# ATP Regulation of Recombinant Type 3 Inositol 1,4,5-Trisphosphate Receptor Gating

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ABSTRACT A family of inositol 1,4,5-trisphosphate ($InsP_3$) receptor ($InsP_3R$) $Ca^{2+}$ release channels plays a central role in $Ca^{2+}$ signaling in most cells, but functional correlates of isoform diversity are unclear. Patch-clamp electrophysiology of endogenous type 1 (*X*-$InsP_3R$-1) and recombinant rat type 3 $InsP_3R$ (r-$InsP_3R$-3) channels in the outer membrane of isolated *Xenopus* oocyte nuclei indicated that enhanced affinity and reduced cooperativity of $Ca^{2+}$ activation sites of the $InsP_3$-liganded type 3 channel distinguished the two isoforms. Because $Ca^{2+}$ activation of type 1 channel was the target of regulation by cytoplasmic ATP free acid concentration ($[ATP]_i$), here we studied the effects of $[ATP]_i$ on the dependence of r-$InsP_3R$-3 gating on cytoplasmic free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). As $[ATP]_i$ was increased from 0 to 0.5 mM, maximum r-$InsP_3R$-3 channel open probability ($P_o$) remained unchanged, whereas the half-maximal activating $[Ca^{2+}]_i$ and activation Hill coefficient both decreased continuously, from 800 to 77 nM and from 1.6 to 1, respectively, and the half-maximal inhibitory $[Ca^{2+}]_i$ was reduced from 115 to 39 μM. These effects were largely due to effects of ATP on the mean closed channel duration. Whereas the r-$InsP_3R$-3 had a substantially higher $P_o$ than *X*-$InsP_3R$-1 in activating $[Ca^{2+}]_i$ (<1 μM) and 0.5 mM ATP, the $Ca^{2+}$ dependencies of channel gating of the two isoforms became remarkably similar in the absence of ATP. Our results suggest that ATP binding is responsible for conferring distinct gating properties on the two $InsP_3R$ channel isoforms. Possible molecular models to account for the distinct regulation by ATP of the $Ca^{2+}$ activation properties of the two channel isoforms and the physiological implications of these results are discussed. Complex regulation by ATP of the types 1 and 3 $InsP_3R$ channel activities may enable cells to generate sophisticated patterns of $Ca^{2+}$ signals with cytoplasmic ATP as one of the second messengers.

KEY WORDS: allosteric regulation• calcium release channel • single-channel electrophysiology • patch clamp • *Xenopus* oocyte

## INTRODUCTION

Modulation of free cytoplasmic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) is a ubiquitous cellular signaling system. In many cell types, binding of ligands to plasma membrane receptors activates the hydrolysis of phosphatidylinositol 4,5-bisphosphate by membrane-bound phospholipase C, generating inositol 1,4,5-trisphosphate ($InsP_3$).[1] $InsP_3$ causes the release of $Ca^{2+}$ from the endoplasmic reticulum (ER) by binding to its receptor ($InsP_3R$), which itself is a $Ca^{2+}$ channel (Taylor and Richardson, 1991; Berridge, 1993; Putney and St. J. Bird, 1993). A family of $InsP_3R$ isoforms (types 1, 2, and 3) with different primary sequences derived from different genes and alternatively spliced isoforms has been identified. The different isoforms have distinct and overlapping patterns of expression in different tissues. Most, if not all, mammalian cells express multiple isoforms whose absolute and relative expression levels can be modified by cell differentiation and physiological status, and which may associate as heterotetramers.

The functional correlates of this impressive diversity of $InsP_3R$ expression are largely unknown (see INTRODUCTION of Mak et al., 2001, in this issue). Expression and single-channel recording of the recombinant rat type 3 $InsP_3R$ (r-$InsP_3R$-3) channels in *Xenopus* oocyte nuclear membrane patches (Mak et al., 2000, 2001) demonstrated that they have remarkably similar ion permeation and channel gating properties as the *Xenopus* type 1 $InsP_3R$ (*X*-$InsP_3R$-1). Of note, r-$InsP_3R$-3 gating also exhibits a biphasic dependence on $[Ca^{2+}]$, with properties of the inhibitory $Ca^{2+}$ site and allosteric tuning of that site by $InsP_3$ highly similar to those properties of the endogenous *X*-$InsP_3R$-1. In contrast, the r-$InsP_3R$-3 channel is uniquely distinguished from the type 1 channel by enhanced $Ca^{2+}$ sensitivity of, and lack of cooperativity between, the $Ca^{2+}$ activation sites (see Mak et al., 2001, in this issue; Fig. 1). Interestingly, studies of the regulation by ATP of the *X*-$InsP_3R$-1 channel revealed that the mechanism by which cytoplasmic free ATP stimulates its gating in low $[Ca^{2+}]_i$ (<1 μM) is by specifically increasing the affinity of the $Ca^{2+}$ activating site of the channel without affecting the degree of co-

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[1]*Abbreviations used in this paper:* ER, endoplasmic reticulum; $InsP_3$, inositol 1,4,5-trisphosphate; MWC, Monad-Wyman-Changeux; $P_o$, open probability; r-$InsP_3R$-3, rat type 3 $InsP_3R$; *X*-$InsP_3R$-1, *Xenopus* type 1 $InsP_3R$.

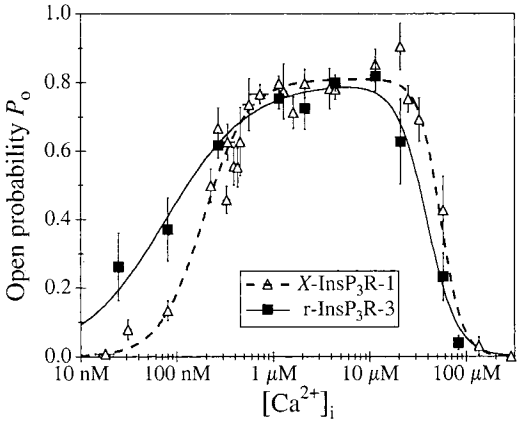


FIGURE 1. $Ca^{2+}$ dependencies of types 1 and 3 $InsP_3R$ channel $P_o$ in the presence of 0.5 mM ATP. 10 μM $InsP_3$ was present in the pipet solutions used in experiments presented in all figures. Open triangles represent data for *X*-$InsP_3R$-1 obtained from uninjected oocytes. Closed squares represent data for r-$InsP_3R$-3 obtained from cRNA-injected oocytes. The curves (dashed for *X*-$InsP_3R$-1 and solid for r-$InsP_3R$-3) are the biphasic Hill equation fits from Mak et al. (2001).

operativity for $Ca^{2+}$ activation or the maximum open probability ($P_o$; Mak et al., 1999). Although channel $P_o$ decreases when $[ATP]_i$ is decreased, this can be fully reversed by increasing $[Ca^{2+}]_i$, demonstrating that ATP is not a necessary agonist for activation of the $InsP_3R$, but is rather an allosteric regulator, tuning the efficacy of $Ca^{2+}$ to stimulate the activity of the $InsP_3$-liganded $InsP_3R$ over a limited range of $[Ca^{2+}]_i$ (10 nM–1 μM). Therefore, $InsP_3$ and ATP are complementary allosteric regulators of $InsP_3R$ gating: $InsP_3$ is a specific regulator of $Ca^{2+}$ inhibition of $Ca^{2+}$ release, tuning the functional affinity of the inhibitory $Ca^{2+}$ binding sites of the channel (Mak et al., 1998, 2001), whereas ATP is a regulator of $Ca^{2+}$-induced $Ca^{2+}$ release, tuning the functional affinity of the activating $Ca^{2+}$ binding sites.

