Stimulation of Inositol 1,4,5-Trisphosphate (IP$_3$) Receptor Subtypes by Adenophostin A and Its Analogues

Huma Saleem$^1$, Stephen C. Tovey$^1$, Andrew M. Riley$^2$, Barry V. L. Potter$^2$, Colin W. Taylor$^1$

$^1$Department of Pharmacology, Cambridge, United Kingdom, $^2$Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom

Abstract

Inositol 1,4,5-trisphosphate receptors (IP$_3$R) are intracellular Ca$^{2+}$ channels. Most animal cells express mixtures of the three IP$_3$R subtypes encoded by vertebrate genomes. Adenophostin A (AdA) is the most potent naturally occurring agonist of IP$_3$R and it shares with IP$_3$ the essential features of all IP$_3$R agonists, namely structures equivalent to the 4,5-bisphosphate and 6-hydroxyl of IP$_3$. The two essential phosphate groups contribute to closure of the clam-like IP$_3$-binding core (IBC), and thereby IP$_3$R activation, by binding to each of its sides (the $\alpha$- and $\beta$-domains). Regulation of the three subtypes of IP$_3$R by AdA and its analogues has not been examined in cells expressing defined homogenous populations of IP$_3$R. We measured Ca$^{2+}$ release evoked by synthetic adenophostin A (AdA) and its analogues in permeabilized DT40 cells devoid of native IP$_3$R and stably expressing single subtypes of mammalian IP$_3$R. The determinants of high-affinity binding of AdA and its analogues were indistinguishable for each IP$_3$R subtype. The results are consistent with a cation-$\pi$ interaction between the adenine of AdA and a conserved arginine within the IBC $\alpha$-domain contributing to closure of the IBC. The two complementary contacts between AdA and the $\alpha$-domain (cation-$\pi$ interaction and 3'-phosphate) allow activation of IP$_3$R by an analogue of AdA (3’-dephyso-AdA) that lacks a phosphate group equivalent to the essential 5-phosphate of IP$_3$. These data provide the first structure-activity analyses of key AdA analogues using homogenous populations of all mammalian IP$_3$R subtypes. They demonstrate that differences in the Ca$^{2+}$ signals evoked by AdA analogues are unlikely to be due to selective regulation of IP$_3$R subtypes.

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* E-mail: cwt10009@cam.ac.uk

Introduction

Inositol 1,4,5-trisphosphate receptors (IP$_3$R) are intracellular Ca$^{2+}$ channels that are expressed in almost all animal cells. They allow release of Ca$^{2+}$ from intracellular stores in response to the many stimuli that activate phospholipase C [1,2]. The genomes of vertebrates encode three closely related IP$_3$R subtypes (IP$_3$R1-3). The physiological significance of this IP$_3$R diversity is poorly understood, and nor are there ligands that allow release of Ca$^{2+}$ from intracellular stores in response to the many stimuli that activate phospholipase C [1,2]. The genomes of vertebrates encode three closely related IP$_3$R subtypes (IP$_3$R1-3), and most cells from vertebrates express functional IP$_3$R that are and later synthesized [11], is a potent agonist of IP$_3$R. It is also resistant to degradation by the enzymes that degrade IP$_3$ via phosphorylation or dephosphorylation [10]. Although AdA is based on a glucose ring, rather than the inositol ring of IP$_3$, its structure retains the key functional groups of IP$_3$ that are known to be essential for IP$_3$R activity at IP$_3$R [12] (Figure 1A). Considerable evidence supports the original suggestion [10] that the essential 4,5-bisphosphate and 6-hydroxyl of IP$_3$ are effectively mimicked by the 4”,3’-bisphosphate and 2’-hydroxyl of AdA (red highlights in Figure 1A). The interactions that allow AdA to bind to IP$_3$R with about 10-fold greater affinity than IP$_3$ have been more difficult to resolve. One view was that the 2’-phosphate of AdA is equivalent to the 1-phosphate of IP$_3$ and, like the latter [13] (blue in Figure 1A), contributes to high-affinity binding to the IBC. The suggestion was that the 2’-phosphate of AdA forms a stronger interaction with the IBC than does the 1-phosphate of IP$_3$. Our recent analyses have challenged this idea and instead suggest that a cation-$\pi$ interaction between the adenine ring of AdA and a guanidinium side chain of an arginine residue within the $\alpha$-domain of the IBC (R504 in IP$_3$R1) may be a more important determinant of the increased affinity of AdA for IP$_3$R [12].
The high-affinity and metabolic stability of AdA have generated considerable interest in both the synthesis of AdA analogues and their application to analyses of IP₃R activation and associated changes in cytosolic Ca²⁺ signalling [12]. There has, however, been no systematic analysis of the activities of AdA or its analogues with defined populations of homogenous IP₃R subtypes. The need for such analyses is particularly important in attempting to explain results in which Ca²⁺ signals evoked by IP₃ differ from those evoked by AdA [14,15,16,17,18,19,20,21], or where different analogues of AdA evoke different cellular responses [reviewed in 12,22]. Here we use DT40 cells in which all endogenous IP₃R have been genetically inactivated [5] to stably express homogenous populations of mammalian IP₃R subtypes and thereby define structure-activity relationships for AdA and its key analogues for each IP₃R subtype.

