DOES *MYO*-INOSITOL SPECIFICALLY INTERACT WITH BRAIN MICROTUBULES?

M. R. PICKARD and J. N. HAWTHORNE

Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, England

Received 26 June 1978

1. Introduction

Evidence that chick brain microtubule-associated phospholipids, particularly phosphatidylinositol, were rapidly labelled with ^{32}P in vivo [1] suggested a functional association between microtubules and neuronal membranes [2]. *myo*-Inositol, 20–100 mM, significantly reduced the temperature-dependent assembly and disassembly of rat brain microtubules in vitro [3] and it was suggested that intracellular free inositol, part of which may arise from the hydrolysis of phosphatidylinositol, may directly influence the functional state of microtubules, and hence regulate various cell activities. Although it was stated [3] that the ratio of 100 μmol inositol/mg tubulin that they required to obtain an effect was of the same order as that found in vivo, normal intracellular levels, calculated from a free inositol concentration in rat brain of 6–7 μmol/g wet wt [4] and a tubulin content of ~10% total soluble protein [5], which accounts for ~2% wet wt [6], are considerably less, ~3 μmol inositol/mg tubulin.

The initial products of phosphatidylinositol hydrolysis, which occurs in a variety of cell types in response to a number of stimuli [7], are inositol-1-phosphate and inositol-1,2-cyclic phosphate. These compounds are therefore more likely candidates for a 'messenger' role and might affect microtubule formation at a lower concentration than inositol.

In a series of experiments testing this hypothesis it was found that 1 mM inositol-1,2-cyclic phosphate had little effect on microtubule formation and that a wide variety of compounds, at 100 mM, affected microtubule polymerization in a similar manner to inositol. Binding studies, with *myo*-[2-^{3}H]inositol, also indicated that inositol binding to tubulin was attributable to non-specific interactions.

2. Materials and methods

Inositol-2-phosphate was prepared from sodium phytate by the method in [8] and inositol-1,2-cyclic phosphate was prepared from this product by the method in [9]. Both compounds were identified and checked for purity by descending paper chromatography in either propan-2-ol : 6 M NH_3 (7:3, v/v) or ethanol : 13.5 M NH_3 (3:2, v/v) [10].

Microtubular protein was isolated by one cycle of assembly/disassembly from adult sheep brain by the procedure in [11] using MES (2-(*N*-morpholino)-ethanesulphonic acid) in the buffer solution as in [3]. In some experiments, indicated in the text, brains from 6 week rats were used. Microtubule polymerization was assayed by a turbidimetric procedure [12] at 1 mg/ml protein incubated in the presence or absence of test compound added in the cold at zero time as in [3].

Binding of *myo*-[2-^{3}H]inositol to microtubular protein was measured using the filtration assay [13]. Non-specific binding of the ligand to the DEAE-cellulose filter paper discs was measured by filtering the complete assay mixture, minus protein; this value was subtracted from the results obtained when tubulin was present. All assays were done in quadruplicate and the results averaged to give the reported values.

3. Results and discussion

It was found that inositol decreased the rates of both assembly and disassembly of microtubular

Elsevier/North-Holland Biomedical Press

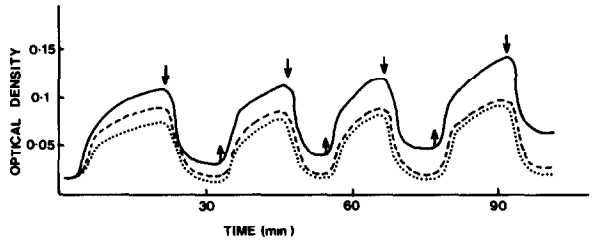

Fig.1. Turbidity changes during successive cycles of assembly/disassembly of sheep brain microtubular protein in the absence (——) and presence of either 250 mM glucose (– –) or 250 mM *myo*-inositol (· · · ·) added in the cold at zero time. Turbidity changes were monitored simultaneously. Arrows indicate a temperature increase to 37°C (↑) or decrease (↓) to 2°C.

protein from sheep brain (fig.1) and acted in a concentration-dependent manner with a maximum effect at ~200 mM (fig.2); cf. [3] for tubulin from rat brain. This experiment was repeated 3 times with microtubular protein isolated from both sheep brain and rat brain in order that a direct comparison could be made. There was no significant species difference.

