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Myo-inositol 1,3,4,5-tetrakisphosphate can independently mobilise intracellular calcium, via the inositol 1,4,5-trisphosphate receptor: studies with *myo*-inositol 1,4,5-trisphosphate-3-phosphorothioate and *myo*-inositol hexakisphosphate

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Myo-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] acts as a full agonist for Ca^{2+} release in saponin-permeabilised SH-SY5Y neuroblastoma cells. Studies were conducted in the presence of *myo*-inositol hexakisphosphate ($InsP_6$, 10 μM), to inhibit the Ins(1,3,4,5)P_4-3-phosphatase catalysed back conversion of Ins(1,3,4,5)P_4 to Ins(1,4,5)P_3. HPLC analysis confirmed that Ins(1,3,4,5)P_4 releases the entire content of Ins(1,4,5)P_3-sensitive intracellular Ca^{2+} stores, independent of 3-phosphatase activity. Further we utilised racemic *myo*-inositol 1,4,5-trisphosphate-3-phosphorothioate [DL-Ins(1,3,4,5)P_4-3S], a novel intrinsically Ins(1,3,4,5)P_4-3-phosphatase resistant Ins(1,3,4,5)P_4 analogue. DL-Ins(1,3,4,5)P_4-3S specifically displaced [^{3}H]Ins(1,4,5)P_3 from bovine adrenal cortex Ins(1,4,5)P_3 binding sites (IC_{50} = 889 nM, compared to Ins(1,4,5)P_3, IC_{50} = 4.4 nM and Ins(1,3,4,5)P_4, IC_{50} = 152 nM). DL-Ins(1,3,4,5)P_4-3S was a full agonist for Ca^{2+} release (EC_{50} = 4.7 μM), being 90- and 2-fold less potent than Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 (with $InsP_6$), respectively. DL-Ins(1,3,4,5)P_4-3S will be an important tool for identification of potentially exclusive Ins(1,3,4,5)P_4 second messenger functions, since its resistance to 3-phosphatase action precludes the inconvenient artefact of steady state Ins(1,4,5)P_3 generation.

Inositol 1,3,4,5-tetrakisphosphate analogue; Calcium mobilization; Ins(1,4,5)P_3 receptor; SH-SY5Y cell; Inositol 1,3,4,5-tetrakisphosphate-3-phosphatase

1. INTRODUCTION

Many cell surface receptors activate phosphoinositidase C via G-proteins, which catalyse the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce the second messengers, Ins(1,4,5)P_3 (Fig. 1 (1)) and diacylglycerol [1]. Ins(1,4,5)P_3 interacts specifically with a family of Ins(1,4,5)P_3 receptor-operated Ca^{2+} channels to mobilise non-mitochondrial intracellular Ca^{2+} stores [1, 2]. Ins(1,4,5)P_3 is rapidly metabolised by 5-phosphatase and 3-kinase activities to form Ins(1,4)P_2 and Ins(1,3,4,5)P_4, respectively (Fig. 1 (2)) [3]. Considerable controversy exists as to whether Ins(1,3,4,5)P_4 possesses an independent or accessory second messenger role. Some evidence suggests that Ins(1,3,4,5)P_4 may modulate in calcium entry across the plasma membrane (reviewed [4–6]), indeed Ins(1,3,4,5)P_4-activated Ca^{2+} channels have been recently identified in the plasma membrane of endothelial cells [7] and Ins(1,3,4,5)P_4-activated Ca^{2+} mobilisation has been observed using crude microsomes and enriched vesicular plasma membranes prepared from T-lymphocyte and monocyte cell lines [8].