Materials and Methods

Materials

Sources of most reagents were provided in a previous publication [7]. The structures of the ligands used and their abbreviations are shown in Figure 1A. IP₃ was from Alexi Biochemicals (Nottingham, UK). AdA [23], imidophostin [24], ribophostin [25], furanophostin [26], manno-AdA and xylo-AdA [27], 3°-dephospho AdA and 4°-dephospho AdA [28], and 2°-dephospho AdA were synthesized, purified and characterized as previously described.

Measurement Ca²⁺ Release by IP₃ Receptors

From quantitative analyses of western blots using antiserca that selectively recognise each IP₃R subtype or react equally with all three subtypes, we established that in the DT40 cells used, levels of IP₃R expression (relative to IP₃R3) were IP₃R1 (71±8%, n = 3), IP₃R2 (48±5%) and IP₃R3 (100%) [7]. It is impracticable to achieve identical levels of IP₃R expression for each cell line, and differences (albeit modest in our cell lines) may affect both the size of the IP₃-sensitive Ca²⁺ pool and its sensitivity to IP₃ [29]. The different levels of IP₃R expression do not compromise the analyses reported here, which are entirely concerned with relative potencies of AdA analogues for each IP₃R subtype (see below).

A comprehensive description of the methods used to measure free [Ca²⁺] within the endoplasmic reticulum of permeabilized DT40 cells was provided in preceding publications [7,30]. Briefly, the endoplasmic reticulum of DT40 cells stably expressing each of the three mammalian IP₃R subtypes was loaded with a low-affinity Ca²⁺ indicator (Mag fluo-4) [30]. After permeabilization of the plasma membrane with saponin (10 µg/mL, ~4 min, 37°C), the permeabilized cells in cytosol-like medium (CLM) were distributed into 96-well plates at 20°C. Addition of MgATP (1.5 mM) then allowed active Ca²⁺ accumulation, which was monitored at intervals of ~1 s using a FlexStation 3 fluorescence plate-reader (MDS Analytical Devices). CLM had the following composition: 140 mM KCl, 20 mM NaCl, 1 mM EGTA, 20 mM Pipes, pH 7, free [Ca²⁺] ~220 nM (after addition of MgATP), and carbonyl cyanide 4-trifluoromethoxy-phenyl hydrazone (FCCP, 10 µM) to inhibit mitochondrial Ca²⁺ uptake. After 150 s, when the stores had loaded to steady-state with Ca²⁺, IP₃, AdA or its analogues was added with thapsigargin (1 µM) to inhibit further Ca²⁺ uptake, and after a further 30 s, the response was recorded. Agonist-evoked Ca²⁺ release was expressed as a fraction of that released by ionomycin (1 µM) [30]. All experiments were performed at 20°C.

