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Quantitative analysis of inositol lipids and inositol phosphates in synaptosomes and microvessels by column chromatography: comparison of the mass analysis and the radiolabelling methods

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ABSTRACT

Chromatographic methods that measure both the mass and the radiolabelling of various inositol lipids and inositol phosphates in tissues have been developed. The mass of phosphatidylinositol (PtdIns), phosphatidylinositol-4-monophosphate [PtdIns(4)P] and phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] was quantitated by measuring the inorganic phosphate, whereas inositol monophosphate (IP), inositol bisphosphate (IP_2), inositol trisphosphate (IP_3) and inositol tetrakisphosphate (IP_4) were quantitated by using an enzymic method. The radiolabelling of various inositol lipids and inositol phosphates was determined by incubating the tissue samples with [^{3}H]myo-inositol, separating individual inositol lipids and inositol phosphates, and measuring the radioactivity in each compound. Although the mass analysis method was sensitive enough to measure low levels of inositol lipids or inositol phosphates, the method was laborious and time-consuming. Compared with the enzymic method, the radiolabelling method was simple and fast, but it gave variable results. This study demonstrated differences in inositol lipid and inositol phosphate levels by radiolabelling and mass measurements, and agonist-stimulated phosphatidylinositol turnover of synaptosomes *versus* the blood–brain barrier as represented by microvessels. Although the mass of PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 was comparable in synaptomsomes and microvessels, the incorporation of [^{3}H]myo-inositol into phosphorylated PtdIns in microvessels was less than that in synaptosomes.

INTRODUCTION

Phosphatidylinositol (PtdIns) serves as an anchor for various membrane-bound enzymes and other proteins, and as the precursor for phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] which undergoes a receptor-mediated hydrolysis to release various myo-inositol (MI) phosphates [1,2]. Previous studies have shown that the agonists of cholinergic [3], serotoninergic [4], noradrenergic [5,6], and adenosine/guanine [7] receptors present in various neuronal and non-neuronal cells stimulate the hydrolysis of PtdIns(4,5)-P_2. Because inositol trisphosphate (IP_3) and inositol tetrakisphosphate (IP_4) regulate the level of intracellular Ca^{2+} in neural cells [8], alterations in the metabolism of PtdIns(4,5)P_2 may also modulate the Ca^{2+}-dependent physiological processes, such as neurotransmission, endocrine functions and growth [9].

The receptor-mediated mobilization of inositol phosphates is commonly studied by labelling PtdIns with [^{3}H]MI and quantitating the formation of ^{3}H-labelled inositol phosphates. Several

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recent studies have indicated that the radiolabelling method has many disadvantages, such as (1) it does not measure the true intracellular level of the inositol phosphates, and (2) [^{3}H]MI must be labelled to an isotopic steady-state that is difficult to achieve [10–13]. The observations that IP_3 may not be the sole source of IP_2 [14,15] and that depolarization may influence the receptor-mediated release of inositol phosphates from PtdIns-(4,5)P_2 in neural cells [16] complicate the interpretation of the radiolabelling data. Recently, Challiss and Nahorski [17] reported that mass measurement provided a more reliable estimate of the mobilization of PtdIns(4,5)P_2 in tissues. Therefore, it may be important to measure both the mass and the radiolabelling of inositol lipids and inositol phosphates to understand the overall mechanism of cell signaling.

Chromatographic techniques such as thin-layer chromatography (TLC) [18,19], neomycin affinity column chromatography [20,21], high-performance liquid chromatography (HPLC) [22–25] and anion-exchange chromatography [26,27] are available for the analysis of various inositol phospholipids and inositol phosphates. However, the methods are not suitable for the simultaneous measurement of the mass and radiolabelling of individual compounds.

The aim of the present investigation was to develop chromatographic methods that determined both the mass and the radiolabelling of various inositol lipids and inositol phosphates in tissues. The performances of mass analysis and radiolabelling methods in studying the mobilization of inositol phosphates were also compared.

EXPERIMENTAL

Materials

PtdIns, phosphatidylinositol-4-monophosphate [PtdIns(4)P], PtdIns(4,5)P_2, inositol phosphate (IP), inositol disphosphate (IP_2), IP_3, IP_4, myo-inositol dehydrogenase (IDH), scyllo-inosose, diphorase, β-NAD, hexokinase, alkaline phosphatase, resazurin, triethylammonium bicarbonate (TEAB), imidazole, bovine serum albumin (BSA), ATP, norepinephrine (NE), and carbamylcholine (CA) were obtained from Sigma (St. Louis, MO, USA). Sep-Pak Accel Plus QMA columns (3 ml containing 500 mg of packed material, Cat. No. 20815) were obtained from the Millipore (Bedford, MA, USA). The ^{3}H-labelled MI was obtained from Amerisam (Chicago, IL, USA). Resazurin was purified by TLC as described by Maslanski and Busa [28].

Loading of cells with [^{3}H]MI

Male rats (n = 100) weighing *ca.* 150 g were sacrificed by decapitation, and each brain was immediately removed and placed in 0.32 *M* sucrose solution at 4°C and processed for the isolation of synaptosomes (n = 50) and microvessels (n = 50) as described previously [29,30]. Samples from ten rats were pooled to achieve n = 5. The pooled samples were resuspended in Ringer buffer and stored at 4°C. For loading the samples with [^{3}H]MI, an aliquot (100 μg of protein) of each pooled sample was incubated for 60 min at 37°C with 0.5 ml of Ringer buffer [bubbled with oxygen–carbon dioxide (95:5)] and 0.75 μM [^{3}H]MI (15 Ci/mmol) and 10 m*M* LiCl. After incubation, the reaction medium was centrifuged (30 000 *g* for synaptosomal samples and 58 000 *g* with sucrose gradient for microvessels as described by Shimon *et al.* [29]) at 4°C for 60 min. The supernatant was discarded and the pellets (which contain synaptosomes or microvessels) were resuspended in Ringer buffer. The radioactivity level in each sample was determined as described previously [31].

