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Effects of Na Readmission on Cellular ^{45}Ca Fluxes in Na-Depleted Guinea Pig Taenia Coli

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Summary. The removal of Na from the medium causes a cellular Ca uptake in the smooth muscle of the guinea pig taenia coli which is rapidly reversed if medium Na is readmitted. This net extrusion was characterized in tissues which were first Na-depleted in a zero-Na (sucrose) solution. Li was able to substitute for Na in mediating this effect. K was also able to mimic Na in this respect if the depolarization-mediated Ca influx caused by the isotonic K solution was blocked with 10^{-5} M D-600. The net Ca extrusion upon Na readmission was due to a small decrease in Ca influx, as well as a marked increase in the transmembrane Ca efflux rate, as revealed by ^{45}Ca washout experiments. The increased ^{45}Ca efflux upon Na readmission could be mimicked by Li, K, choline and tris. We conclude that the Na/Ca-exchange hypothesis is insufficient to explain these data, in that both Ca extrusion and ^{45}Ca efflux can be stimulated in the absence of a Na gradient, or in the absence of any monovalent cationic gradient. These observations are discussed in terms of a possible intracellular competition of Ca and monovalent cations for anionic binding sites, as well as with regard to a possible direct stimulation of a plasmalemmal CaATPase by monovalent cations.

Key words Na/Ca-exchange · smooth muscle · ^{45}Ca fluxes · Ca homeostasis · Na substitution · guinea pig taenia coli

Introduction

Removal of medium Na induces a cellular uptake of Ca in guinea pig taenia coli. This uptake is caused by an increase in Ca influx and a decrease in Ca efflux [1]. Upon readmission of Na into the medium, cellular Ca returns rapidly to control levels. This net extrusion could not be induced by submaximal concentrations of the Ca influx inhibitor D-600, leading Ma and Bose [21] to conclude that it was mediated solely by an increase in Ca extrusion. However, recent work in guinea pig aorta has established that a concentration of D-600 capable of completely inhibiting a depolarization-mediated stimulation of Ca influx does not block the influx seen upon reduction of the Na gradient [25]. D-600 may therefore not interact with Ca transport systems which are specifically Na sensitive.

Manipulations of the Na gradient are generally thought to influence Ca transport via a Na/Ca-exchange carrier present in vascular and gastrointestinal smooth muscle cell membranes [10, 26], although the role of such a system is in dispute [11, 14]. The observations that replacement of Na into the medium following incubation of isolated smooth muscles in a Na-free medium causes net Ca extrusion, and a stimulation of ^{45}Ca efflux, provide the most compelling evidence supporting Na/Ca-exchange that is presently available [10, 26].

In this report, we show that following an incubation of tissues in a zero Na (sucrose) medium, readmission of Na into the medium causes a concentration-dependent net Ca extrusion. This extrusion is due both to a small inhibition of unidirectional Ca influx and a large concentration-dependent stimulation of Ca efflux. Both Li and K are able to mimic Na in causing stimulation of Ca extrusion and efflux. We conclude that the previously observed stimulation of Ca efflux upon reinstatement of the Na gradient is a nonspecific effect which does not provide evidence for a Na/Ca-exchange carrier in guinea pig taenia coli. Preliminary reports of these data have been presented previously [2, 12].

Materials and Methods

The taenia coli was dissected from the caecum of female English Shorthair guinea pigs. The tissue was cleaned and cut into short strips while being bathed in a physiological saline solution (PSS) containing (in mM): Na 142.0, K 5.0, Ca 1.5, Mg 1.0, Cl 149.6, glucose 10.0, HEPES (N-2-hydroxyethylpiperazine N′-2-ethanesulfonic acid) 5.0, and bubbled with 100% O_2, pH 7.2, temperature 36–37 °C. Tissues were allowed to equilibrate in this medium for at least one hour before flux studies were initiated.

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Measurement of Cellular Ca

Following equilibration, tissues were placed in PSS containing $1-5\times10^6$ cpm/ml ^{45}Ca for at least 120 min to maximally label all exchangeable cellular Ca pools. After labeling, tissues were moved to various experimental media, each containing the same specific activity of ^{45}Ca as PSS, so that any changes in tissue ^{45}Ca were due to a net Ca uptake or loss by the tissue.

In order to measure cellular Ca during experimental manipulations, tissues were removed at appropriate times and incubated for 45 min in an ice-cold medium identical to PSS except that it contained 2 mM EGTA (ethyleneglycol-bis-(β-aminoethyl ether)N,N′-tetraacetic acid) and no added Ca. We have previously shown that this washing step selectively removes extracellular free and bound ^{45}Ca, while leaving intracellular Ca pools largely intact [1]. Tissues were subsequently left overnight in scintillation vials containing 3 ml of a 5 mM EDTA (ethylenediamine-tetraacetic acid) solution in order to disperse tissue ^{45}Ca. Seven ml of a triton X-containing scintillation fluid was added to each vial. Blanks and samples of all radioactive media were similarly prepared. Label was quantitated in a scintillation counter (Tracor Analytic, model 43) and cellular Ca calculated using the tissue counts and specific activities of labeled media. Ca was expressed as μmoles/kg tissue wet weight.

Measurement of Unidirectional ^{45}Ca Influx

Initial ^{45}Ca uptake into the cellular compartment of taenia coli is characterized by a linear phase (*see* Table 1), suggesting that measurement of the initial ^{45}Ca uptake rate will provide an estimate of the unidirectional ^{45}Ca influx rate. Tissues in PSS or experimental media were therefore removed at appropriate times, exposed to an identical medium containing ^{45}Ca for 3 min, washed in ice-cold Ca-free PSS containing 2 mM EGTA, and then processed for quantitation of cellular ^{45}Ca uptake during this short "pulse-labeling" period.