Because the properties of the $Ca^{2+}$ activation sites represented the only significant distinction between the types 1 and 3 $InsP_3R$ channels that could be discerned in our previous studies, and $Ca^{2+}$ activation of *X*-$InsP_3R$-1 channel gating is modulated by ATP, here we have investigated the effects of ATP on the gating properties of the recombinant r-$InsP_3R$-3 channel. Surprisingly, our results reveal that the presence of ATP is absolutely necessary to confer distinct $Ca^{2+}$ activation properties to the types 1 and 3 channels because, in its absence, $Ca^{2+}$ activation of the two channels is very similar. ATP activates type 3 channel gating in a dramatically different manner compared with its effects on the type 1 channel: whereas maximal $P_o$ of both isoforms is similarly unaffected by $[ATP]_i$, ATP abolishes the cooperativity of $Ca^{2+}$ activation of r-$InsP_3R$-3 besides increasing the functional affinity of the activating $Ca^{2+}$ binding sites. Thus, complex allosteric dependencies on $[ATP]_i$ of the gating of types 1 and 3 $InsP_3R$ channels, coupled with differential levels of expression and subcellular localization, may enable cells to generate sophisticated $Ca^{2+}$ signals, with cytoplasmic ATP as one of the second messengers.

## MATERIALS AND METHODS

*Xenopus* oocytes were obtained and selected for a low functional expression level of the endogenous *X*-$InsP_3R$-1 as described previously (Mak et al., 2001). Of 89 nuclear patches obtained from isolated nuclei of selected uninjected oocytes, only 1 channel was detected. The mean number of $InsP_3R$ channels per nuclear patch was 0.011. Selected oocytes were microinjected with r-$InsP_3R$-3 cRNA and used for nuclear patch-clamp experiments 4–5 d after injection. The mean number of $InsP_3R$ channels per nuclear patch for the cRNA-injected oocytes increased dramatically to 1.65. In 330 nuclear patches, 544 channels were detected in 167 patches, with 105 patches exhibiting multiple $InsP_3R$ channels. Assuming random, binomial association of *X*-$InsP_3R$-1 and r-$InsP_3R$-3 to form tetrameric channels (Mak et al., 2000), most (97.4%) of the channels detected in cRNA-injected oocytes are predicted to be homotetrameric r-$InsP_3R$-3 channels.

Nuclear patch-clamp experiments were performed on isolated nuclei from cRNA-injected oocytes as described in the companion paper (see Mak et al., 2001, in this issue), except the pipet solutions used in this study contained a saturating concentration of 10 μM of $InsP_3$ (used without further purification; Molecular Probes), various concentrations of $Na_2ATP$, and either 0 or 3 mM $MgCl_2$ as stated. Because of chelation of $Mg^{2+}$ by ATP, the actual free $Mg^{2+}$ in the solutions was 0 or 2.5 mM. The bath solutions used in all experiment had 140 mM KCl, 10 mM HEPES, 300 μM $CaCl_2$, and 500 μM BAPTA ($[Ca^{2+}]$ = 500 nM), pH 7.3.

Data acquisition and analysis were performed as described in the companion paper (see Mak et al., 2001, in this issue).

## RESULTS

### *ATP Activation of Recombinant Type 3 $InsP_3R$*

To study the effects of $[ATP]_i$ on gating of single recombinant r-$InsP_3R$-3 channels in their native ER membrane environment, we performed patch-clamp experiments on nuclei isolated from oocytes injected with cRNA of r-$InsP_3R$-3. Although the oocyte expresses endogenously a type 1 $InsP_3R$, under the conditions of our experiments, >90% of the channels recorded were contributed by type 3 homotetramers (Mak et al., 2000). To facilitate comparisons with experimental results obtained for the type 1 $InsP_3R$ (Mak et al., 1999), the patch-clamp experiments were performed for the $InsP_3R$-3 using similar experimental conditions. The pipet solutions contained various $[Ca^{2+}]_i$ with 0.5 mM ATP alone, 3 mM $Mg^{2+}$ alone, 0.5 mM ATP and 3 mM $Mg^{2+}$ (calculated $[ATP]_i$ = 12 μM; calculated $[Mg^{2+}]_i$ = 2.5 mM), or no ATP or $Mg^{2+}$. To avoid possible effects of $Ca^{2+}$ on $InsP_3$ binding (Hagar and Ehrlich, 2000; Meas et al., 2000), a functionally saturating $InsP_3$ concentration of 10 μM was used.

r-$InsP_3R$-3 channel activities with a high $P_o$ of 0.6 (Fig. 2 A) and gating kinetics similar to those of the r-$InsP_3R$-3 reported previously (see Mak et al., 2001, in this issue) were observed in pipet solutions containing 0.5 mM

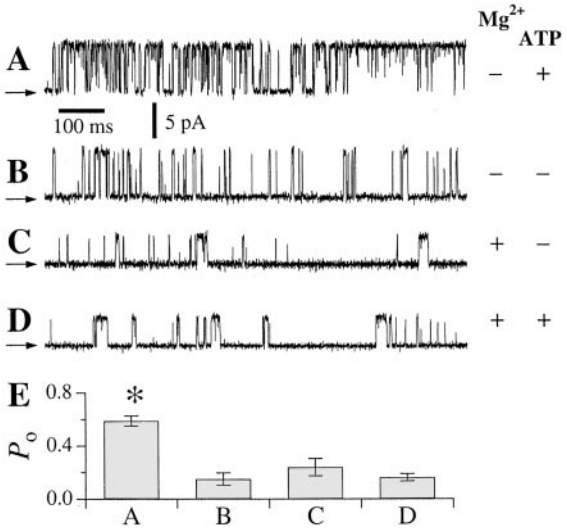


FIGURE 2. (A–D) Typical single-channel current traces of r-InsP$_3$R-3 channels in the outer membrane of nuclei isolated from r-InsP$_3$R-3 cRNA-injected oocytes under suboptimal $[Ca^{2+}]_i$, with various $[ATP]_i$ and $[Mg^{2+}]_i$. Arrows indicate closed channel current levels. (A) $[Ca^{2+}]_i$ = 224 nM, $[ATP]_i$ = 0.5 mM; $[Mg^{2+}]_i$ = 0 mM. (B) $[Ca^{2+}]_i$ = 255 nM, $[ATP]_i$ = 0 mM; $[Mg^{2+}]_i$ = 0 mM. (C) $[Ca^{2+}]_i$ = 274 nM, $[ATP]_i$ = 0 mM; $[Mg^{2+}]_i$ = 3.0 mM. (D) $[Ca^{2+}]_i$ = 286 nM, $[ATP]_i$ = 12 μM; $[Mg^{2+}]_i$ = 2.5 mM (0.5 mM total ATP; 3 mM total $Mg^{2+}$). Reduction of InsP$_3$R channel conductance in the presence of $Mg^{2+}$ (C and D) is caused by permeant ion block of the channel by the divalent cation (Mak and Foskett, 1998). (E) Single-channel $P_o$ of the r-InsP$_3$R-3 channel in conditions specified in A–D. Asterisk represents $P < 0.05$.