Analysis of PtdIns, PtdIns(4)P and PtdIns(4,5)-P_2

The [^{3}H]MI-loaded synaptosomes and microvessels were extracted by using the method described by Folch *et al.* [32]. Fraction I was collected and further extracted to separate phospholipids from glycolipids as described by Dugan *et al.* [33]. The phospholipid fraction was subjected to DEAE-cellulose chromatography (separation sequence 4) as described by Rouser *et al.* [34] to purify further the inositol lipids. The purified extract was transferred to a Sep-Pak cartridge. Various inositol lipids were eluted from the column

with a linear gradient of ammonium acetate prepared by using two Beckman solvent pumps and a solvent programmer (Model 126). The solvent controller was programmed to produce a linear gradient of ammonium acetate and water from 0.5% of ammonium acetate (200 m*M*) and 99.5% of water to 75% of ammonium acetate and 25% of water in 60 min. The flow-rates from the pump and the column were adjusted to 0.5 ml/min. The column eluate for 120 min was collected in 1-ml fractions. Each fraction was divided into two aliquots: one was assayed for organic phosphorus [35] and the other was used for radioactivity determination. Different peaks were identified by using PtdIns, PtdIns(4)P and PtdIns-(4,5)P_2 standards. To check the purity of PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 peaks, the fractions containing individual inositol lipid were pooled and analysed by HPLC as described by Geurts van Kessel *et al.* [36]. Individual phospholipids were detected at 206 nm by using a diode-array detector programmed to scan from 200 to 350 nm. The UV absorption curve for each phospholipid was determined and compared with the UV absorption curve of the respective standard.

Quantitative analysis of inositol phosphates

The [^{3}H]MI-loaded cells were incubated with Ringer buffer and LiCl (10 m*M*) in the presence or absence of CA (100 μM) or NE (100 μM). The incubation was stopped at 5 min after the agonist addition by adding 500 μl of perchloric acid (10%, v/v). Each sample was incubated for 20 min at 4°C and centrifuged at 30 000 *g* at 4°C for 10 min. The supernatant was collected and mixed with 50 μl of 10 m*M* EDTA and 10 *M* KOH to adjust the pH to greater than 7.0. The mixture was incubated for 20 min at 4°C and centrifuged at 30 000 *g* for 30 min. The supernatant was transferred to a Dowex-50W column, and MI was separated with other inositol phosphates as described by Maslanski and Busa [28]. The eluate containing inositol phosphates was concentrated and transferred to a Sep-Pak column. Individual inositol phophates were eluted by using a linear gradient of the increasing concentration of TEAB (1.0 *M*). The initial solvent proportion was 10% TEAB and 90% water. The percentage of TEAB was increased to 100% in 60 min by using a solvent programmer as described above.

The column eluate was collected for 60 min in 1.0-ml fractions. Each TEAB fraction was dried and various inositol phosphates were dephosphorylated by the addition of 50 μl of alkaline phosphatase [10 U in 0.1 *M* Tris buffer (pH 9.0) and $ZnCl_2$]. In our experience, IP took 1 h, IP_2 and IP_3 took 2 to 3 h, and IP_4 took *ca.* 5 h for complete dephosphorylation. The dephosphorylated samples were mixed with 5 μl of hexokinase (200 U/ml containing 50 m*M* Tris, 10 m*M* $MgCl_2$, 0.1 *M* ATP and 0.02% BSA, pH 9.0) and the mixture was incubated for 30 min. After incubation, each sample was mixed with 10 μl of 100 μM NAD and 5 μl of IDH (5 U of IDH were mixed with 1 ml of 10 m*M* phosphate buffer and 0.02% BSA at pH 6.8). The mixture was incubated at 37°C for 15 min. The reaction was stopped by adding 10 μl of 1.0 *M* HCl. The incubation mixture was mixed with 10 μl of 20 μM resazurin and 5 μl of diphorase. The mixture was stored in dark for 15 min, and the fluorescence was measured at an excitation wavelength of 565 nm and an emission wavelength of 585 nm, as described previously [28].

To determine the incorporation of [^{3}H]MI into various inositol phosphates, the final TEAB eluates were subjected to radioactivity determinations by using a scintillation counter. Although the enzymic method was sensitive enough to measure low levels of MI, IP, IP_2, IP_3 and IP_4 in synaptosomes and microvessels, the method was laborious and time-consuming. Compared with the enzymic method, the radiolabelling method was simpler and quicker. However, the radiolabelling method gave variable results.

Quantitation

Inositol lipids were quantitated by analysing various concentrations of individual lipid standards and constructing a standard curve by plotting the concentration of individual compounds added to a sample (x-axis) against the fluorescence value for each concentration (y-axis). The recovery was determined by extracting known

amounts of the lipids and determining the amount recovered. Inositol lipids were measured by extracting simultaneously blank, spiked standard and samples as described by Maslanski and Busa [28]. The precision was determined as described by Van Loenhout *et al.* [37]. Correlation coefficients were calculated as described by Lin *et al.* [38].

