Stereoselectivity of Ins(1,3,4,5)P_4 recognition sites: implications for the mechanism of the Ins(1,3,4,5)P_4-induced Ca^{2+} mobilization

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Ins(1,3,4,5)P_4 was able to mobilize the entire Ins(1,4,5)P_3-sensitive intracellular Ca^{2+} store in saponin-permeabilized SH-SY5Y human neuroblastoma cells in a concentration-dependent manner, yielding an EC_{50} value of 2.05 ± 0.45 μM, compared with 0.14 ± 0.03 μM for Ins(1,4,5)P_3. However, L-Ins(1,3,4,5)P_4 [= D-Ins(1,3,5,6)P_4] failed to cause mobilization of intracellular Ca^{2+} at concentrations up to 100 μM. Binding studies using pig cerebellar membranes as a source of both Ins(1,4,5)P_3/Ins(1,3,4,5)P_4-specific binding sites have revealed a marked contrast in their stereospecificity requirements. Ins(1,4,5)P_3-receptors from pig cerebella exhibited stringent stereospecificity, L-Ins(1,4,5)P_3 and L-Ins(1,3,4,5)P_4 were > 1000-fold weaker, whereas Ins(1,3,4,5)P_4 (IC_{50} 762 ± 15 nM) was only about 40-fold weaker than D-Ins(1,4,5)P_3 (IC_{50} 20.7 ± 9.7 nM) at displacing specific [^{3}H]Ins(1,4,5)P_3 binding from an apparently homogeneous Ins(1,4,5)P_3 receptor population. In contrast, the Ins(1,3,4,5)P_4-binding site exhibited poor stereoselectivity. Ins(1,3,4,5)P_4 produced a biphasic displacement of specific [^{32}P]Ins(1,3,4,5)P_4 binding, with two-site analysis revealing K_D values for high- and low-affinity sites of 2.1 ± 0.5 nM and 918 ± 161 nM respectively. L-Ins(1,3,4,5)P_4 also produced a biphasic displacement of specific [^{32}P]Ins(1,3,4,5)P_4 binding which was less than 10-fold weaker than with D-Ins(1,3,4,5)P_4 (IC_{50} values for the high- and low-affinity sites of 17.2 ± 3.7 nM and 3010 ± 542 nM respectively). Therefore, although L-Ins(1,3,4,5)P_4 appears to be a high-affinity Ins(1,3,4,5)P_4-binding-site ligand in pig cerebellum, it is a very weak agonist at the Ca^{2+}-mobilizing receptors of permeabilized SH-SY5Y cells. We suggest that the ability of D-Ins(1,3,4,5)P_4 to access intracellular Ca^{2+} stores may derive from specific interaction with the Ins(1,4,5)P_3- and not the Ins(1,3,4,5)P_4-receptor population.

INTRODUCTION

Many cell-surface receptors activate phosphoinositide-specific phospholipase C via G-proteins, to catalyse the hydrolysis of phosphatidylinositol 4,5-bisphosphate and produce the second messengers Ins(1,4,5)P_3 and diacylglycerol [1]. Ins(1,4,5)P_3 specifically interacts with a family of Ins(1,4,5)P_3-receptor-gated channels to mobilize non-mitochondrial intracellular Ca^{2+} stores [1,2]. In animal cells Ins(1,4,5)P_3 is rapidly metabolized by 5-phosphatase and 3-kinase activities, to form Ins(1,4)P_2 and Ins(1,3,4,5)P_4 respectively [1]. Although controversy exists as to whether Ins(1,3,4,5)P_4 also plays a role in cell signalling, evidence has accumulated suggesting it may have a role in Ca^{2+} entry across the plasma membrane (reviewed in [3–5]). Ins(1,3,4,5)P_4-activated Ca^{2+} channels have been recently identified in the plasma membrane of endothelial cells [6], and Ins(1,3,4,5)P_4-activated Ca^{2+} mobilization has been observed using crude microsomes and enriched vesicular plasma membranes prepared from T-lymphocyte and monocyte cell lines [7]. Furthermore, Ins(1,3,4,5)P_4 has also been reported to induce a heparin-insensitive Ca^{2+} sequestration into intracellular pools of the 261B rat liver epithelial cell line [8,9].

