Alkali Metal Ion Dependence of Inositol 1,4,5-Trisphosphate-induced Calcium Release from Rat Cerebellar Microsomes

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The effects of the alkali metal ions Na\(^+\), K\(^+\), Rb\(^+\), and Cs\(^+\) on ATP-dependent Ca\(^{2+}\) uptake, \(^{3}H\)inositol 1,4,5-trisphosphate (InsP\(_3\)) binding, and quantal InsP\(_3\)-induced Ca\(^{2+}\) release were investigated using rat cerebellar microsomes. Both the ion species and concentration affected the ability of the microsomes to support Ca\(^{2+}\) uptake with K\(^+\) being most effective (3.8 nmol of Ca\(^{2+}\)/min/mg at 100 mM K\(^+\)). The order of efficacy of the other ions was as follows: K\(^+\) > Na\(^+\) > Rb\(^+\) > Cs\(^+\) > Li\(^+\). The binding of \(^{3}H\)InsP\(_3\) to cerebellar microsomes was, however, affected little by the presence of these ions. All these alkali metal ions (except Li\(^+\)) supported InsP\(_3\)-induced Ca\(^{2+}\) release at concentrations above 25 mM; however, the extent of Ca\(^{2+}\) release (expressed as a percent Ca\(^{2+}\) release compared with that released by the ionophore A23187) was dependent upon the ion species present. Again K\(^+\) was more potent than the other ions at facilitating InsP\(_3\)-induced Ca\(^{2+}\) release (order of efficacy K\(^+\) > Rb\(^+\) > Na\(^+\) > Cs\(^+\) > Li\(^+\)). The IC\(_{50}\) of InsP\(_3\) required to induce half-maximal Ca\(^{2+}\) release (IC\(_{50}\)) was not significantly altered. Over the ion concentration range tested (25–100 mM), the extent of InsP\(_3\)-induced Ca\(^{2+}\) release with both K\(^+\) and Rb\(^+\) increased in a linear fashion, while Na\(^+\) showed only a slight increase and Cs\(^+\) showed no increase over this range. The effect of K\(^+\) concentration on quantal Ca\(^{2+}\) release was to alter the extent of release rather than the IC\(_{50}\) InsP\(_3\) concentration. Using stopped-flow techniques, the effects of InsP\(_3\) and K\(^+\) concentrations on the kinetics of InsP\(_3\)-induced Ca\(^{2+}\) release were shown to exhibit a monoeponential process in this microsomal preparation. The rate constants for Ca\(^{2+}\) release increased with InsP\(_3\) concentration (0.11 s\(^{-1}\) at 0.02 \(\mu\)M InsP\(_3\) to 0.5 s\(^{-1}\) at 40 \(\mu\)M InsP\(_3\)), however, the relationship between the fractional extent of release and rate constants for release did not change in a similar way with InsP\(_3\) concentration. Although the fractional extent of Ca\(^{2+}\) release increased with K\(^+\) concentration, the rate constants for release over this K\(^+\) concentration range were unaffected. This observation leads us to question the role of K\(^+\) as a counter ion required for Ca\(^{2+}\) release, and we therefore postulate a role for K\(^+\) (and the other alkali metal ions) as a “co-factor” required for channel opening.

The abbreviation used is: InsP\(_3\), d-myoinositol 1,4,5-trisphosphate.

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GLOSSARY

MATERIALS AND METHODS

Fluo-3 was obtained from Sigma, InsP\(_3\) from Calbiochem, and \(^{3}H\)InsP\(_3\) from DuPont NEN. All alkali metal chlorides were obtained from Aldrich.

