Carbohydrate Research, 234 (1992) 237–246
Elsevier Science Publishers B.V., Amsterdam

The role of the 2- and 3-hydroxyl groups of 1D-*myo*-inositol 1,4,5-trisphosphate in the mobilisation of calcium from permeabilised human 1321N1 astrocytoma cells

Robert A. Wilcox [a], Stefan R. Nahorski [a], Deborah A. Sawyer [b], Changsheng Liu [b,c] and Barry V.L. Potter [b,c]

[a] *Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN (United Kingdom)*
[b] *Department of Chemistry, University of Leicester, Leicester LE1 7RH (United Kingdom)*
[c] *School of Pharmacy and Pharmacology and Institute for Life Sciences, University of Bath, Claverton Down, Bath BA2 7AY (United Kingdom)*

(Received January 4th, 1992; accepted April 7th, 1992)

ABSTRACT

The functional significance of the 2- and 3-hydroxyl groups of 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] was probed by using Ins(1,4,5)P_3 analogues variously modified at positions 2 and 3 or elsewhere. The intrinsic activities of these compounds were compared to that of Ins(1,4,5)P_3, using the calcium-mobilising receptor of the 1321N1 human astrocytoma cell line. The ligand-binding affinities were also determined using membrane preparations from rat cerebellum and bovine adrenal cortex. The results show that HO-2 and HO-3 of Ins(1,4,5)P_3 have a relatively insignificant role in receptor binding and calcium release. However, the possibility of a regulatory role for the 3-position of Ins(1,4,5)P_3 in these processes is proposed.

INTRODUCTION

1D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] is now well characterised as a second messenger responsible[1] for Ca^{2+}-mobilising activity from non-mitochondrial intracellular stores of Ca^{2+}. Recently, the cerebellar binding site of Ins(1,4,5)P_3 has been purified, reconstituted, and cloned[2–5]. However, there is a paucity of synthetic pharmacological agents which affect the interaction of Ins(1,4,5)P_3 with either its intracellular receptor or the enzymes involved in its metabolism, namely, Ins(1,4,5)P_3 3-kinase and 5-phosphatase. Elucidation of the molecular interactions of Ins(1,4,5)P_3 with these three proteins could guide the

Correspondence to: Dr. R.A. Wilcox, Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN, UK.

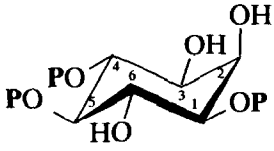
chemical design of novel Ins(1,4,5)P_3 agonists, antagonists, and enzyme inhibitors, which may have therapeutic utility.

Several ring- and phosphate-modified analogues of Ins(1,4,5)P_3 have been synthesised[6,7] and some progress has been made in understanding the role of the three phosphate and three hydroxyl groups in determining receptor binding and Ins(1,4,5)P_3-induced release of Ca^{2+}. The critical importance of the 4,5-phosphate groups of Ins(1,4,5)P_3 in receptor binding was recognised in studies that used stereoisomers and positional isomers, while the presence of the 1-phosphate further enhances receptor affinity[8]. The significance of the hydroxyl (HO) groups to Ins(1,4,5)P_3 receptor interaction is less well characterised. However deoxy-Ins(1,4,5)P_3 analogues have been synthesised in order to probe the role(s) of HO-2,3,6. These analogues include the 2,3,6-trideoxy[9], 2-deoxy[10], 3-deoxy[11], and 6-deoxy[12] derivatives of Ins(1,4,5)P_3.

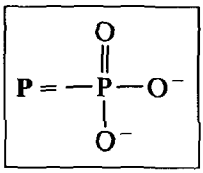
One model for the binding of Ins(1,4,5)P_3 to its receptor proposes that the phosphate groups make the dominant contribution to the binding energy, by interacting with pockets of positive charge on the receptor protein[13]. The potential for HO-2,3,6 to form intermolecular hydrogen bonds with the receptor protein and to fix the conformation of Ins(1,4,5)P_3 in solution, via intramolecular hydrogen bonds to the neighbouring phosphate groups, was also emphasised[13].

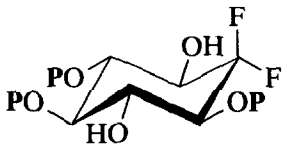
A well recognised approach for probing the interaction of a hydroxyl group with a receptor protein involves isosteric replacement with fluorine[14]. The size and electronegativity of a fluorine substituent lie between those of a hydrogen and a hydroxyl group, and the C–F and C–OH bonds have similar lengths and polarisation[15]. However, a fluorine substituent can only accept and not donate hydrogen bonds[15]. Several fluorinated *myo*-inositol and *myo*-inositol phosphate analogues have been synthesised[16–26].