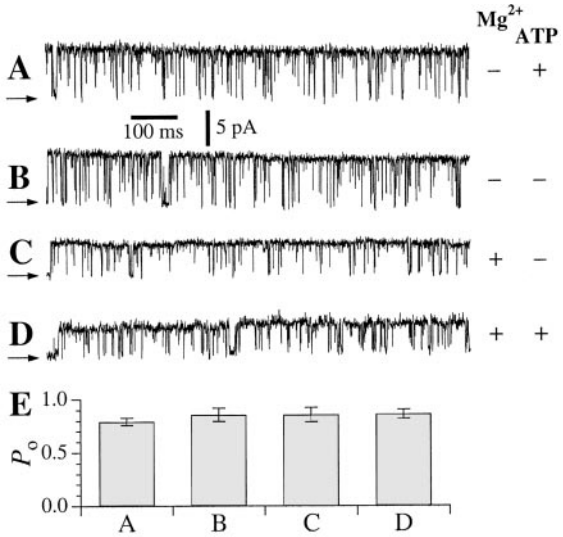


FIGURE 3. (A–D) Typical single-channel current traces of r-InsP$_3$R-3 channels under optimal $[Ca^{2+}]_i$ with various $[ATP]_i$ and $[Mg^{2+}]_i$. Arrows indicate closed channel current levels. (A) $[Ca^{2+}]_i$ = 4.4 μM, $[ATP]_i$ = 0.5 mM; $[Mg^{2+}]_i$ = 0 mM. (B) $[Ca^{2+}]_i$ = 5.6 μM, $[ATP]_i$ = 0 mM; $[Mg^{2+}]_i$ = 0 mM. (C) $[Ca^{2+}]_i$ = 2.5 μM, $[ATP]_i$ = 0 mM; $[Mg^{2+}]_i$ = 3.0 mM. (D) $[Ca^{2+}]_i$ = 2.5 μM, $[ATP]_i$ = 12 μM; $[Mg^{2+}]_i$ = 2.5 mM (0.5 mM total ATP; 3 mM total $Mg^{2+}$). Reduction of InsP$_3$R channel conductance in the presence of $Mg^{2+}$ (C and D) is caused by permeant ion block of the channel by the divalent cation (Mak and Foskett, 1998). (E) Single-channel open probability ($P_o$) of the r-InsP$_3$R-3 channel in conditions specified in A–D.

free ATP, and ~250 nM free $Ca^{2+}$. In the absence of ATP, in contrast, the r-InsP$_3$R-3 channel had significantly lower $P_o$ of 0.2, either in the presence or absence of 3 mM $Mg^{2+}$ (Fig. 2, B and C). ATP did not affect the conductance of the r-InsP$_3$R-3 channel (Fig. 2, A and B). The time course of channel inactivation of the r-InsP$_3$R-3 channel was not substantially different in the presence and absence of ATP.

To examine in more detail if the presence of the MgATP complex affects the $P_o$ of the r-InsP$_3$R-3, similar experiments were performed using pipet solutions containing 3 mM total $Mg^{2+}$ and 0.5 mM total ATP, so that the calculated [MgATP], free $Mg^{2+}$ concentration ($[Mg^{2+}]_i$), and $[ATP]_i$ were 0.5 mM, 2.5 mM, and 12 μM, respectively. The $P_o$ of the r-InsP$_3$R-3 channel remained low under these conditions (Fig. 2 D). Thus, the r-InsP$_3$R-3 is activated by ATP free acid ($ATP^{3-}$ or $ATP^{4-}$), but not by the MgATP complex (Fig. 2 E), suggesting that ATP hydrolysis or phosphorylation is not involved in ATP activation of the type 3 InsP$_3$R. Therefore, this behavior is similar to that of the type 1 InsP$_3$R channel (Mak et al., 1999).

This activation of r-InsP$_3$R-3 by ATP was prominently observed only at low $[Ca^{2+}]_i$ (<1 μM). At optimal $[Ca^{2+}]_i$ (>2 μM), the channel $P_o$ achieved the maximum value of ~0.8 in both 0 or 0.5 mM free $[ATP]_i$ (Fig. 3). Thus, as in the case for *X*-InsP$_3$R-1 (Mak et al., 1999), ATP is not essential for maximal activation of r-InsP$_3$R-3.

### *Effects of ATP on the $Ca^{2+}$ Dependence of Types 1 and 3 InsP$_3$R Gating*

To determine the mechanisms by which ATP activates r-InsP$_3$R-3 channel gating, we investigated systematically the effects of cytoplasmic ATP on the channel kinetics over a wide range of $[Ca^{2+}]_i$. In the absence of ATP, $Ca^{2+}$ dependence of channel $P_o$ of the InsP$_3$R-3 was biphasic (Fig. 4 A) and well fitted with the biphasic Hill equation (Mak et al., 1998):

$$P_o = P_{max}\{1 + (K_{act}/[Ca^{2+}]_i)^{H_{act}}\}^{-1} \{1 + ([Ca^{2+}]_i/K_{inh})^{H_{inh}}\}^{-1}, \quad (1)$$

with a maximum open probability ($P_{max}$) of 0.84 ± 0.02, a half-maximal activating $[Ca^{2+}]_i$ ($K_{act}$) of 800 ± 50 nM, an activation Hill coefficient ($H_{act}$) of 1.6 ± 0.3, a half-maximal inhibitory $[Ca^{2+}]_i$ ($K_{inh}$) of 115 ± 15 μM, and an inhibition Hill coefficient ($H_{inh}$) of 2 ± 0.5 (Table I).

This behavior is dramatically different from the $Ca^{2+}$ dependence of gating of the r-InsP$_3$R-3 in the presence of 0.5 mM cytoplasmic free ATP (Fig. 4 A). At high, inhibitory $[Ca^{2+}]_i$, the presence of 0.5 mM ATP decreased the value of $K_{inh}$ from 115 to 39 μM such that, in $[Ca^{2+}]_i$ > 50 μM, the r-InsP$_3$R-3 channel activity was