Furthermore, Ins(1,3,4,5)P_4 can apparently directly mobilise intracellular Ca^{2+} stores, in cerebellar [9] and adrenal [10] microsomes, microinjected *Xenopus* oocytes [11] and permeabilised SH-SY5Y neuroblastoma cells [12, 13], contradicting earlier reports that suggested that Ins(1,3,4,5)P_4 failed to affect intracellular Ca^{2+} stores or modulate Ins(1,4,5)P_3-induced Ca^{2+} mobilisation [8, 14–16]. However, categorical interpretation of these studies may have been complicated by a number of factors, notably Ins(1,4,5)P_3 contamination of the Ins(1,3,4,5)P_4 [13,17] and evidence that endogenous 3-phosphatase activity in the cells was producing steady state generation of Ins(1,4,5)P_3 from exogenous Ins(1,3,4,5)P_4 [18,19]. Significantly, Ins(1,3,4,5)P_4 3-phosphatase activity has only been detected in vitro in broken and permeabilised cells, or purified enzyme preparations [3,20]. It has re-

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Abbreviations: *myo*-Inositol 1,4,5-trisphosphate, Ins(1,4,5)P_3; *myo*-Inositol 1,3,4,5-tetrakisphosphate, Ins(1,3,4,5)P_4; *myo*-Inositol hexakisphosphate, $InsP_6$; L-*chiro*-inositol 2,3,5-trisphosphorothioate, L-chiro-Ins(2,3,5)PS_3; DL-*myo*-inositol 1,4,5-trisphosphate-3-phosphorothioate, DL-Ins(1,3,4,5)P_4-3S; *myo*-Inositol 1,4,5-trisphosphate receptor, IP_3R; calcium, Ca^{2+}; HEPES, 4-(2-hydroxylethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether *N,N,N',N'*-tetraacetic acid, HPLC, high performance liquid chromatography.

cently become apparent that both Ins(1,3,4,5,6)P_5 and $InsP_6$ potently inhibit Ins(1,3,4,5)P_4 3-phosphatase activity [21,22]. Thus, considering the endogenous cellular concentration range of Ins(1,3,4,5,6)P_5 and $InsP_6$ (5 μM to 60 μM) [23,24], there is probably negligible Ins(1,3,4,5)P_4 3-phosphatase activity in intact cells [21,22].

We have recent used the low intrinic activity partial agonist, L-*chiro*-Ins(2,3,5)PS_3, to provide compelling evidence that Ins(1,3,4,5)P_4 is a weak but full agonist at the Ca^{2+} mobilising Ins(1,4,5)P_3 receptor (IP_3R) of saponin-permeabilised SH-SY5Y cells [25,26]. Here utilising HPLC analysis of Ins(1,3,4,5)P_4-metabolism in permeabilised SH-SY5Y cells, we have confirmed that the limited 3-phosphatase activity present could be totally suppressed by inositol hexakisphosphate ($InsP_6$) or by conducting the experiment at 4°C. This suggests that Ins(1,3,4,5)P_4 can release the entire content of Ins(1,4,5)P_3-sensitive intracellular Ca^{2+} stores, independent of detectable 3-phosphatase catalysed Ins(1,4,5)P_3 generation. Furthermore, we demonstrate that the novel intrinsically Ins(1,3,4,5)P_4-3-phosphatase resistant Ins(1,3,4,5)P_4 analogue, DL-*myo*-inositol 1,4,5-trisphosphate-3-phosphorothioate [DL-Ins(1,3,4,5)P_4-3S, (3, Fig. 1.)] is also a weak full agonist at the Ca^{2+} mobilising IP_3R of saponin-permeabilised SH-SY5Y cells.

2. EXPERIMENTAL

2.1. *Materials*

The following reagents were used; $^{45}CaCl_2$ (approximately 1,000 Ci/mmol, Amersham, UK), [^{3}H]Ins(1,3,4,5)P_4 (45 Ci/mmol, Amersham, UK). Disodium ATP, fura-2, carbachol and EGTA were from Sigma (UK), all other reagents were of the highest purity available. DL-Ins(1,3,4,5)P_4-3S was synthesised from 3-*O*-propenyl-2,6-di-*O*-benzyl-*myo*-inositol (prepared by isomerisation of the corresponding 3-*O*- allyl derivative [27]) by trisphosphorylation using a P(III) approach [28], followed by removal of the propenyl group and thiophosphorylation [29] at the 3-position followed by deblocking using sodium in liquid ammonia [29] and purification by ion exchange chromatography on Q-Sepharose Fast Flow using a gradient of triethylammonium bicarbonate. Ins(1,3,4,5)P_4-3S was quantified by Briggs phosphate assay and was used as its triethylammonium salt. Full synthetic details will be published elsewhere. Chemically synthesised Ins(1,4,5)P_3 [30] and Ins(1,3,4,5)P_4 [31] as K^+ salts, were obtained from the University of Rhode Island Foundation Chemistry Group, USA. Both compounds were extensively characterised by ^{31}P- and ^{1}H-NMR, and found to be >99% pure with no other detectable inositol polyphosphate contaminants.