We now report on the use of several fluorinated derivatives of Ins(1,4,5)P_3 and analogues of Ins(1,4,5)P_3, based on *chiro*- and *scyllo*-inositol, to examine the contribution of HO-2,3 to (*a*) the binding of Ins(1,4,5)P_3 to the receptor and (*b*) the induction of the release of intracellular Ca^{2+}. The compounds used are the 2-deoxy-2,2-difluoro derivatives (**2**, **3**, and **2/3**, respectively) of 1D-, 1L-, and DL-*myo*-inositol 1,4,5-trisphosphate [2,2F_2-D-, -L-, and -DL-*myo*-Ins(1,4,5)P_3][25,26], 2-deoxy-2-fluoro-DL-*scyllo*-inositol 1,4,5-trisphosphate [**4**, 2F-DL-*scyllo*-Ins(1,4,5)P_3][25,26], 1D-3-deoxy-3-fluoro-*myo*-inositol 1,4,5-trisphosphate [**5**, 3F-D-*myo*-Ins(1,4,5)P_3][16], L-*chiro*-inositol 2,3,5-trisphosphate [**6**, L-*chiro*-Ins(2,3,5)P_3][27,42], and L-*chiro*-inositol 1,4,6-trisphosphate [**7**, L-*chiro*-Ins(1,4,6)P_3][28]. The intrinsic activity of each of these derivatives was tested at the Ca^{2+}-mobilising Ins(1,4,5)P_3 receptor of 1321N1 human astrocytoma cells, and for receptor-binding affinity using rat cerebellar and bovine adrenal cortex Ins(1,4,5)P_3 receptors.

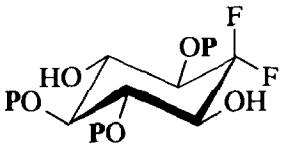


1

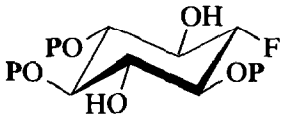




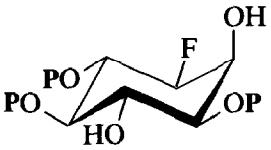
2



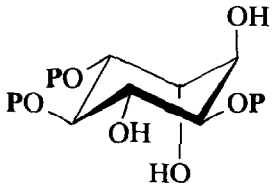
3



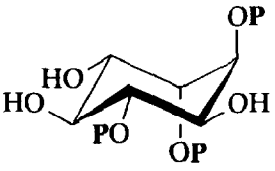
4



5



6



7

The 1321N1 astrocytoma cell line exhibits well characterised potent muscarinic and histaminergic receptor responses, which result[29–31] in the turnover of inositol lipids and the mobilisation of Ca^{2+}. These cells provide an appropriate model system for assessing the efficacies of Ins(1,4,5)P_3 analogues, since maximal doses of Ins(1,4,5)P_3 can release $> 80\%$ of preloaded $^{45}Ca^{2+}$ from the mobilisable intracellular stores of Ca^{2+}. In contrast, most other cell types[32] have Ins(1,4,5)P_3-sensitive stores of calcium which represent only 25–60% of their total mobilisable Ca^{2+} pools and, thus, the indirect effects of agents on the Ins(1,4,5)P_3-insensitive Ca^{2+} stores could complicate the analysis of potential agonists.

Hitherto, all Ca^{2+}-mobilising inositol trisphosphates have been observed to exhibit binding affinities for the Ins(1,4,5)P_3 receptor which broadly correlate[7,33] with their intrinsic activities for the release of Ca^{2+}. Consequently, receptor-binding studies were also undertaken, using microsomal membrane preparations from rat cerebellum[34,35] and bovine adrenal cortex[36–40], each of which possess well characterised, specific, high-affinity binding sites for Ins(1,4,5)P_3.

EXPERIMENTAL

Inositol trisphosphate analogues.—The following inositol trisphosphate analogues were synthesised as previously described: 2F-DL-*scyllo*-Ins(1,4,5)P_3[26], 2,2F_2-

DL-*myo*-Ins(1,4,5)P_3[26], 2,2F_2-D-*myo*-Ins(1,4,5)P_3[26,41], 2,2F_2-L-*myo*-Ins(1,4,5)P_3[26,41], L-*chiro*-Ins(2,3,5)P_3[27,42], L-*chiro*-Ins(1,4,6)P_3[28], and 1D-6-deoxy-*myo*-inositol 1,4,5-trisphosphate [6-deoxy-D-*myo*-Ins(1,4,5)P_3][12]. Each analogue was purified by anion-exchange chromatography on DEAE Sephadex A-25 and used as the triethylammonium salt. 3F-D-*myo*-Ins(1,4,5)P_3 was obtained from Mind Labs. (USA). Prior to biological evaluation, an aqueous solution of each analogue was passed through Chelex-100 (Na^+ form, 100–200 mesh, BioRad) in order to remove contaminating Ca^{2+}.