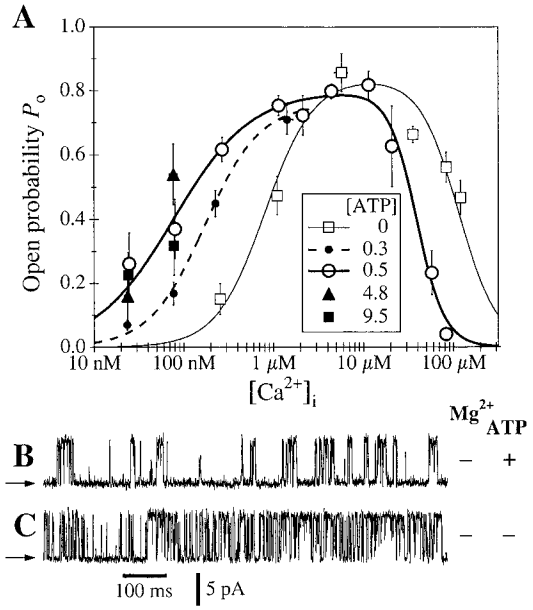


FIGURE 4. (A) $Ca^{2+}$ dependencies of the r-$InsP_3R$-3 channel $P_o$ in the presence of various $[ATP]_i$. The symbols correspond to $P_o$ data in various $[ATP]_i$ (in millimolars) as tabulated in the graph. The curves for 0 and 0.5 mM ATP represent theoretical fits to the data using the biphasic Hill equation (Eq. 1) whereas the curve for 0.3 mM ATP represents a fit using the activation Hill equation (Eq. 2). Hill equation parameters used are tabulated in Table I. (B–C) Typical single-channel current traces of r-$InsP_3R$-3 channels under inhibiting $[Ca^{2+}]_i$. Arrows indicate closed channel current levels. (B) $[Ca^{2+}]_i$ = 58 μM, $[ATP]_i$ = 0.5 mM; $[Mg^{2+}]_i$ = 0 mM. (C) $[Ca^{2+}]_i$ = 83 μM, $[ATP]_i$ = 0 mM; $[Mg^{2+}]_i$ = 0 mM.

lower in the presence of 0.5 mM free ATP than in the absence of ATP (Fig. 4, B and C). The value of $H_{inh}$ was somewhat higher in the presence of ATP. Of likely greater physiological significance, ATP induced a much lower $K_{act}$ of 77 ± 10 nM and reduced the value of $H_{act}$ to 1.0 ± 0.1 (Fig. 1 and Table I). Thus, cytoplasmic ATP decreased both the half-maximal activating $[Ca^{2+}]_i$ as well as the activation Hill coefficient, with the result that the $P_o$ of the r-$InsP_3R$-3 at $[Ca^{2+}]_i < 500$ nM is substantially higher in the presence of ATP than in its absence (Fig. 4 A). It is this dramatic increase of the $Ca^{2+}$ affinity of the r-$InsP_3R$-3 activating site(s) and loss of cooperativity between these sites in the tetrameric channel that give rise to the observed difference in channel activities of the r-$InsP_3R$-3 and *X*-$InsP_3R$-1 in activating $[Ca^{2+}]_i$ (<1 μM) in the presence of ATP (Fig. 1).

In sharp contrast, the biphasic $Ca^{2+}$ dependence of the r-$InsP_3R$-3 in the absence of cytoplasmic free ATP is remarkably similar to the biphasic $Ca^{2+}$ dependence of the *X*-$InsP_3R$-1 in the absence of ATP (Fig. 5), with $P_{max}$ = 0.80 ± 0.02, $K_{act}$ = 550 ± 50 nM, $H_{act}$ = 1.9 ± 0.6, $K_{inh}$ = 110 ± 15 μM, and $H_{inh}$ = 4.0 ± 0.7 (Table I). Thus, in the absence of cytoplasmic ATP, there are no significant differences between the responses to $Ca^{2+}$ of the types 1 and 3 $InsP_3R$ channels.

TABLE I

*Hill Equation Parameters for $[Ca^{2+}]_i$ Dependence of $InsP_3R$ Isoforms*

| | $InsP_3R$ isoform | $[ATP]_i$ | $P_{max}$ | $K_{act}$ | $H_{act}$ | $K_{inh}$ | $H_{inh}$ |
|---|---|---|---|---|---|---|---|
| | | *mM* | | *nM* | | *μM* | |
| A | Type 3 | 0.0 | 0.84 ± 0.02 | 800 ± 50 | 1.6 ± 0.3 | 115 ± 15 | 2.0 ± 0.5 |
| B | Type 3 | 0.3 | 0.76 ± 0.04 | 180 ± 30 | 1.4 ± 0.2 | N/A | N/A |
| C | Type 3 | 0.5 | 0.80 ± 0.03 | 77 ± 10 | 1.0 ± 0.1 | 39 ± 7 | 2.8 ± 0.4 |
| D | Type 1 | 0.0 | 0.80 ± 0.02 | 550 ± 50 | 1.9 ± 0.6 | 110 ± 15 | 4.0 ± 0.7 |
| E | Type 1 | 0.3 | N/A | 230 ± 30 | N/A | N/A | N/A |
| F | Type 1 | 0.5 | 0.81 ± 0.02 | 190 ± 20 | 1.9 ± 0.3 | 54 ± 3 | 3.9 ± 0.7 |

Parameters for the biphasic Hill equations (Eq. 1) that fit the $Ca^{2+}$ dependence of the $P_o$ of $InsP_3R$ channels under various experimental conditions. $[ATP]_i$ refers to the concentration of ATP free acid. Parameters for C are from Mak et al. (2001), parameters for E are from Mak et al. (1999). N/A, not available.

Examination of the gating kinetics of the r-$InsP_3R$-3 channel revealed that the mean open channel duration ($\tau_o$) in the absence of cytoplasmic ATP lay within a narrow range between 3 and 12 ms over the range of $[Ca^{2+}]_i$ studied (0.2–120 μM; Fig. 6). Within this range, $\tau_o$ varied with $[Ca^{2+}]_i$ in a biphasic fashion, increasing as $[Ca^{2+}]_i$ increased from 0.2 to 2 μM, and decreasing as $[Ca^{2+}]_i$ further increase from 6 to 120 μM. This partly mirrored the variation of r-$InsP_3R$-3 channel $P_o$ with $[Ca^{2+}]_i$ (Fig. 4 A). In contrast, the mean closed channel duration ($\tau_c$) decreased nearly an order of magnitude, from 18 to 2 ms, as $[Ca^{2+}]_i$ was increased from 0.2 to 6 μM, and then remained low (~2 ms) in high $[Ca^{2+}]_i$ (6–120 μM). The changes in $\tau_c$ accounted for the major part of the $Ca^{2+}$ activation of channel activity. These $Ca^{2+}$ dependencies of $\tau_c$ and $\tau_o$ of the type 3 channel in the absence of ATP are reminiscent of those of *X*-$InsP_3R$-1 in 0 mM ATP (Mak et al., 1999).

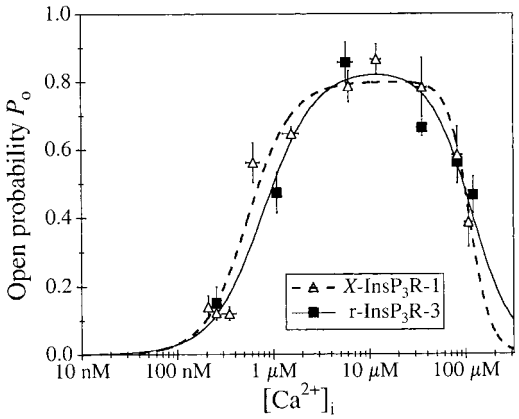


FIGURE 5. $Ca^{2+}$ dependencies of types 1 and 3 $InsP_3R$ channel $P_o$ in the absence of ATP. Open triangles represent data for *X*-$InsP_3R$-1 obtained from uninjected oocytes. Closed squares represent data for r-$InsP_3R$-3 obtained from cRNA-injected oocytes. The curves (dashed for *X*-$InsP_3R$-1 and solid for r-$InsP_3R$-3) are the biphasic Hill equation fits using Eq. 1 and parameters tabulated in Table I.