Rat cerebellar microsomes were prepared essentially as described in Refs. 14 and 15 with minor modifications. Briefly, 15–20 cerebella were minced and homogenized in 10 volumes of cold buffer (0.32 M sucrose, 5 mM Hepes, 0.1 mM phenylmethylsulfonyl fluoride, 0.03 mM benzami...
dine, 5 μl leupeptin A, and 10 μl pepstatin, pH 7.2). The homogenate was centrifuged at 500 × g for 10 min. The resulting pellet was homogenized in 5 volumes of buffer and again centrifuged at 500 × g for 10 min. The supernatants were pooled and the mitochondria removed by centrifugation at 10,000 × g for 20 min. The microsomal pellet was obtained by centrifuging the remaining supernatant for 1 h at 100,000 × g. The pellet was resuspended in the Hepes-sucrose buffer for a concentration of ~15 mg/ml, snap-frozen in liquid nitrogen, and stored at ~70 °C.

Ca²⁺ uptake and InsP₃-induced release from cerebellar microsomes were measured using fluo-3 as described elsewhere (16), with some modifications. Rat cerebellar microsomes (0.3 mg/ml) were suspended in a buffer containing Tris phosphate (40 mm), creatine kinase (10 μg/ml), phosphocreatine (10 mm), Tris salt, fluo-3 (250 nm), and the appropriate concentration of alkali metal ion as the chloride salt, pH 7.2, at 37 °C. Ca²⁺ uptake was initiated by the addition of 1.5 mM Mg-ATP and the fluorescence change monitored on a Perkin-Elmer LS-50B spectrophotometer, with excitation at 506 nm and detecting the emission at 526 nm. After ATP-dependent Ca²⁺ loading, further Ca²⁺ uptake was inhibited by the addition of between 0.2 and 0.5 mM sodium orthovanadate (which inhibited >90% of the Ca²⁺ pumps) (7) and InsP₃ at the appropriate concentration was added. Total Ca²⁺ accumulated within the microsomes was measured by permeabilization with Ca²⁺ ionophore A23187 (12.5 μg/ml).

Fluorescence intensity was related to [Ca²⁺] by the following equation given in Ref. 16, as shown in the dissociation constant for Ca²⁺ binding to fluo-3 at pH 7.2 and 37 °C. F is the fluorescence intensity of the sample and F_max and F_min are the fluorescence intensities of the sample in 1 mM EGTA and 2.5 mM CaCl₂, respectively.

\[
[Ca^{2+}] = K_d \times (F - F_{\text{min}})/(F_{\text{max}} - F) \quad (\text{Eq. 1})
\]

where \( K_d \) is the dissociation constant for Ca²⁺ binding to fluo-3 at pH 7.2 and 37 °C. F is the fluorescence intensity of the sample and F_max and F_min are the fluorescence intensities of the sample in 1 mM EGTA and 2.5 mM CaCl₂, respectively.

Under standard conditions (100 mM KCl, pH 7.2 and 37 °C) we have shown the dissociation constant for Ca²⁺ binding to fluo-3 to be 900 nm (17); however, both the alkali metal ion present and its concentration also affect this dissociation constant. The dissociation constants for fluo-3 binding to Ca²⁺ were therefore measured in the appropriate alkali metal salt at a variety of concentrations by monitoring the change in fluorescence in a 10 mM Hepes-Tris buffer, pH 7.2, 37 °C using 250 μM fluo-3 and varying the free Ca²⁺ concentration by known concentrations of CaCl₂ and EGTA as calculated by the 'ION' computer program developed by Fabiato (18). It must be noted that the pH of the EGTA and CaCl₂ solutions were adjusted with Aristar Tris (from BDH) to avoid alkali metal ion contamination.

The rapid measurements of InsP₃-induced Ca²⁺ release were monitored using an Applied Photophysics stopped-flow spectrophotometer (model SX 17 MV), exciting the sample at 505 nm and measuring the emission above 515 nm using a cut-off filter. The microsomes were in the same buffer as described previously and Ca²⁺ accumulation was followed on a conventional spectrophotometer. Once sufficient Ca²⁺ loading had occurred, further accumulation was inhibited with orthovanadate and microsomal/fluoro-3 suspension added to syringe A of the stopped-flow apparatus. Syringe B was filled with InsP₃ at 30 times the experimental concentration required as the mixing ratio of syringe A to B was 10:1, to avoid introducing substantial Ca²⁺ contamination when mixing the microsomes/fluoro-3 suspension with InsP₃. The fluorescence data were initially adjusted by comparing the changes on the stopped-flow apparatus with identical experiments undertaken on a conventional fluorometer, such that these traces could be related to fractional Ca²⁺ release. These traces were then analyzed using nonlinear regression analysis programs supplied by Applied Photophysics. The time courses for Ca²⁺ release for the microsomal preparation used in this study could be fitted well to a monoeponential process using the following equation,