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[43]. 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°.

Cerebella (200–250 mg) from male Wistar rats were homogenised at −4°, using a Polytron (setting 3, 2 × 10 s), in 10 vol of a buffer consisting of 20 mM $NaHCO_3$ and mM dithiothreitol pH 8.0 (buffer *A*.). The homogenate was centrifuged (5000*g*, 10 min, 4°), and the supernatant solution was harvested and then centrifuged (40000*g*, 20 min) at 4°. The resulting supernatant solution was discarded and the pellet was washed twice (40000*g*, 20 min) in buffer *A*, then resuspended in buffer *A* at 6–8 mg/mL, and stored at ≤ −20°. The cerebellar membranes were diluted in buffer *A* to yield a working concentration of 50 μg of protein/assay tube. Equilibrium binding was fast, and bound and free [^{3}H]-Ins(1,4,5)P_3 were separated by rapid centrifugation (14000*g*, 4 min) after incubation for 30 min at 4°. The supernatant solutions were rapidly aspirated, the pellets were solubilised using Lumosolve (100 μL, May and Baker), and the radioactivity was then determined by scintillation counting.

In each binding assay, specifically bound [^{3}H]-Ins(1,4,5)P_3 (~ 2500 dpm/assay tube) was displaced by increasing doses (0 to 1 μM) of each inositol trisphosphate analogue. The non-specific binding was defined by the addition of 10 μM Ins(1,4,5)P_3.

Cell culture, permeabilisation, and calcium release.—1321N1 human astrocytoma cells, obtained from the American Type Culture Collection, were routinely cultured at 37° with a 5% CO_2 atmosphere, in Hepes-buffered Dulbecco's modified Eagles media supplemented with foetal-calf serum (10% v/v), penicillin (100 IU/mL), streptomycin (100 μg/mL), fungizone (5 μg/mL) and 2 mM glutamine (Gibco). The cell monolayers grown in 175-cm^2 flasks (Nunc, UK) were passaged using trypsin–EDTA solution (Gibco, UK), with a split ratio between 1:4 and 1:5, and then used for experiments when confluent. Prior to permeabilisation, 1321N1 cells were harvested using EDTA(0.02%)/NaCl(0.9%) solution buffered with 10 mM Hepes (pH 7.4). The $^{45}Ca^{2+}$-release experiments were performed using saponin-permeabilised cells[44], and 50 μM ionomycin free acid (Calbiochem, UK) was used to define the size of the total releasable $^{45}Ca^{2+}$ pool.

The mobilisation of Ca^{2+} was also monitored in electropermeabilised 1321N1 cells[45], using a Ca^{2+}-specific electrode[46], which was utilised to confirm the inactiv-

ity of compounds previously shown to exhibit weak binding affinity for the Ins(1,4,5)P_3 receptor. These putatively inactive compounds were tested at concentrations in the range of 10 to 30 μM.

Data analysis.–The K_d and B_{max} values for receptor binding were estimated by the computer-assisted equilibrium-binding analysis program EBDA/LIGAND[47,48]. EC_{50} and K_i values were estimated using GraphPad INPLOT version 3.1 (GraphPad Software, USA). The combined data from the independent experiments are expressed as mean ± SEM, where $n \geq 3$.

Reagents.—The following reagents were used: Ins(1,4,5)P_3 (Semat Technical), $^{45}CaCl_2$ (~ 1,000 Ci/mmol, Amersham), [^{3}H]-Ins(1,4,5)P_3 (17–30 Ci/mmol, New England Nuclear), and ATP and Quin-2 (Sigma).

RESULTS AND DISCUSSION

The permeabilised human 1321N1 astrocytoma cell line has been used to assess the relative potencies of a series of analogues of Ins(1,4,5)P_3, modified at positions 2 or 3 and elsewhere. A single cell type was used in order to avoid intercellular variation in the Ca^{2+}-mobilising efficacies of the Ins(1,4,5)P_3 receptor. To our knowledge, 1321N1 astrocytoma cells are the most responsive cell type to Ins(1,4,5)P_3, with maximal doses mobilising > 80% of the Ca^{2+} from the intracellular stores.