In some cell types, Ins(1,3,4,5)P_4 apparently fails to mobilize intracellular Ca^{2+} stores or to modulate Ins(1,4,5)P_3-induced Ca^{2+} mobilization [10–13]. However, several studies have reported either Ins(1,4,5)P_3-independent or Ins(1,4,5)P_3-synergistic effects of Ins(1,3,4,5)P_4 on intracellular Ca^{2+} mobilization, although the problems of Ins(1,4,5)P_3 contamination of the Ins(1,3,4,5)P_4 used [14,15], back-conversion into Ins(1,4,5)P_3 by endogenous 3-phosphatase activity in the cells [16,17] or indirect effects of Ins(1,3,4,5)P_4 by protection of Ins(1,4,5)P_3 from 5-phosphatase metabolism [18], have not always been convincingly addressed. Ins(1,3,4,5)P_4 has been shown directly to mobilize the Ca^{2+} stores in cerebellar [19] and adrenal [20] microsomes, permeabilized SH-SY5Y neuroblastoma cells [15,21] and microinjected *Xenopus* oocytes [22]. In all these studies the maximal concentrations of Ins(1,3,4,5)P_4 (20–30 μM) used mobilized significantly less intracellular Ca^{2+} than could be achieved with Ins(1,4,5)P_3.

At present it is not clear how, or indeed if, the different physiological effects of Ins(1,3,4,5)P_4 (i.e. stimulation of Ca^{2+} entry and intracellular Ca^{2+} mobilization and sequestration), are mediated via the specific high-affinity Ins(1,3,4,5)P_4-binding sites detected in certain tissues. Here we have assessed where Ins(1,3,4,5)P_4-binding sites might be linked to Ca^{2+} mobilization from intracellular stores. Ins(1,3,4,5)P_4 and L-Ins(1,3,4,5)P_4 were used to characterize the stereospecific requirements of ligand binding in pig cerebellar membranes and Ca^{2+} mobilization in permeabilized SH-SY5Y neuroblastoma cells.

MATERIALS AND METHODS

Materials

$^{45}CaCl_2$ (approx. 1000 Ci/mmol; Amersham International), [^{3}H]Ins(1,4,5)P_3 (17 Ci/mmol) and [^{32}P]Ins(1,3,4,5)P_4 (116–162 Ci/mmol) were generously given by NEN DuPont. Heparin (M_r 4000–5000), disodium ATP, fura-2 and EGTA were from Sigma; all other reagents were of the highest purity

Abbreviation used: CLB, 'cytosol-like' buffer.

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available. L-Ins(1,4,5)P_3 [23] and L-Ins(1,3,4,5)P_4 [24] were synthesized as previously described.

Chemically synthesized Ins(1,4,5)P_3 [25] and Ins(1,3,4,5)P_4 [26] were obtained from the University of Rhode Island Foundation Chemistry Group, U.S.A., and were used for all the $^{45}Ca^{2+}$-release assays. Both compounds were extensively characterized by ^{31}P- and ^{1}H-n.m.r., and were found to be > 99% pure, with no other detectable inositol polyphosphates, reflecting that the respective synthetic pathways effectively exclude the possibility of Ins(1,4,5)P_3/Ins(1,3,4,5)P_4 cross-contamination [26].