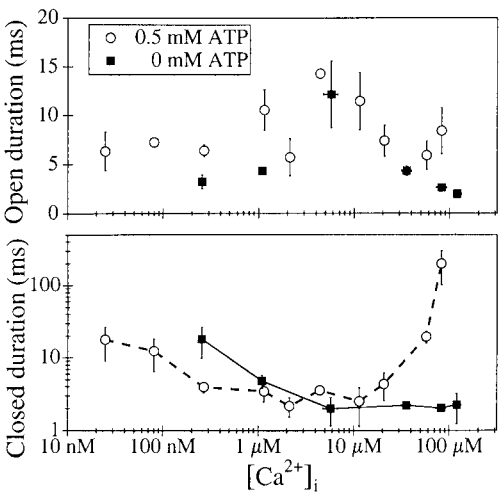


FIGURE 6. $Ca^{2+}$ dependencies of the mean open and closed channel durations of the r-$InsP_3R$-3 channels in the presence of 0 (■) or 0.5 mM (○) cytoplasmic free ATP. In the closed channel duration graph, data points obtained with the same $InsP_3$ concentrations are connected with a line for clarity.

The presence of ATP enhanced r-$InsP_3R$-3 channel activity at low $[Ca^{2+}]_i$ (<2 μM) (Figs. 2 and 3) by both stabilizing the open channel kinetic state(s) and destabilizing the closed kinetic state(s) (Fig. 6). As $[Ca^{2+}]_i$ increased from 10 to 83 μM, ATP reduced r-$InsP_3R$-3 channel activity by stabilizing closed kinetic state(s) so that $\tau_c$ increased dramatically (Figs. 4 and 6).

### *Effects of ATP Concentration on $Ca^{2+}$ Activation of r-$InsP_3R$-3*

The effects of cytoplasmic ATP concentration on the $Ca^{2+}$ activation of the r-$InsP_3R$-3 were studied in more detail in a series of patch-clamp experiments using various $[ATP]_i$ between 0 and 9.5 mM, over a wide range of $[Ca^{2+}]_i$ between 20 nM and 6 μM. The pipet solutions again contained 10 μM $InsP_3$, sufficient to saturate the $InsP_3R$ (Mak et al., 2001). For each ATP concentration used, the channel $P_o$ data over the range of $[Ca^{2+}]_i$ employed could be well described by the activation Hill equation:

$$P_o = P_{max}\{1 + (K_{act}/[Ca^{2+}]_i)^{H_{act}}\}^{-1}, \quad (2)$$

with $P_{max} \sim 0.8$ for all $[ATP]_i$ used (0–9.5 mM). Between $[ATP]_i$ of 0 and 500 μM, both $K_{act}$ and $H_{act}$ changed continuously (Fig. 4 A and Table I). At concentrations of ATP >500 μM, the activation of r-$InsP_3R$-3 by $Ca^{2+}$ exhibited no further systematic change (Fig. 4 A).

## DISCUSSION

Inositol trisphosphate–mediated intracellular $Ca^{2+}$ signaling is under complex regulation because the gating of the $InsP_3R$ $Ca^{2+}$ release channel is sensitive to $[Ca^{2+}]_i$ as well as to $[InsP_3]_i$ (Bezprozvanny and Ehrlich, 1995; Joseph, 1995; Taylor and Traynor, 1995). Gating of the $InsP_3$-liganded channel requires $Ca^{2+}$ binding to activation sites, whereas it is inhibited by $Ca^{2+}$ binding to inhibition sites (Taylor and Marshall, 1992; Iino and Tsukioka, 1994; Mak et al., 1998), resulting in a biphasic dependence on $[Ca^{2+}]_i$. In patch-clamp studies of the endogenous *X*-$InsP_3R$-1 channel (Mak and Foskett, 1994, 1997, 1998; Mak et al., 1998) or the recombinant r-$InsP_3R$-3 channel (see Mak et al., 2001, in this issue) in the outer membrane of isolated *Xenopus* oocyte nuclei, gating of both the $InsP_3$-liganded types 1 and 3 channels under optimal conditions exhibits robust activity, with a $P_{max}$ of ~0.8 over a wide range of $[Ca^{2+}]_i$ (Mak et al., 1998, 2001). In addition, $InsP_3$ binding was found to activate both isoforms of the $InsP_3R$ by allosterically reducing the $Ca^{2+}$ affinity of the inhibitory binding sites on the channel (Mak et al., 1998). When $[InsP_3]_i$ is low, the channel is inhibited by $Ca^{2+}$ because the $Ca^{2+}$ affinity of the inhibitory site is higher than that of the activating site. At higher $[InsP_3]_i$, the channel becomes less sensitive to $Ca^{2+}$ inhibition. When the affinity of the $Ca^{2+}$ inhibitory site(s) falls below that of the activating site(s), the channel can become fully activated. Furthermore, the properties of the $InsP_3$ binding site and the $Ca^{2+}$ inhibition site are similar for the *Xenopus* type 1 and recombinant type 3 channels in the same nuclear membrane system: the functional affinity for $InsP_3$ is ~50 nM for both channels, and both channel isoforms exhibit $K_{inh}$ of ~40–50 μM with Hill coefficient of 3–4 in the presence of 0.5 mM free ATP and saturating concentrations (~10 μM) of $InsP_3$.

In contrast, the properties of the $Ca^{2+}$ activation sites differ between the two isoforms. In nuclear patch-clamp studies, the type 3 channel is uniquely distinguished from the type 1 channel by enhanced sensitivity of ($K_{act}$ of 77 nM instead of 190 nM) and lack of cooperativity between the $Ca^{2+}$ activation sites ($H_{act}$ of 1 instead of ~2) in the presence of 0.5 mM free ATP and saturating concentrations (10 μM) of $InsP_3$ (Mak et al., 1998, 2001). As a result, the r-$InsP_3R$-3 has a substantially higher $P_o$ than the endogenous *X*-$InsP_3R$-1 in activating $[Ca^{2+}]_i$ (<1 μM) in the presence of 0.5 mM ATP (Fig. 1). We have suggested that these properties endow the $InsP_3R$-3 with high gain $InsP_3$–induced $Ca^{2+}$ release and low gain $Ca^{2+}$–induced $Ca^{2+}$ release properties, features which are complementary to the low gain $InsP_3$–induced $Ca^{2+}$ release and high gain $Ca^{2+}$–induced $Ca^{2+}$ release properties of $InsP_3R$-1.

Our previous study of the regulation by ATP of the *X*-$InsP_3R$-1 channel (Mak et al., 1999) revealed that the mechanism by which ATP stimulates gating of the $InsP_3R$-1 involved increasing the affinity of the $Ca^{2+}$ activating site of the channel specifically (i.e., decreasing

the $K_{act}$), without affecting $H_{act}$ or $P_{max}$ (Mak et al., 1999). Although channel $P_o$ decreased when $[ATP]_i$ was decreased, this could be fully reversed by increasing $[Ca^{2+}]_i$, demonstrating that ATP is not a necessary agonist for activation of the $InsP_3R$, but is rather an allosteric regulator, tuning the efficacy of $Ca^{2+}$ to stimulate the activity of the $InsP_3$-liganded $InsP_3R$-1 over a limited range of $[Ca^{2+}]_i$ (10 nM to 1 μM).