\[
[Ca^{2+}]_{\text{release}} = A \times (1 - \exp^{-t/K}) \quad (\text{Eq. 2})
\]

where A is the fractional amount or extent of release and k is the rate constant which defines this release process. Maximal amount of Ca²⁺ release (1.0) was defined as that released by 40 μM InsP₃. Over the Ca²⁺ concentration range in which InsP₃-induced release was monitored, the fluorescence change, when related to Ca²⁺ concentrations, were around the K_d value for Ca²⁺ binding to fluo-3. Over this range of fluorescence is linearly related to Ca²⁺ concentration (linear regression correlation r = 0.99).

The binding of [³H]InsP₃ to cerebellar microsomes was carried out as described in Refs. 14–16. 0.5 mg of rat cerebellar microsomes was suspended in 0.5 ml of buffer containing 50 mM Tris/HCl, pH 8.3, 1 mM EDTA, with the appropriate concentration of alkali metal chloride salt and InsP₃. Over the Ca²⁺ concentration range of 230 nM in the absence of added ion to 900 nM in the presence of 10 mM excess cold InsP₃, K_d values were measured at pH 7.2, 37 °C in the presence of the appropriate metal ion and concentration. The K_d was calculated by altering the [Ca²⁺] free, by varying the EGTA and CaCl₂ concentration and measuring the changes in fluo-3 fluorescence as described in Ref. 17. Each Ca²⁺ titration was carried out three times and the mean value plotted. The K_d values for the metal ions were represented as follows: K⁺, Na⁺; Rb⁺, Cs⁺; and Li⁺.

RESULTS

Before using fluo-3 to measure InsP₃-induced Ca²⁺ release in the presence of different alkali metal ions at different concentrations, the effects of these ions on the dissociation constant of Ca²⁺ binding to fluo-3 were determined. Fig. 1 shows that the K_d value for Ca²⁺ binding to fluo-3 is dependent upon the ionic concentration and type of metal ion present. The K_d value varies from 230 nM in the absence of added ions to 900 nM in the presence of 100 mM KCl, the latter being identical to the previously determined value (17). Both K⁺, Na⁺, and Li⁺ ions affected the K_d for Ca²⁺ binding to fluo-3 in a similar fashion. The change in K_d for Ca²⁺ binding to fluo-3 in the presence of Cs⁺ and Rb⁺ at concentrations up to 100 mM salt was substantially lower than the values obtained with K⁺ (varying from 230 nM to 550 nM). In all subsequent experiments the changes in Ca²⁺ concentrations were calculated using the appropriate K_d for the metal ion and concentration used in each experiment. However, in buffers containing cerebellar microsomes, no additional effect on the K_d for Ca²⁺ binding to fluo-3 was observed.

Prior to monitoring the effects of alkali metal ions on InsP₃-induced Ca²⁺ release, the microsomes were first loaded with Ca²⁺ by activating the microsomal Ca²⁺-pump (Ca²⁺-ATPase) with ATP. If no alkali metal ions were present in the assay buffer, little or no Ca²⁺ uptake could be measured (<0.1 nmol of Ca²⁺/min/mg), also extremely poor Ca²⁺ uptake was also observed in the presence 100 mM Li⁺ (~0.2 nmol of Ca²⁺/min/mg). In our system K⁺ was the most effective alkali metal ion (3.8 ± 0.2 nmol of Ca²⁺/min/mg at 100 mM K⁺); however, all other alkali metal ions used (except Li⁺) could support Ca²⁺ pump activity to a level which could sufficiently load the microsomes with Ca²⁺ prior to performing release experiments.