^{45}Ca Efflux

Tissues were labeled in PSS for at least 150 min and the further labeled for 30 min in the appropriate zero Na-medium (*see* Results). Tissues were then washed for 40–60 min in an ice-cold medium identical to PSS except that 5.0 mM CaEGTA was present in addition to the normal 1.5 mM Ca. We have previously presented evidence that this washing procedure removes extracellular bound and free ^{45}Ca while maintaining cellular labeled stores largely intact [3]. The presence of an excess of free unlabeled Ca during this wash should insure the replacement of bound labeled ^{45}Ca with ^{40}Ca thus maintaining plasmalemmal stability. ^{45}Ca washout into various media at 37 °C was then monitored by successive "dipping" of tissues into vigorously oxygenated 3-ml volumes. Residual tissues ^{45}Ca was quantitated at the end of each efflux procedure. Washout data are presented as the fraction of tissue ^{45}Ca lost/min (the apparent rate constant of ^{45}Ca efflux).

Experimental Media

The Na-free media were prepared by omission of NaCl from PSS and addition of one of the following: LiCl 140 mM, KCl 140 mM, choline Cl 142 mM, Tris Cl (tris(hydroxymethyl) aminomethane) 142 mM, or sucrose 260 mM. The pH was adjusted to 7.2 with 10% LiOH for Li-PSS and with 50% KOH for K-PSS, choline-PSS, and sucrose-PSS. In the latter two media, KCl was reduced accordingly to maintain [K] at 5.0 mM.

In one experiment, a zero Na (sucrose) PSS containing 2 mM EGTA instead of 1.5 mM Ca was used. This solution was buffered to pH 7.2 with 5 mM Tris instead of 5 mM HEPES.

Results

Effects of Na Repletion on Cellular Ca

Substitution of medium Na with sucrose resulted in a cellular Ca gain of about 200 μmoles/kg tissue. Subsequent readmission of Na led to a rapid, concentration-dependent decrease in cellular Ca. As little as 20 mM $[Na]_e$ sufficed to reduce cellular Ca back to control levels (Fig. 1). In 15 mM $[Na]_e$, there was only a transitory net Ca extrusion. The net extrusion observed upon replacement of sucrose-PSS with PSS was not significantly inhibited by either 10^{-6} M D-600 or 10 mM $LaCl_3$. The former was added simultaneously with PSS while the La was present both during the last 30 min of the zero Na incubation and during Na replacement (*data not shown*).

Incubation of taenia coli in choline-PSS also resulted in a large uptake of cellular Ca which could be reversed by replacement of tissues in PSS. For example, during 60 min in choline-PSS, cellular Ca increased from 240 ± 20 ($n=5$) to 565 ± 56 ($n=5$) μmoles/kg tissue. Replacement of tissues in PSS at this time caused cellular Ca to decrease to 383 ± 50 μmoles/kg after 5 min and to 308 ± 15 μmoles/kg after 30 min.

If tissues were allowed to accumulate Ca during a 30-min period in sucrose-PSS and were then placed in isotonic Li-PSS (Fig. 2), cellular Ca decreased to the control level within 3 min and was maintained at this low value for the remainder of the experiment (70 min). The replacement of sucrose-PSS with a medium containing 40 mM Li caused a slow net Ca extrusion which became significant after 40 min.

If medium Na is replaced by K, taenia coli cells gain a large amount of Ca. This uptake is caused by a transient increase in unidirectional Ca influx [4] which may be due to the opening of potential sensitive Ca channels [7]. D-600, a Ca influx inhibitor, is known to block the K-mediated Ca gain [22]. If tissues were exposed sequentially to sucrose-PSS and then to K-PSS, there was a cellular Ca gain in the K solution on top of the Ca uptake which had already occurred in sucrose-PSS, as shown in the upper set of points in Fig. 3. The lower set of points represent tissues which were exposed to an identical sequence of media, except that 10^{-5} M D-600 was present in PSS for an hour prior to Na removal and also in both substituted media. Note that although this agent significantly reduced the cellular Ca content in PSS, it did not affect the Ca gain in sucrose-PSS. However, in the presence of D-600 replacement of sucrose with K caused a net loss of cellular Ca analogous to that observed upon Na resubstitution.

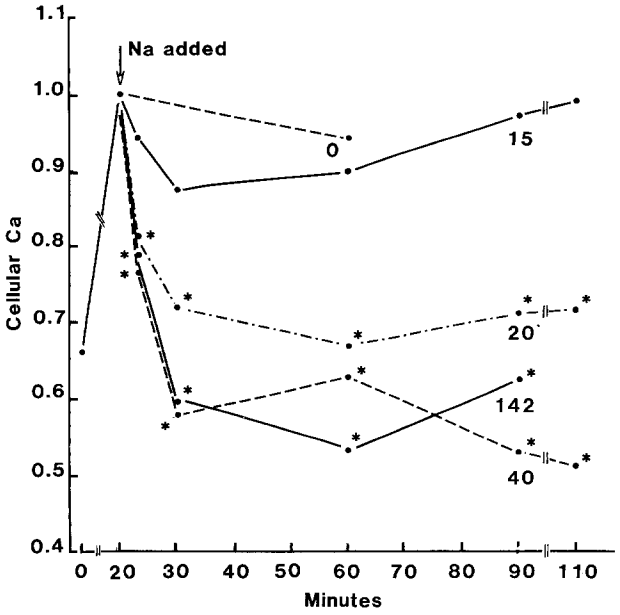

Fig. 1. Effect upon total cellular exchangeable Ca of Na removal from, and subsequent replacement into, the medium. Results from four separate experiments were normalized to the cellular Ca content measured after 20-min incubation in zero Na (sucrose)-PSS, as shown. Tissues were moved from PSS to sucrose-PSS at time zero, and then placed in a Na-containing medium at 20 min. Asterisks indicate that cellular Ca had decreased significantly from that value seen just prior to Na readdition ($p<0.05$). The numbers next to each line represent the mM Na readmitted at 20 min