Several of the analogues tested were full and potent agonists at the Ca^{2+}-mobilising receptor of 1321N1 cells, with a rank order of Ins(1,4,5)P_3 (**1**) > 3F-D-*myo*-Ins(1,4,5)P_3 (**5**) > 2,2F_2-DL-*myo*-Ins(1,4,5)P_3 (**2/3**) > 2F-DL-*scyllo*-Ins(1,4,5)P_3 (**4**) > L-*chiro*-Ins(2,3,5)P_3 (**6**) (Fig. 1 and Table I). The rank order of ligand affinity in both adrenal cortex and cerebellum correlated well with the Ca^{2+}-release profiles for each compound. The reasons for the discrepancy between the K_d and K_i values and the EC_{50} values are due largely to differences in the assay procedures, are well characterised, and have been discussed extensively[49].

L-*chiro*-Ins(1,4,6)P_3 (**7**) has a structure far removed from that of Ins(1,4,5)P_3, and it is not surprising that it was a very poor agonist, failed to induce Ca^{2+} release, and did not bind specifically at the receptor (Tables I and II). Although **7** possesses a vicinal bisphosphate group, presumably each phosphate group is axial; however, no data are available to confirm this conformation in solution. Nevertheless, L-*chiro*-Ins(1,4,6)P_3 was tested, since Ca^{2+}-mobilisation, albeit weak, can be induced by 1D-*myo*-inositol 1,3,4,6-tetrakisphosphate, which does not possess a 4,5 vicinal bisphosphate group[50].

2,2F_2-DL-*myo*-Ins(1,4,5)P_3 (**2/3**) and 2F-DL-*scyllo*-Ins(1,4,5)P_3 (**4**) act as full agonists for Ca^{2+} release in SH-SY5Y cells[25]. Other reports of biological activity for Ins(1,4,5)P_3 analogues modified at position 2 have appeared[9,10,51,52] and some general principles have emerged. Position-2 does not appear to be significantly involved in the recognition of, and the interaction with, either the Ins(1,4,5)P_3 receptor or Ins(1,4,5)P_3-3-kinase, but substitution of HO-2 with such bulky groups

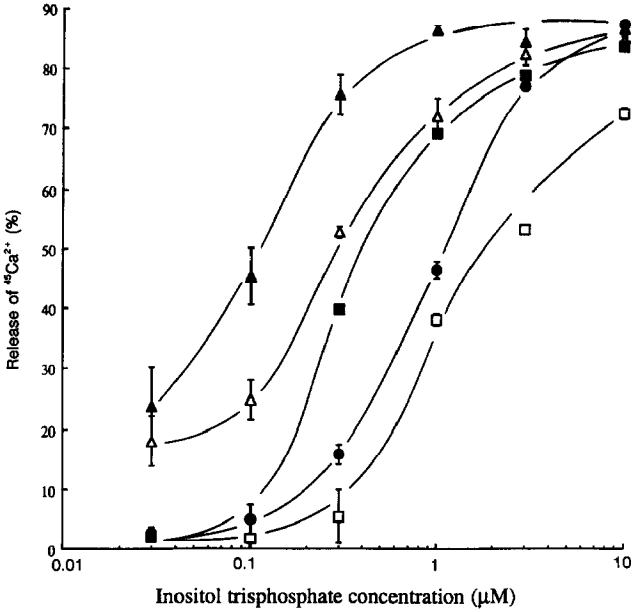


Fig. 1. The dose response curves for the release of $^{45}Ca^{2+}$ from saponin-permeabilised 1321N1 astrocytoma cells induced by the inositol trisphosphates: ▲, Ins(1,4,5)P_3; △, 3F-D-*myo*-Ins(1,4,5)P_3; □, 2F-DL-*scyllo*-Ins(1,4,5)P_3; ■, 2,2F_2-DL-*myo*-Ins(1,4,5)P_3; ●, L-*chiro*-Ins(2,3,5)P_3.

as 4-aminocyclohexanecarbonyl or 4-aminobenzyl[10,51] or its replacement by fluorine[25] produces Ins(1,4,5)P_3 analogues which are poor substrates for the 5-phosphatase. Indeed, 2,2F_2-DL-*myo*-Ins(1,4,5)P_3 inhibited the 5-phosphatase with a high affinity (K_i 26 μM)[25].

