced by Ba^{2+} or TEA$^+$

We observed that plants of *S. maritima* wilted severely after adding Ba^{2+} (5 mM) for 48 h in the absence of NaCl, even at 100% relative humidity; in the presence of 25 mM NaCl, Ba^{2+} treatment brought about only slight wilting and in 150 mM NaCl the plants did not wilt at all when treated with Ba^{2+}. This observation was in accordance with leaf water status shown in Figure 5, where for plants grown in the presence of Ba^{2+}, leaf water content was higher, the higher

external Na$^+$. Na$^+$ concentrations in leaf, stem, and root of 25NaBa treatment increased 2.3-, 3.6-, and 5.5-fold in comparison with the corresponding parts of 0NaBa treatment, and for 150NaBa treatment, Na$^+$ concentrations increased by 5.2-, 7.1-, and 10.3-fold, respectively (Fig. 6). However, K$^+$ concentrations in leaf and root of 25NaBa or 150NaBa treatment were not significantly different from the corresponding parts of plants in the 0NaBa treatment; only stem K$^+$ concentrations were lowered by 15% for the 25NaBa treatment and by 17% for the 150NaBa treatment (data not shown). It appears that Na$^+$ alleviates the wilt symptom of *S. maritima* caused by Ba^{2+}.

A similar phenomenon was also observed in the presence of TEA$^+$ (10 mM). With decreasing external NaCl concentrations from 150 to 0 mM wilting increased: Na$^+$ alleviated TEA$^+$-induced wilting of *S. maritima*. These visible symptoms again reflected changes in leaf water content (Fig. 9): Leaf water content increased 7-fold in 75 mM NaCl and 18-fold in 150 mM NaCl in comparison with the plants growing in the presence of TEA$^+$ but the absence of external NaCl. These changes in wilting and water content were correlated with changes in the Na$^+$ concentration in the leaves (the Na$^+$ concentration of roots and stems also increased with increasing external Na$^+$; Fig. 10; there was, however, no effect of increasing external Na$^+$ on the concentration of K$^+$; Supplemental Fig. S5).

DISCUSSION

S. maritima Is Valuable for Characterizing Na$^+$ Uptake and Transport

Net accumulation of Na$^+$ in a plant is the end result of the balance of influx and efflux, moderated by the capacity for storage synonymous with the vacuolar volume in leaf cells. Among 15 species of the Chenopodiaceae tested for Na$^+$ and K$^+$ accumulation under similar conditions, *S. maritima* had among the highest accumulation of Na$^+$ and the lowest K$^+$/Na$^+$ ratio (Reimann and Breckle, 1993). In this strongly Na$^+$-accumulating species growing in low external NaCl (25 mM), ^{22}Na$^+$ influx (0.23 ± 0.04 μmol g^{-1} fresh weight root min^{-1}; Fig. 8) measured over 2 min was virtually the same as net flux (0.20 ± 0.02 μmol g^{-1} fresh weight root min^{-1}; Table II) determined over 48 h. At higher salinity (150 mM external NaCl), close

Table III. *Effect of Ba^{2+} (5 mM) on whole plant Na$^+$ content (μmol/plant) and root net Na$^+$ flux (μmol g^{-1} fresh weight root min^{-1}) of S. maritima*

The data were calculated from those of Figures 5 and 6. Net uptake was calculated, using the 25Na data as an example, as (Na$^+$ content in whole plant of 25Na − Na$^+$ content in whole plant before NaCl and inhibitor treatments)/(fresh weight of 25Na root × 48 × 60). Different letters within the same row indicate significant differences at $P <$ 0.05 (Duncan test); BT, before NaCl and inhibitor treatment.

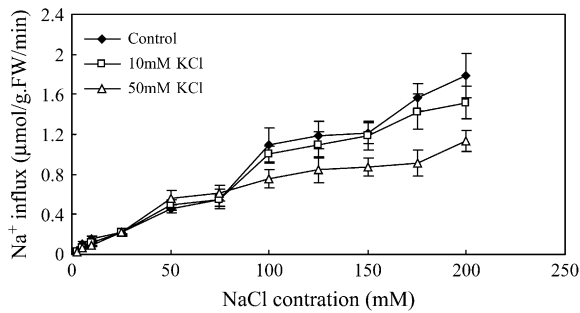|	BT	25Na	25NaBa	150Na	150NaBa
Na$^+$ content	7.0 ± 0.56 d	50 ± 5.61 b	22 ± 1.4 c	100 ± 7.7 a	51 ± 4.4 b
Na$^+$ net flux		0.19 ± 0.02 c	0.10 ± 0.01 d	0.43 ± 0.02 a	0.31 ± 0.03 b