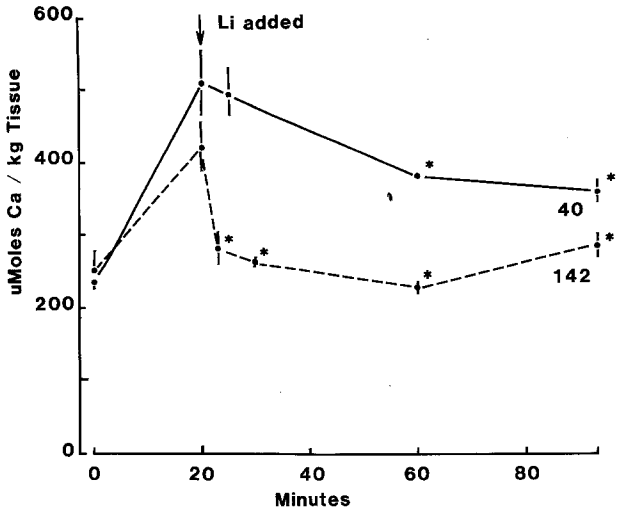

Fig. 2. Effect on total cellular exchangeable Ca of Na substitution by sucrose, followed by full or partial replacement of sucrose by Li. Asterisks denote a singificant decrease in cellular Ca from that value seen just prior to Li addition. The numbers next to each line represent the mM Li readmitted at 20 min

Thus, if the depolarization-mediated uptake in K-PSS was blocked by D-600, K was able to mimic Na in reversing the Ca gain which had previously taken place in sucrose-PSS.

These experiments suggest strongly that the type of net Ca extrusion seen upon replacement of Na into a Na-free medium is not dependent upon the reimposition of a Na gradient but is a nonspecific effect caused by the replacement of a monovalent cation into the medium.

Unidirectional ^{45}Ca Influx Following Na Repletion

The unidirectional Ca influx rate was estimated by measuring ^{45}Ca uptake during a 3-min exposure of tissues to labeled media. In taenia coli, the initial ^{45}Ca uptake is linear for at least 3 min, suggesting that during a 3-min pulse period reflux of label out of cells should not affect Ca uptake significantly.

Taenia coli was incubated for 30 min in sucrose-PSS and then exposed to labeled sucrose-PSS for 3 min. During this period, cells took up 75.5 ± 3.5 μmoles ^{45}Ca/kg tissue ($n=6$). If tissues were first incubated in sucrose-PSS for 30 min and then placed into PSS, the ^{45}Ca uptake during a 3-min pulse period was reduced to 69.0 ± 3.8 μmoles/kg ($n=6$)

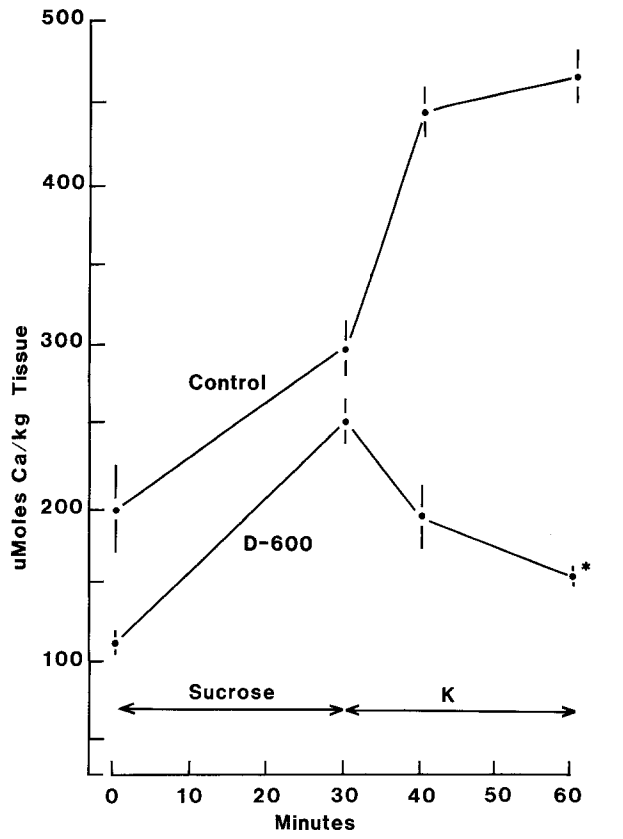

Fig. 3. Effect on total cellular exchangeable Ca of Na substitution by sucrose (at time zero) followed by isotonic replacement of sucrose by K (at 30 min). This protocol was followed in the absence (upper line) or the presence (lower line) of 10^{-5} M D-600. When used, D-600 was present during the last hour of labeling in PSS, as well as during sucrose and K incubations. The loss of Ca in K-PSS in the presence of D-600 was significant after 30 min (asterisk)

Table 1. Effect of medium Na upon early cellular ^{45}Ca uptake in tissues previously depleted of Na[a]

Na_e in (mM)	Cellular ^{45}Ca (μmoles/kg tissue)				
	1 min	3 min	7 min	15 min	30 min
0.5	19.9±1.6	67.3± 1.2	191.2±17.1	275.6±15.1	448.4±24.4
0	18.4±0.8	69.8± 5.9	203.3±13.4	286.4±21.3	453.4±10.1
2.0	30.1±1.8	93.7± 8.5	173.1±13.7	268.3± 9.6	423.4±30.2
0	27.7±1.4	89.8± 8.5	197.4±10.7	319.3±47.4	444.4±52.9
4.0	58.2±5.6*	178.3±10.2	342.4± 4.1	525.4±12.2	756.4±36.4
0	74.5±4.5	194.4± 3.3	343.7±22.7	490.4±21.4	793.6±42.0
10.0	25.3±1.7	72.4± 5.2	164.0± 3.5	240.3±11.6	423.0±47.4
0	20.1±0.7	74.7± 3.3	164.2±11.5	264.4±20.8	369.6±11.9
30.0	33.7±2.2*	89.6± 4.5*	175.0±11.7*	297.3±19.5*	362.3±26.7*
0	38.0±0.9	111.7± 4.5	218.2± 8.5	378.2± 6.8	500.7±22.1
142.0	32.1±4.5	57.3± 1.6*	111.4± 4.1*	174.6± 7.4*	275.3±15.2*
0	28.5±0.8	75.5± 1.8	183.9± 1.5	339.6±17.1	394.9±12.9

[a] Tissues were incubated in sucrose-PSS for 20 min, and were then placed into either labeled sucrose-PSS, or into an isotonic labeled Na-containing medium. Cellular ^{45}Ca uptake was subsequently measured at 1, 3, 7, 15 and 30 min. Each value shown represents the mean and SEM from a group of 4–7 tissues. Asterisks indicate that cellular ^{45}Ca uptake in a Na-containing medium was significantly lower than that in the paired tissues exposed to sucrose-PSS.

after 5 min in PSS and to 60.2±2.8 μmoles/kg ($n=5$) after 30 min. This latter value represented a significant decrease in the ^{45}Ca influx rate.