2,2F_2-DL-*myo*-Ins(1,4,5)P_3 and the enantiomers (**2** and **3**) were tested for binding to the Ins(1,4,5)P_3 receptor. Predictably, the L enantiomer (**3**) was a poor ligand, whereas the D enantiomer (**2**), which possesses the appropriate 4,5-bisphosphate

TABLE I

The EC_{50} values [a] for the release of $^{45}Ca^{2+}$ from saponin-permeabilised 1321N1 cells ($n = 4$)

Inositol trisphosphate	EC_{50} (μM)
Ins(1,4,5)P_3	0.10 ±0.01
3F-D-*myo*-Ins(1,4,5)P_3	0.28 ±0.02
2F-DL-*scyllo*-Ins(1,4,5)P_3	1.23 ±0.02
2,2F_2-DL-*myo*-(1,4,5)P_3	0.35 ±0.03
2,2F_2-D-*myo*-(1,4,5)P_3	Not tested
2,2F_2-L-*myo*-(1,4,5)P_3	> 10 μM (calcium electrode)
L-*chiro*-Ins(2,3,5)P_3	1.00 ±0.07
L-*chiro*-Ins(1,4,6)P_3	≫ 30 μM (calcium electrode)

[a] Estimated using GraphPad INPLOT version 3.1 (see Experimental).

TABLE II

K_i values for inhibition of [^{3}H]-Ins(1,4,5)P_3 binding to rat cerebellar and bovine adrenal cortical membranes [a]

Inositol trisphosphate	Adrenal cortex	Cerebellum
Ins(1,4,5)P_3	K_d 6.3 ±0.5 nM B_{max} 454.4 ±14.2 [b] K_i (μM)	K_d 9.1 ±1.3 nM B_{max} 3261.9 ±350 [b] K_i (μM)
3F-D-*myo*-Ins(1,4,5)P_3	13.3 ±0.6	21.3 ±0.9
2F-DL-*scyllo*-Ins(1,4,5)P_3	100.5 ±9.8	58.1 ±6.0
2,2F_2-DL-*myo*-(1,4,5)P_3	38.0 ±3.0	57.8 ±5.4
2,2F_2-D-*myo*-(1,4,5)P_3	22.1 ±1.6	25.1 ±0.4
2,2F_2-L-*myo*-(1,4,5)P_3	1940.0 ±202.7	1865.5 ±298.1
L-*chiro*-Ins(2,3,5)P_3	161.6 ±2.9	159.0 ±4.9
L-*chiro*-Ins(1,4,6)P_3	≫ 1000	≫ 1000
D-6-deoxy-*myo*-(1,4,5)P_3	not tested	3772.5 ±391.5

[a] Displacement isotherms were constructed using at least seven concentrations (nM–μM) of each displacing agent per experiment ($n = 4$). K_d and B_{max} values were estimated using EBDA/LIGAND, and K_i values using GraphPad INPLOT version 3.1 (see EXPERIMENTAL). [b] fmol/mg of protein.

group for recognition, was only slightly less potent than 3F-D-*myo*-Ins(1,4,5)P_3 (**5**). Indeed, it has been demonstrated, using permeabilised SH-SY5Y cells, that virtually all of the Ca^{2+}-mobilising activity of 2,2F_2-DL-*myo*-Ins(1,4,5)P_3 evolves from the D enantiomer[40].

In 2F-DL-*scyllo*-Ins(1,4,5)P_3 (**4**), the single fluorine substituent is equatorial, whereas the HO-2 of Ins(1,4,5)P_3 is axial. Consequently, 2F-DL-*scyllo*-Ins(1,4,5)P_3 mimics the stereochemistry and electronic environment at position 2 of Ins(1,4,5)P_3 less efficiently than does 2,2F_2-DL-*myo*-Ins(1,4,5)P_3. This difference was reflected by the relatively elevated EC_{50} value for Ca^{2+} release from 1321N1 cells and the moderate binding affinities (Fig. 1, Tables I and II).

3F-D-*myo*-Ins(1,4,5)P_3 (**5**) was the most potent ligand and agonist of the analogues tested and, clearly, the F-3 group therefore appropriately mimics both the electronic environment and stereochemistry of HO-3 of Ins(1,4,5)P_3. Position 3 of Ins(1,4,5)P_3 is the site of 3-kinase phosphorylation that produces the putative second messenger 1D-*myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4]. Both 3F-D-*myo*-Ins(1,4,5)P_3 (**5**)[16] and 3-deoxy-D-*myo*-Ins(1,4,5)P_3[11] are intrinsically resistant to the 3-kinase and are only slightly poorer agonists than Ins(1,4,5)P_3 in several cell types[11,16,53]. The binding studies reported here support these findings and demonstrate that 3F-D-*myo*-Ins(1,4,5)P_3 is a very potent ligand for the Ins(1,4,5)P_3 receptor.