Figure 7. Effects of NaCl and KCl concentration on root ^{22}Na$^+$ influx of *S. maritima* seedlings. Seventeen-day-old seedlings were transferred to non-KNO$_3$ Hoagland solution supplemented with three concentrations (0, 10, and 50 mM) of KCl and various concentrations (2.5 to 200 mM) of NaCl for 3 d. For measurements of influx, the seedlings were transferred to equivalent solutions labeled with ^{22}Na$^+$. Values are means ± SD ($n = 6$) and bars indicate SD.

to the optimal concentration for growth (approximately 200 mM NaCl; Flowers, 1972; Yeo and Flowers, 1980; Khan et al., 2000; Lu et al., 2003), ^{22}Na$^+$ influx (0.76 ± 0.10 μmol g^{-1} fresh weight root min^{-1}; Fig. 8) exceeded net flux (0.56 ± 0.04 μmol g^{-1} fresh weight root min^{-1}; Table II; determined in 75 mM NaCl for 24 h + 150 mM NaCl for 24 h). However, the difference between influx and net accumulation is relatively small, especially in comparison with the situation in Arabidopsis, where influx (1.88 μmol g^{-1} fresh weight root min^{-1}) exceeded uptake over 3 weeks (0.03 μmol g^{-1} fresh weight root min^{-1}; both in 50 mM NaCl and 0.2 mM Ca^{2+}) by more than 60-fold (Essah et al., 2003).

Since for *S. maritima* growing in 150 mM NaCl, 95% of the Na$^+$ that is accumulated is in the shoot (78% in the leaves and 17% in the stems; Table I) and since there is evidence that little or no Na$^+$ is retranslocated from the shoots (Yeo, 1981), then this suggests that virtually all the sodium that enters roots is transported to the shoots where it is accumulated. *S. maritima* has succulent leaves with enlarged cells in which the vacuoles, where Na$^+$ is accumulated, occupy most of the volume (Hajibagheri et al., 1984). In contrast to *S. maritima*, glycophytes (Läuchli, 1984; Huang et al., 2006; James et al., 2006) and some halophytes, such as *T. halophila* (Volkov et al., 2003; Inan et al., 2004; Taji et al., 2004) and *P. tenuiflora* (Wang et al., 2002, 2004) do not accumulate such high ion concentrations in the leaves, except when those leaves are dying or dead. To date, several mechanisms of limiting Na$^+$ accumulation in leaves of salt-excluding plants have been proposed. It has been suggested that SOS1 mediates not only Na$^+$ extrusion to the soil solution but also Na$^+$ retrieval from the xylem into the surrounding parenchyma cells and so affects long-distance Na$^+$ transport (Shi et al., 2002, 2003). Arabidopsis AtHKT1;1 and rice OsHKT1;5 (OsHKT8) are also proposed to function in Na$^+$ unloading from xylem vessels to xylem parenchyma cells (Berthomieu et al., 2003; Ren et al., 2005; Sunarpi et al., 2005) and AtHKT1;1 in retrieval of Na$^+$ from the root xylem (Rus et al., 2001; Davenport et al.,