Results

Quantal Ca²⁺ Release Evoked by AdA and IP₃

The kinetics of IP₃-evoked Ca²⁺ release from intracellular stores are unexpectedly complex. It is widely observed that under conditions where Ca²⁺ uptake into the endoplasmic reticulum (ER) is inhibited, submaximally effective concentrations of IP₃ rapidly release only a fraction of the IP₃-sensitive Ca²⁺ stores [32]. Thereafter, there is either no, or a massively reduced, effect of IP₃ on the rate of Ca²⁺ release. The mechanisms underlying this pattern of response, known as quantal Ca²⁺ release [33], remain
### Table 1. Effects of AdA analogues on Ca\(^{2+}\) release by subtypes of IP\(_3\) receptor.

<table>
<thead>
<tr>
<th>IP(_3)R</th>
<th>(1,4,5)IP(_3)</th>
<th>AdA</th>
<th>Imidophostin</th>
<th>Ribophostin</th>
<th>Furanophostin</th>
<th>Manno-AdA</th>
<th>Xylo-AdA</th>
<th>2'-dephospho-AdA</th>
<th>3'-dephospho-AdA</th>
<th>4'-dephospho-AdA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC(_{50})</td>
<td>pEC(_{50})</td>
<td>h</td>
<td>Ca(^{2+})release</td>
<td>n</td>
<td>EC(_{50})</td>
<td>pEC(_{50})</td>
<td>h</td>
<td>Ca(^{2+})release</td>
<td>n</td>
</tr>
<tr>
<td>IP(_3)R1</td>
<td>87</td>
<td>7.06 ± 0.05</td>
<td>0.99 ± 0.05</td>
<td>75 ± 1</td>
<td>31</td>
<td>145</td>
<td>6.84 ± 0.06</td>
<td>1.29 ± 0.09</td>
<td>61 ± 2</td>
<td>34</td>
</tr>
<tr>
<td>IP(_3)R2</td>
<td>8.3</td>
<td>8.08 ± 0.09</td>
<td>1.17 ± 0.09</td>
<td>72 ± 3</td>
<td>10</td>
<td>18.2</td>
<td>7.74 ± 0.06</td>
<td>1.79 ± 0.21</td>
<td>56 ± 2</td>
<td>13</td>
</tr>
<tr>
<td>IP(_3)R3</td>
<td>37</td>
<td>7.43 ± 0.28</td>
<td>1.17 ± 0.21</td>
<td>78 ± 5</td>
<td>3</td>
<td>68</td>
<td>7.17 ± 0.14</td>
<td>1.84 ± 0.50</td>
<td>59 ± 3</td>
<td>3</td>
</tr>
<tr>
<td>IP(_3)R4</td>
<td>40</td>
<td>7.40 ± 0.29</td>
<td>1.34 ± 0.16</td>
<td>77 ± 4</td>
<td>3</td>
<td>102</td>
<td>6.99 ± 0.11</td>
<td>1.60 ± 0.50</td>
<td>61 ± 2</td>
<td>3</td>
</tr>
<tr>
<td>IP(_3)R5</td>
<td>51</td>
<td>7.29 ± 0.25</td>
<td>0.90 ± 0.10</td>
<td>79 ± 6</td>
<td>3</td>
<td>76</td>
<td>7.12 ± 0.01</td>
<td>1.73 ± 0.20</td>
<td>60 ± 3</td>
<td>3</td>
</tr>
<tr>
<td>IP(_3)R6</td>
<td>34</td>
<td>7.47 ± 0.19</td>
<td>1.33 ± 0.30</td>
<td>75 ± 7</td>
<td>3</td>
<td>69</td>
<td>7.16 ± 0.07</td>
<td>1.33 ± 0.22</td>
<td>57 ± 3</td>
<td>3</td>
</tr>
<tr>
<td>IP(_3)R7</td>
<td>5.9</td>
<td>8.23 ± 0.17</td>
<td>1.27 ± 0.27</td>
<td>73 ± 7</td>
<td>3</td>
<td>79</td>
<td>8.10 ± 0.10</td>
<td>1.52 ± 0.40</td>
<td>52 ± 6</td>
<td>3</td>
</tr>
<tr>
<td>IP(_3)R8</td>
<td>275</td>
<td>6.56 ± 0.13</td>
<td>1.31 ± 0.15</td>
<td>66 ± 7</td>
<td>3</td>
<td>575</td>
<td>6.24 ± 0.10</td>
<td>0.85 ± 0.07</td>
<td>63 ± 2</td>
<td>3</td>
</tr>
<tr>
<td>IP(_3)R9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>15 ± 8(^a)</td>
<td>7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>6 ± 2(^b)</td>
<td>6</td>
</tr>
<tr>
<td>IP(_3)R10</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>6 ± 4(^b)</td>
<td>6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>6 ± 2(^b)</td>
<td>6</td>
</tr>
</tbody>
</table>

*Inactive at 300 µM.

