5-phosphatases show a similar pattern on SDS/PAGE, in agreement with kinetic behaviour previously determined for both isoforms of the enzyme [3].

Frédéric HOLLANDE, Benoît VERJANS and
Christophe ERNEUX

Institute of Interdisciplinary Research, Free University of Brussels, Campus Erasme, Bâtiment C, 808 route de Lennik, B-1070 Brussels, Belgium

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Is decavanadate a specific inositol 1,4,5-trisphosphate receptor antagonist?

It is now well established that inositol 1,4,5-trisphosphate (InsP_3) interacts with a specific intracellular receptor to mobilize Ca^{2+} and plays a crucial rôle in signalling initiated by a variety of cell-surface receptors [1]. The purification and sequencing of the InsP_3 receptor [2,3], and the characterization of the structural elements of inositol polyphosphates required for receptor binding and opening of associated Ca^{2+} channels, have begun to provide leads for potential pharamacological tools that might manipulate Ca^{2+} homeostasis [4]. In particular, an InsP_3 receptor antagonist would be useful in allowing dissection of the primary rôle of InsP_3 from other associated events, such as Ca^{2+} entry.

The polysulphated polysaccharide heparin has been established as an InsP_3 receptor antagonist [5–7]. However, heparin also inhibits InsP_3 3-kinase activity [7], the specific binding of inositol 1,3,4,5-tetrakisphosphate (InsP_4) to cerebellar membranes [8] and the ability of InsP_4 to release Ca^{2+} from cerebellar microsomes [9].

More recently, Föhr et al. (1989) have shown that the poly-oxoanion decavanadate inhibits (IC$_{50}$ 5 μM) InsP_3-induced Ca^{2+} mobilization in permeabilized rat insulinoma and PC12 cells [10]. Thus we evaluated the potential antagonist properties of decavanadate in a number of experimental systems. In agreement with previous work [10] we find that decavanadate competitively antagonizes InsP_3-induced Ca^{2+} mobilization from permeabilized SH-SY5Y human neuroblastoma cells (K_i 1.2 μM). In addition, we find that this polyoxoanion inhibits the specific binding of [^{3}H]InsP_3 to its receptor in cerebellar and adrenal cortical membranes with K_{50} values (concentration of decanavadate inhibiting InsP_3 binding by 50%, corrected for the mass of competing radioligand) of 2.6$\pm$0.6 μM and 2.2$\pm$0.4 μM respectively. However, although this ability to bind to the InsP_3 receptor was not shared by orthovanadate, at concentrations up to 100 μM, the specificity of decavanadate was poor, since the polyoxoanion also competed with [^{3}H]InsP_4 binding to positionally-specific sites in cerebellum [8] (K_{50} 4.5$\pm$0.4 μM).

Furthermore decavanadate can also suppress InsP_4-induced Ca^{2+} release from permeabilized SH-SY5Y cells (D. J. Gawler & S. R. Nahorski, unpublished work). Finally, decavanadate also interacts with and inhibits human erythrocyte ghost InsP_3 5-phosphatase, (K_i 1.5$\pm$0.5 μM) rat cerebral InsP_3 3-kinase (K_i 5.0$\pm$1.7 μM) and SH-SY5Y cell InsP_4 5-phosphatase (K_i 0.6$\pm$0.2 μM).

Unfortunately therefore, although decavanadate is a potent and competitive antagonist at the InsP_3 receptor, its specificity is low. It also binds to all known recognition sites for InsP_3 and probably InsP_4. This may relate to the ability of decavanadate to place charged oxygen atoms of vanadate octahedra at sites potentially occupied by the 4,5 vicinal phosphate pair of InsP_3 and InsP_4. It would seem from these observations that decavanadate will not be as useful as first envisaged [10] as a tool to investigate the second messenger rôle of InsP_3.

James STRUPISH,* Richard J. H. WOJCIKIEWICZ,*
R. A. John CHALLISS,* Stephen T. SAFRANY,*
Alan L. WILLCOCKS,* Barry V. L. POTTER† and
Stefan R. NAHORSKI*

* Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester LE1 9HN, and
† School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

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Distinction between endo-oligopeptidase A (EC 3.4.22.19) and soluble metalloendopeptidase (EC 3.4.24.15)

These comments arose after reading the article of Barrett & Brown (1990) in which they conclude that endo-oligopeptidase A, metalloendopeptidase and Pz-peptidase activities are due to a single enzyme. Other experimental data concerning the features of these three endopeptidase activities do not support this hypothesis. A number of significant properties of brain endo-oligopeptidase A obtained since this enzyme was first described (Camargo et al., 1973) can be used to distinguish this enzyme from metalloendopeptidases. However, the main argument against Barrett and Brown's hypothesis is that endo-oligopeptidase A can be selectively separated from endopeptidase 24.15. This was performed in an enzyme preparation containing both activities by immunoprecipitation using a polyclonal antibody against endo-oligopeptidase A. This procedure did not affect the soluble metalloendopeptidase activity which remains in the supernatant (Toffoletto et al., 1988). Alternatively, a brain cytosolic endo-oligopeptidase A preparation lacking soluble metalloendopeptidase activity can be obtained by DEAE-Sepharose chromatography as illustrated by fraction A in Fig. 1. Differences in M_r and electrical charge of endo-oligopeptidase A