RESULTS AND DISCUSSION

PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 levels in synaptosomes and microvessels

Typical elution profiles for the mass and the radiolabelling of these phospholipids in synaptosomes and microvessels are shown in Figs. 1 and 2, respectively. The chromatographic conditions used in this study provided clear separation of PtdIns, PtdIns(4)P and PtdIns(4,5)P_2. Phosphatidylglycerol was not retained by the column and was eluted before phosphatidylcholine (peak 1 in Figs. 1 and 2). The PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 fractions were homogeneous because the UV absorption spectra for each PtdIns peak obtained from the sample was identical with the UV absorption spectra for the respective standards (Fig. 3–5). Alternative techniques for the quantitation of inositol lipids are TLC [18,19], HPLC [22–25] and neomycin affinity column chromatography [20,21]. The method described in this study was simpler and quicker than the TLC methods described by Sun [18] and Sun and Lin [19]. Although the neomycin column provided excellent separation of phospholipids, some studies have indicated that the method may not effectively separate the three inositol phospholipids [39,40].

Although the HPLC methods may be simpler than the chromatographic method described in this study, most HPLC methods do not effectively separate PtdIns, PtdIns(4)P and PtdIns(4,5)P_2.

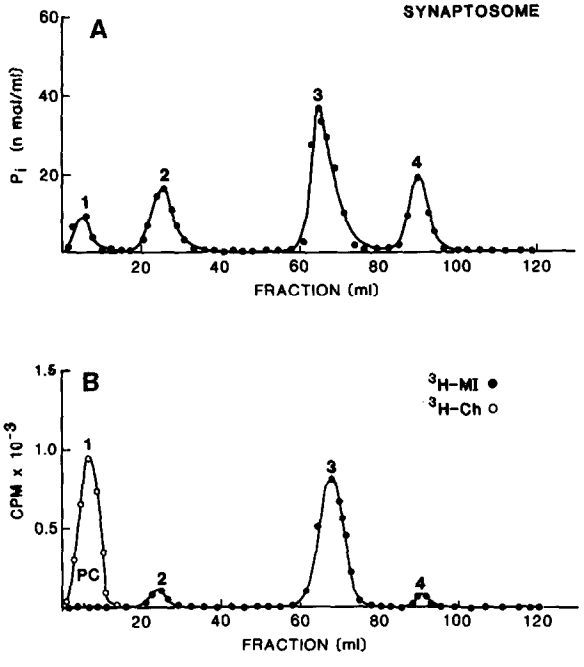

Fig. 1. Chromatographic separation and relative proportions of phosphatidylinositol (PtdIns) (peak 2), phosphatidylinositol-4-monophosphate [PtdIns(4)P] (peak 3) and phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] (peak 4) in synaptosomes. (A) Quantitation of various phospholipids by mass analysis method; (B) incorporation of [^{3}H]choline ([^{3}H]Ch) into PtdCh and incorporation of [^{3}H]MI into PtdIns, PtdIns(4)P and PtdIns (4,5)P_2, peaks 1 to 4, respectively.

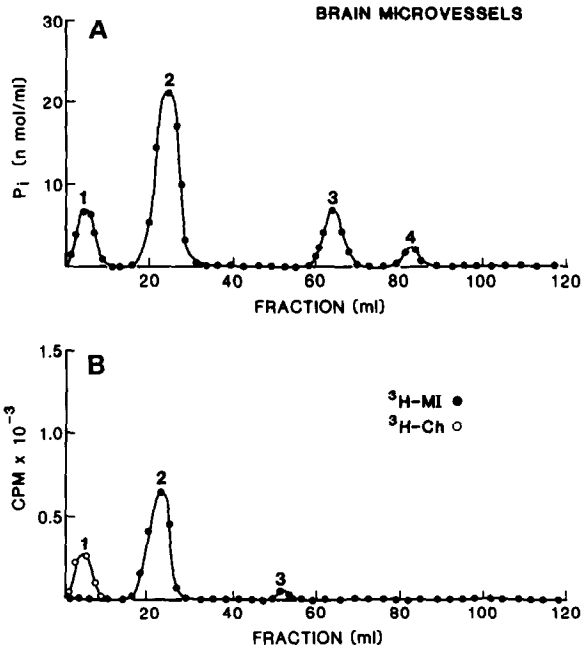

Fig. 2. Chromatographic separation, mass analysis and radiolabelling of PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 (peaks 2 to 4, respectively) in microvessels. (A) Quantitation by mass analysis; (B) radiolabelling in PtdCh, PtdIns, PtdIns(4)P and PtdIns(4,5)-P_2.

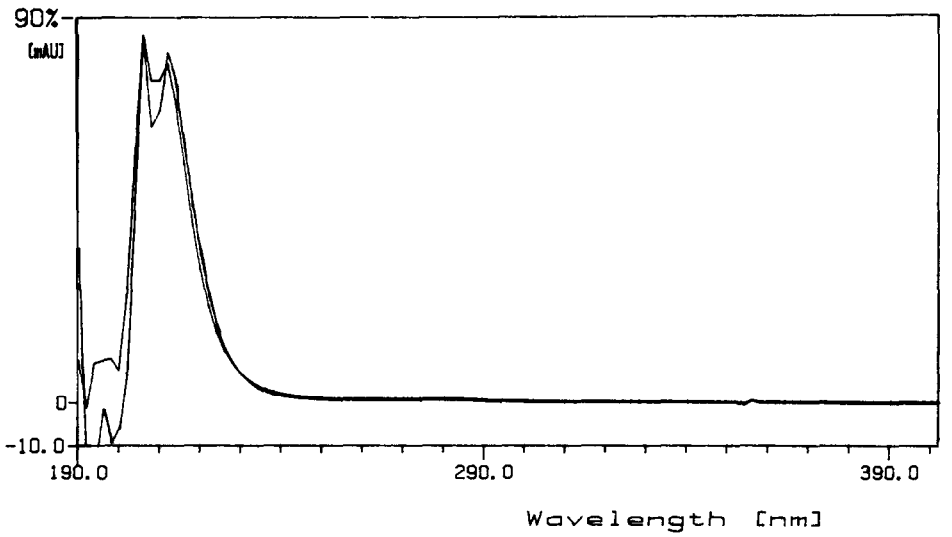

Fig. 3. Comparison of the UV absorption curves for a PtdIns standard and PtdIns extracted from a synaptosomal sample. PtdIns-containing samples were analysed by HPLC. The UV absorption curve was determined at the apex of the PtdIns peak.