However, very similar results could be obtained with 200 mM glucose (fig.1,2) and sorbitol (fig.2) and although certain phosphorylated compounds, sodium phytate, inositol-2-phosphate and inositol-1,2-cyclic phosphate, produced a maximum effect at 1 μmol/mg

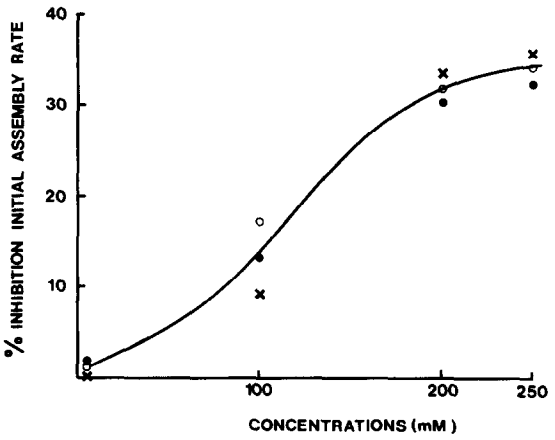

Fig.2. Effect of inositol (○), sorbitol (×) and glucose (●) on initial rates of assembly of sheep brain microtubular protein. Initial rates are expressed in arbitrary units calculated from the initial maximum slope of the polymerization curve obtained with tubulin only. Protein concentration was 1 mg/ml.

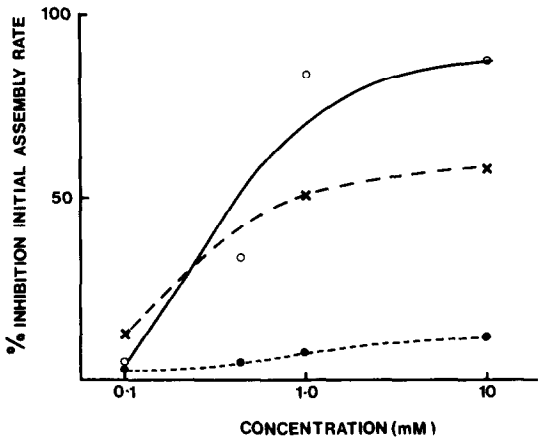

Fig.3. Effect of sodium phytate (○), inositol-2-phosphate (×) and inositol-1,2-cyclic phosphate (●) on initial rate of assembly of sheep brain microtubular protein. For details see fig.2 legend.

tubulin (fig.3) this value is too high to assign a physiological role. The concentration of inositol-1,2-cyclic phosphate in rat brain has been estimated as 90 nmol/g tissue [14] i.e., ~45 nmol/mg tubulin, and sodium phytate, the most potent compound tested, is not normally present in mammalian cells.

The ability of microtubular protein subunits to form microtubules is extremely sensitive to environmental conditions [15] and in view of the fact that sucrose is also known to stabilize microtubules [16] and that glycerol probably exerts its stabilizing effect by altering the water structure in the vicinity of the protein [15] it is likely that the effect of inositol, and the other compounds tested is due to a similar, non-specific, interaction altering the subunit–polymer equilibrium.