2007). Durum wheat TmHKT7-A2 and TmHKT1;5-A withdraw Na$^+$ from the xylem in the roots, and also TmHKT7-A2 withdraws Na$^+$ from the xylem in the leaf (Huang et al., 2006; James et al., 2006; Byrt et al., 2007). Whatever the mechanism, limiting Na$^+$ accumulation in leaves of salt-excluding plants is central to tolerance (Munns, 2002; Wang et al., 2002). However, in *S. maritima* because the bulk of Na$^+$ (95%) entering the plant is transported to shoot this is valuable material for characterizing the pathway of Na$^+$ transport from root to shoot.
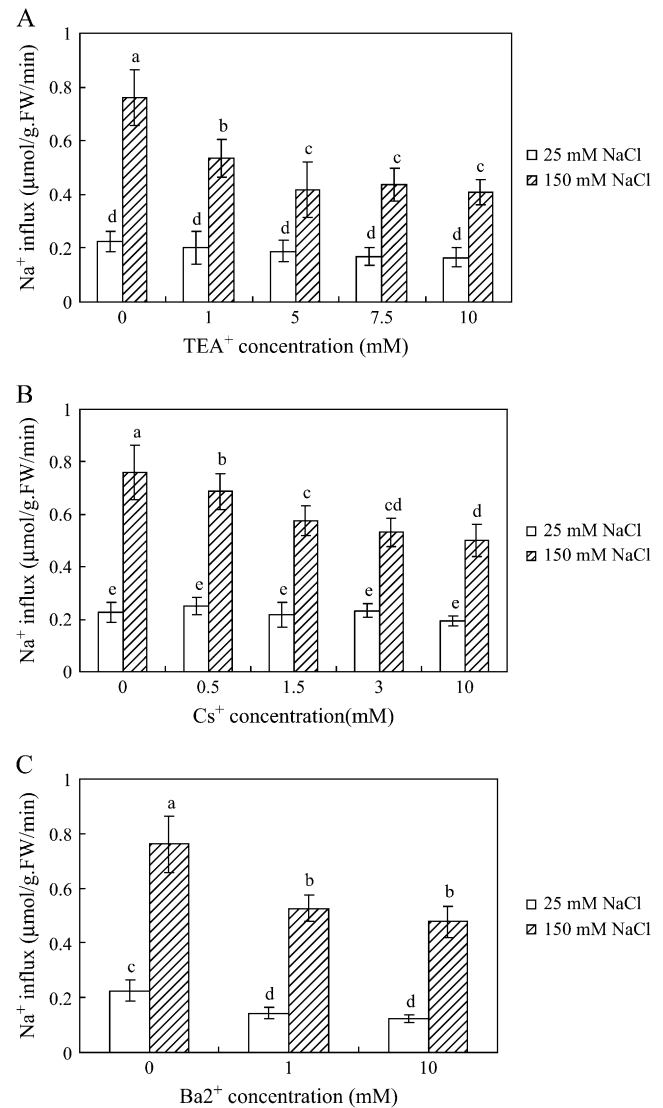

Figure 8. Root ^{22}Na$^+$ influx of *S. maritima* seedlings treated with TEA$^+$ (A), Cs$^+$ (B), and Ba^{2+}(C). Seventeen-day-old seedlings were transferred to Hoagland solution supplemented with 25 mM NaCl and 150 mM NaCl for 4 d, respectively. Then seedlings were transferred to Hoagland solution supplemented with corresponding concentrations of NaCl and TEA$^+$, Cs$^+$, or Ba^{2+} for 10 min before they were transferred into the above corresponding solution labeled with ^{22}Na$^+$. Values are means ± SD ($n = 6$) and bars indicate SD. Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

Figure 9. Tissue water content in *S. maritima* seedlings treated with 10 mM TEA$^+$ and different concentrations of NaCl. Three-week-old seedlings were transferred to Hoagland solution supplemented with 10 mM TEA$^+$ and different concentrations of NaCl for 48 h (150 mM NaCl was added at 75 mM NaCl/24 h) in 100% relative humidity chamber. Values are means ± SD (n = 24) and bars indicate SD. Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test). [See online article for color version of this figure.]
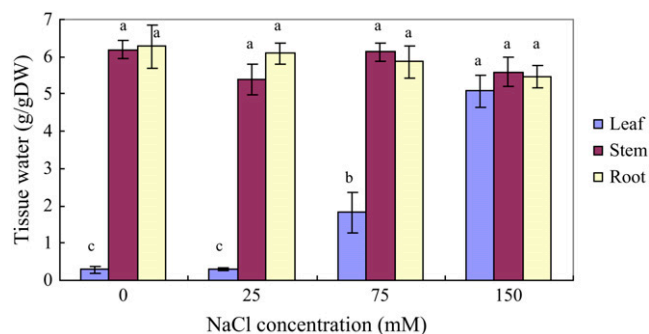
The Evidence in Planta Did Not Support NSCCs and LCT as the Major Pathways for Na$^+$ Entry into Root Cells