Table I lists the linear regression parameters for the standard curves constructed for various inositol lipids. The recovery of PtdIns, PtdIns-(4)P and PtdIns(4,5)P_2 ranged from 70 to 80% when the phospholipid concentration was 100 or 500 pmol. However, the recovery was somewhat lower when the phospholipid concentration was 1.0 nmol (Table I). In synaptosomes, the mass profile of individual phospholipids was PtdIns < PtdIns(4,5)P_2 < PtdIns(4)P (Fig. 1A), whereas the radiolabelling profile was PtdIns(4,5)P_2 < PtdIns < PtdIns(4)P (Fig. 1B). However, in mi-

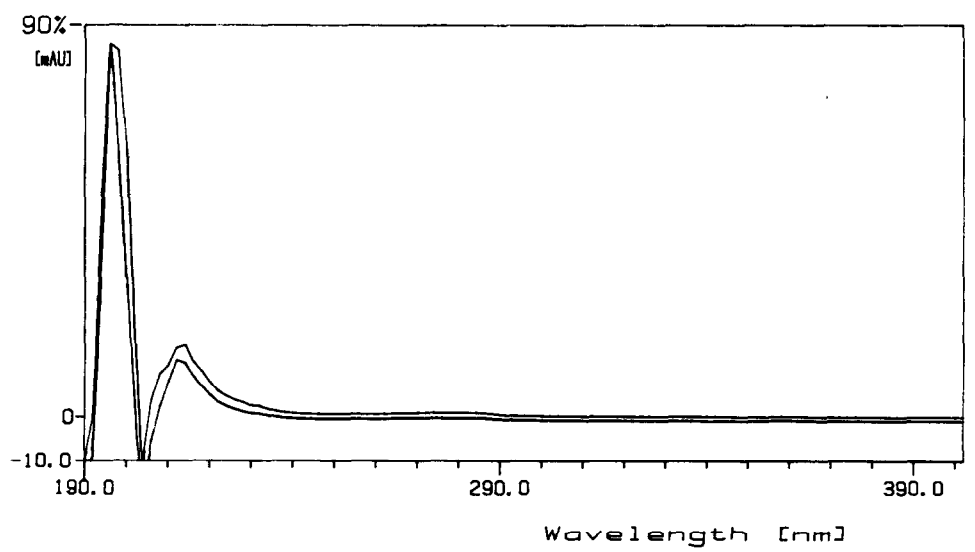

Fig. 4. Comparison of the UV absorption curves for a PtdIns(4)P standard and PtdIns(4)P extracted from a synaptosomal sample. PtdIns(4)P-containing samples were analysed by HPLC. The UV absorption curve was determined at the apex of the PtdIns(4)P peak.

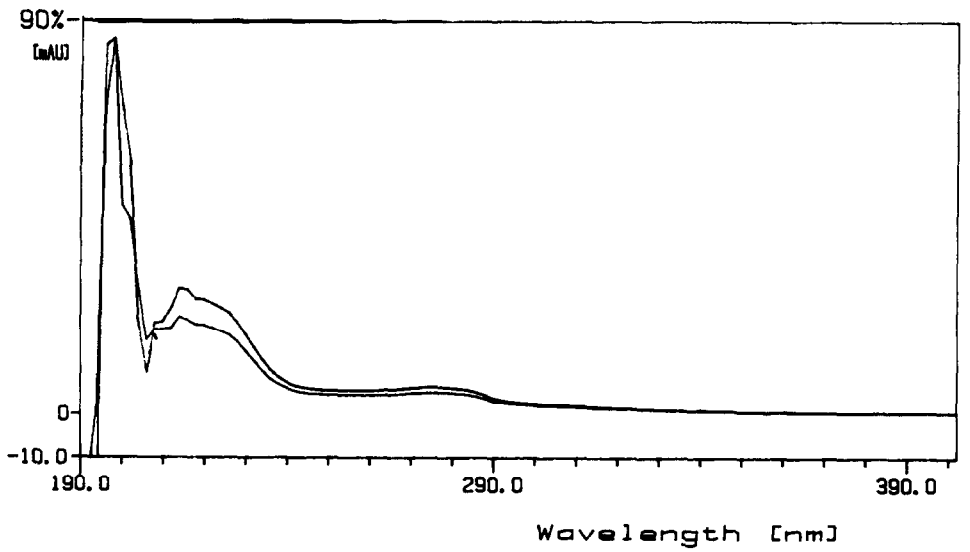

Fig. 5. Comparison of the UV absorption curves for a PtdIns(4,5)P_2 standards and PtdIns(4,5)P_2 extracted from a synaptosomal sample. PtdIns(4,5)P_2-containing samples were analysed by HPLC. The UV absorption curve was determined at the apex of the PtdIns(4,5)P_2 peak.