\(^a\)Ca\(^{2+}\) release evoked by 300 µM 3'-dephospho AdA.

\(^b\)Refer to Table 2 for relative potencies of 3'-dephospho AdA. ND, not determined.

The EC\(_{50}\) (nM), pEC\(_{50}\) (/M), Hill coefficient (h) and fraction (%) of the intracellular Ca\(^{2+}\) stores released by a maximally effective concentration of each analogue are shown for each IP\(_3\)R subtype. All results (except EC\(_{50}\)) are shown as means ± SEM from n independent experiments.
unclear. It may require desensitization of IP3R as the Ca\(^{2+}\) content of the ER declines [34] or heterogeneity among IP3-sensitive Ca\(^{2+}\) stores [35]. The results shown in Figures 1B and C confirm that the Ca\(^{2+}\) release evoked by submaximal concentrations of either IP3 or AdA from permeabilized DT40-IP3R1 cells is quantal. These observations provide the justification for all subsequent experiments in which the concentration-dependent effects of IP3 or AdA were measured 30 s after their addition (see Methods).

**AdA is a Potent Agonist of All Three IP3 Receptor Subtypes**

The results shown in Figure 2 and Tables 1 and 2 demonstrate that AdA is \(\sim 10\)-times more potent than IP3 at each IP3R subtype, and for each subtype, maximally effective concentrations of IP3 and AdA release the same fraction of the intracellular Ca\(^{2+}\) stores. This is consistent with many analyses of IP3 and AdA in a variety of cell types using both functional and binding assays, in which AdA behaves as a full agonist with \(\sim 10\)-fold greater affinity than IP3 [reviewed in 8]. Our results do, however, provide the first direct demonstration that AdA interacts similarly with all three IP3R subtypes. Subsequent experiments examine the interactions between key analogues of IP3 and AdA with each IP3R subtype.

**Trimming the Adenosine Moiety of AdA Reduces its Potency at All IP3 Receptor Subtypes**

Systematic trimming of the adenosine moiety of AdA successively produces imidophostin (which lacks the pyrimidine ring of AdA), ribophostin (in which a methoxy group replaces the adenine moiety of AdA) and furanophostin (in which only the furanoid ring remains) (Figure 1A). Maximally effective concentrations of each of these analogues released the same fraction of the intracellular Ca\(^{2+}\) stores as AdA in cells expressing each of the three IP3R subtypes, and each analogue was \(\sim 5\)-to-10-fold less potent than AdA (Figure 3, Tables 1 and 2). These results are consistent with previous analyses of IP3R in hepatocytes, which express predominantly IP3R2 [24,36], with analyses of binding of ribophostin and furanophostin to an N-terminal fragment of IP3R1 [12], and with evidence from other analogues that trimming the adenosine

| Table 2. Relative potencies of AdA analogues at different IP3 receptor subtypes. |
|--------------------------------|------------------|-----------------|------------------|
| IP3R1 | IP3R2 | IP3R3 |
| IP3 | 1.02±0.02 | 0.9±0.30 | 1.1±0.30 |
| Imidophostin | 0.78±0.15 | 0.78±0.08 | 0.81±0.04 |
| Ribophostin | 0.82±0.18 | 0.96±0.20 | 1.06±0.07 |
| Furanophostin | 0.92±0.13 | 0.83±0.14 | 1.25±0.05 |
| Manno-AdA | 0.74±0.08 | 0.79±0.18 | 0.98±0.08 |
| Xylo-AdA | –0.01±0.07 | –0.3±0.27 | 0.05±0.08 |
| 2′-dephospho-AdA | 1.24±0.33 | 1.60±0.18 | 1.68±0.16 |
| 3′-dephospho-AdA | 4.03±0.09 | 4.47±0.30 | 4.13±0.14 |