Although it appears that the HO-3 of Ins(1,4,5)P_3 does not contribute significantly to binding, the receptor may have a poor tolerance for bulky charged substituents at position 3. For example, Ins(1,3,4,5)P_4, despite possessing appropriately positioned 1,4,5-trisphosphate groups, binds with low affinity to the Ins(1,4,5)P_3 receptor[50]. Thus, HO-3 may act as a weak anchoring substituent such that changes in its orientation or its replacement may adversely affect binding to

the receptor. Indeed, this view is supported by the finding that L-*chiro*-Ins(2,3,5)P_3 (**6**), which differs structurally from Ins(1,4,5)P_3 only by virtue of the axial-*chiro*-HO-1 (or pseudo-*myo*-HO-3) group, remains a full agonist for Ca^{2+} release in 1321N1 and SH-SY5Y cells[5]. However, it is 10-fold less potent than Ins(1,4,5)P_3, compared to the 3-fold lower potency of 3F-D-*myo*-Ins(1,4,5)P_3 (Tables I and II)[53].

Predictably, 3F-D-*myo*-Ins(1,4,5)P_3 (**5**) and L-*chiro*-Ins(2,3,5)P_3 (**6**) are both resistant to, and potent inhibitors of, the 3-kinase (K_i 7.1 and 8.6 μM, respectively)[53]. 3F-D-*myo*-Ins(1,4,5)P_3 was hydrolysed by the 5-phosphatase at a rate similar to that of Ins(1,4,5)P_3, but inhibited dephosphorylation of [^{3}H]-Ins(1,4,5)P_3. L-*chiro*-Ins(2,3,5)P_3 had a high affinity for the receptor, but was resistant to hydrolysis[53]. Therefore, position 3 of Ins(1,4,5)P_3, which is crucial for 3-kinase phosphorylation and, perhaps, for hydrolysis by the 5-phosphatase, does not appear to play a direct role in binding to the Ins(1,4,5)P_3-receptor (however, see ref. 54).

In contrast to the HO-2 and HO-3, HO-6 of Ins(1,4,5)P_3 appears to have a more significant role in binding to the Ins(1,4,5)P_3 receptor and the interaction with the enzymes. 6-Methoxy-DL-*myo*-Ins(1,4,5)P_3 and 6-deoxy-D-*myo*-Ins(1,4,5)P_3 each binds with a moderate affinity to the 5-phosphatase, but both compounds are resistant to 5-phosphatase-catalysed dephosphorylation[9,12]. Polokoff et al.[9] proposed that the minimum structural requirement for 5-phosphatase activity against inositol polyphosphate substrates includes phosphate groups at positions 4 and 5 and a free HO-6. We concluded that HO-6 is critical for 5-phosphatase activity, but not for binding of the substrate. Thus, whereas 6-methoxy-DL-*myo*-Ins(1,4,5)P_3 and 6-deoxy-D-*myo*-Ins(1,4,5)P_3 are relatively potent inhibitors of 5-phosphatase activity[9,12], 6-deoxy-D-*myo*-Ins(1,4,5)P_3, in contrast to 6-methoxy-DL-*myo*-Ins(1,4,5)P_3, is one of the few modified inositol phosphates which can bind to the 3-kinase enzyme with a high affinity, and it is a weak substrate for the enzyme[12]. We have proposed[12] that the relatively poor binding of 6-methoxy-DL-*myo*-Ins(1,4,5)P_3 to the 3-kinase is due to either reduction in the hydrogen bonding to the neighbouring phosphate groups or to steric effects associated with the methylation.

Ins(1,4,5)P_3 binding and Ins(1,4,5)P_3-induced release of Ca^{2+} appear to be facilitated by the presence of HO-6. Thus, 6-deoxy-D-*myo*-Ins(1,4,5)P_3 is a 400-fold weaker ligand than Ins(1,4,5)P_3 in rat cerebellar membranes (Table II). 6-Deoxy-D-*myo*-Ins(1,4,5)P_3 and 6-methoxy-DL-*myo*-Ins(1,4,5)P_3 are full agonists at the Ins(1,4,5)P_3 receptor, albeit 70- and 200-fold less potent, respectively, at mobilising Ca^{2+} than Ins(1,4,5)P_3[9,12]. Thus, removal of the HO-6 of Ins(1,4,5)P_3 may affect the optimal conformation of the neighbouring 1- and 5-phosphate groups required for high-affinity receptor binding[12].

The data presented here and previous results indicate that, compared to the HO-6, HO-2 and HO-3 of Ins(1,4,5)P_3 have a relatively insignificant direct contribution to receptor interaction and Ca^{2+} release. However, the substituent status of the 3 position of Ins(1,4,5)P_3 may be the major regulatory mechanism for binding to the Ins(1,4,5)P_3 receptor in vivo, since phosphorylation of Ins(1,4,5)P_3 by the

3-kinase, to produce Ins(1,3,4,5)P_4, results in effective exclusion from the Ins(1,4,5)P_3 receptor[55]. Further work is necessary in order to identify the structural features at position 3 which result in exclusion from the Ins(1,4,5)P_3 receptor and concomitantly the structural transitions required for the development of effective interaction with the Ins(1,3,4,5)P_4 receptor.