Because the $Ca^{2+}$ activation properties of channel gating was the major feature distinguishing the types 1 and 3 channels, and these properties of the type 1 channel were regulated by $[ATP]_i$, we therefore investigated the effects of ATP on the gating of the type 3 channel.

### *ATP Tuning of the Affinities of the $InsP_3R$ Inhibitory $Ca^{2+}$ Binding Sites*

Our patch-clamp experimental data indicate that ATP decreases $K_{inh}$ of both types 1 and 3 $InsP_3R$ in very similar manner, whereas $P_{max}$ values remained unchanged (Table I). Thus, ATP reduces the channel activities of both $InsP_3R$ channels at $[Ca^{2+}]_i > 10$ μM by decreasing $K_{inh}$, from ~110 μM in the absence of ATP to ~45 μM at 0.5 mM ATP (Figs. 1, 4, and 5). This reduction of $K_{inh}$ could not be reversed by the application of supersaturating $[InsP_3]_i$ (10 μM) that was substantially higher than the half-maximal $[InsP_3]_i \sim 50$ nM for *X*-$InsP_3R$-1 (Mak et al., 1998) and r-$InsP_3R$-3 (Mak et al., 2001). Thus, the more efficacious inhibition of $InsP_3R$ channel activities by high $[Ca^{2+}]_i$ (>10 μM) in the presence of ATP is independent of any possible competitive inhibition of $InsP_3$ binding to the $InsP_3R$ by ATP (Bezprozvanny and Ehrlich, 1993; Hagar and Ehrlich, 2000; Meas et al., 2000), and may be a mechanism through which $[ATP]_i$ can regulate feedback inhibition of $InsP_3R$-mediated $Ca^{2+}$ release.

Although binding assays have indicated that ATP can competitively inhibit binding of $InsP_3$ to $InsP_3R$-3 (Meas et al., 2000), no inhibition of the recombinant r-$InsP_3R$-3 channel activities was observed in $[Ca^{2+}]_i < 1$ μM by even 9.5 mM ATP under our experimental conditions. This is likely due to our use of a supersaturating $[InsP_3]_i$ (10 μM) that far exceeds the $K_{IP3}$ of r-$InsP_3R$-3 (~55 nM; Mak et al., 2001). As $InsP_3$ affects the $InsP_3R$-3 channel activity solely by tuning the affinity of the inhibitory $Ca^{2+}$ binding site(s) and has no effect on $Ca^{2+}$ activation of the channel (Mak et al., 2001), effective $InsP_3$ binding must be reduced to <0.3% (Mak et al., 2001) before effects of competitive inhibition of $InsP_3$ binding by ATP would be observed in activating $[Ca^{2+}]_i$ (<1 μM) in our experiments. This requires $[ATP]_i > 10$ mM (Meas et al., 2000), which is higher than the range of $[ATP]_i$ used in our experiment. An inhibition by 7–10 mM ATP of $InsP_3R$-3 single-channel activities in lipid bilayers in 160 nM $Ca^{2+}$ and 2 μM $InsP_3$ (Hagar and Ehrlich, 2000) was probably caused by the reduced functional sensitivity to activation by $InsP_3$ of the type 3 channel reconstituted into bilayers ($EC_{50}$ of 3.2 μM in 160 nM $Ca^{2+}$) compared with that observed in our experiments (see Mak et al., 2001, in this issue). In fact, enhancement, not inhibition, of $InsP_3R$-3 channel activity by 10 mM ATP was also observed in the presence of 10 μM $InsP_3$ in permeabilized cells using $Ca^{2+}$ imaging (Miyakawa et al., 1999). Similarly, no evidence of ATP inhibition of $InsP_3$ binding to $InsP_3R$-1 was observed in our characterization of regulation by ATP (0–9.5 mM) of $Ca^{2+}$ activation of *X*-$InsP_3R$-1 (Mak et al., 1999), again because of application of supersaturating $[InsP_3]$ (Mak et al., 1998). Therefore, differential inhibition of $InsP_3$ binding to types 1 and 3 $InsP_3R$ reported in Meas et al. (2000) has no impact on our characterization of ATP regulation of $InsP_3R$ channel activities in Mak et al. (1999) and this study.

### *ATP Enhancement of $Ca^{2+}$ Activation of r-$InsP_3R$-3 Channel*

Our systematic single-channel patch-clamp experimental data demonstrated that, in the nuclear membrane system, cytoplasmic free ATP, but not MgATP, enhanced the activation by $Ca^{2+}$ (<1 μM) of recombinant type 3 $InsP_3R$ channel through an increase of the $Ca^{2+}$ affinity and decrease of the cooperativity of the activating sites of the channel.

The effects of cytoplasmic ATP on the activity of the $InsP_3R$-3 have been studied previously primarily by $Ca^{2+}$ release assays using cells that expressed endogenous type 3 $InsP_3R$ as the only (Miyakawa et al., 1999) or major (Missiaen et al., 1998; Meas et al., 2000) $InsP_3R$ isoform. Enhancement of $Ca^{2+}$ release by ATP was observed in all the studies. The half-maximal ATP concentrations of 341 and 177 μM reported for ATP activation of $Ca^{2+}$ release (Missiaen et al., 1998) and ATP inhibition of photoaffinity labeling (Meas et al., 2000), respectively, of $InsP_3R$-3 are comparable to our experimental results with the activation of r-$InsP_3R$-3 channel saturated by 0.5 mM ATP. Our patch-clamp data indicate that ATP decreases the apparent Hill coefficient of $Ca^{2+}$ activation of the $InsP_3R$-3 channel, whereas ATP did not apparently have such an effect on $Ca^{2+}$ release in permeabilized 16HBE14o- cells (Missiaen et al., 1998). This difference is probably due to the very different experimental systems used. Whereas our patch-clamp experiments measure directly the single-channel activities of the $InsP_3R$ under rigorously controlled experimental conditions, measurements of $Ca^{2+}$ flux characterize the activities of heterogeneous populations of unknown numbers of $InsP_3R$ containing various isoforms (Sienaert et al., 1998) and possibly heterooligomers, from which the single-channel activities of the $InsP_3R$ can only be inferred indirectly. The effects of cytoplasmic free ATP on $InsP_3$-induced $Ca^{2+}$ release in permeabilized B cells genetically engineered to express individual $InsP_3R$ isoforms was reported in Mi-

yakawa et al. (1999). Under the experimental conditions used in that study (in 300 nM $Ca^{2+}$), the reduction in $InsP_3$-induced $Ca^{2+}$ release when $[ATP]_i$ was decreased from 10 to 0 mM was smaller in cells expressing $InsP_3R$-3 only than in cells expressing $InsP_3R$-1 only. This result agrees qualitatively with our single-channel results. A similar change of $[ATP]_i$ decreased $P_o$ from ~0.6 to ~0.2 in r-$InsP_3R$-3 (Fig. 4 A), whereas $P_o$ changed from 0.8 to 0.2 in *X*–$InsP_3R$-1 (Mak et al., 1999).