FIG. 1. The effects of alkali metal ion concentration and species on the dissociation constant (K_d) of Ca²⁺ binding to fluo-3. Each K_d value was measured at pH 7.2, 37 °C in the presence of the appropriate metal ion and concentration. The K_d was calculated by altering the [Ca²⁺] free, by varying the EGTA and CaCl₂ concentration and measuring the changes in fluo-3 fluorescence as described in Ref. 17. Each Ca²⁺ titration was carried out three times and the mean value plotted. The K_d values for the metal ions are represented as follows: K⁺, Na⁺; Rb⁺; Cs⁺; and Li⁺.
with K
orthovanadate.

The effects of alkali metal ions on quantal InsP3-induced Ca2+
release are dependent upon the type of metal ion present, with K+
able to support the greatest amount of release (15.7% Ca2+
release at maximal InsP3 concentration). RB+ was the next most potent ion (causing 11.8% InsP3-induced Ca2+
release), while Na+ and Cs+ supported lower levels of Ca2+
release (9.3 and 6.7% release, respectively). Although the concentration of InsP3 required to cause half-maximal InsP3-in-
duced Ca2+
release (IC50) varied between 1.0 and 2.0 µM for the metal ions tested at 100 mM (Na+, 1.0 ± 0.2 µM; K+, 1.3 ± 0.3
µM; RB+, 2.0 ± 0.8 µM; Cs+, 1.1 ± 0.4 µM), the standard errors for the IC50 values were such that no significance could be
detected on these small variations. Fig. 2 also shows that in this
preparation the concentration of InsP3 required to reach max-
imal release differed with the type of metal ion present. Both K+
and RB+ required ~ 10 µM InsP3 to reach maximal levels of
Ca2+
release, while Na+ and Cs+ appear to require lower InsP3
concentrations (~ 3 µM) to attain their maximal levels.

Table I shows that different alkali metal ions do not signif-
icantly alter the affinity of the receptor for InsP3, since little or
no effect was observed on the amount of [3H]InsP3 bound to the
cerebellar membranes in the presence of these ions when meas-
ured using 40 nM InsP3 (the Kd value for InsP3 binding to
cerebellar microsomes under our experimental conditions).

Several studies looking at the time course for Ca2+
release induced by InsP3 using permeabilized cells have shown it to be
biphasic in nature, comprising a fast and slow component (19, 20). Here an investigation of rapid InsP3-induced Ca2+
release from rat cerebellar microsomes was undertaken. Fig. 5A shows the effects of increasing InsP3 concentration from 0.02 to 40 µM on Ca2+
release measured using a stopped-flow spectrophotometer at 37°C and 100 mM KCl. The time courses for Ca2+
release were plotted as fractional InsP3-induced Ca2+
release, where maximal release was set to the percent Ca2+
release observed at 40 µM InsP3. As shown in Fig. 5A the Ca2+
release data can be simply fitted to a monoexponential process (solid line). However, the data presented here could also equally well be fitted to a biexponential processes comprising two rate con-
stants and two amplitudes where the values are similar in both
cases. As a monoexponential equation could be used to fit all experimental conditions described here (i.e. varying InsP3 and K+
concentrations), our analysis was confined to using the
simplest mathematical function describing this process. At low
InsP3 concentrations maximal release is reached between 10
and 15 s after addition, while at high concentrations maximal
release is reached after about 5 s. Fig. 5B shows that the rates
and amplitudes of InsP3-induced Ca2+
release are dependent
upon the InsP3 concentration added. The rate constants deter-
mined here appear to be 5–10-fold lower than previously re-
TABLE I

| Ion concentration | Li+ | Na+ | K+ | Rb+ | Cs+ |
|-------------------|-----|-----|----|-----|-----|
| mW                |     |     |    |     |     |
| 100               | 14.7 ± 1.3 | 14.6 ± 1.8 | 14.7 ± 1.5 | 13.7 ± 1.0 | 13.4 ± 1.3 |
| 75                |     |     |    |     |     |
| 50                |     |     |    |     |     |
| 25                |     |     |    |     |     |
| 0.0               |     |     |    |     |     |