ACKNOWLEDGMENTS

This work was supported by the S.E.R.C. Molecular Recognition Initiative. R.A.W. was a British Council A.L.I.S scholar, and B.V.L.P. is a Lister Institute Fellow. We thank the University of Leicester for a Research Scholarship to D.A.S.

REFERENCES

1 M.J. Berridge and R.F. Irvine, *Nature (London)*, 341 (1989) 197–205.
2 C.D. Ferris, R.L. Huganir, S. Supattapone, and S.H. Snyder, *Nature (London)*, 342 (1989) 87–89.
3 T. Furuichi, S. Yoshikawa, A. Miyawaki, K. Wada, N. Maeda, and K. Mikoshiba, *Nature (London)*, 342 (1989) 32–38.
4 N. Maeda, M. Niinobe, and K. Mikoshiba, *EMBO J.*, 9 (1990) 61–68.
5 S. Supattapone, P.F. Worley, J.M. Baraban, and S.H. Snyder, *J. Biol. Chem.*, 263 (1988) 1530–1534.
6 D.C. Billington, *Chem. Soc. Rev.*, 18 (1989) 83–122.
7 B.V.L. Potter, *Nat. Prod. Rept.*, 7 (1990) 1–24.
8 M.J. Berridge and R.F. Irvine, *Nature (London)*, 312 (1984) 315–321.
9 M.A. Polokoff, G.H. Bencen, J.P. Vacca, J. de Solm, S.D. Young, and J.R. Huff, *J. Biol. Chem.*, 263 (1988) 11922–11927.
10 M. Hirata, Y. Watanabe, T. Ishimatsu, T. Ikebe, Y. Kimura, K. Yamaguchi, S. Ozaki, and T. Koga, *J. Biol. Chem.*, 264 (1989) 20303–20308.
11 M.J. Seewald, I.A. Aksoy, G. Powis, A.H. Fauq, and A.P. Kozikowski, *J. Chem. Soc., Chem. Commun.*, (1990) 1638–1639.
12 S.T. Safrany, R.J.H. Wojcikiewicz, J. Strupish, S.R. Nahorski, D. Dubreuil, J. Cleophax, S.D. Gero, and B.V.L. Potter, *FEBS Lett.*, 278 (1991) 252–256.
13 S.R. Nahorski and B.V.L. Potter, *Trends Pharmacol. Sci.*, 10 (1989) 139–143.
14 M. Schlosser, *Tetrahedron*, 34 (1978) 3–17.
15 P.J. Card, *J. Carbohydr. Chem.*, 4 (1985) 451–487.
16 A.P. Kozikowski, A.H. Fauq, I.A. Aksoy, M.J. Seewald, and G. Powis, *J. Am. Chem. Soc.*, 112 (1990) 7403–7404.
17 A.P. Kozikowski, A.H. Fauq, and J.M. Rusnak, *Tetrahedron Lett.*, 30 (1989) 3365–3368.
18 A.P. Kozikowski, A.H. Fauq, G. Powis, and D.C. Melder, *J. Am. Chem. Soc.*, 112 (1990) 4528–4531.
19 J.D. Moyer, O. Reizes, A. Surender, C. Jiang, N. Malinowski, and D.C. Baker, *Mol. Pharmacol.*, 33 (1988) 683–689.
20 J.D. Moyer, O. Reizes, N. Malinowski, C. Jiang, and D.C. Baker, in N.F. Taylor (Ed.), *Fluorinated Carbohydrates, Chemical and Biochemical Aspects*, Am. Chem Soc., Washington, DC, 1988, pp 43–58.
21 J.L. Offer, J.C. Metcalfe, and G.A. Smith, *J. Chem. Soc., Chem. Commun.*, (1990) 1312–1313.
22 G. Lowe and F. McPhee, *J. Chem. Soc., Perkin. Trans. 1*, (1991) 1249–1253.
23 S.V. Ley, M. Parra, A.J. Redgrave, F. Sternfeld, and A. Vidal, *Tetrahedron Lett.*, 30 (1989) 3557–3560.
24 J.F. Maracek and G.D. Prestwich, *Tetrahedron Lett.*, 30 (1989) 5401–5404.
25 S.T. Safrany, D. Sawyer, R.J.H. Wojcikiewicz, S.R. Nahorski, and B.V.L. Potter, *FEBS Lett.*, 276 (1990) 91–94.
26 D. Sawyer and B.V.L. Potter, *J. Chem. Soc., Perkin. Trans. 1*, (1992) 923–932.