crovessels, the mass profile of various phospholipids was PtdIns(4,5)P_2 < PtdIns(4)P < PtdIns (Fig. 2A), whereas the radiolabelling profile was PtdIns(4)P < PtdIns (Fig. 2B). The PtdIns(4,5)-P_2 fraction did not incorporate [^{3}H]MI in microvessels. The relative levels of PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 in synaptosomes were different from those in microvessels. The PtdIns/PtdIns-(4)P ratio was 0.5 and 2.5 in synaptosomes and microvessels, respectively; the PtdIns/PtdIns(4,5)P_2 ratio in the two tissues was 1.0 and 7.5, respectively (Figs. 1 and 2). Although [^{3}H]PtdIns(4)P and [^{3}H]PtdIns(4,5)P_2 in synaptosomes accounted for more than 70% of the total radioactivity incorporated into phospholipids, [^{3}H]PtdIns(4)P and [^{3}H]PtdIns(4,5)P_2 in microvessels accounted for less than 5% of the total radioactivity incorporated into phospholipids (Figs. 1 and 2).

TABLE I

LINEAR REGRESSION PARAMETERS FOR THE STANDARD CURVES AND RECOVERY DATA FOR VARIOUS INOSITOL PHOSPHOLIPIDS AND INOSITOL PHOSPHATES

Values are mean ± S.D., n = 5.

Compound	Standard curve		Recovery		
	Slope	r	100 pmol	500 pmol	1 nmol
PtdIns	2.0 ± 0.1	0.9990	79 ± 5	430 ± 50	0.65 ± 0.1
PtdIns(4)P	1.3 ± 0.2	0.9998	69 ± 3	390 ± 40	0.60 ± 0.2
PtdIns(4,5)P_2	1.4 ± 0.1	0.9990	80 ± 4	410 ± 20	0.60 ± 0.3
IP	2.3 ± 0.1	9.9995	76 ± 8	436 ± 40	0.61 ± 0.1
IP_2	1.7 ± 0.05	0.9954	71 ± 3	390 ± 30	0.59 ± 0.2
IP_3	2.0 ± 0.1	0.9895	69 ± 2	360 ± 35	0.60 ± 0.1
IP_4	0.9 ± 0.05	0.9800	60 ± 3	310 ± 40	0.51 ± 0.2

Quantitation and mobilization of various inositol phosphates in synaptosomes and microvessels

The chromatographic method described in this study provided excellent separation of IP, IP_2, IP_3, and IP_4 in synaptosomes (Fig. 6) and microvessels (Fig. 7), and both the mass and radiolabelling of each inositol phosphate were determined. In previous studies, the separation of inositol phosphates was achieved by HPLC [41–43] or anion-exchange chromatography [26,27] and the mobilization or inositol phosphates was studied by measuring the radioactivity incorporation into each compound. Because the radiolabelling method may not accurately represent the total amount of inositol phosphates present in the sample, it is essential to measure the mass of the