Radioligand-binding studies using pig cerebellar membranes

Preparation of cerebellar 'P_2' membrane fraction

Pig cerebella were obtained from a local abattoir. Portions of cerebellum were either used immediately or frozen in liquid nitrogen and stored at −70 °C. Cerebellum was chopped with scissors at 4 °C and homogenized in 20 vol. of 20 mM $NaHCO_3$/1 mM dithiothreitol, pH 8.0, with a Polytron (setting 5, 2 × 15 s). The homogenate was centrifuged (4000 *g*, 10 min, 4 °C) and the supernatant kept. The pellet was re-homogenized and centrifuged as above. The pooled supernatants from the low-speed centrifugation steps were then centrifuged (35000 *g*, 20 min, 4 °C). The resulting pellets were then homogenized and the high-speed centrifugation step was repeated twice. The final pellet was resuspended in homogenization buffer at 6–8 mg of protein/ml, 'snap'-frozen in liquid nitrogen and stored at −20 °C until required.

Characterization of Ins(1,4,5)P_3- and Ins(1,3,4,5)P_4-binding sites

Increasing concentrations (0.5–1000 nM) of Ins(1,4,5)P_3 were incubated in a total assay volume of 120 μl with 2–3 nM [^{3}H]Ins(1,4,5)P_3 in a buffer containing 25 mM Tris/HCl, 5 mM $NaHCO_3$, 1 mM EDTA and 0.25 mM dithiothreitol, pH 8.0. Incubations were initiated by addition of 50–70 μg of cerebellar membrane protein and continued for 30 min at 4 °C. Bound and free ligand were separated by centrifugation (12000 *g*, 4 min). Pellets were dissolved in 2% SDS. Residual bound radioactivity in the presence of 10 μM D-Ins(1,4,5)P_3 (Research Biochemicals Inc., St. Albans, Herts., U.K.), 1 mM InsP_6, 100 μg/ml heparin or 3 mM 2,3-bisphosphoglycerate was similar (< 5% of total binding) and was defined as non-specific binding.

Increasing concentrations (0.1–10000 nM) of Ins(1,3,4,5)P_4 were incubated in a total assay volume of 120 μl with 0.2–0.4 nM [^{32}P]Ins(1,3,4,5)P_4 in a buffer containing 25 mM sodium acetate, 25 mM KH_2PO_4, 5 mM $NaHCO_3$, 1 mM EDTA and 0.25 mM dithiothreitol, pH 5.0. Incubations were initiated by addition of 200–250 μg of cerebellar membrane protein and continued for 30 min at 4 °C. Bound and free ligand were separated by rapid vacuum filtration over GF/B filters, with 3 × 3 ml washes with 25 mM sodium acetate/25 mM KH_2PO_4/5 mM $NaHCO_3$, pH 5.0. Residual bound radioactivity in the presence of 100 μM DL-Ins(1,3,4,5)P_4 (generously given by Dr. D. C. Billington, Merck Sharp and Dohme Research, Harlow, Essex, U.K.), 1 mM InsP_6, 100 μg/ml heparin or 3 mM 2,3-bisphosphoglycerate was similar (10–15% of total binding) and was defined as non-specific binding. Where indicated, competition curves were constructed for other inositol polyphosphates, by using the assays described above.

Cell culture

SH-SY5Y human neuroblastoma cell monolayers (passage 70–90), initially a gift from Dr. J. L. Biedler (Sloane–Kettering Institute, New York, NY, U.S.A.), were subcultured and maintained as described [27].

$^{45}Ca^{2+}$-mobilization assays

SH-SY5Y cell monolayers were harvested in 25 ml of Hepes-buffered saline, consisting of 10 mM Hepes, 15 mM NaCl and 0.02% (w/v) EDTA (pH 7.2). The cell suspension was centrifuged at 500 *g* for 2 min, and the resulting pellet resuspended in a 'cytosol-like' buffer (CLB), consisting of 120 mM KCl, 2 mM Na_2ATP, 2.4 mM $MgCl_2$,$6H_2O$, 2 mM KH_2PO_4, 5 mM sodium succinate and 20 mM Hepes (pH 7.2). The free Ca^{2+} concentration of the CLB was buffered between 80 and 150 nM by addition of 1–3 μM EGTA; this was confirmed fluorimetrically in 2 ml samples by using fura-2 (250 nM) as described [28]. The cells were washed twice in CLB, by spinning at 500 *g* for 1 min, and then resuspended in CLB containing 100 μg/ml saponin and a cell protein concentration of 1.5–2 mg/ml. After exactly 1 min the cells were centrifuged (500 *g*, 1 min) and the pellet was resuspended to 0.3–0.4 mg/ml in CLB containing 1 μCi of $^{45}Ca^{2+}$/ml.