2.2. *Cell culture*

SH-SY5Y human neuroblastoma cell monolayers (passage 70–90), initially a gift from Dr. J.L. Biedler (Sloane-Kettering Institute, New York, USA) were subcultured and maintained, as described [32], except that the culture media was supplemented with 10% (v/v) new-born calf serum (Gibco, UK).

2.3. *$^{45}Ca^{2+}$-mobilisation assays*

$^{45}Ca^{2+}$-mobilisation assays were conducted using saponin-permeabilised SH-SY5Y cell at 20–22°C as previously described [25]. Each experiment was performed in duplicate with ionomycin (10 μM, free acid, Calbiochem, UK) to define the total releasable $^{45}Ca^{2+}$ pool and Ins(1,4,5)P_3 (20–30 μM) to define the Ins(1,4,5)P_3-sensitive $^{45}Ca^{2+}$ pool, included as internal standards.

2.4. *Preparation and analysis of HPLC samples*

SH-SY5Y cells were prepared exactly as for the $^{45}Ca^{2+}$ release assay in CLB or CLB supplemented with $InsP_6$ (10 μM), however, $^{45}Ca^{2+}$ was replaced by an identical concentration of Ca^{2+}. On completing the 15 min loading period, 100 μl of the permeabilised cell suspension was added to 100 μl of CLB (± $InsP_6$, 10 μM) containing 3 μM Ins(1,3,4,5)P_4 spiked with [^{3}H]Ins(1,3,4,5)P_4 (6.7 nM or 30 nCi per tube) in 1.5 ml microcentrifuge tubes. The cells were allowed to incubate for 2 min, and terminated by the addition of 7% (w/v) ice-cold perchloric acid. Boiled cells were included as an appropriate control. Preparation of samples and HPLC analysis of the inositol polyphosphates was performed as previously described [33], 5 ml of Flo-Scint IV (Canberra Packard, UK) was added to each sample and vortexed, prior to scintillation counting.

2.5. *Binding studies*

Preparation of bovine adrenal cortices and the [^{3}H]Ins(1,4,5)P_3 binding and displacement assays were performed as described [34]. Bound and free [^{3}H]Ins(1,4,5)P_3 were separated by rapid filtration through Whatman GF/B glass fibre filters after incubation for 30 min at 4°C. Non-specific binding (approximately 150 dpm/assay) was defined by addition of 10 μM Ins(1,4,5)P_3. Specifically bound [^{3}H]Ins(1,4,5)P_3 (approximately 2,500 dpm/replicate) was readily displaced by Ins(1,4,5)P_3.

2.6. *Data analysis*

EC_{50} and IC_{50} values (concentrations producing half maximal stimulation and inhibition, respectively) and slope factors were estimated by computer assisted curve fitting using GraphPad INPLOT version 3.1 (GraphPad Software, USA). Combined data from the independent experiments were expressed as mean ± S.E.M., where $n \geq 3$.