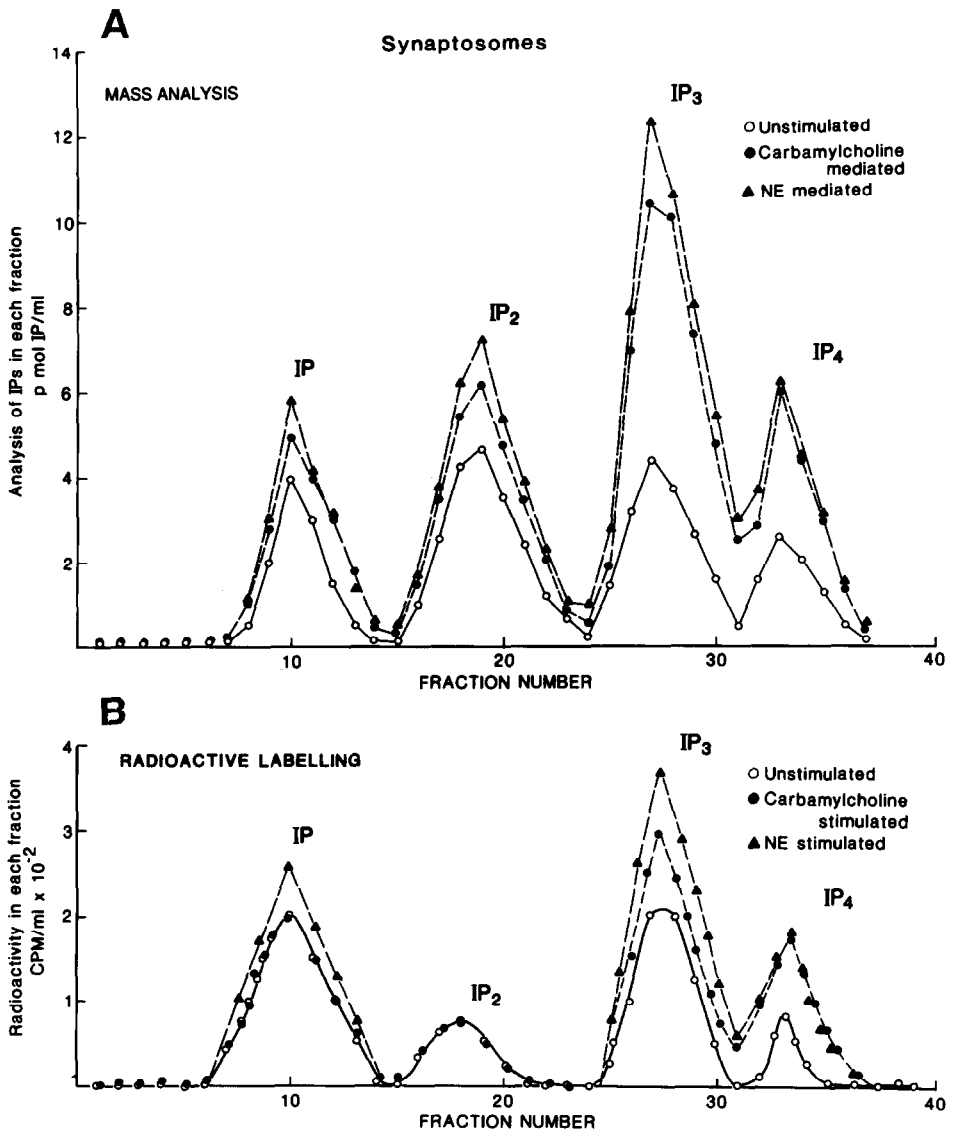

Fig. 6. Chromatographic separation and relative proportions of IP, IP_2, IP_3 and IP_4 in synaptosomes. (A) Quantitation of inositol phosphates by the mass analysis method; (B) mobilization of radiolabelled inositol phosphate in synaptosomes. (○) Unstimulated tissue; (●) stimulated with CA; (▲) stimulated with NE.

inositol phosphates. The methods that have been commonly used for the mass analysis of inositol phosphates are gas chromatography–mass spectrometry (GC–MS) [44], HPLC with post-column derivatization [45,46], HPLC with ion measurements [45] or enzyme fluorometry [46] and radioreceptor assay [47,48]. Although the GC–MS method is sensitive, the method is time-consuming, requires derivatization of the samples, and cannot be used for the analysis of heigher inositol phosphates. The HPLC method with post-column derivatization by yttrium and 4-(2-

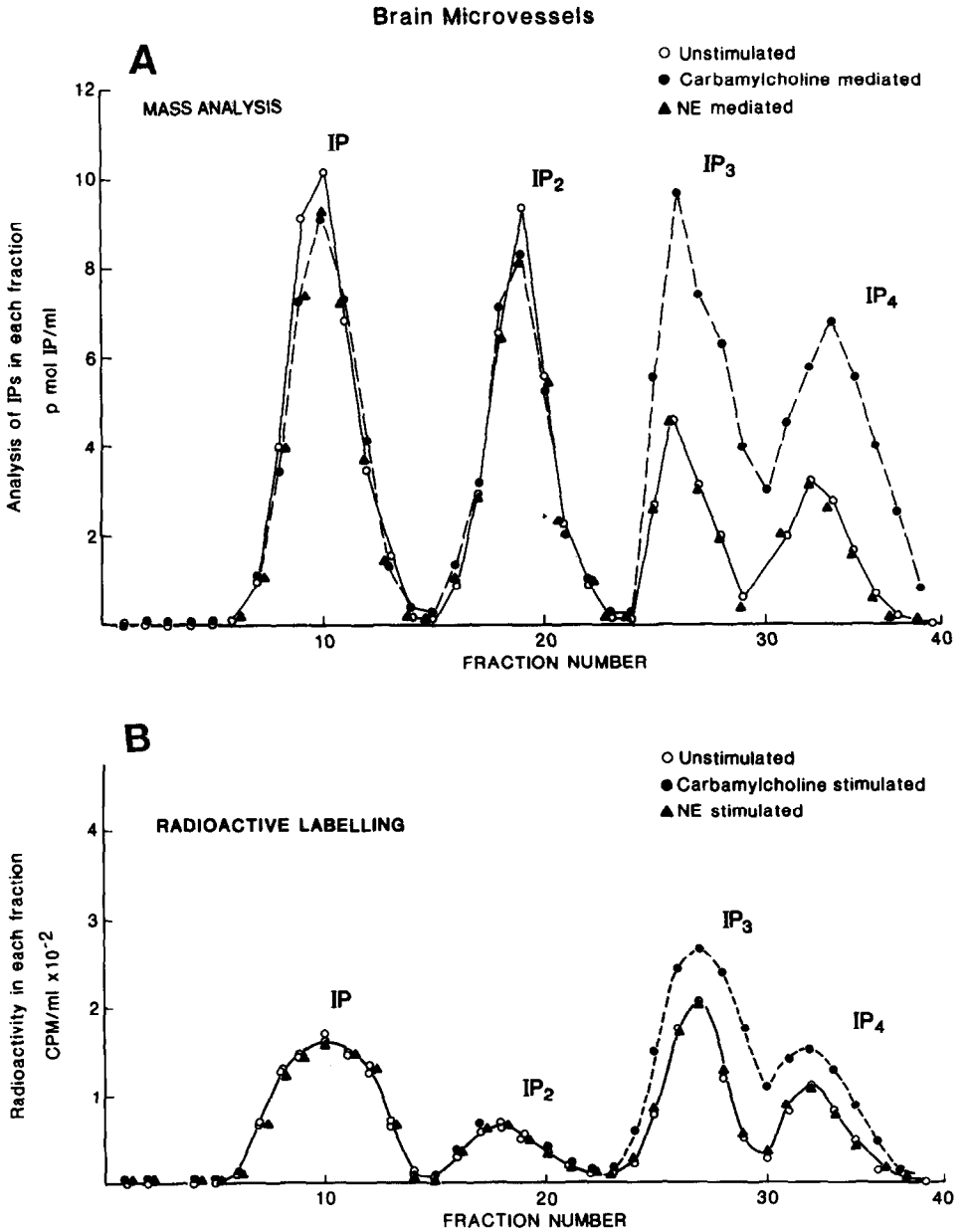

Fig. 7. Chromatographic separation and relative proportions of IP, IP_2, IP_3 and IP_4 in microvessels. (A) Quantitation of inositol phosphates by the mass analysis method; (B) mobilization of radiolabelled inositol phosphate in microvessels. (○) Unstimulated tissue, (●) stimulated with CA; (▲) stimulated with NE.