It has been suggested that voltage-independent channels or NSCCs and a LCT are the major pathways for Na$^+$ uptake (Schachtman et al., 1997; Amtmann and Sanders, 1999; Tyerman and Skerrett, 1999; Amtmann et al., 2001; Demidchik et al., 2002). NSCCs and the LCT are characterized by Ca^{2+}, cAMP, and cGMP inhibition of ion conductance and Na$^+$ influx (Clemens et al., 1998; Maathuis and Sanders, 2001; Demidchik and Tester, 2002; Tyerman, 2002; Essah et al., 2003; Antosiewicz and Hennig, 2004). However, our results indicate that 10 mM Ca^{2+} and 500 μM cAMP did not significantly affect whole plant Na$^+$ contents of *S. maritima* under either 25 mM or 150 mM NaCl treatment for 48 or 144 h (Supplemental Fig. S1A). Furthermore, neither 5 mM nor 10 mM Ca^{2+} have any inhibitory effects on Na$^+$ influx of *S. maritima* in either 25 mM or 150 mM external NaCl (Supplemental Fig. S4B). The absence of any effects of 5 mM or 10 mM Ca^{2+} suggests that LCT is not involved in the uptake of sodium by *S. maritima*. Even though *S. maritima* was grown in a medium with 0.5 mM Ca^{2+} in our whole plant experiments (except for the special cases of the 10 mM Ca^{2+} treatments), LCT is unlikely to be the major pathway of Na$^+$ influx in *Suaeda*, since in most soils calcium levels are high enough to inhibit Na$^+$ transport through LCT substantially (Schachtman and Liu, 1999; Hirschi, 2004). Ca^{2+} is more common than Na$^+$, being the fifth most abundant element in the earth's crust and true Ca^{2+} deficiencies in soils are rather rare; seawater calcium concentrations are about 10 mM (Harvey, 1966). Furthermore, adding 5 mM or 10 mM Ca^{2+} still did not have any significant effect on Na$^+$ influx of *S. maritima* cultured in a medium with 0.1 mM Ca^{2+} (Supplemental Fig. S4B). As far as NSCCs are concerned, these are also unlikely conduits for Na$^+$ in *S. maritima* as they are not inhibited by Cs$^+$ and TEA$^+$ (Demidchik and

Tester, 2002; Table I shows that both Cs$^+$ and TEA$^+$ permeate the channel, so they do not block it and in *T. halophila*, neither TEA$^+$ nor Cs$^+$ block the voltage-independent current; V. Volkov and A. Amtmann, personal communication).

Pathway 1 of Low-Affinity Na$^+$ Uptake

Over 48 h in 25 mM NaCl, the K$^+$ channel blocker Ba^{2+} (5 mM) significantly decreased (by 47%; Table III) the net flux of Na$^+$ by the roots of *S. maritima*, but two other K$^+$ channel blockers, TEA$^+$ (10 mM) and Cs$^+$ (3 mM), were without effect (Table II). ^{22}Na$^+$ influx measurements made over 2 min exhibited exactly the same characteristics: Ba^{2+} (1 mM) significantly reduced ^{22}Na$^+$ influx (by 36%; Fig. 8C), but TEA$^+$ (1–10 mM) and Cs$^+$ (0.5–10 mM) had no effect (Fig. 8, A and B). This finding is in agreement with previous reports about the influx of ^{22}Na$^+$ into the roots of Arabidopsis where neither TEA$^+$ nor Cs$^+$ had any inhibitory effect, but Ba^{2+} significantly reduced uptake in 50 mM NaCl (Essah et al., 2003). In the yeast (*Saccharomyces cerevisiae*) heterologous expression system and in Na$^+$-depletion experiments, Garciadeblas et al. (2003) found that Ba^{2+} inhibited Na$^+$ uptake mediated by OsHKT2;1 or OsHKT1;1 and completely inhibited Na$^+$ uptake in rice roots, but TEA$^+$ showed no effect under low external Na$^+$ concentrations. EcHKT1;1 and EcHKT1;2 mediating K$^+$ uptake were also strongly blocked by Ba^{2+} but less so by Cs$^+$; TEA$^+$ was ineffective (Fairbairn et al., 2000; Liu et al., 2001). Influx was also inhibited by K$^+$ although the nature of the inhibition has yet to be fully resolved. The uptake of ^{22}Na$^+$ from a low external concentration (5 mM) measured over a period of 24 h had previously been shown to be inhibited by K$^+$ (by 5%, 9%, and 44% in 1, 10, and 100 mM K$^+$; Yeo, 1974 [The uptake of ^{42}K from a 5 mM solution was also inhibited by the presence of Na, by 37%, 47%, and 67% in 1, 10, and 100 mM Na$^+$.]). These results
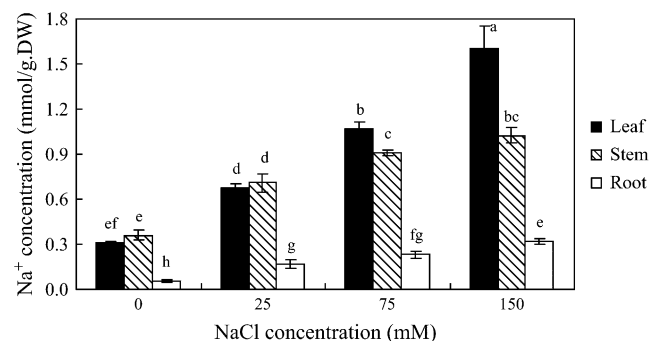