3. RESULTS AND DISCUSSION

3.1. *HPLC studies*

HPLC analysis of the boiled cell preparations revealed a small quantity of contaminating Ins(1,3,4)P_3 (1.4%) and Ins(1,4,5)P_3 (1.2%) present in the [^{3}H]Ins(1,3,4,5)P_4 ($n = 3$). In control preparations from saponin permeabilised SH-SY5Y cells allowed to incubate for 2 min with ~6.7 nM [^{3}H]Ins(1,3,4,5)P_4 and 3 μM Ins(1,3,4,5)P_4 only 1.7% was metabolised to [^{3}H]Ins (1,4,5)P_3 and 6.8% to [^{3}H]Ins(1,3,4)P_3 ($n = 3$). The addition of $InsP_6$ (10 μM) completely obliterated Ins(1,3,4,5)P_4-3-phosphatase catalysed generation of [^{3}H]Ins(1,4,5)P_3 (1.1%, $n = 2$) and also inhibited [^{3}H]Ins(1,3,4)P_3 generation (2.2% $n = 2$). No inositol bisphosphate peaks were detected above background (Fig. 2A).

A more significant percentage of the [^{3}H]Ins (1,3,4,5)P_4 was metabolised during the 15 min incubations allowing more accurate assessment of the 3-phosphatase inhibition by $InsP_6$. In control permeabilised cells about 39% ($n = 3$) of the [^{3}H]Ins(1,3,4,5)P_4 was metabolised with

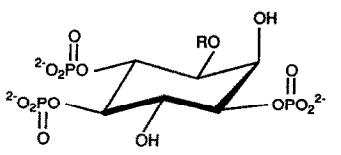

Fig. 1. Structures of Ins(1,4,5)P_3, Ins(1,3,4,5)P_4 and DL-Ins(1,3,4,5)P_4-3S (only D-isomers are shown). (1) Ins(1,4,5)P_3, R = H; (2) Ins(1,3,4,5)P_4, R = PO_3^{2-}; (3) Ins(1,3,4,5)P_4-3S, R = PSO_2^{2-}.