Ins(1,3,4,5)P_4-induced $^{45}Ca^{2+}$ mobilization was performed at 4 °C to preclude the possibility of 3-phosphatase activity causing Ins(1,4,5)P_3 formation in the permeabilized SH-SY5Y cells [21]. Cells were preincubated for 20 min at 25 °C to allow ATP-dependent loading of intracellular Ca^{2+} stores, cooled to 4 °C in ice/water, and then 50 μl of cell suspension was added to 50 μl of CLB containing the inositol polyphosphates or other agents in 1.5 ml microcentrifuge tubes. After 3 min incubation at 4 °C, cells were pelleted by centrifugation (16000 *g*, 2 min); then 250 μl of a silicone oil mixture (Dow–Corning 556/550; 1:1, v/v) was added and the tubes were re-centrifuged (16000 *g*, 1 min). Buffer and oil were removed by aspiration, and the tubes allowed to drain for 20 min. The resultant cell pellets were solubilized in 1 ml of Optiphase X scintillation fluid for 6–8 h at 4 °C, and the radioactivity was then counted. All experiments were performed in duplicate; 20 μM ionomycin (free acid; Calbiochem) was used to define the total releasable $^{45}Ca^{2+}$ pool and 20–30 μM Ins(1,4,5)P_3 to define the Ins(1,4,5)P_3-sensitive $^{45}Ca^{2+}$ pool.

Data analysis

EC_{50}, IC_{50}, K_D and slope values were estimated by computer-assisted curve fitting by using GraphPad INPLOT version 3.1 (GraphPad Software, U.S.A.) and Allfit [29]. Where slope factors were significantly less than unity, computer-assisted one- and two-site analyses were compared [30]. Where data were best fitted by two-site analysis, the concentrations of agents required to cause 50% displacement from high (K_H)- and low (K_L)-affinity populations are given. Combined data from the independent experiments were expressed as means ± S.E.M., where $n \geq 3$.

RESULTS

Inositol polyphosphate binding to pig cerebellar membranes

Ins(1,4,5)P_3 displaced specific [^{3}H]Ins(1,4,5)P_3 binding from an apparently homogeneous single site (slope 0.97 ± 0.03). Scatchard transformation of isotope-dilution data yielded K_D 17.5 ± 1.8 nM and $B_{max.}$ 20.8 ± 2.4 pmol/mg of protein ($n = 5$). As reported previously for rat cerebellar membranes prepared in an identical fashion, L-Ins(1,4,5)P_3 was > 1000-fold weaker in its displacing activity [30], as was L-Ins(1,3,4,5)P_4 in pig cerebellum, in this study. The other inositol polyphosphates tested exhibited similar IC_{50} values to those reported previously for rat cerebellar membranes [30] (Table 1).

Displacement of specific [^{32}P]Ins(1,3,4,5)P_4 binding by

Table 1 Inhibition of specific [^{3}H]Ins(1,4,5)P_3 and [^{32}P]Ins(1,3,4,5)P_4 binding to pig cerebellar membranes

Results are shown as means ± S.E.M. ($n \geq 3$) for IC_{50} (M) and slope factors (h) where appropriate. Abbreviation: ND, not determined.