From paired comparisons with AdA, the potency (\(\Delta\text{pEC}_{50}\)) of the analogues relative to AdA is shown for each IP3R subtype. Results are means ± SEM, with \(n\) provided in Table 1. ND, not determined. *Because the very low affinity of 3′-dephospho AdA for IP3R made it impracticable to stimulate cells with a maximally effective concentration, \(\Delta\text{pEC}_{50}\) for 3′-dephospho AdA was estimated by comparing concentrations of it and AdA that evoked the same sub-maximal Ca\(^{2+}\) release.

doi:10.1371/journal.pone.0058027.t002

**Figure 2. AdA is a potent agonist of all three IP3 receptor subtypes.** (A) Concentration-dependent effects of AdA on Ca\(^{2+}\) release from the intracellular stores of cells expressing IP3R1, IP3R2 or IP3R3. All results are expressed as percentages of the Ca\(^{2+}\) release evoked by ionomycin. The same colour codes are used in all subsequent figures. (B) Comparison, for each IP3R subtype, of the Ca\(^{2+}\) release evoked by IP3 and AdA. Results are means ± SEM from the number of independent experiments given in Table 1. Here, and in many subsequent figures, some error bars are smaller than the symbols.

doi:10.1371/journal.pone.0058027.g002
Hydroxyl Moieties that are Important for IP₃ Binding are Less Important for Binding of AdA

The 5’-CH₂OH and 2’-OH substituents of the glucose ring of AdA are thought to mimic the 3’-OH and 6’-OH of IP₃, respectively (Figure 1A). A structure equivalent to the 6’-OH of IP₁ is an essential feature of all inositol phosphate analogues that bind to IP₁R [13,39,39] and inversion of its orientation from equatorial to axial reduces affinity by more than 100-fold at all IP₁R subtypes [40]. It is therefore surprising, but consistent with previous analyses of native hepatic IP₁R [36], that mann-AdA, which differs from AdA only in the orientation of its 2’-OH, should be only 5- to 10-fold less potent than AdA at each IP₁R subtype (Figures 4A and B, Tables 1 and 2). Why, when the 6’-OH of IP₁ and 2’-OH of AdA seem to be analogous in the ligand structures, should these moieties make such different contributions to the interactions of IP₁ and AdA with IP₁R?

The 6’-OH of Ip₁ interacts, through a water molecule, with a lysine residue (K569) in the IBC [41] and, by interacting with the adjacent 1-phosphate, it has also been proposed to influence the behaviour of the 4,5-bisphosphate moiety of IP₁ [42]. The latter interaction is unlikely to contribute to AdA binding because the structures equivalent to the 6’-OH (2’-OH of AdA) and the 1-phosphate of IP₁ (2’-phosphate of AdA) are in different rings in AdA (Figure 1A). We suggest that the lesser importance in AdA of a structure equivalent to the essential 6’-OH of IP₁ comes from this hydroxyl mediating a relatively minor interaction with K569 in AdA, whereas for IP₁ it contributes also to appropriately orienting the critical 4,5-bisphosphate moiety.

The 3’-OH group, although less important than the 6’-OH, is another feature of IP₁ that contributes to high-affinity binding [43]. Our recent analyses of the functional effects of 3-deoxy-IP₁ established that it was ~40-fold less potent than IP₁ at all three IP₁R subtypes [7]. This is consistent with earlier work showing that 3-deoxy-IP₁ and analogues with other modifications of the 3’-position have reduced affinity for the three IP₁R subtypes [40]. However, the equivalent modification of AdA, removal of its 5’-CH₂OH to give xylo-AdA (Figure 1A), had no significant effect on its potency at any IP₁R subtype (Figures 4C and D, Tables 1 and 2). This is consistent with a previous functional analysis of hepatic IP₁R, where xylo-AdA was only marginally less potent than AdA (ΔpEC₅₀ = -0.28) [36]. Our results suggest that despite the apparent structural similarity between the 3’-OH of IP₁ and the 5’-CH₂OH of AdA (Figure 1A), the two hydroxyl groups do not contribute similarly to ligand binding. Previous analyses of IP₁R analogues suggested that replacing the 3’-OH with the larger CH₂OH moiety caused the affinity to decrease by no more than 7-fold [40]. A partial explanation for the lack of effect of removing the 5’-CH₂OH of AdA may therefore be that this moiety is less readily accommodated than a hydroxyl group in the IBC. This would suggest that an analogue of AdA in which the 5’-CH₂OH is replaced by 3’-OH might bind with increased affinity. We are unaware of such an analogue having been synthesized. The larger substituent at the 5’-position of AdA is, however, unlikely to provide the sole explanation for it making no discernible contribution to binding.