The dependency of ^{45}Ca influx inhibition upon restitution of $[Na]_e$ was studied using a similar type of procedure. Tissues were first incubated in sucrose-PSS for 20 min and were then divided into two groups. One group was placed into labeled sucrose-PSS and the other was placed into a labeled medium containing a particular Na concentration. The uptake of ^{45}Ca was subsequently measured at 1, 3, 7, 15 and 30 min; these data are shown in Table 1. Each row represents the paired data from an experiment comparing ^{45}Ca uptake in sucrose-PSS to that in a medium containing the $[Na]_e$ shown. Note that the inclusion of low (≦10 mM) concentrations of Na in the medium did not affect the uptake of ^{45}Ca in a consistent manner. However, a clear pattern of Na-mediated inhibition of ^{45}Ca uptake was apparent at 30 and 142 mM $[Na]_e$. This inhibition was obvious at 3 min for both media, indicating that the inclusion of Na at these concentrations decreased the unidirectional ^{45}Ca influx rate. These results are consistent with the pulse labeling data, suggesting that Na replacement after a sucrose-PSS incubation caused a small but significant decrease in the Ca influx rate. Upon replacement of 142 mM Na, the inhibition was on the order of 5–6 μmoles Ca/kg tissue/min, a value which is much too small to alone explain the rapid net extrusion of cellular Ca occurring at this time (Fig. 1).

^{45}Ca Efflux Following Replacement of Sucrose by Na, Li or K

We have previously shown that the efflux of ^{45}Ca from the taenia coli cells may be demonstrated if after labeling, the tissues are washed for 40–60 min in an ice-cold medium identical to PSS, except that it contains 6.5 mM $CaCl_2$ and 5.0 mM EGTA instead of 1.5 mM $CaCl_2$. Subsequent ^{45}Ca efflux at 37 °C is almost exclusively transmembrane since extracellularly bound ^{45}Ca is replaced by ^{40}Ca during the cold wash [3].

In order to study the effect of Na replacement upon ^{45}Ca efflux, tissues were labeled in PSS for 150 min and then in sucrose-PSS for 30 min. Tissues were then washed for 40–60 min in ice-cold Ca-EGTA-PSS, and efflux into unlabeled sucrose-PSS was commenced at 37 °C. After 24 min, some tissues were switched to Na-containing media. Figure 4 illustrates the results of this procedure. $[Na]_e$ caused a concentration-dependent increase in the apparent rate constant of ^{45}Ca efflux. At all concentrations, this potentiation of efflux was transient, suggesting that Na was displacing an intracellular Ca fraction of limited size.

These data are consistent with the hypothesis that net Ca extrusion during Na replacement is dependent mainly upon an increase in Ca efflux at this time. Since both Li and K were able to mediate a net Ca extrusion in a manner analogous to Na, the effect of these cations upon ^{45}Ca efflux was investigated.

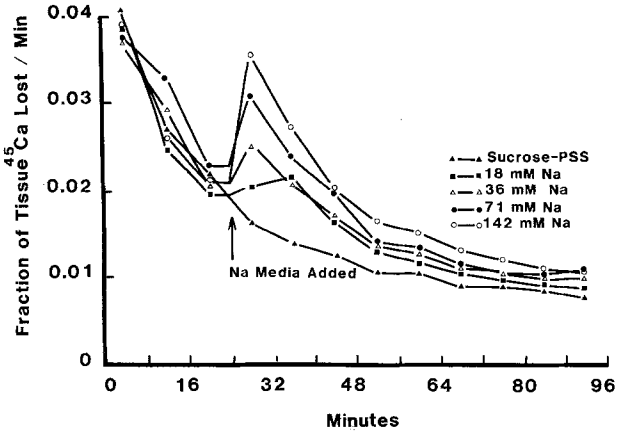

Fig. 4. Effect on ^{45}Ca efflux of replacing Na into the medium after a previous incubation in zero Na (sucrose)-PSS. Tissues were exposed to sucrose-PSS during the last 30 min of labeling, and were then washed for 45–47 min in ice-cold Ca-EGTA-PSS. Efflux into sucrose-PSS at 37°C was initiated, and after 24 min, some tissues were moved to isotonic efflux media containing 18, 36, 71 or 142 mM Na. Each curve represents the mean of 4–6 separate washouts ($n=4$–6)

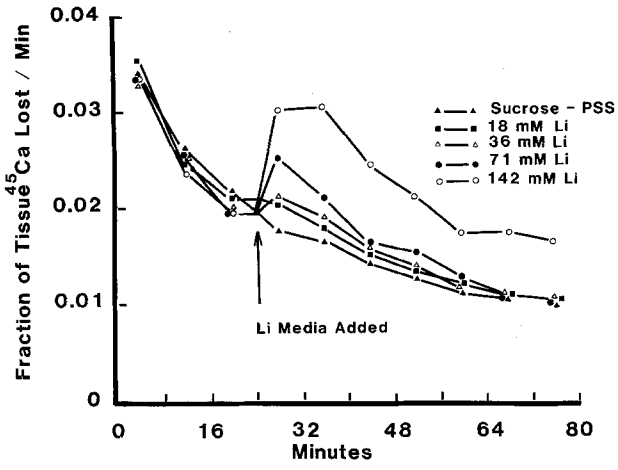

Fig. 5. Effect on ^{45}Ca efflux of successive exposure of taenia coli to isotonic zero Na media containing sucrose, and Li. Tissues were labeled and washed as described for Fig. 4, and efflux was initiated in sucrose-PSS. After 24 min, tissues were switched to isotonic efflux media containing 18, 36, 71 or 142 mM LiCl ($n=4$)