Fig. 2. The effect of alkali metal ions on quantal InsP3-induced Ca2+
release. Each curve represents the effect of 100 mM: ○, K+; ■, Na+;
K+; +, RB+; and , Cs+ on InsP3-induced Ca2+
release measured as a
percent of Ca2+
released by InsP3 (0.01–20 µM) compared with that released by A23187 (12.5 µg/ml). The data are presented as the mean ±
S.E. of three or more determinations.
Fig. 3. Metal ion concentration on InsP₃-induced Ca²⁺ release. Each curve represents the effect of increasing the metal ion concentrations of: K⁺, Na⁺, Rb⁺, and Cs⁺ on Ca²⁺ release induced by 20 μM InsP₃. Fig. 6A shows a variation in the relationship between the amplitudes and the rate constants with InsP₃ concentration. In the microsomal preparation used for this part of the study, the maximum amount of Ca²⁺ release (amplitude) required ~1 μM InsP₃; however, the rate constant for this process still had not reached its maximum level by 40 μM InsP₃.

Fig. 6A shows the InsP₃-induced Ca²⁺ release time course (using 1 μM InsP₃) at different K⁺ concentrations (25–100 mM). In some of the time courses in this figure a split time base was used to enhance the amount of data points collected within the first 2 s. A 1-mS time filter was also used to reduce the signal-to-noise ratio. Again all the data could be fitted to a monoexponential equation. Fig. 6B shows that although the amplitude (fractional amount of Ca²⁺ release) increases in a linear relationship with K⁺ concentration (see also Fig. 2), the rate constants for Ca²⁺ release are essentially unaffected.

Discussion

Fluo-3 is a commonly used fluorophore for measuring Ca²⁺ fluxes in intact and permeabilized cells as well as subcellular fractions (14–16, 22). The affinity of this dye for Ca²⁺ is greatly dependent upon both ion species and ion concentration present in the medium. Most workers in the field using fluo-3 tend to use either 400 nM (21) or 900 nM (16, 17) as the dissociation constant for Ca²⁺ binding in order to calculate changes in Ca²⁺ concentration. However, as illustrated, here the Kᵥ values can vary substantially from 225 to 900 nM dependent upon the ion present and its concentration and as such will affect the calculated free Ca²⁺ concentrations. As pH and Mg²⁺ concentration also affect the affinity of fluo-3 for Ca²⁺, we must stress the importance of using the appropriate Kᵥ values for fluo-3 depending on the experimental conditions used. These observations should also serve as a warning to experimentalists attempting to draw conclusions from small differences in the calibrated Ca²⁺ concentrations inside cells.

The rate of ATP-dependent Ca²⁺ uptake into cerebellar microsomes is dependent upon the ion species and concentration.

2 F. Michelangeli, unpublished observation.

K⁺ was the best ion for Ca²⁺ uptake being twice as effective as Rb⁺ at the same concentration. Li⁺ was extremely poorly at eliciting uptake (approximately 5% of the rate as that for K⁺), and therefore no InsP₃-induced Ca²⁺ release experiments were undertaken with this ion. We noted that at least 20 mM alkali metal ion concentration was required to attain a sufficient level of Ca²⁺ uptake into the microsomes in order for Ca²⁺ release experiments to be undertaken. These results are comparable with those observed by Mualem et al. (2) using rat liver microsomes and probably relate to the fact that alkali metal ions, in particular K⁺, stimulate the microsomal Ca²⁺-ATPase. K⁺ ions have been shown to stimulate the sarcoplasmic reticulum SERCA1 isoform of the Ca²⁺-ATPase by increasing the rate of the dephosphorylation step (E₂P → E₁) (23). Since the kinetic properties of the endoplasmic reticulum Ca²⁺-ATPase are similar to the SR type (24), it is likely that K⁺ stimulation of ATP-dependent Ca²⁺ uptake by the microsomal Ca²⁺-ATPase is by a similar mechanism.