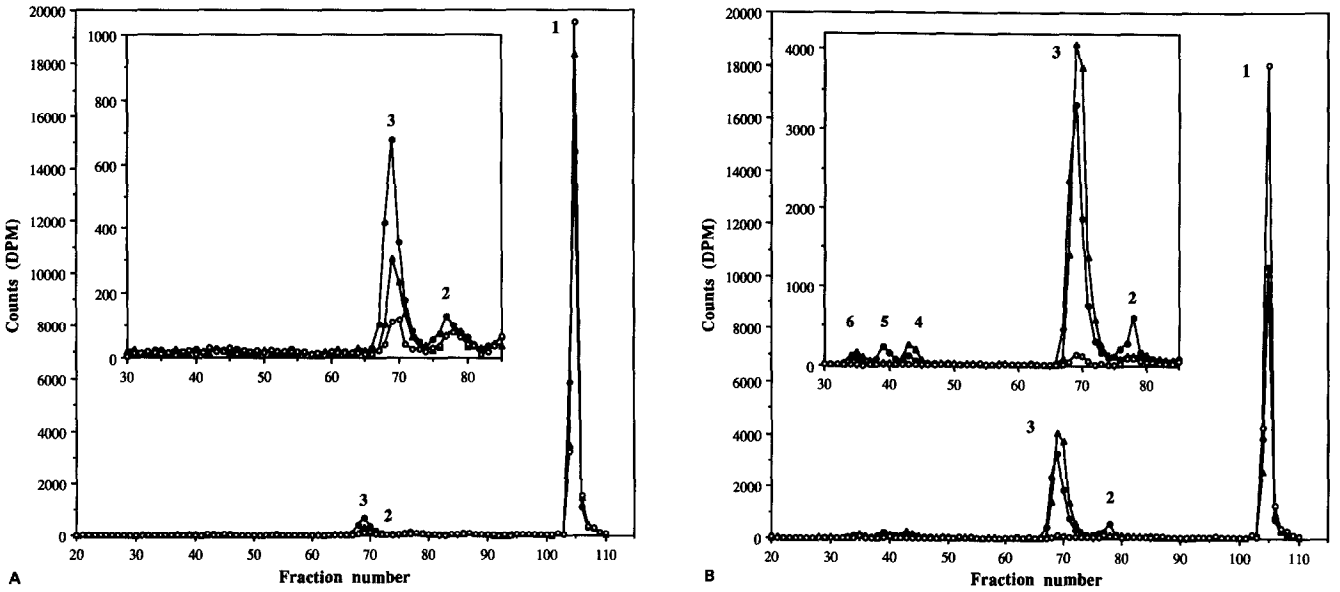

Fig. 2. HPLC analysis of the metabolism of Ins(1,3,4,5)P_4 (3 μM) spiked with [^{3}H]Ins(1,3,4,5)P_4 (60 nCi, ≈ 6.7 nM) in SH-SY5Y cells at 20–22°C, after 2 min (A) and 15 min (B) incubations. The peak counts corresponding to the various inositol poly-phosphate isomers were determined and corrected for background counts. Data from typical experiments are shown expressed as DPM counts, as an estimation of relative metabolism of the [^{3}H]Ins(1,3,4,5)P_4 added. Total counts recovered were in the range 25–26 × 10^3 DPM. ^{3}H-labelled inositol bisphosphate isomers were not detected in the boiled cells or following the 2 min incubations. Permeabilised SH-SY5Y cells were incubated with control CLB (●), CLB supplemented with $InsP_6$ (10 μM) (△) or boiled prior to incubation in control CLB (○), see section 2 for further details. Inositol polyphosphates are numbered as indicated: (1) Ins(1,3,4,5)P_4, (2) Ins(1,4,5)P_3, (3) Ins(1,3,4)P_3, (4) Ins(3,4)P_2, (5) Ins(1,4)P_2 and (6) Ins(1,3)P_2.

32% appearing as [^{3}H]Ins(1,3,4)P_3 and 3% as [^{3}H]Ins(1,4,5)P_3. Additionally, a small amount of label was detected in the major $InsP_2$ isomers (Fig. 2B). The $InsP_6$ treated cells exhibited a similar total metabolism (37%, n = 3), however the ^{3}H-label appeared predominantly as [^{3}H]Ins(1,3,4)P_3 (33%) and its metabolites Ins(1,3)P_2 and Ins(3,4)P_3, no significant counts were detected in the eluted fractions normally corresponding to [^{3}H]Ins(1,4,5)P_3 or [^{3}H]Ins(1,4)P_2. These data suggest that even after 15 min, $InsP_6$ (10 μM) completely inhibits 3-phosphatase back-conversion of Ins(1,3,4,5)P_4 to Ins(1,4,5)P_3 in our saponin permeabilised SH-SY5Y cells. Since our Ca^{2+}-mobilisation assays involve the exposure of permeabilised cells ≤ 2 min at 20–22°C, the inclusion of $InsP_6$ (10 μM) in the CLB insures that steady state generation of contaminating Ins(1,4,5)P_3 will be negligible. $InsP_6$ has no intrinsic Ca^{2+} mobilising ability at concentrations up to 100 μM, nor does $InsP_6$ (10 μM) significantly shift the Ins(1,4,5)P_3-concentration response curve [25].

The metabolism of Ins(1,3,4,5)P_4 to Ins(1,3,4)P_3, catalysed via Ins(1,4,5)P_3/Ins(1,3,4,5)P_4-5-phosphatase isoenzymes is well characterised, but recently active Ins(1,3,4,5)P_4-3-phosphatase catalysed metabolism of Ins(1,3,4,5)P_4 to Ins(1,4,5)P_3 has been detected in several cells and tissues, including saponin permeabilised SH-SY5Y cells [3,12]. Since Ins(1,4,5)P_3 exhibits a more potent intrinsic activity for Ca^{2+} mobilisation, 3-phosphatase catalysed generation of contaminating Ins(1,4,5)P_3 from exogenous Ins(1,3,4,5)P_4 should produce a leftward shift of the Ins(1,3,4,5)P_4 concentration response curve. Indeed the EC_{50} of Ins(1,3,4,5)P_4-induced Ca^{2+} release in SH-SY5Y cells was shifted from 0.89 to 2.54 μM in the presence of 10 μM $InsP_6$ [25], and a similar EC_{50} value (2.05 μM) was obtained Ins(1,3,4,5)P_4-induced Ca^{2+} mobilisation was conducted at 4°C to inhibit total cellular metabolism [26].