Figure 10. Na$^+$ concentrations in *S. maritima* seedlings treated with 10 mM TEA$^+$ and different concentrations of NaCl. Three-week-old seedlings were transferred to Hoagland solution supplemented with 10 mM TEA$^+$ and different concentrations of NaCl for 48 h (150 mM NaCl was added at 75 mM NaCl/24 h) in 100% relative humidity chamber. Values are means ± SD (n = 8) and bars indicate SD. Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

demonstrate that HKT possesses the pharmacological property of insensitivity to TEA$^+$ and Cs$^+$ and great sensitivity to Ba^{2+}. This makes HKT a prime candidate for mediating the uptake of Na$^+$ through pathway 1.

Pathway 2 of Low-Affinity Na$^+$ Uptake

At a higher concentration of NaCl (150 mM), TEA$^+$ (10 mM), Cs$^+$ (3 mM), and Ba^{2+} (5 mM) significantly reduced net Na$^+$ fluxes in *S. maritima* by 54%, 24%, and 29% (Tables II and III); after 48 h treatment, whole plant Na$^+$ content was decreased by 61%, 25%, and 49%, respectively (Tables I and III); TEA$^+$ (10 mM), Cs$^+$ (3 mM), and Ba^{2+} (1 mM) significantly reduced Na$^+$ influx by 47%, 30%, and 31% (Fig. 8). The K_m for Na$^+$ influx of 243 mM was some three times higher than that for pathway 1—80 mM estimated from influx rates between 2.5 and 75 mM NaCl. This result is in contrast to the situation in *T. halophila*, where TEA$^+$ (20 mM) and Cs$^+$ (5 mM) both increased rather than decreased the influx of Na$^+$ from a solution of 100 mM NaCl (Wang et al., 2006). As mentioned above, HKT is insensitive to TEA$^+$ and Cs$^+$, so it could not be involved in Na$^+$ absorption in *S. maritima* in conditions of high salinity. The inward-rectifying K$^+$ channel AKT1 is sensitive to TEA$^+$, Cs$^+$, and Ba^{2+}, and is involved in plant root K$^+$ uptake from the soil solution (Maathuis et al., 1997; Hirsch et al., 1998). It also has been proposed that AKT1 could mediate a significant Na$^+$ uptake with an increase in external Na$^+$ concentrations (Amtmann and Sanders, 1999; Blumwald et al., 2000). This viewpoint was supported by the coincidence of *OsAKT1* expression with Na$^+$ accumulation in rice: In the relatively salt-tolerant rice varieties Pokkali and BK, *OsAKT1* transcripts disappeared from the exodermis and endodermis in plants treated with 150 mM NaCl for 48 h but *OsAKT1* transcription was not changed in these cells in the salt-sensitive variety IR29; at this time the plants of IR29 accumulated Na$^+$ in leaf tissue to concentrations of 1,400 μmol per gram fresh weight; in contrast, Pokkali had accumulated 200 μmol/g Na$^+$ in the leaves, and BK about 100 μmol/g Na$^+$ (Golldack et al., 2003). However, cell specificity of the expression pattern of the *OsAKT1* was identical in all tissues for the three rice lines irrespective of whether the plants were grown in 100 μM or 4 mM K$^+$ (Golldack et al., 2003). Therefore, Golldack et al. (2003) concluded that *OsAKT1* expression did not respond to external K$^+$ concentrations and that Na$^+$ accumulation or exclusion in the whole plant depended on the *OsAKT1* expression in specific cells of plants exposed to 150 mM NaCl. Furthermore, plant AKT1-type K$^+$ channels show homology to animal Shaker-type K$^+$ channels, both at the sequence and structure levels (Very and Sentenac, 2003). Several studies have shown that substantial Na$^+$ can permeate animal Shaker K$^+$ channels at very positive potentials; this Na$^+$ permeation is often most prominent in the C-type inactivated state of the channels (Lopez-Barneo et al., 1993; Callahan and Korn, 1994; Starkus et al.,