pyridylazo)resorcinol permits the quantitation of inositol phosphates in picomole range [49,50]. However, the HPLC method requires expensive equipment and may not be suitable for radioactivity determinations. The radioreceptor method specifically quantitates IP_3 or IP_4 and, therefore, the levels of other inositol phosphates cannot be determined. The chromatographic method developed in this study has the advantage of simultaneous determination of the mass and radiolabelling of various inositol phosphates. However, the method may be more laborious and time consuming than the HPLC methods.

Table I lists the linear regression parameters for the standard curves for various inositol phosphates. The recovery of IP, IP_2, and IP_3 ranged from 69 to 76%; IP_4 exhibited a lower recovery, possibly because it is difficult to dephosphorylate [39]. Maslanski and Busa [28] reported a dephosphorylation of less than 30% by using alkaline phosphatase. This difficulty was overcome in this study by comparing the radioactivity before and after dephosphorylation and by using spiked standards. The mass of inositol phosphates in unstimulated synaptosomes exhibited the following concentration profile: $IP_4 < IP < IP_2 < IP_3$ (Fig. 6A), whereas the radiolabelling in unstimulated synaptosomes exhibited the following profile: $IP_4 < IP_2 < IP < IP_3$ (Fig. 6B). Although CA and NE increased the mass of all four inositol phosphates (Fig. 6), these two compounds increased the radiolabelling of only IP_2 and IP in synaptosomes (Fig.6). The mass of inositol phosphates in unstimulated microvessels exhibited the following concentration profile: $IP_4 < IP_3 < IP_2 < IP$ (Fig. 7A), whereas the radioactivity labelling exhibited the following concentration profile: $IP_4 < IP_2 < IP < IP_3$ (Fig. 7B). Although CA stimulated the mobilization of IP_4 and IP_3 in microvessels, NE did not (Fig. 7). Both CA and NE failed to increase the levels of IP_2 and IP in microvessels.

Comparison of the mass analysis and the radiolabelling methods

The mobilization of inositol phosphates is commonly studied by using the radiolabelling method but this has several disadvantages [10–13] and may not give an accurate account of PtdIns metabolism [14,15]. To circumvent these disadvantages, Challiss and Nahorski [17] used a radioreceptor method for the mass analysis of IP_3 and IP_4 which, they suggested, provided more accurate information than the radiolabelling method regarding the mobilization of inositol phosphates. However, the radioreceptor method has limited scope because it specifically quantitates IP_3 or IP_4 and, therefore, the mobilization of other inositol phosphates cannot be studied.

The method described in this study simultaneously measures both the mass and the radiolabelling of various inositol phosphates in tissue samples. The results indicate that the mass of inositol phosphate in cells is not always represented accurately when cells are labelled with [^{3}H]MI and the radioactivity of each compound is used as the method of quantitation. Therefore, various inositol phosphates and phospholipids may not be in a steady state or in equilibrium with respect to each other. The observations that the mass of IP was higher than that of IP_3 and that the specific activity of ^{3}H in IP was less than the specific activity in IP_3 suggest that IP_3 may not be the only source of IP in the blood–brain barrier cells. The mass and radioactivity measurements gave variable results when synaptosomes (Fig. 6) and microvessels (Fig. 7) were stimulated by CA or NE. This indicates that the two methods may be measuring different pools of inositol phosphates.

CONCLUSION

The chromatographic method used in this study measured the mass and radiolabelling of inositol lipids and inositol phosphates. Differences in the metabolism of inositol lipids and the receptor-mediated mobilization of inositol phosphates were observed in synaptosomes and microvessels. The results also demonstrate differences in the mass and radiolabelling profiles of inositol lipids and inositol phosphates in synaptosomes and the blood–brain barrier as represented by microvessels.