27 C. Liu, S.R. Nahorski, and B.V.L. Potter, *J. Chem. Soc., Chem. Commun.*, (1991) 1014–1016.
28 C. Liu, S.R. Nahorski, and B.V.L. Potter, unpublished work.
29 E.A. Kunysz, A.D. Michel, R.L. Whiting, and K. Woods, *Br. J. Pharmacol.*, 96 (1989) 271–278.
30 P.M. McDonough, J.H. Eubanks, and J.H. Brown, *Biochem. J.*, 249 (1988) 135–141.
31 P.M. McDonough and D.C. Button, *Cell Calcium*, 10 (1989) 171–180.
32 M.J. Berridge, *Annu. Rev. Biochem.*, 56 (1987) 159–193.
33 S.R. Nahorski, *Trends Neurosci.*, 11 (1988) 444–448.
34 A.L. Willcocks, A.M. Cooke, B.V.L. Potter, and S.R. Nahorski, *Biochem. Biophys. Res. Commun.*, 146 (1987) 1071–1078.
35 P.F. Worley, J.M. Baraban, S. Supattapone, V.S. Wilson, and S.H. Snyder, *J. Biol. Chem.*, 262 (1987) 12132–12136.
36 A.J. Baukal, G. Guillemette, R.P. Rubin, A. Spät, and K.J. Catt, *Biochem. Biophys. Res. Commun.*, 133 (1985) 532–538.
37 G. Guillemette, T. Balla, A.J. Baukal, A. Spät, and K.J. Catt, *J. Biol. Chem.*, 262 (1987) 1010–1015.
38 S. Palmer, K.T. Hughes, D.Y. Lee, and M.J. Wakelam, *Cell Signalling*, 1 (1989) 147–156.
39 S. Palmer and M.J. Wakelam, *Biochem. J.*, 260 (1989) 593–596.
40 A.L. Willcocks, R.A.J. Challiss, and S.R. Nahorski, *Eur. J. Pharmacol.*, 189 (1990) 185–193.
41 S.T. Safrany, D.A. Sawyer, S.R. Nahorski, and B.V.L. Potter, *Chirality*, (1992) submitted.
42 C. Liu, S.R. Nahorski, and B.V.L. Potter, *Carbohydr. Res.*, 234 (1992) 107–115.
43 R.A.J. Challiss, E.R. Chilvers, A.L. Willcocks, and S.R. Nahorski, *Biochem. J.*, 265 (1990) 421–427.
44 J. Strupish, A.M. Cooke, B.V.L. Potter, R. Gigg, and S.R. Nahorski, *Biochem. J.*, 253 (1988) 901–905.
45 R.J.H. Wojcikiewicz, D.G. Lambert, and S.R. Nahorski, *J. Neurochem.*, 54 (1990) 676–685.
46 D.L. Clapper and H.C. Lee, *J. Biol. Chem.*, 260 (1985) 13947–13954.
47 G.A. McPherson, *Computer Prog. Biomed.*, 17 (1983) 107–114.
48 P.J. Munson and D. Rodbard, *Anal. Biochem.*, 107 (1980) 220–239.
49 D.L. Nunn and C.W. Taylor, *Biochem. J.*, 270 (1990) 227–232.
50 D.J. Gawler, B.V.L. Potter, R. Gigg, and S.R. Nahorski, *Biochem. J.*, 276 (1991) 163–167.
51 M. Hirata, F. Yanaga, T. Koga, T. Ogasawara, Y. Watanabe, and S. Ozaki, *J. Biol. Chem.*, 265 (1990) 8404–8407.
52 S.T. Safrany, R.J.H. Wojcikiewicz, J. Strupish, J. McBain, A.M. Cooke, B.V.L. Potter, and S.R. Nahorski, *Mol. Pharmacol.*, 39 (1991) 754–761.
53 S.T. Safrany, R.A. Wilcox, C. Liu, B.V.L. Potter, and S.R. Nahorski, *Eur. J. Pharmacol. (Molecular Section)*, 226 (1992) 265–272.
54 C. Schultz, G. Gebauer, T. Metschies, L. Rensing, and B. Jastorff, *Biochem. Biophys. Res. Commun.*, 166 (1990) 1319–1327.
55 R.A.J. Challiss, A.L. Willcocks, B. Mulloy, B.V.L. Potter, and S.R. Nahorski, *Biochem. J.*, 274 (1991) 861–867.