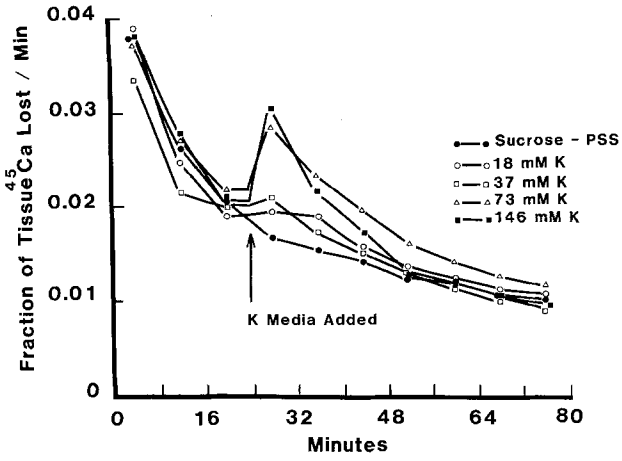

Fig. 6. Stimulation of ^{45}Ca efflux by K. Tissues were labeled and washed as described for Fig. 4, and efflux was initiated in sucrose-PSS. After 24 min, tissues were switched to isotonic efflux media containing 18, 37, 73 or 146 mM KCl ($n=4$)

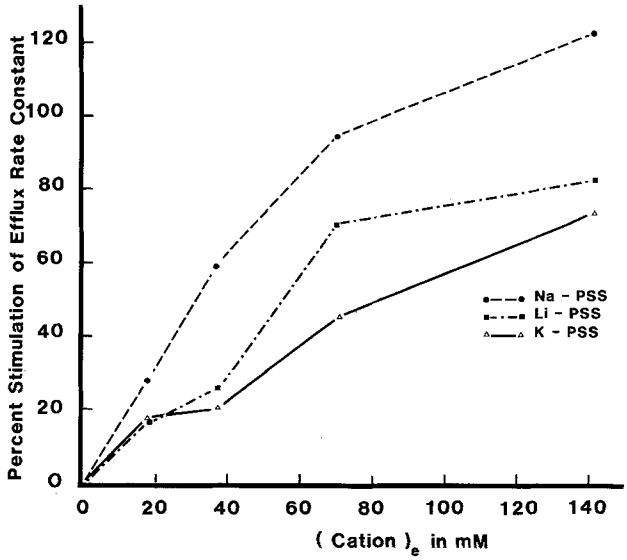

Fig. 7. Initial stimulation of ^{45}Ca efflux by monovalent cations. Percent stimulation was calculated by subtracting the rate constant of efflux seen in sucrose-PSS during the 24–32 min washout period from that seen in the media containing monovalent cation. This value was then expressed as a percent of the rate constant in sucrose PSS during this time

Tissues were labeled in PSS and sucrose-PSS and then washed in Ca-EGTA-PSS, as described in the previous experiment. Efflux in warm sucrose-PSS was commenced. After 24 min, some tissues were switched to Li-containing media. Figure 5 shows that Li caused a stimulation of ^{45}Ca efflux which appeared smaller than that seen in Na; this difference was most obvious at lower $[Li]_e$. Note that isotonic Li-PSS caused a sustained increase in the apparent rate constant of ^{45}Ca efflux.

A similar protocol was used to study the effect of K upon ^{45}Ca efflux. Figure 6 shows that transfer of tissues from sucrose-PSS to K-containing media caused a transient efflux stimulation. K was similar to Li in that at the lower concentrations used the stimulation was small. Inclusion of 10^{-5} M D-600 in sucrose-PSS and in the K-containing media, during efflux, did not affect ^{45}Ca washout in any medium.

The percent stimulation of efflux by each cation was roughly estimated by comparing the apparent rate constants of efflux seen during the 24- to 32-min washout period of each experiment. Stimulated

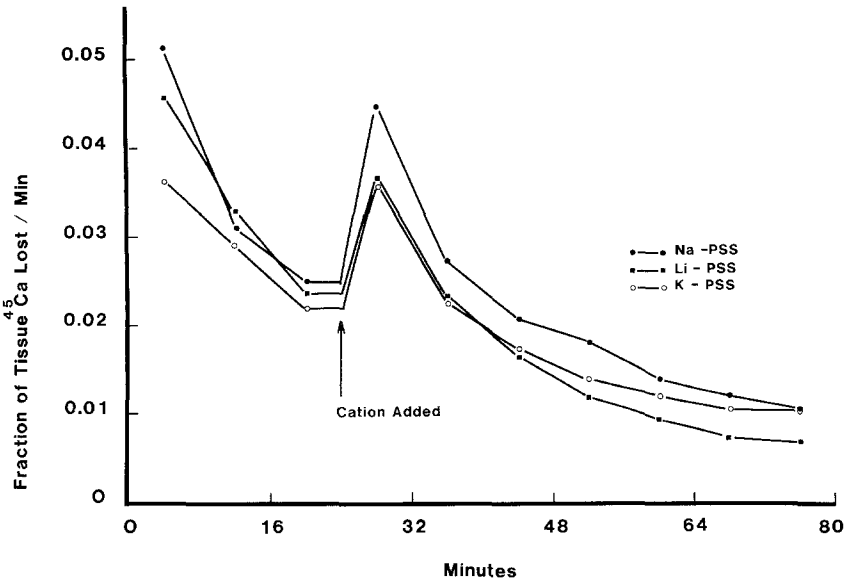

Fig. 8. ^{45}Ca efflux in choline-PSS and its stimulation upon replacement of choline by isotonic media containing Na, Li or K. Tissues were placed in choline-PSS during the last 30 min of labeling, and were then washed for 55 min in ice-cold Ca-EGTA-PSS. Efflux was initiated into choline-PSS. After 24 min, tissues were switched to isotonic efflux media containing 142 mM NaCl or LiCl, or 146 mM KCl (n=2–3)