However, it has been shown that inositol readily binds to assembly-competent tubulin oligomers [3], although only one inositol concentration was used. This is not enough to demonstrate specific binding and in an attempt to establish this, the binding of *myo*-[2-^{3}H]inositol to microtubular protein at different inositol concentrations was measured at 37°C and 0°C in the presence and absence of GTP (fig.4). If the binding of a radiolabelled compound to a fraction represents specific binding then a component of that binding should saturate with increasing concentrations of ligand and as can be seen in fig.4, no such satu-

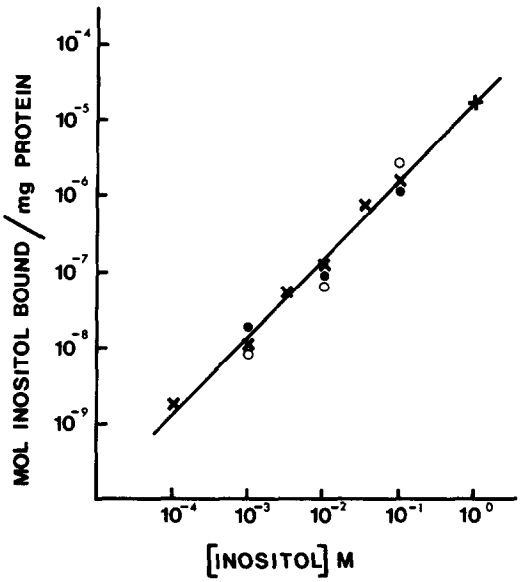

Fig.4. Binding of *myo*-[2-^{3}H]inositol to sheep brain microtubular protein at 37°C (×), 0°C (○) and in the presence of 1 mM GTP (●). For details see text.

rability was observed. The straight line with a slope very close to one (1.08) indicates that the binding of inositol to tubulin is entirely due to non-specific interactions.

It can be concluded therefore that although inositol directly affects the subunit–polymer equilibrium, probably by promoting a pool of 'stabilized' microtubular protein [3], the fact that a variety of other compounds have a similar effect and that specific binding of inositol to microtubular protein cannot be demonstrated, indicates that this is a non-specific effect of little evident physiological relevance.

Acknowledgements

The authors wish to thank Glen Dooley for technical assistance. M.R.P. thanks the Medical Research Council for the award of a training fellowship.

References

[1] Kirazov, E. P. and Lagnado, J. R. (1976) Biochem. Soc. Trans. 4, 734.
[2] Lagnado, J. R. and Kirazov, E. P. (1975) in: Microtubules and microtubule inhibitors (Borgers, M. and De Brabander, M. eds) pp. 127–140, North-Holland, Amsterdam.
[3] Kirazov, E. P. and Lagnado, J. R. (1977) FEBS Lett. 8, 173–178.
[4] Palmano, K. P., Whiting, P. H. and Hawthorne, J. N. (1977) Biochem. J. 167, 229–235.
[5] Johnson, K. A. and Borisy, G. G. (1975) in: Molecules and cell movement (Inoue, S. and Stephens, R. E. eds) pp. 119–141, Raven Press, New York.
[6] Ansell, G. B. (1961) Biochemists Handbook (Long, C. ed) p. 652, Spon, London.
[7] Michell, R. H. (1975) Biochim. Biophys. Acta 415, 81–147.
[8] Desjobert, A. (1954) Bull. Ste. Chim. Biol. 36, 1293–1299.
[9] Pizer, F. L. and Ballou, C. E. (1959) J. Am. Chem. Soc. 81, 915–921.
[10] Dawson, R. M. C. and Clarke, N. (1972) Biochem. J. 127, 113–118.
[11] Borisy, G. G., Marcum, G. M., Olmsted, J. B., Murphy, D. B. and Johnson, K. A. (1975) Ann. NY Acad. Sci. 253, 771–779.
[12] Gaskin, F. and Cantor, C. R. (1974) J. Mol. Biol. 89, 737–758.
[13] Weisenberg, R. G., Borisy, G. and Taylor, E. (1968) Biochemistry 7, 4466–4479.
[14] Majumder, A. L. and Eisenberg, F. (1974) Biochem. Biophys. Res. Commun. 60, 133–139.
[15] Gaskin, F. (1976) in: Essays in Biochemistry (Campbell, P. N. and Aldrige, W. eds) vol. 12, pp. 115–147, Academic Press, London.
[16] Frigon, R. P. and Lee, J. C. (1972) Arch. Biochem. Biophys. 153, 587–589.