Significant Ins(1,3,4,5)P_4-3-phosphatase activity has only been observed in permeabilised cells and not in intact cell preparations [3]. Indeed Ins(1,3,4,5)P_4-3-phosphatase activity may not be physiologically relevant since the inositol pentakisphosphate ($InsP_5$) isomers and $InsP_6$ which are present in cells at 5–60 μM [23,24], inhibit Ins(1,3,4,5)P_4-3-phosphatase with n_i values ≤ 60 nM and ≤ 3 nM respectively [22,35]. In fact $InsP_5$ isomers and $InsP_6$ are apparently the physiological substrates for the enzyme and their K_m values have been estimated using purified rat liver 3-phosphatase at 40 nM and 0.3 nM, respectively [35]. Thus Ins(1,3,4,5)P_4-3-phosphatase activity is probably an artefact which develops only when $InsP_5$ isomers and $InsP_6$ have been sufficiently diluted by experimental procedures, such as the detergent permeabilisation and centrifugation washing steps used during our $^{45}Ca^{2+}$-release assays.

There are a number of metabolic precedents indicating that inositol polyphosphates can perturb the metabolism of other inositol polyphosphates; Ins(3,4,5,6)P_4 potently inhibits Ins(1,3,4)P_3 6-kinase [36], while Ins(1,3,4,5,6)P_5 and $InsP_6$ also inhibit Ins(1,4,5)P_3/Ins-

(1,3,4,5)P_4-5-phosphatase activity with 50% inhibition occurring at 10–20 μM [35]. Indeed, we saw some evidence for $InsP_6$ inhibition of 5-phosphatase metabolism in our 2 min, but not the 15 min Ins(1,3,4,5)P_4 HPLC study. Perhaps the inclusion of $InsP_6$ and $InsP_5$ isomers at appropriate physiological concentrations should be an important consideration for the design of any 'cytosol like' buffers used for inositol polyphosphate studies.

3.2. *$^{45}Ca^{2+}$-mobilisation and binding studies*

Ins(1,4,5)P_3 mobilised about 70% of pre-loaded $^{45}Ca^{2+}$ from saponin-permeabilised SH-SY5Y cells at 20–22°C, with an EC_{50} of 52 nM. We have recently shown Ins(1,3,4,5)P_4 was able to mobilise the entire Ins(1,4,5)P_3-sensitive intracellular calcium store of saponin-permeabilised SH-SY5Y with an EC_{50} of 879 nM, and that the EC_{50} value was significantly increased in the presence of 10 μM $InsP_6$ (EC_{50} = 2536 nM) [25]. Here our HPLC data confirm that the decreased potency of exogenous Ins(1,3,4,5)P_4 in the presence of $InsP_6$, was due to inhibition of 3-phosphatase catalysed steady state generation of Ins(1,4,5)P_3.

To assess further our hypothesis that Ins(1,3,4,5)P_4 is a weak but full agonist acting directly on the IP_3R we synthesised a novel intrinsically 3-phosphatase resistant Ins(1,3,4,5)P_4 analogue; DL-Ins(1,3,4,5)P_4-3S. DL-Ins-(1,3,4,5)P_4-3S was able to fully displace [^{3}H]Ins(1,4,5)P_3 from bovine adrenal cortex membranes (IC_{50} = 889 nM), but it was considerably less potent than Ins(1,4,5)P_3 (200-fold) and slightly less potent than Ins-(1,3,4,5)P_4 (5-fold) (Table I, Fig. 3). DL-Ins(1,3,4,5)P_4-3S was also a weak but full agonist for Ca^{2+} mobilisation in saponin permeabilised SH-SY5Y cells (EC_{50} = 4.6 μM). DL-Ins(1,3,4,5)P_4-3S was much weaker agonist than Ins(1,4,5)P_3 (90-fold), but its action was only 2-fold less potent than Ins(1,3,4,5)P_4 in the presence of $InsP_6$ (Table I, Fig. 4). Additionally, the EC_{50} of DL-Ins(1,3,4,5)P_4-3S was not significantly shifted in the presence $InsP_6$ (10 μM) which our HPLC studies had shown completely obliterates 3-phosphatase activity (Table I, Fig. 4). This was wholly expected because the 3-position phosphorothioate group should be intrinsically resistant to Ins(1,3,4,5)P_4-3-phosphatase activity, just as the 5-phosphorothioate groups of inositol 1,4,5-trisphosphorothioate and inositol 1,4-bisphosphate 5-phosphorothioate were resistant to Ins(1,4,5)P_3-5-phosphatase activity [37]. In contrast, the concentration response curve of Ins(1,3,4,5)P_4 [but not Ins(1,4,5)P_3] was significantly shifted to the right by $InsP_6$ [25] (Table I). Although DL-Ins(1,3,4,5)P_4-3S is a racemic mixture, the L-isomer is very probably inactive, because the IP_3R displays stringent stereospecificity with L-Ins(1,4,5)P_3 [38–40] and L-Ins(1,3,4,5)P_4 [26] both exhibiting exceeding poor ligand and agonist profiles.