The 2’-phosphate of AdA is not a Super-optimal Mimic of the 1-phosphate of IP₃

It has been suggested that the 2’-phosphate of AdA interacts with the IBC in a manner that allows it to behave as a super-optimal mimic of the 1-phosphate of IP₃ [44,43]. However, our recent study combining structure-activity analyses with mutagenesis of the binding site suggest that the 1-phosphate of IP₃ is more important for binding than is the 2’-phosphate of AdA [12]. Removal of the 1-phosphate from IP₃ (to give (4,5)IP₂) caused its potency and affinity for IP₁R to decrease by ~100-fold [12], whereas removal of the 2’-phosphate from AdA (2’-dephospho AdA) causes a decrease in potency of ~17-fold in IP₁R (Figure 5) and ~40-fold decreases in potency were obtained with 2’-dephospho AdA and IP₁R2 and IP₁R3 (Figure 5, Table 1 and 2). These results establish that for all three IP₁R subtypes, the enhanced affinity of AdA is not due to its 2’-phosphate interacting more effectively than the 1-phosphate of IP₃ with the IBC.

A Bisphosphate Moiety is not Essential for Activation of IP₃ Receptors by AdA

All known active analogues of IP₃ have structures equivalent to its 4,5-bisphosphate moiety [13]. Structures of the IBC with and without IP₃ bound provide a rationale for this requirement by revealing that these two phosphate groups contact opposite sides (the α- and β-domains) of the clam-like IBC, closure of which initiates IP₁R activation [4,41]. Substantial evidence suggests that the 4,5-bisphosphate moiety of AdA mimics the critical 4,5-bisphosphate of IP₃ [8] (Figure 1A).

3’-dephospho-AdA at concentrations up to 300 μM failed to evoke Ca²⁺ release via any IP₁R subtype (Figure 6A). This is consistent with previous analyses by both functional and binding assays of IP₁R [28,46]. 3’-dephospho-AdA did, however, cause detectable Ca²⁺ release albeit with much reduced potency (Figure 6B). The synthetic route used to prepare 3’-dephospho-AdA makes it extremely unlikely that the activity could be due to minor contamination with AdA or related structures with a vicinal bisphosphate moiety. Maximal attainable concentrations of 3’-dephospho-AdA (300 μM) failed to release the entire IP₁R-sensitive Ca²⁺ store, but comparison of the concentrations required to achieve the same submaximal Ca²⁺ release suggests that 3’-dephospho-AdA is ~10,000-fold less potent than AdA at all three IP₁R subtypes. With such a massive reduction in potency the lesser sensitivity of DT10-IP₁R3 cells to AdA means that even the highest practicable concentration of 3’-dephospho-AdA (300 μM) is close to the threshold for detecting Ca²⁺ release (Figure 6B).
Figure 4. Hydroxyl groups within the glucose ring of AdA are unimportant. (A–D) Effects of manno-AdA (A) and xylo-AdA (C) on Ca²⁺ release via each IP₃R subtype, and the same analogues compared with AdA (B and D). Results are means ± S.E.M. from 3 independent experiments. doi:10.1371/journal.pone.0058027.g004
The inability of high concentrations of 3'-dephospho-AdA to release the entire IP3-sensitive Ca$^{2+}$ store is likely to be due solely to its reduced affinity rather than reduced efficacy. A concentration of 3'-dephospho-AdA (30 μM) that caused detectable Ca$^{2+}$ release via IP$_3$R1 (~21 ± 5%) had no effect on the sensitivity of the Ca$^{2+}$ release evoked by a subsequent addition of IP$_3$. The pEC$_{50}$ was 7.00 ± 0.02 and 7.04 ± 0.06 (n = 3) for (1,4,5)IP$_3$ alone and in the presence of 3'-dephospho-AdA, respectively (Figure 6C). A partial agonist would be expected to shift the sensitivity to higher concentrations of IP$_3$. These results suggest that 3'-dephospho-AdA is a low-affinity full agonist of IP$_3$R.