Displacing ligand	[^{3}H]Ins(1,4,5)P_3 IC_{50} (M)/h	[^{32}P]Ins(1,3,4,5)P_4 IC_{50} (M)/h
Ins(1,4,5)P_3	$2.07 (\pm 0.21) \times 10^{-8}$/$0.97 \pm 0.03$	$1.40 (\pm 0.25) \times 10^{-5}$/$0.96 \pm 0.02$
L-Ins(1,4,5)P_3	$2.24 (\pm 0.39) \times 10^{-5}$/$0.90 \pm 0.05$	ND
Ins(1,3,4,5)P_4	$7.62 (\pm 0.15) \times 10^{-7}$/$0.95 \pm 0.04$	K_H $2.10 (\pm 0.50) \times 10^{-9}$ K_L $9.18 (\pm 1.62) \times 10^{-7}$
L-Ins(1,3,4,5)P_4	$2.38 (\pm 0.84) \times 10^{-5}$/$0.89 \pm 0.07$	K_H $1.72 (\pm 0.37) \times 10^{-8}$ K_L $3.01 (\pm 0.54) \times 10^{-6}$
Ins(1,3,4,6)P_4	$8.31 (\pm 0.38) \times 10^{-7}$/$0.90 \pm 0.09$	$1.03 (\pm 0.09) \times 10^{-6}$/$0.88 \pm 0.08$

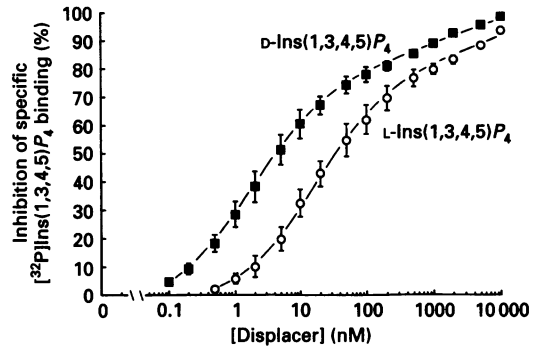

Figure 1 Inhibition of specific [^{3}H]Ins(1,4,5)P_3 and [^{32}P]Ins(1,3,4,5)P_4 binding to pig cerebellar membranes by increasing concentrations of Ins(1,3,4,5)P_4 (■) and L-Ins(1,3,4,5)P_4 (○)

Results are shown as means ± S.E.M. for $n \geq 3$ independent experiments.

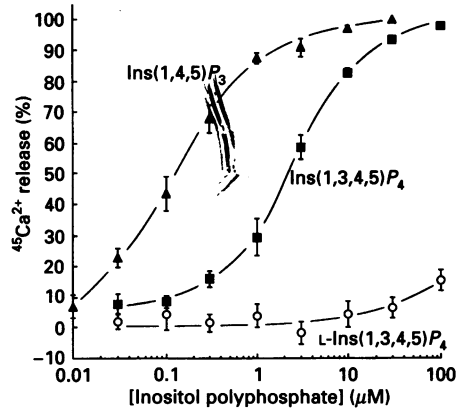

Figure 2 Inositol polyphosphate-induced $^{45}Ca^{2+}$ mobilization in saponin-permeabilized SH-SY5Y cells

Results are shown as means ± S.E.M. for $n = 4$ independent experiments using Ins(1,4,5)P_3 (▲), Ins(1,3,4,5)P_4 (■) and L-Ins(1,3,4,5)P_4 (○). The respective mean EC_{50} and slope values are reported in the Results section.