Ins(1,3,4,5)P_4 and Ins(1,3,4,5)P_4-3S possess all the critical structural motifs requisite for effective interaction with the Ins(1,4,5)P_3-receptor [41, 42], and we propose that the respective addition to Ins(1,4,5)P_3 of a 3-phosphate or 3-phosphorothioate group, simply produces a weaker ligand and agonist at the IP_3R in SH-SY5Y cells. Being the first 3-phosphatase resistant Ins(1,3,4,5)P_4 analogue, DL-Ins(1,3,4,5)P_4-3S will be a crucial tool for probing and confirming exclusive second messenger roles for Ins(1,3,4,5)P_4, since it precludes

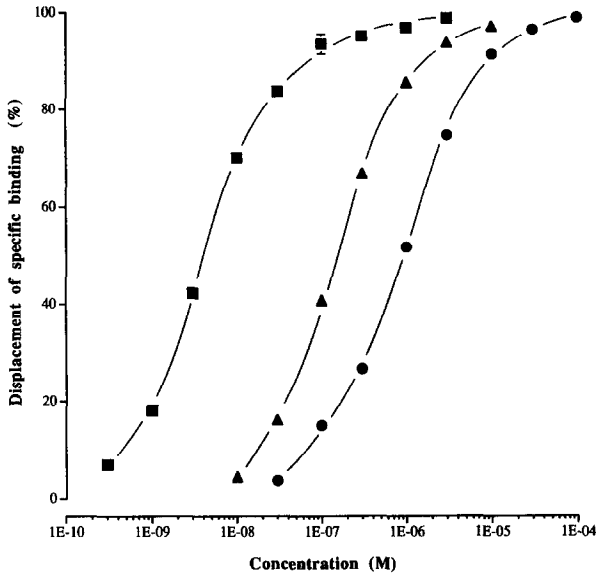

Fig. 3. Displacement of specific [^{3}H]Ins(1,4,5)P_3 binding by Ins(1,4,5)-P_3-, DL-Ins(1,3,4,5)P_4-3S and Ins(1,3,4,5)P_4 from bovine adrenal cortex membranes Ins(1,4,5)P_3 binding sites. Data indicate the percentage displacement of specific of [^{3}H]Ins(1,4,5)P_3 binding by increasing concentrations of Ins(1,4,5) (■), Ins(1,3,4,5)P_4 (▲) and DL- Ins(1,3,4,5)P_4-3S (●). Results are shown as mean ± S.E.M. of 3–4 experiments. Non-specific binding was defined using 10 μM Ins(1,4,5)P_3 (100% displacement); see section 2 for further details.