1997, 1998, 2000; Kiss et al., 1998, 1999; Ogielska and Aldrich, 1998, 1999; Yellen, 1998; Wang et al., 2000). Taken together, the available data, including our study showing classical K$^+$ channel blockers TEA$^+$, Cs$^+$, or Ba^{2+} strongly inhibit root Na$^+$ uptake, indicate involvement of AKT1-type channel in the absorption of Na$^+$ at high external salt concentration.

Alleviation by Na$^+$ of the Effects of K$^+$ Channel Blockers on Tissue Water Relations

Our data show that TEA$^+$, Cs$^+$, and Ba^{2+} significantly reduced leaf water content (TEA$^+$ by 95%, Cs$^+$ by 23%, and Ba^{2+} by 42% in 25 mM NaCl; and by 40%, 31%, and 25%, respectively, in 150 mM NaCl for 48 h; Figs. 1, 3, and 5). This suggests that the three typical K$^+$ channels blockers either reduced the hydraulic conductivity of *S. maritima* and cause wilting of the leaves or caused an increase in the ion concentrations in the leaf cell walls, a process that reduces leaf turgor (Clipson et al., 1985). Our results show that under TEA$^+$ and Ba^{2+} treatments, with increasing external NaCl concentrations, the leaf Na$^+$ concentrations in *S. maritima* increased (Figs. 6 and 10), improving leaf water status (Figs. 5 and 9) and alleviating wilting. Perhaps at the low external Na$^+$ concentration, Na$^+$ and/or K$^+$ uptake is blocked at the leaf plasma membrane, leaving these ions in the cell walls and reducing turgor. At higher external sodium concentrations, the higher vacuolar ion concentrations alter the balance between cell walls and the protoplast: Such changes are not reflected in tissue ion concentrations owing to the small capacity of the cell wall fraction. However, it has been demonstrated that TEA$^+$ blocks the water permeability through some aquaporin channels expressed in *Xenopus* oocytes (Brooks et al., 2000; Detmers et al., 2006), so this effect may also influence the movement of water into the leaf cells.

CONCLUSION

Our results provide clear evidence for differences in the characteristics of Na$^+$ uptake with increasing external concentration. It is too early to speculate why different pathways might have evolved, as little is known of how Na$^+$ uptake is effected in other halophytes. *S. maritima* represents an extreme in terms of Na/K selectivity, where the supply of Na$^+$ ions appears obligately coupled to growth rate (Yeo and Flowers, 1986), where the ability to maintain sodium uptake for growth and osmotic adjustment at high external salt concentrations is essential for survival in seawater and on drying salt marshes. A comparison with other members of the Chenopodiaceae and the Poaceae should be informative.

MATERIALS AND METHODS

Plant Materials and Treatments

about 6 d, seedlings were transplanted to sand irrigated with modified Hoagland nutrient solution containing 6 mM KNO$_3$, 1 mM NH$_4$H$_2$PO$_4$, 0.5 mM MgSO$_4$, 0.5 mM Ca(NO$_3$)$_2$, 60 μM Fe citrate, 92 μM H$_3$BO$_3$, 18 μM MnCl$_2$, 1.6 μM ZnSO$_4$, 0.6 μM CuSO$_4$, and 0.7 μM (NH$_4$)$_6$Mo$_7$O$_{24}$. Solutions were changed every 3 d. After 18 d in sand culture, the plants were transferred to beakers containing 70 mL of the same modified Hoagland solution and left for 3 d to acclimatize before being used in experiments: Each beaker contained three plants. The solution was changed everyday, but was not aerated; separate measurements showed that the oxygen concentrations in these beakers was approximately 4.4 mg/L and changed little in 2 d. The plants were grown in a room where the temperature ranged from 23°C to 28°C and the relative humidity averaged 65%/75% (day/night); the daily photoperiod was 16/8 h (light/dark and the light flux density during the light period was between 220 and 240 μmol m^{-2} s^{-1}).