Ins(1,3,4,5)P_4 yielded curves with slope factors significantly less than unity [0.53 ± 0.04 ($n = 3$)]. These were poorly fitted by single-site analysis, but could be accurately modelled by using a two-site analysis. Such analysis yielded K_D values for high- and low-affinity binding sites of 2.1 ± 0.5 nM and 918 ± 161 nM respectively ($n = 3$): at the concentration of [^{32}P]Ins(1,3,4,5)P_4 used, the high-affinity site accounted for $75.4 \pm 2.5\%$ of total binding and corresponded to a density of sites of 212 ± 30 fmol/mg of protein (Figure 1). In contrast with the stereospecificity exhibited by the Ins(1,4,5)P_3-binding site, L-Ins(1,3,4,5)P_4 [= Ins(1,3,5,6)P_4] also caused a biphasic displacement of specific [^{32}P]Ins(1,3,4,5)P_4 binding and was < 10-fold weaker in its displacing activity relative to the D-isomer (IC_{50} for high-affinity site 17.2 ± 3.7 nM; IC_{50} for low-affinity site 3010 ± 542 nM). The proportional distribution of high- and low-affinity sites observed in the L-Ins(1,3,4,5)P_4-displacement studies (high-affinity $72.4 \pm 3.1\%$) was similar to that estimated from Ins(1,3,4,5)P_4 isotherms. Comparison of the ability of other inositol polyphosphates to displace specific [^{32}P]Ins(1,3,4,5)P_4 binding from pig cerebellar membranes was entirely consistent with the rank-order of potency previously determined by using rat cerebellar membranes [30] (Table 1; Figure 1).

Ca^{2+} mobilization by inositol polyphosphates

Ins(1,4,5)P_3 mobilized $56.4 \pm 1.4\%$ ($n = 20$) of pre-loaded $^{45}Ca^{2+}$ from saponin-permeabilized SH-SY5Y cells at 4 °C, with a mean EC_{50} (and slope) value of 141 ± 27 nM (0.91 ± 0.03). Ins(1,3,4,5)P_4 (100 μM) was able to mobilize the entire Ins(1,4,5)P_3-sensitive intracellular Ca^{2+} store of saponin-permeabilized SH-SY5Y with a mean EC_{50} (and slope) value of 2.05 ± 0.45 μM (1.03 ± 0.09), which was only about 15-fold weaker than Ins(1,4,5)P_3 (Figure 2).

However, L-Ins(1,3,4,5)P_4 did not significantly mobilize intracellular Ca^{2+} even at concentrations up to 100 μM (Figure 2). Also, co-incubation of the SH-SY5Y cells with 10 μM L-Ins(1,3,4,5)P_4 failed to produce a significant shift of either the Ins(1,4,5)P_3- or Ins(1,3,4,5)P_4-induced Ca^{2+}-release concentration–response curves. These were superimposable, with their control curves yielding mean EC_{50} values for the Ins(1,4,5)P_3- or Ins(1,3,4,5)P_4-induced Ca^{2+}-release concentration–response curves of 170 ± 53 nM and 1.70 ± 0.58 μM respectively. These results suggest that L-Ins(1,3,4,5)P_4 fails to interact functionally with any Ca^{2+}-mobilizing inositol polyphosphate receptor(s) in SH-SY5Y cells.

DISCUSSION

In previous studies we have demonstrated that the binding of Ins(1,4,5)P_3 to its receptor(s) [31] and its ability to mobilize intracellular Ca^{2+} [32] are highly stereospecific, with the D-isomer greater than 300-fold more potent than L-Ins(1,4,5)P_3. The value of comparing stereoisomers is also emphasized in the present study, where we have been able to dissociate clearly the ability of Ins(1,3,4,5)P_4 to mobilize Ca^{2+} in SH-SY5Y cells from its activity at specific binding sites in pig cerebellum. Thus the marked stereospecificity of D- over L-Ins(1,3,4,5)P_4 to mobilize Ca^{2+} mirrors its activity at Ins(1,4,5)P_3- but not Ins(1,3,4,5)P_4-binding sites. Although it remains possible that SH-SY5Y cells contain specific Ins(1,3,4,5)P_4 sites with totally distinct properties from those in pig cerebellum, they were not detectable by using [^{32}P]Ins(1,3,4,5)P_4. Indeed, although we have previously detected Ins(1,4,5)P_3-receptor binding sites in membranes prepared from SH-SY5Y cells [33], we have not been able to detect any specific binding sites for [^{32}P]Ins(1,3,4,5)P_4 under conditions identical with those used in cerebellum studies. We believe that the most parsimonious interpretation of the data at present is that Ins(1,3,4,5)P_4 releases Ca^{2+} by interacting with Ins(1,4,5)P_3 receptors, at least in SH-SY5Y cells.