These results extend our previous analyses of IP$_3$R1 by demonstrating that for all IP$_3$R subtypes, the 4'-phosphate group of AdA is essential for activity, whereas the 3'-phosphate is important but not essential. 3'-dephospho-AdA is the only known agonist of IP$_3$R to lack a structure equivalent to the 4,5-bisphosphate moiety of IP$_3$.

Discussion

AdA is a high-affinity full agonist of IP$_3$R that has been extensively used to explore the behaviour of IP$_3$R [reviewed in8]. The activity of AdA has been confirmed in many cell types, but hitherto there has been no assessment of its activity in homogenous populations of IP$_3$R subtypes. We have demonstrated that AdA is ~10-fold more potent than IP$_3$R at each IP$_3$R subtype (Figure 2, Tables 1 and 2), and the structural determinants of its high-affinity interaction with IP$_3$R are similar for all three IP$_3$R subtypes. Contrary to an earlier suggestion that the 2'-phosphate of AdA mediates its enhanced affinity by forming a stronger interaction with the IBC than the analogous 1-phosphate of IP$_3$, we find that the 1-phosphate makes a greater contribution to IP$_3$ binding than does the 2'-phosphate of AdA (Figure 5) [12]. A more likely explanation for the enhanced affinity of AdA is a cation-π interaction between its adenine moiety and R504 within the α-subunit of the IBC (Figure 3G) [28]. That explanation is supported by results for each IP$_3$R subtype showing that truncation of the adenosine moiety of AdA brings the potency of the resulting analogues (imidophostin, ribophostin and furanophostin) close to that of IP$_3$ (Figure 3).

A key step in the initial activation of IP$_3$R by IP$_3$ appears to be closure of its clam-like IBC as the 4-phosphate of IP$_3$ contacts one side of the clam (its β-domain) and the 5-phosphate contacts the other side (α-domain) [4]. That mechanism provides a satisfying explanation for the long-standing observation that all inositol phosphates that activate IP$_3$R share this essential 4,5-bisphosphate moiety. AdA is different in that its 4'-phosphate (analogous to the 4-phosphate of IP$_3$, Figure 1A) is essential, but 3'-dephospho-AdA retains activity at all three IP$_3$R subtypes, albeit with very low affinity (Figure 6). We suggest that for AdA, the need for the bisphosphate moiety to cause closure of the IBC can be partially replaced for all IP$_3$R subtypes by having an interaction between the adenine of AdA and the α-domain substitute for the interaction between the 3'-phosphate (analogous to the 5-phosphate of IP$_3$) and the α-domain [28]. Finally, whereas the 6-OH and, to a lesser extent, the 3-OH of IP$_3$ are important for IP$_3$ binding, the equivalent structures within AdA play lesser roles.
Both store-operated Ca^{2+} entry, which is triggered by depletion of IP_{3}-sensitive Ca^{2+} stores [47], and the spatial organization of subcellular Ca^{2+} signals have been reported to be differentially affected by IP_{3}, AdA or its analogues [14,16,17,19,21,22]. Our present results, which demonstrate that AdA structure-activity relationships are similar for all IP_{3,R} subtypes, suggest that different physiological effects of IP_{3}, AdA or its analogues are more likely to result from differences in their affinities, kinetics or rates of degradation than from selective interactions with different IP_{3,R} subtypes.

**References**


43. Wilcox RA, Challiss RAJ, Traynor JR, Faq H, Ognayanov VI, et al. (1994) Molecular recognition at the myo-inositol 1,4,5-trisphosphate receptor. 3-position substituted myo-inositol 1,4,5-trisphosphate analogues reveal the binding and Ca\(^{2+}\) release requirements for high affinity interaction with the myo-inositol 1,4,5-trisphosphate receptor. J Biol Chem 269: 26815–26821.