Seedlings, which were 21 to 23 d old (with shoots that were about 6 cm in height with young lateral branches) were used to evaluate the effect of inhibitors of ion transport on growth and ion accumulation. The choice of concentrations of inhibitors was based on previous studies: 500 μM 8-bromo-cAMP (Maathuis and Sanders, 2001), 10 mM CaCl$_2$ (Demidchik and Tester, 2002), 10 mM LiCl (Rubio et al., 2004), 10 mM TEA-Cl (Essah et al., 2003), 3 mM CsCl (Hampton et al., 2004), and 5 mM BaCl$_2$ (Garciadeblas et al., 2003). Each treatment, which lasted between 48 and 144 h (see figure and table legends or the text for details) under the conditions described above, had eight replicates with three plants per beaker. Where TEA-Cl, CsCl, or BaCl$_2$ was included in the treatment, the relative humidity of the growth chamber (Sanyo MLR-350HT growth cabinet; Sanyo Electrical and Biomedical; photosynthetically active radiation of 200 μmol m^{-2} s^{-1}) was maintained at 100% to minimize the effect of transpiration on Na$^+$ accumulation (compare with Nublat et al., 2001; Shi et al., 2002), since these three inhibitors caused plants to wilt. For treatments with BaCl$_2$, MgCl$_2$ (0.5 mM) replaced MgSO$_4$ (0.5 mM) in the Hoagland nutrient solution.

Growth Measurements, and Na$^+$ and K$^+$ Concentration Determination

At the end of the treatments, plant roots were washed twice for 8 min in ice-cold 20 mM CaCl$_2$ to exchange cell wall-bound Na$^+$ and the shoots rinsed in deionized water to remove surface salts. Root, stem, and leaf were separated and blotted; fresh weights were determined immediately and samples oven dried at 70°C for 3 d to obtain dry weights. Tissue water content was calculated from the difference between fresh and dry weights. Na$^+$ and K$^+$ were extracted from dried plant tissue in 100 mM acetic acid at 90°C for 2 h and ion analysis was performed using an atomic absorption spectrophotometer (Unicam SP919).

^{22}Na$^+$ Influx Experiments

S. maritima seedlings were grown in Hoagland nutrient solution [except for the experiment using Ca^{2+} as an inhibitor where the external Ca(NO$_3$)$_2$ concentration was reduced to 0.1 mM] in sand for 14 d and in beakers (without sand) for 3 d. They were subsequently transferred to Hoagland solution supplemented with 25 mM NaCl or 150 mM NaCl for 4 d. The seedlings were then used to evaluate ^{22}Na$^+$ influx according to the method described by Essah et al. (2003). Briefly, roots with 4 cm of attached main stem were excised and pretreated for 10 min in nutrient solution (except for the experiment of Ba^{2+} inhibitor with 0.5 mM MgSO$_4$ replaced by 0.5 mM MgCl$_2$) containing inhibitors and 25 mM or 150 mM unlabeled NaCl, with a change of solution after 5 min, and then transferred to above corresponding solution (10 mL) labeled with 185 to 370 kBq L^{-1} of ^{22}Na$^+$ as uptake solution. After 2 min, roots were removed from the uptake solution, blotted, and transferred to 200 mL of ice-cold NaCl (25 or 150 mM plus 20 mM CaCl$_2$) for two successive rinses of 2 min and then a further rinse of 3 min. Finally, the roots were blotted gently, weighed, and transferred to glass vials containing 2.5 mL of Optiphase Hisafe (Fisher Chemicals) and Na uptake was determined using a scintillation counter (Wallac Rackbeta 1217, LKB Wallac).

Statistical Analysis

Results of growth, ion concentration, water status, and ^{22}Na$^+$ influx rate of plants are presented as means with SDs. Statistical analyses, one-way ANOVA, and Duncan's multiple range test were performed using software (SPSS).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Whole plant Na$^+$ and K$^+$ contents in *S. maritima* seedlings treated with NaCl, cAMP, Li$^+$, or Ca^{2+}.

Supplemental Figure S2. Na$^+$ and K$^+$ concentrations in *S. maritima* seedlings treated with NaCl, cAMP, Li$^+$, or Ca^{2+}.

Supplemental Figure S3. Double reciprocal plots showing the effects of KCl concentration on root Na$^+$ influx of *S. maritima* seedlings at different NaCl concentrations.

Supplemental Figure S4. Root Na$^+$ influx of *S. maritima* seedlings treated with Li$^+$ and Ca^{2+}.

Supplemental Figure S5. K$^+$ concentrations in *S. maritima* seedlings treated with 10 mM TEA$^+$ and different concentrations of NaCl.

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