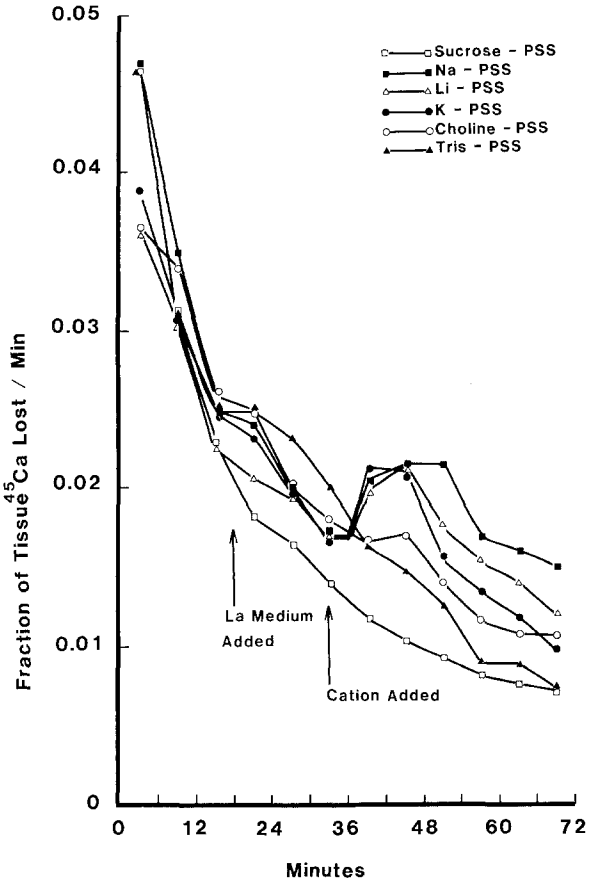

Fig. 9. Stimulation of ^{45}Ca efflux by monovalent cations in the presence of 10 mM $LaCl_3$. Tissues were labeled and washed as described for Fig. 4. Efflux was commenced into sucrose-PSS. After 18 min, all tissues were switched to zero-Ca sucrose-PSS containing 10 mM $LaCl_3$. After 12 min in this medium, tissues were switched to various isotonic zero-Ca media containing 10 mM $LaCl_3$ (n=5–6)

efflux was taken as the rate constant in the cation-containing medium, less the rate constant in sucrose-PSS, during this time. Figure 7 illustrates that Na was more effective than either Li or K in potentiating ^{45}Ca efflux. This difference was most obvious at 36 mM cation.

The stimulation of the efflux rate observed upon replacement of sucrose with NaCl, LiCl and KCl might be related to changes in ionic strength or chloride concentration. In order to test for this possibility, tissues were first labeled for 150 min in PSS and were then placed in labeled choline-PSS for 30 min. After a subsequent wash in cold CaEGTA-PSS, efflux was commenced in choline-PSS at 37 °C. After 24 min, choline was replaced by Na, K or Li. In this situation, the increase in ^{45}Ca efflux upon replacement of choline with the alkali earth cations, which is shown in Fig. 8, cannot be due to changes in ionic strength or the Cl concentration of the medium.

The stimulation of ^{45}Ca efflux by Na may also be mimicked by choline Cl or Tris Cl if these are substituted for sucrose during efflux in a manner analogous to that shown in Figs. 4–6. However, such stimulation is less pronounced than that caused by Na, Li or K, amounting to 30–40%, as defined for Fig. 7, for both cations during the efflux period immediately following their substitution for sucrose. Stimulation by either cation was not inhibited by 5×10^{-6} M D-600.

Although the CaEGTA washing procedure is designed to remove extracellularly bound label, it is possible that some residual extracellular ^{45}Ca remains in the tissue upon initiation of efflux. In ad-

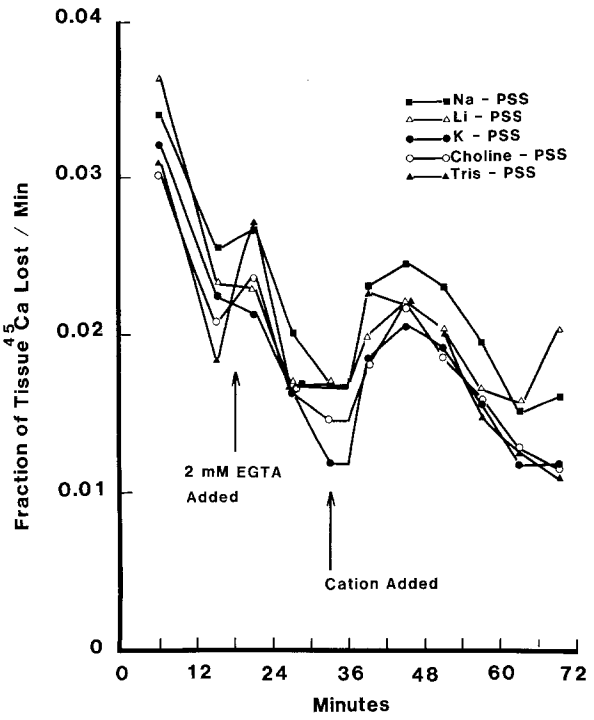

Fig. 10. Stimulation of ^{45}Ca efflux by monovalent cations in the presence of 2 mM EGTA. Tissues were labeled and washed as described for Fig. 4. Efflux was commenced into sucrose-PSS. After 18 min, all tissues were switched to zero-Ca sucrose-PSS containing 2 mM EGTA. After 12 min in this medium, tissues were switched to various isotonic zero-Ca media containing 2 mM EGTA (n=2–4)