Under our experimental conditions, only free ATP, not the MgATP complex, enhanced r-$InsP_3R$-3 channel activity, as in the case for the *X*-$InsP_3R$-1 (Mak et al., 1999). However, $Ca^{2+}$ flux measurements in permeabilized cells suggested that MgATP also enhanced $Ca^{2+}$ release mediated by the $InsP_3R$-3 (Meas et al., 2000). This discrepancy may be due to the fact that single-channel $P_o$ is directly measured in our nuclear patch-clamp experiments, whereas $Ca^{2+}$ flux measurements are affected by the $Ca^{2+}$ conductance of the $InsP_3R$ channels as well as their $P_o$. Because $Mg^{2+}$ is a permeant blocking ion of the $InsP_3R$ (Mak and Foskett, 1998; Mak et al., 2000), the presence of $Mg^{2+}$ would be expected to reduce $InsP_3$-induced $Ca^{2+}$ flux through the $InsP_3R$, as observed in Meas et al. (2000). Thus, addition of ATP could generate an apparent increase in the observed $Ca^{2+}$ flux because the added ATP lowered the concentration of free $Mg^{2+}$ by forming the MgATP complex, thus alleviating the $Mg^{2+}$ blockage of the $InsP_3R$.

Recently, the effects of ATP on $InsP_3R$-3 channel properties were studied (Hagar and Ehrlich, 2000) by reconstituting into planar lipid bilayers $InsP_3R$ in microsomes isolated from RIN-m5F cells that express mainly type 3 $InsP_3R$ (77–96%; Wojcikiewicz and He, 1995; Swatton et al., 1999). Under the lipid bilayer experimental conditions, ATP (<6 mM) activated the channels by decreasing the mean closed channel durations and increasing the mean open channel durations, but did not affect the channel conductance. This agrees qualitatively with our patch-clamp results (Figs. 2, 4, and 6). However, the reconstituted $InsP_3R$-3 in planar lipid bilayers exhibited a half-maximal activating $[ATP]_i$ of 2.8 mM, whereas the r-$InsP_3R$-3 in our nuclear membrane patches was fully activated by 0.5 mM ATP (Fig. 4 A). The cause of the discrepancy between the two experimental systems is uncertain; the microenvironment (lipid membranes, buffer solutions, and transmembrane voltages) experienced by the $InsP_3R$-3 channel in the two studies were very different. However, as the planar lipid bilayer system consistently recorded a significantly lower maximum $P_o$ (~0.05) for both the type 3 (Hagar et al., 1998; Hagar and Ehrlich, 2000) and type 1 (Kaftan et al., 1997) $InsP_3R$ than was observed in our experimental system (0.8 for both types 1 and 3 $InsP_3R$), it is possible that cellular factors, like phosphatidylinositol 4,5-bisphosphate (Lupu et al., 1998), may be associated with the $InsP_3R$ reconstituted into the planar lipid bilayer system, and reduce the channel activities of the $InsP_3R$ and the efficacy of ATP to activate it.

### *Molecular Models for ATP Regulation of $Ca^{2+}$ Activation of the $InsP_3R$*

In a previous study of ATP regulation of the single-channel activity of the *X*-$InsP_3R$-1 (Mak et al., 1999), it was demonstrated that ATP activates the *X*-$InsP_3R$-1 channel not by increasing the $P_{max}$, but by increasing the apparent affinity of the activating $Ca^{2+}$ binding site(s), i.e., decreasing $K_{act}$. Within the range of $[ATP]_i$ used in that study (0–9.5 mM), $[ATP]_i$ had no observable effect on the value of $H_{act}$. The data could be interpreted by an empirical model described by a modified Michaelis-Menten equation, in which $[ATP]_i$ only affects the functional affinity of the activating $Ca^{2+}$ binding site(s) of the *X*-$InsP_3R$-1 with no effects on the cooperativity of those sites (Mak et al., 1999).

In contrast, the regulation by ATP of the r-$InsP_3R$-3 observed in this study is dramatically different. Whereas $P_{max}$ of the r-$InsP_3R$-3 was similarly unaffected by $[ATP]_i$, both $H_{act}$ and $K_{act}$ of the type 3 channel were reduced by ATP. Furthermore, these effects of ATP on the $Ca^{2+}$ activation of the r-$InsP_3R$-3 were saturated by 0.5 mM ATP (Fig. 4 A), whereas increasing $[ATP]_i$ up to several mM continued to further decrease $K_{act}$ of the type 1 channel (Mak et al., 1999). Of particular interest is that the $Ca^{2+}$ activation responses of the two isoforms become essentially the same in the absence of ATP (Fig. 5). Remarkably, therefore, the major feature distinguishing the types 1 and 3 channel isoforms (Mak et al., 2001) is dependent on the presence of ATP. In the absence of ATP, the permeation and gating behaviors of the two isoforms are indistinguishable in our nuclear patch-clamp studies.

How can we account for the distinct regulation by ATP of the $Ca^{2+}$ activation properties of the two channel isoforms? Analysis of the primary sequence of the type 1 $InsP_3R$ (Mignery et al., 1990) revealed two putative ATP binding sites (Yamada et al., 1994), only one of which is conserved in the sequence of the type 3 $InsP_3R$ (Maranto, 1994; Yamada et al., 1994; Yamamoto-Hino et al., 1994). Glutathione-S-transferase (GST)–fusion proteins containing the putative type 1–specific ATP-binding sequence or the ATP-binding sequence present in both types 1 and 3 $InsP_3R$ have both been shown to bind ATP in vitro (Maes et al., 1999). Therefore, it is possible that the functional ATP binding sites responsible for the regulation by ATP are distinct between the types 1 and 3 channels. Accordingly, whereas ATP binds with a functional affinity of ~0.27 mM to the functional ATP binding site in type 1 $InsP_3R$ and increases the sensitivity of the channel to $Ca^{2+}$ activation without affecting the cooperativity of $Ca^{2+}$ activation (Mak et al., 1999), it might

bind with a higher affinity to a different functional ATP binding site in the type 3 $InsP_3R$. In this model, binding to this distinct site in the type 3 channel would change, through a different molecular mechanism, the number and cooperativity of $Ca^{2+}$ binding site(s) involved in the $Ca^{2+}$ activation of the r-$InsP_3R$-3, as well as the sensitivity of the channel to $Ca^{2+}$ activation.

Alternately, the regulation of $InsP_3R$ by ATP and $Ca^{2+}$ can be accounted for by the molecular Monad-Wyman-Changeux (MWC) model (Monod et al., 1965) for allosteric systems. In this allosteric model, the $InsP_3R$ channel can exist in two conformations, one active and one inactive. In the absence of ligands, the channel mostly exists in the inactive conformation. Both ATP and $Ca^{2+}$ regulate the $InsP_3R$ channel as activating heterotropic ligands (Monod et al., 1965) by preferentially binding to and stabilizing the active conformation of the channel. Although not a general feature of the MWC model, our experiment results showed that ATP and $Ca^{2+}$ are not equivalent heterotropic ligands of the $InsP_3R$ channel. The $InsP_3R$ channel had low $P_o$ at low $[Ca^{2+}]_i$ despite the presence of saturating $[ATP]_i$, whereas the channel exhibited high $P_o$ at optimal $[Ca^{2+}]_i$ even in the absence of ATP (Figs. 4 A and 5; Mak et al., 1999). To account for this non-equivalence in a modified MWC model, we assume that $Ca^{2+}$ must bind to one or more of the activating $Ca^{2+}$ binding sites in the channel before the channel can be active, whereas ATP binding is not necessary.