K⁺ is also most effective at stimulating InsP₃-induced Ca²⁺ release when measured at 100 mM concentration. However, the potency of K⁺ compared with the other ions tested was diminished at concentrations below 50 mM. The stimulation of InsP₃-induced Ca²⁺ by K⁺ increased linearly with concentration up to 100 mM, which was the maximum concentration tested in this study. This observation directly contrasts with the study of Joseph and Williamson (3), which showed that in rat permeabilized hepatocytes K⁺ stimulated InsP₃-induced Ca²⁺ release optimally at 40 mM, while at higher concentration Ca²⁺ release was inhibited. The only significant effects of the metal ion species and ion concentration on quantitative InsP₃-induced Ca²⁺ release was on the percent or extent of release, as the IC₅₀ values for Ca²⁺ release with InsP₃ concentration and [³H]InsP₃ binding levels were affected little. The fact that here we observe little effect of K⁺ on [³H]InsP₃ binding, while in the paper by Hannert-Merah et al. (28) shows a 2–3-fold decrease in affinity with K⁺, most probably reflects differences in experimental conditions used in both studies (i.e. binding studies were undertaken at 4 °C rather than 20 °C).

In this study the rate of InsP₃-induced Ca²⁺ release from rat cerebellar microsomes was resolved using stopped-flow techniques and shown to be slower than earlier reports using permeabilized rat basophilic leukemia cells and rat hepatocytes.
but considerably faster than the rates observed for the cerebellar InsP₃ receptor reconstituted into liposomes (8–10). The differences in the rates of Ca²⁺ release using different cell types may reflect differences in InsP₃ receptor isoforms present in these cells. From immunological studies using isoform specific antibodies, the cerebellum appears to express mainly the type I isoform, while hepatocytes express mainly the type II isoform (25). It is as yet unknown what isoforms are present in basophilic leukemia cells. The differences observed between the Ca²⁺ release rates from cerebellar microsomes compared with cerebellar InsP₃ receptors reconstituted into liposomes may well reflect a difference in the receptor density in membrane vesicles between the two systems, which in turn may affect the rates of release (26). The rate of InsP₃-induced Ca²⁺ release was calculated to be >0.1 s⁻¹.

In this preparation the release process with low InsP₃ concentration reached completion after 10 s, whereas with high InsP₃ concentration (40 mM) the maximal amount of release was reached after 5 s. The fact that the rate of Ca²⁺ release is more sensitive to InsP₃ concentration than the extent or amplitude of Ca²⁺ release (illustrated by the fact that in Fig. 5B the amplitude or extent of Ca²⁺ release was affected little whether the Ca²⁺ pumps were fully inhibited or 90% inhibited, since even at lowest InsP₃ concentration used the rate constant for Ca²⁺ release was calculated to be >0.1 s⁻¹.

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release has reached a maximum by $1\,\mu M$ InsP$_3$, while the rate constant still appears to increase beyond $40\,\mu M$ InsP$_3$ would also argue against a more elaborate version of the all-or-none model for quantal Ca$^{2+}$ release which assumes that the stores are not only heterogeneous with respect to their sensitivities to InsP$_3$ but also have heterogeneous receptor densities. In this case the concentration of InsP$_3$ required to reach both the maximum amount of Ca$^{2+}$ release and the maximum rate of Ca$^{2+}$ release should be the same.