dition, label leaving cells may be bound in the ECS (extracellular space) during the initial efflux periods in sucrose-PSS. In either case, replacement of sucrose with a cation might cause an apparent stimulation of transmembrane ^{45}Ca efflux which would actually be due to a displacement of extracellularly bound label by cation. In order to investigate this possibility, tissues were labeled in PSS and sucrose, and washed in Ca-EGTA-PSS, as shown in Figs. 4–6. Efflux was commenced into sucrose-PSS. After 18 min, sucrose-PSS was replaced by a similar medium containing no Ca and 10 mM $LaCl_3$. The La should displace extracellularly bound label. Subsequently, various media, all containing 10 mM La and no Ca, were substituted for the sucrose medium. Figure 9 shows that La did not eliminate the stimulation of ^{45}Ca efflux by Na, Li or K. The choline-mediated stimulation was still present, although very much decreased by the La treatment, and the Tris-mediated efflux stimulation appeared to be completely suppressed.

A similar experiment was performed which utilized EGTA instead of La in order to remove extracellular label. In this case, sucrose-PSS was replaced after 18 min of efflux by a sucrose medium containing no Ca and 2 mM EGTA. Subsequently, sucrose was replaced by Na, Li, K, choline or Tris. Figure 10 shows that each cation caused a large stimulation of the ^{45}Ca efflux rate.

These results indicate that Na, Li and K are in fact causing a potentiation of transmembrane efflux rather than simply displacing extracellular label. Removal of extracellular Ca with La or EGTA does not suppress efflux stimulation by these cations. The data are somewhat more ambiguous with respect to choline and Tris, since La and EGTA give opposite results; possible explanations for this dichotomy will be included in the Discussion section.

Discussion

The cellular Ca gain observed upon replacement of medium Na with sucrose is reversed if Na is returned to the medium. This effect is caused by a large increase in the Ca efflux rate as well as a modest decrease in the Ca influx rate. The potentiation of ^{45}Ca efflux during Na repletion has been previously noted in Na-loaded taenia coli cells in the presence of La [10]. This phenomenon has been explained in terms of a Na/Ca-exchange carrier. According to this model, removal of medium Na should prevent $Na_{in}-Ca_{out}$ exchange, while reimposition of the Na gradient should stimulate this process. In addition, the readdition of Na would be expected to inhibit Ca influx either through a direct competition between Na and Ca for binding to a shuttle type carrier [6] or by shifting the transmembrane distribution of the Na binding sites of a rotating carrier as proposed by Mullins [24].

Several of the observations reported above are not consistent with the Na/Ca-exchange model as it has been typically applied to smooth muscle. For example, Fig. 1 shows that following incubation of tissues in a Na-free medium, replacement of as little as 20 mM Na reduces cellular Ca to the level observed in PSS. Cellular Ca is subsequently maintained at this level. Although the Na gradient immediately following the replacement of 20 mM Na into the medium should be large enough to allow for net Ca extrusion by a Na/Ca-exchange carrier, the ability of such a process to maintain cellular Ca at control levels over an appreciable time interval in this medium is doubtful. We have shown that the ratio $[Na]_e/[Na]_i$ reaches a steady-state value of <2 in 20 mM Na-PSS [1]. The Na/Ca-exchange hypothesis as described by Blaustein [5] for arterial muscle would predict that cellular Ca

should be significantly elevated above control in the presence of this reduced Na gradient.

This discrepancy may be explained if taenia coli utilizes another mechanism for active Ca extrusion which can itself regulate cellular Ca in the partial or total absence of a Na gradient. It has been suggested that an ATP-driven Ca pump is involved in the maintenance of the Ca gradient in this tissue [14], and there is evidence that a Ca-ATPase is present in the plasmalemmal fraction of membranes isolated from vascular and intestinal smooth muscles [16, 19]. The existence of such a process is strongly indicated for taenia coli since tissues relax completely in a zero Na medium at a time when the Ca influx rate is not depressed [1, 8]. Furthermore, the fact that other cations can substitute for Na in mediating both a net Ca extrusion and a concentration-dependent increase in the ^{45}Ca efflux rate brings into question the existence of a highly selective Na/Ca-exchange carrier in this tissue. It may be argued that K, Li and to a lesser extent, choline and Tris, may substitute for Na on the carrier sites. Although this possibility can explain the ^{45}Ca efflux data, it is insufficient in regard to the observation that the K stimulates net Ca extrusion after tissues have been incubated in a zero Na medium. Substitution of sucrose for Na causes a slow loss of cellular K such that after 30 min cellular K is not seriously depleted [9]. Therefore, a subsequent replacement of sucrose with K will not produce an appreciable electrochemical K gradient especially since the membrane should be profoundly depolarized at a $[K]_e$ of 146 mM. Therefore, the net Ca extrusion at this time occurs in the absence of a monovalent cationic gradient as a potential energy source. In addition, if K can substitute for Na in a Na/Ca-exchange process, the efficiency of the carrier in maintaining a Ca gradient would be decreased by its involvement in a net Na/K-exchange.

The ability of Li to substitute for Na in reversing a zero Na-induced Ca gain (Fig. 2) is also difficult to explain in terms of a gradient effect. Although the initial inward Li gradient subsequent to Li addition could conceivably provide energy for a net Ca extrusion, this gradient is not maintained due to the inability of taenia coli cells to actively extrude Li [12]. The maintenance of cellular Ca at a control level for at least 70 min following Li addition can therefore not be dependent on an inward monovalent cationic gradient.