Here we have demonstrated that Ins(1,3,4,5)P_4 can mobilize the entire Ins(1,4,5)P_3-sensitive Ca^{2+} store in SH-SY5Y cells, using highly pure synthetic D-isomer and under conditions that we have previously shown preclude the possibility of enzymic conversion into Ins(1,4,5)P_3 [21]. We had previously reported that chemically synthesized DL-Ins(1,3,4,5)P_4 mobilizes intracellular Ca^{2+} in SH-SY5Y cells [15,21], but that the presumed maximal concentration of DL-Ins(1,3,4,5)P_4 (10–30 μM) utilized in these studies mobilized at best only 62% of the intracellular Ins(1,4,5)P_3-sensitive store. Additionally, 10 μM DL-Ins(1,3,4,5)P_4 failed to affect significantly the Ins(1,4,5)P_3-induced Ca^{2+} mobilization [15]. These previous data led us to speculate that Ins(1,3,4,5)P_4 might therefore be mobilizing Ca^{2+} via a receptor distinct from the Ins(1,4,5)P_3 receptor. In contrast, the present study indicates that maximal concentrations of synthetic D-Ins(1,3,4,5)P_4 between 30 and 100 μM are required to mobilize fully the Ins(1,4,5)P_3-sensitive intracellular Ca^{2+} store. Since we now know L-Ins(1,3,4,5)P_4 to be a very weak Ins(1,4,5)P_3-receptor ligand and Ca^{2+}-mobilizing agent, the affects that we previously observed with DL-Ins(1,3,4,5)P_4 are probably mediated exclusively by the D-isomer, suggesting that 5–15 μM was the maximal effective concentration present in our earlier studies [15,21]. This may at least partially explain why we only observed 60% release of the Ca^{2+} stores, since Ins(1,3,4,5)P_4 concentrations of 3–30 μM span the 50–90% Ca^{2+}-release range on the Ins(1,3,4,5)P_4-concentration/response relationship. We now also suggest that the inability of DL-Ins(1,3,4,5)P_4 (10 μM) to shift the Ins(1,4,5)P_3 concentration/response curve [21] is consistent with Ins(1,3,4,5)P_4 being a weak but full agonist at the Ins(1,4,5)P_3 receptor. Clearly Ins(1,3,4,5)P_4 shares key structural motifs with Ins(1,4,5)P_3, including the 6-OH group, the 1-phosphate and the crucial D-vicinal 4,5-phosphate grouping that are apparently requisite for receptor binding and Ca^{2+}-mobilizing activity [34–36]. Analysis of our Ins(1,4,5)P_3-binding and Ins(1,3,4,5)P_4-induced Ca^{2+}-mobilization data show that Ins(1,3,4,5)P_4 is a 40-fold weaker ligand, and 20-fold weaker agonist than Ins(1,4,5)P_3 (Table 1). We have also observed a similar Ca^{2+}-mobilizing profile in 1321N1 human astrocytoma cells, at 4 °C (R. A. Wilcox, unpublished work), and Parker and Ivorra [22] have estimated a similar potency in microinjected *Xenopus* oocytes. Collectively, these data in conjunction with the critical behaviour of the stereoisomers of Ins(1,3,4,5)P_4 indicate that Ins(1,3,4,5)P_4-induced Ca^{2+} mobilization may occur via the Ins(1,4,5)P_3-receptor population, at least in some cell types. We are currently utilizing 3-position-modified Ins(1,4,5)P_3/Ins(1,3,4,5)P_4 analogues and Ins(1,4,5)P_3-receptor partial agonist(s) to test the validity of our hypothesized interaction of Ins(1,3,4,5)P_4 with the Ins(1,4,5)P_3 receptor.

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