In this preparation of rat cerebellar microsomes we found that InsP$_3$-induced Ca$^{2+}$ release could be fitted assuming a simple monoeponential process at all InsP$_3$ concentrations and K$^+$ concentrations used; however, a bieponential process with similar rate components and amplitudes for the two components could also be fitted equally well. In investigations of the rate constants of InsP$_3$-induced Ca$^{2+}$ release using different preparations of cerebellar microsomes, we have concluded that the rate constants are consistently lower than those previously reported in other studies, using permeabilized cells (19, 20). However, they are consistently similar between cerebellar microsomal preparations (varying between 0.5 and 1.7 s$^{-1}$ with 20–40 $\mu M$ InsP$_3$). In some micromolar preparations InsP$_3$-induced Ca$^{2+}$ release can only be successfully fitted to a biexponential process consisting of two independent monoeponential components (27), while other preparations, such as the one used in this study, can be fitted equally well to a monoeponential process. As yet the reason for this variation between preparations remains unknown, although there is also a variation in the levels of Ca$^{2+}$ released by InsP$_3$, the IC$_{50}$ values and the cooperativity of InsP$_3$-induced Ca$^{2+}$ release between cerebellar microsomal preparations, the variability may be due to subtle differences in membrane preparations. A related phenomenon was recently reported by Hannaert-Merah et al. (28), who showed that the kinetics of InsP$_3$ binding and dissociation to cerebellar microsomes was either monophasic or biphasic depending on the cerebellar microsome preparation used.

From the kinetic data presented here, the only effect of varying K$^+$ concentrations seems to be on the extent of Ca$^{2+}$ release rather than on any effects on the rate constants for release. Since it has been reported previously that there is heterogeneity between InsP$_3$-sensitive Ca$^{2+}$ stores (5), one plausible explanation for this observation might be that these stores are also heterogeneous in their sensitivities to K$^+$, such that some stores will be able to respond to InsP$_3$ and release Ca$^{2+}$ at low K$^+$ concentration while other stores require higher K$^+$ concentration before Ca$^{2+}$ release occurs. An alternative explanation, which at present cannot be ruled out, is the possibility that as InsP$_3$-sensitive Ca$^{2+}$ channels slowly desensitize after being opened by the addition of InsP$_3$ (29), K$^+$ could slow down this desensitization step, thus increasing the amount of Ca$^{2+}$ release without necessarily affecting the rate of release. It is unlikely that the effects of K$^+$ we have observed on quantal InsP$_3$-induced Ca$^{2+}$ release are due substantially to an increase in the rate of InsP$_3$ dissociation from its receptor as suggested by Hannaert-Merah et al. (28).

As K$^+$ concentration does not affect the rate of InsP$_3$-induced Ca$^{2+}$ release, this may have implications in assessing the possible role of K$^+$ as a counter ion. Although some studies have tried to monitor changes in $^{86}$Rb$^+$ uptake into cerebellar microsomes upon addition of InsP$_3$, such changes have not been detected (6). We have also tried to monitor changes in K$^+$ uptake into microsomes upon exposure to InsP$_3$, using flame spectrophotometry with no success. Since the cerebellar InsP$_3$ receptor can be purified and reconstituted into sealed vesicles and still retain Ca$^{2+}$ channel activity (8–10), this must imply that K$^+$ ions are required as a counter ion during Ca$^{2+}$ release then the channel itself must be an antipporter allowing Ca$^{2+}$ to flow in one direction, while K$^+$ moves in the opposite direction (11). Although several studies using electrophysiological approaches have shown the InsP$_3$ receptor to be weakly permeable to both K$^+$ and Na$^+$ (30, 31), they have only been shown to move in the same direction as Ca$^{2+}$ and not in the opposite direction as would be required here. If the InsP$_3$ receptor was an antipporter, then by analogy with other co-transporters such as the the Na$^+$/Ca$^{2+}$ exchanger (32, 33), changing the concentration of one ion should have a direct effect on the rate at which the other ion is transported as long as neither are at saturating concentrations. However, the fact that changing the concentration of K$^+$ has no effect on the rate of Ca$^{2+}$ release (measured at InsP$_3$ concentrations where the rate of Ca$^{2+}$ release is not maximal) must imply that K$^+$ is unlikely to be acting as a counter ion for Ca$^{2+}$ release. This therefore leaves us to postulate a more direct role for K$^+$ and the other alkali metal ions in affecting the mechanism of channel opening, possibly by acting as a co-factor. This possibility of the InsP$_3$ receptor containing a putative K$^+$ binding site which affects channel function obviously requires further investigation.

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