REFERENCES

1 C. P. Downes, *Biochem. Soc.*, 17 (1989) 259.
2 R. M. Michell, A. H. Drummond and C. P. Downes, *Inositol Lipids in Cell Signalling*, Academic Press, New York, 1984.
3 P. Adamson, T. Mantzouridis, J.-Z. Xiang, I. Hajimohamadreza, M. J. Brammer and I. C. Campbell, *J. Neurochem.*, 53 (1989) 1077.
4 I. Hide, T. Kato and S. Yamawaki, *J. Neurochem.*, 53 (1989) 556.
5 C. J. Fowler, I. Fagarville, B. G. Minor and B. S. Ross, *J. Pharm. Pharmacol.*, 38 (1986a) 385.
6 C. Fowler, O. Magnusson, A. K. Mohammed, W. Danysz and T. Archer, *Eur. J. Pharmacol.*, 123 (1986) 401.
7 R. A. Gonzales, and F. T. Crews, *Biochem. J.*, 232 (1985) 799.
8 R. F. Irvine, in J. W. Putney (Editor), *Phosphoinositides and Receptor Mechanisms*, Alan R. Liss, New York, 1988, pp. 89–107.
9 Y. Nishizuka, *Science*, 233 (1986) 305.
10 R. A. J. Challiss, I. H. Batty and S. R. Nahorski, *Biochem. Biophys. Res. Commun.*, 157 (1988) 684.
11 D. A. Horstman, H. Takemura and J. W. Putney, Jr., *J. Biol. Chem.*, 263 (1988) 15297.
12 C. E. King, L. J. Stephens, P. T. Hawkins, G. R. Guy and R. H. Michell, *Biochem. J.*, 244 (1987) 209.
13 K. Koreh and M. E. Monaco, *J. Biol. Chem.*, 261 (1986) 88.
14 J. G. Baird and S. R. Nahorski, *Biochem. J.*, 267 (1990a) 835.
15 M. J. Brammer and K. Weaver, *J. Neurochem.*, 53 (1989) 399.
16 J. G. Baird and S. R. Nahorski, *J. Neurochem.*, 54 (1990b) 555.
17 R. A. J. Challiss and S. R. Nahorski, *J. Neurochem.*, 57 (1991) 1042.
18 G. Y. Sun, in A. A. Boulton, G. B. Baker and L. A. Horrocks (Editors), *Neuromethods: Lipid and Related Compounds*, Humana Press, Clifton, NJ, 1980, pp. 63–82.
19 G. Y. Sun and T. N. Lin, *Life Sci.*, 44 (1989) 689.
20 J. Schacht, *J. Lipid Res.*, 19 (1978) 1063.
21 F. B. Palmer, *J. Lipid Res.*, 22 (1981) 1296.
22 R. F., Irvine, E. E. Arggard, A. J. Letcher and C. P. Downes, *Biochem. J.*, 229 (1985) 505.
23 J. L. Meek and F. Nicoletti, *J. Chromatogr.*, 351 (1986) 303.
24 K. M. Wilson, G. Siobhan and K. P. Minneman, *J. Neurochem.*, 55 (1990) 691.
25 P. R. Haddad and A. L. Heckenberg, *J. Chromatogr.*, 300 (1984) 357.
26 M. J. Berridge, C. P. Downes and M. R. Hanley, *Biochem. J.*, 206 (1982) 587.
27 M. J. Berridge, R. M. Dawson, C. P. Downes, J. P. Heslop and R. F. Irvine, *Biochem. J.*, 212 (1983) 473.
28 J. A. Maslanski and W. B. Busa, in R. F. Irvine (Editor), *Methods in Inositide Research*, Raven Press, New York, 1990, pp. 113–126.
29 M. Shimon, Y. Egozi, Y. Kloog, M. Sokolovsky and S. Cohen, *J. Neurochem.*, 50 (1988) 1719.
30 A. K. Singh, R. J. Zeleznikar and L. R. Drewes, *Life Sci.*, 38 (1986) 165.
31 R. S. Jope, *Brain Res. Rev.*, 1 (1979) 313.
32 J. Folch, M. Lee and S. M. Stanley, *J. Biol. Chem.*, 226 (1957) 497.
33 L. L. Dugan, P. Demediuk, C. E. Pendley and L. A. Horrocks, *J. Chromatogr.*, 378 (1986) 317.
34 G. Rouser, G. Kritchevsky, A. Yamamoto, G. Simon, C. Gallic and A. Bauman, *Methods Enzymol.*, 14 (1969) 272.
35 P. A. Lanzetta, L. J. Alvarez, P. S. Reinach and O. A. Candia, *Anal. Biochem.*, 100 (1979) 95.
36 W. S. M. Geurts van Kessel, W. M. A. Hax, R. A. Demel and J. DeGier, *Biochim. Biophys. Acta*, 486 (1977) 524.
37 J. W. A. van Loenhout, C. A. M. van Ginneker, H. C. J. Ketelars, P. M. Kimenai, Y. Tan and F. W. J. Gribnau, *J. Liq. Chromatogr.*, 5 (1982) 549.
38 E. T. Lin, D. E. Smith, L. Z. Benet, B. Hoener, *J. Chromatogr.*, 163 (1979) 315.
39 J. A. Shayaman, A. R. Morrisom and O. H. Lowry, *Anal. Biochem.*, 162 (1987) 562.
40 R. F. Irvine, in R. F. Irvine (Editor), *Methods in Inositide Research*, Raven Press, New York, 1991, pp. 1–7.
41 R. A. Minear, J. E. Segers, J. W. Elwood and P. J. Mulholland, *Analyst*, 113 (1988) 645.
42 D. Pitted, W. Schlegel, P. D. Lew, A. Monod and G. W. Mayr, *J. Biol. Chem.*, 264 (1989) 489.
43 R. E. Smith and R. A. MacQuarrie, *Anal. Biochem.*, 170 (1988) 308.
44 M. P. Honchar, J. W. Olney and W. R. Sherman, *Science*, 202 (1983) 323.
45 G. Y. Sun, T.-N. Lin, N. Premkumar, S. Carter and R. A. Mac-Quarrie, in R. F. Irvine (Editor), *Methods in Inositide Research*, Raven Press, New York, 1991, pp. 135–143.
46 L. C. MacGregor and F. M. Matschinsky, *Anal. Biochem.*, 141 (1984) 382.
47 R. A. J. Challis, I. R. Batty and S. R. Nahorski, *Biochem. Biophys. Res. Commun.*, 157 (1988) 684.
48 S. Palmer, K. T. Hughes, D. Y. Lee and M. J. O. Wakelam, *Cell. Signalling*, 1 (1989) 147.
49 G. W. Mayer, *Biochem. J.*, 254 (1988) 585.
50 G. W. Mayer, *Wurzburger Chromatographic-Gesprache (Ultrochrome 88)*, Vol. 3, Pharmacia LKB, Freiburg, 1989, pp. 45–52.