In the MWC model, $InsP_3R$ channel activity can exhibit a dependence on the concentration of one of its ligands ($Ca^{2+}$) with a Hill coefficient >1 regardless of the number of $Ca^{2+}$ required to bind to the channel to open it (our unpublished data). The MWC model also predicts that the apparent half-maximal activating concentration ($K_{act}$) of one ligand ($Ca^{2+}$) can vary in the presence of different concentrations of the other ligand (ATP), even though the dissociation constants for the ligands of both conformations of the channel remains unchanged. Furthermore, heterotropic effects of $Ca^{2+}$ and ATP on the $InsP_3R$ channel can change the Hill coefficient for $Ca^{2+}$ activation ($H_{act}$) of the channel without changing the number of $Ca^{2+}$ required to bind to the channel before it can adopt the active conformation. Thus, according to the MWC model, binding of ATP, a heterotropic ligand, to the r-$InsP_3R$-3 channel can abolish the cooperativity of $Ca^{2+}$ and simultaneously decrease its half-maximal activating concentration (Monod et al., 1965). The magnitudes of changes in the observed $K_{act}$ and $H_{act}$ for $Ca^{2+}$ activation of an $InsP_3R$ isoform due to heterotropic effects of ATP, and the range of $[ATP]_i$ over which the changes occur, will depend on relevant parameters of that isoform, including the relative stability of the active and inactive conformations, and the affinities of those conformations for the ligands. With a different set of parameters for the type 1 $InsP_3R$, the binding of ATP can continuously change the observed $K_{act}$ for $Ca^{2+}$ activation over a wide range of $[ATP]_i$ without affecting the value of $H_{act}$ observably. Therefore, despite the observed differences in the regulation by ATP of the $Ca^{2+}$ activation of the types 1 and 3 $InsP_3R$, it is possible that ATP regulates $Ca^{2+}$ activation of the two $InsP_3R$ isoforms through the same MWC allosteric mechanism, with the different channel isoforms possessing different sets of relevant parameters. Because there are a large number of parameters involved in a MWC model for a tetrameric channel interacting with two ligands (Changeux and Edelstein, 1998; Jones, 1999), detailed numerical fittings by the MWC model of the r-$InsP_3R$-3 channel open probability and dwell time distribution data, similar to those performed in Rothberg and Magleby (1999), will be necessary to determine if the regulation by ATP and $Ca^{2+}$ of channel gating of $InsP_3R$ (both types 1 and 3) can be well described by such a model.

### *Differential Regulation by ATP of $Ca^{2+}$ Activation of the Types 1 and 3 $InsP_3R$ Isoforms*

We characterized previously the permeation properties, propensity to cluster, and regulation by $Ca^{2+}$ and $InsP_3$ of the type 3 $InsP_3R$ channel (Mak et al., 2000), and concluded that the only parameter that distinguishes the types 1 and 3 isoforms in the same membrane under identical experimental conditions is their $Ca^{2+}$ activation properties. However, the results of this study reveal that cytoplasmic ATP is critical to establishing this difference between the $Ca^{2+}$ responses of the two isoforms. In the absence of ATP, the biphasic $Ca^{2+}$ responses of the *X*-$InsP_3R$-1 and r-$InsP_3R$-3 are very similar (Fig. 5). This may have important consequences in cells that express both isoforms. As a result of the difference in the regulation of the two $InsP_3R$ isoforms by ATP, the relative level of activation of the two $InsP_3$-liganded isoforms by $[Ca^{2+}]_i$ (between 10 and 1,000 nM) will vary in a complex pattern with changes in $[ATP]_i$, as depicted in Fig. 7. When $[ATP]_i$ is <0.5 mM, the $InsP_3R$-1 channel is mostly less sensitive to activation by $Ca^{2+}$ than is $InsP_3R$-3. This is because ATP decreases the $K_{act}$ of $InsP_3R$-3 to a greater extent than that of $InsP_3R$-1, and decreases $H_{act}$ of $InsP_3R$-3 but does not affect that of $InsP_3R$-1. Whereas increases of $[ATP]_i$ (from 0.5 to 9.5 mM) continue to decrease $K_{act}$ of the type 1 channel (Mak et al., 1999), the effects of ATP on $Ca^{2+}$ activation of the type 3 channel are saturated at 500 μM. Thus, at 4.8 mM ATP, $P_o$ of $InsP_3R$-1 in $[Ca^{2+}]_i$ > 35 nM is higher than that of $InsP_3R$-3, although the type 3 channel is still more active than the type 1 isoform in $[Ca^{2+}]_i$ < 35 nM. At 9.5 mM ATP, $InsP_3R$-1 is more active than $InsP_3R$-3 in most $[Ca^{2+}]_i$.

Whereas the MgATP concentration in the cytoplasm

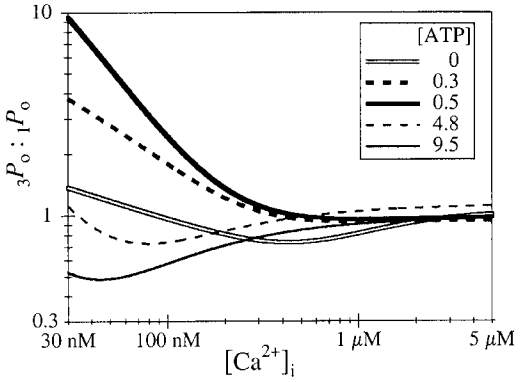


FIGURE 7. Variation of the ratio between the $P_o$ of r-InsP$_3$R-3 ($_3P_o$) and that of *X*–InsP$_3$R-1 ($_1P_o$), with $[Ca^{2+}]_i$ in the presence of different $[ATP]_i$ as tabulated. $P_o$ are calculated with Eq. 2, using the parameters ($P_{max}$, $K_{act}$, and $H_{act}$) tabulated in Table I. $_3P_o$ for 4.8 and 9.5 mM ATP were calculated using the same parameters as in 0.5 mM ATP. $P_{max}$ of *X*-InsP$_3$R-1 was assumed to be 0.8 in 0.3 mM ATP.

is in the range of 3–8 mM (Corkey et al., 1986; Dunne et al., 1988; Flatman, 1991; Kargacin and Kargacin, 1997; Maechler et al., 1998), the concentration of $ATP^{4-}$, the ligand of the InsP$_3$R (Mak et al., 1999; and this study), is in the range of 400–600 μM. Thus, under normal physiological conditions in the cytoplasm, the type 3 channel will be more sensitive than the type 1 channel to activation by $Ca^{2+}$. Nevertheless, with the close physical proximity of the ER and mitochondria (Otsu et al., 1990; Satoh et al., 1990; Simpson et al., 1997; Rizzuto et al., 1998), it is possible that the concentration of free ATP in the microdomains in which the InsP$_3$R are located may vary over a wide range without substantial change in the overall cytoplasmic ATP concentration, so that $[ATP]_i$ detected by the InsP$_3$R may be elevated to levels at which the type 1 InsP$_3$R becomes more active than the type 3 InsP$_3$R. Thus, the complex dependencies of the types 1 and 3 InsP$_3$R channel activities on $[ATP]_i$, coupled with differential levels of expression and subcellular localization, may enable the cell to generate sophisticated patterns of $Ca^{2+}$ signals with cytoplasmic ATP as one of the second messengers.

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