Choline and Tris, two widely used Na substitutes, also stimulate ^{45}Ca efflux after tissues have been incubated in a zero Na (sucrose) medium. This stimulation is inhibited if 10 mM La is included in the medium but is greatly enhanced if 2 mM EGTA is included in the solution. We cannot yet fully explain these results. However, La is thought to stabilize membranes, decreasing permeability [13] while EGTA, by removing external membrane-bound Ca, may destabilize the membrane [16]. Therefore, the permeability of the plasmalemma to choline and Tris may be decreased in La and increased in EGTA. It is possible therefore that the stimulation of ^{45}Ca efflux is dependent upon the ability of a particular cation to enter the cells. At any rate, the stimulation of ^{45}Ca efflux by the various cations employed in the presence of EGTA strongly suggests that this effect is not due to a simple competition for extracellular anionic binding sites. The stimulation of ^{45}Ca efflux by Na, Li and K after tissues have been labeled and allowed to wash out in choline-PSS is indicative of the fact that changes in medium Cl or ionic strength do not play a major role in this phenomenon.

These results clearly show that Na stimulation of both net and unidirectional Ca extrusion cannot constitute acceptable evidence for a specific Na/Ca-exchange in taenia coli. Na stimulation appears to be a specific aspect of a more general phenomenon by which the presence of a monovalent cation in the medium enables cells to extrude Ca more effectively than is possible in the absence of that cation. In this regard, we note that replacement of Na by K does not cause an increase in cellular Ca if the channels opened by depolarization are specifically blocked by D-600 [22]. Also, replacement of Na by Li causes only a small uptake of cellular Ca by guinea pig taenia coli and rabbit aorta [12, 14].

The failure of the Na/Ca-exchange carrier hypothesis to explain our data in this tissue necessitates the development of alternative models for monovalent cation stimulation of Ca extrusion.. We therefore will discuss two types of alternative Na−Ca interactions which may be involved in causing Na-mediated alterations in Ca fluxes, especially with regard to Na-stimulated Ca extrusion. These approaches are admittedly speculative, and are intended to suggest future avenues for experimental endeavor.

a) Competition Between Ca and Na for Intracellular Binding Sites. We have previously shown that Na removal causes a plateau-type gain of Ca by taenia coli cells [1]; the present report shows that replenishment of Na reverses this gain. The potentiation of ^{45}Ca efflux during Na resubstitution is transient, suggesting that Na is causing the loss of a discrete fraction of cell Ca rather than directly stimulating a transport system via a gradient effect.

These observations are consistent with the hy-

pothesis that Na and Ca compete for a class of intracellular binding sites. Na removal would increase the availability of such sites for Ca binding, inducing an uptake of cellular Ca [20]. The observations that various Na substitutes cause a differential Ca uptake in smooth muscles [12, 14], can be explained within this theoretical framework if it is considered that substitutes may differ both in their ability to penetrate the membrane and in their affinity for cellular Ca binding sites.

The increase in net and unidirectional Ca efflux observed upon replacing sucrose or choline with Na, Li or K, and the more limited but similar effect seen upon replacement of sucrose with choline or Tris, can therefore be visualized in terms of a sudden displacement of intracellular Ca from binding sites and a resulting increase in free $[Ca]_i$ available for extrusion by a plasmalemmal Ca pump.

It is interesting that the large Ca uptake subsequent to Na removal is not associated with a sustained contracture, and that Na readmission does not induce an increase in tension [1]. These procedures do, however, cause large changes in cellular Ca, and in transmembrane Ca fluxes. These data suggest that the Na-sensitive Ca pool in guinea pig taenia coli cells may be located superficially, such that $[Ca]_i$ in this area may be regulated independently of $[Ca]_i$ in the bulk cytoplasm containing the myofilaments.

It has previously been proposed that Na/Ca-exchange in smooth muscle may occur between the sarcoplasmic reticulum and the extracellular space, in the region where the peripheral SR is closely apposed to the plasmalemma [10, 11, 26]. In this model, Ca extrusion via exchange would be facilitated by the high Ca activity in the SR lumen. Also, since changes in the Na gradient would directly affect only SR Ca, this hypothesis is consistent with the observation that Na gradient manipulation may cause changes in total cell Ca which are not reflected by myofilament activation.

The data presented above, while inconsistent with the regulation of tension by Na/Ca-exchange, do not rule out the possibility that an SR-ECS exchange might regulate the Ca content of the peripheral SR. It is alternatively possible that Ca is extruded from the SR to the ECS by another type of pump, and that monovalent cations may indirectly affect the activity of this pump by competing with Ca for binding sites in the SR lumen. Therefore, Na removal might increase Ca binding within the SR, thereby decreasing free $[Ca]_{SR}$. Replacement of monovalent cation into the medium would increase the level of cation in the SR, causing a displacement of bound Ca, and an increase in free $[Ca]_{SR}$ which would be reflected in a stimulation of Ca pump activity.

b) Direct Stimulation of a Ca-ATPase by Monovalent Cations. A number of observations in the literature are consistent with the idea that an ATP-driven Ca pump is important in the extrusion of Ca from smooth muscle cells [13, 18]. The activity of (Ca + Mg)-ATPase in isolated SR vesicles from skeletal and cardiac muscle shows a dependency upon the presence of monovalent cations such as Na, Li and K [17]. Chiu and Haynes [15] have recently shown that pump function in skeletal muscle SR is dependent upon the presence of cation on the side of the membrane to which Ca is being pumped and suggests that monovalent cations may serve both to displace Ca from the pump site after transport and to act as counterions to maintain electroneutrality during pump turnover.

Na and K have also been found to enhance the activity of the (Ca + Mg)-ATPase of enriched sarcolemma from dog heart [23] and of reconstituted human red blood cells [27]. Enzyme activity in this latter preparation appeared to be potentiated by Na at the external face of the membrane, and by K on the inner side of the membrane.

If smooth muscle plasmalemmal CaATPase is similar in structure to these (Ca + Mg)-ATPases, it is reasonable to consider the possibility that Na, as the major monovalent cation on the side of the membrane to which Ca is pumped, may directly stimulate pump turnover. Thus, Na removal would inhibit Ca efflux, and its replacement would have the opposite effect. K and Li, and to a lesser degree, choline and Tris, might also serve this role. This type of "Na − Ca exchange" would differ from that proposed by Mullins [24] in that Na would stimulate an ATP-requiring process rather than ATP facilitate a Na gradient-requiring process.

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