Table I

The bovine adrenal cortex IP_3R binding and permeabilised SH-SY5Y cell $^{45}Ca^{2+}$ release profiles of Ins(1,4,5)P_3, DL-Ins(1,3,4,5)P_4-3S and Ins(1,3,4,5)P_4

Inositol polyphosphate	IC_{50} (nM)	EC_{50} (nM)
Ins(1,4,5)P_3	4.4 ± 0.1	52 ± 2[a]
Ins(1,4,5)P_3 + $InsP_6$ (10 μM)	–	58 ± 6[a]
Ins(1,3,4,5)P_4	152 ± 4.4	879 ± 92[a]
Ins(1,3,4,5)P_4 + $InsP_6$ (10 μM)	–	2536 ± 303[a,*]
DL-Ins(1,3,4,5)P_4-3S	889.7 ± 52.7	4647 ± 576
DL-Ins(1,3,4,5)P_4-3S + $InsP_6$ (10 μM)	–	3534 ± 429

(i) IC_{50} estimates (nM) for binding affinity obtained via inositol polyphosphate displacement of specific [^{3}H]Ins(1,4,5)P_3 from Ins(1,4,5)P_3-binding sites on bovine adrenal cortex membranes. Results are shown as mean ± S.E.M. ($n \geq 3$); see section 2 for further details. (ii) EC_{50} estimates (nM) for inositol polyphosphate-induced $^{45}Ca^{2+}$ mobilisation in saponin permeabilised SH-SY5Y cells at 20–22°C. Results are shown as mean ± S.E.M. ($n \geq 4$); see section 2 for further details.

[a] The EC_{50} values for Ins(1,4,5)P_3- and Ins(1,3,4,5)P_4-induced Ca^{2+} release have been previously reported [25] and are included here for comparison. Note only the Ins(1,3,4,5)P_4 concentration response curve was significantly shifted by $InsP_6$ (10 μM) (*) indicates $P < 0.05$.).

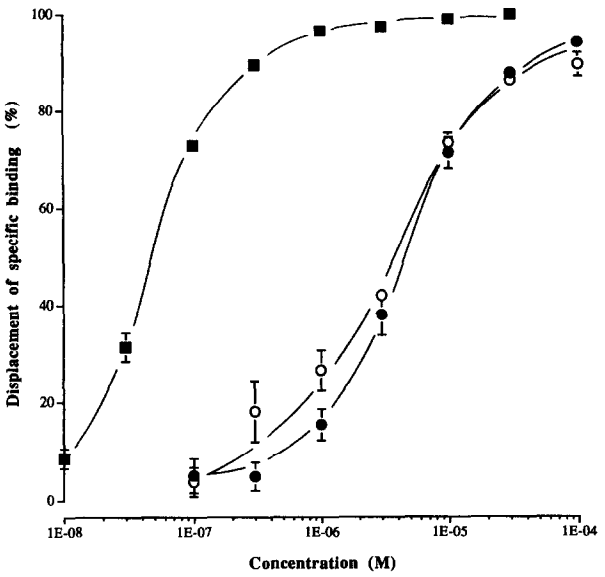

Fig. 4. Ins(1,4,5)P_3- and DL-Ins(1,3,4,5)P_4-3S-induced Ca^{2+} release. Data indicates the percentage of $^{45}Ca^{2+}$ released at 20–22°C from the intracellular Ins(1,4,5)P_3-sensitive Ca^{2+} stores of saponin permeabilised SH-SY5Y cells in the presence of increasing concentrations of Ins(1,4,5) (■), DL-Ins(1,3,4,5)P_4-3S (●) and DL-Ins(1,3,4,5)P_4-3S + $InsP_6$ (10 μM) (○). Results are shown as mean ± S.E.M. error of four experiments. Maximal Ca^{2+} release from intracellular Ins(1,4,5)P_3-sensitive Ca^{2+} stores was defined using an internal standard of 30 μM Ins(1,4,5)P_3 (100% release); see section 2 for a complete description.

the complication of steady state generation of contaminating Ins(1,4,5)P_3. Here DL-Ins(1,3,4,5)P_4-3S has been used to confirm that Ins(1,3,4,5)P_4 independently mobilises intracellular Ca^{2+} via the IP_3R in SH-SY5Y cells. However, DL-Ins(1,3,4,5)P_4-3S should be even more uniquely suited to the investigation of the putative role of the various 'Ins(1,3,4,5)P_4 receptors' (reviewed [43]) in processes such as Ca^{2+} entry across the plasma membrane [5].

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