Biochimica et Biophysica Acta 904 (1987) 392–400
Elsevier

BBA 73751

Compartmentation of phosphorylated precursors of phospholipid biosynthesis in cultured neuroblastoma cells

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(Received 26 January 1987)
(Revised manuscript received 8 June 1987)

Key words Sodium ion, Phosphate uptake, Phospholipid biosynthesis, Cellular compartmentation, (Mouse neuroblastoma)

The continuous turnover of membrane phospholipids requires a steady supply of biosynthetic precursors. We evaluated the effects of decreasing extracellular Na^+ concentration on phospholipid metabolism in cultured neuroblastoma (N1E 115) cells. Incubating cultures with 145 to 0 mM NaCl caused a concentration-dependent inhibition of [^{32}P]phosphate uptake into the water-soluble intracellular pool and incorporation into phospholipid. Phospholipid classes were differentially affected; [^{32}P]phosphate incorporated into phosphatidylethanolamine (PE) and phosphatidylcholine (PC) was consistently less than into phosphatidylinositol (PI) and phosphatidylserine (PS). This could not be attributed to decreased phospholipid synthesis since under identical conditions, there was no effect on arachidonic acid or ethanolamine incorporation, and choline utilization for PC synthesis was increased. The effect of Na^+ was highly specific since reducing phosphate uptake to a similar extent by incubating cultures in a phosphate-deficient medium containing Na^+ did not alter the relative distribution of [^{32}P]phosphate in phospholipid. Of several cations tested only Li^+ could partially (50%) replace Na^+. Incubation in the presence of ouabain or amiloride had no effect on [^{32}P]phosphate incorporation into phospholipid. The differential effects of low Na^+ on [^{32}P]phosphate incorporation into PI relative to PC and PE suggests preferential compartmentation of [^{32}P]phosphate into ATP in pools used for phosphatidic acid synthesis and relatively less in ATP pools used for synthesis of phosphocholine and phosphoethanolamine, precursors of PC and PE, respectively. This suggestion of heterogeneous and distinct pools of ATP for phospholipid biosynthesis, and of potential modulation by Na^+ ion, has important implications for understanding intracellular regulation of metabolism.

Introduction

The outward movement of Na^+ coupled with the inward flux of K^+ that is mediated by ($Na^+ + K^+$)-ATPase maintains the electrochemical gradient across the plasma membrane. Since the intracellular Na^+ concentration is low relative to the level in the extracellular fluid, Na^+ ions move passively into the cell. In electrically excitable cells, Na^+ influx may also be attributed to activation of voltage-sensitive Na^+ channels [1,2], occur as part of Na^+/H^+ exchange [3], or be a component of anion transport [4]. In the latter, anions coupled with Na^+ enter the cell against a concentration gradient; for example, either $H_2PO_4^-$

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or HPO_4^{2-} inorganic phosphate associates with one or two Na^+ ions to form an electroneutral complex which is translocated across the membrane [4]. Thus, by maintaining the asymmetric distribution of Na^+ and K^+, $(Na^+ + K^+)$-ATPase generates the driving force for intracellular phosphate accumulation.

Following incorporation into the ATP pool, phosphate is utilized in a wide variety of regulatory and biosynthetic processes vital to cellular function. In phospholipid metabolism, phosphate is incorporated by two distinctly different pathways. For phosphatidylcholine (PC) and phosphatidylethanolamine (PE), the phosphate is introduced by phosphorylation of choline or ethanolamine by the respective kinases prior to activation by cytidine triphosphate and transfer to diacylglycerol to form the phospholipids. Phosphatidylserine (PS) of eukaryotic cells seems to be derived from PE by base exchange of serine with ethanolamine. In contrast, the phosphate in phosphatidylinositol (PI) is incorporated through phosphatidic acid which is in turn derived from phosphorylated intermediates of the glycolytic pathway.

In this study, the interaction between Na^+-dependent phosphate uptake and membrane phospholipid metabolism was evaluated in cultured neuroblastoma cells. Consistent with previous reports [5–8], we observed a direct relationship between the Na^+ concentration of the medium and both phosphate transport and incorporation into phospholipid. However, we also noted a specific compartmentation of the [^{32}P]phosphate utilized for phospholipid synthesis which resulted in less [^{32}P]phosphate being incorporated into PC and PE, relative to PI and PS, when Na^+-dependent phosphate transport was inhibited.

Materials and Methods

Materials

All chemicals and reagents used were of the finest available grade obtained from various commercial suppliers. Carrier free [^{32}P]orthophosphoric acid, [*methyl*-^{3}H]choline chloride (80 Ci/mmol), [1,2-^{14}C]ethanolamine hydrochloride (4 mCi/mmol), and [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (83 Ci/mmol) were from New England Nuclear (Lachine, Quebec, Canada). Ouabain octahydrate and amiloride hydrochloride were from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Methods

Tissue culture. N1E-115 neuroblastoma cell lines were maintained in 150 cm^2 flask (Corning Glass Works, Corning, NY, U.S.A.) in Dulbecco's modified Eagles medium containing 5% fetal and 5% newborn calf serum (Gibco Canada Ltd., Burlington, ON, Canada) and penicillin: streptomycin (100 units and 100 μg/ml, respectively). Cells were grown at 37 °C in a humidified atmosphere of 95% air, 5% CO_2. Three or four days prior to each experiment, cells were subcultured (passage number 30 to 50) into 60 mm plastic petri dishes at a density of $8 \cdot 10^5$ cells/dish.

Experimental procedures. Cells were pulse-labeled for 1 h in 2 ml of a balanced salt solution which contained 5.4 mM KCl, 1.0 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.1 mM KH_2PO_4, 20 mM glucose and 20 mM Hepes (pH 7.4). Medium osmolarity was maintained by adding NaCl and choline chloride to a final total concentration of 145 mM. In some experiments, NaCl was replaced with 145 mM of either choline chloride, LiCl, KCl, tetraethylammonium chloride or 220 mM sucrose. To examine the effect of phosphate depletion, NaCl and choline chloride were held constant at 20 mM and 125 mM, respectively, while KH_2PO_4 was varied from 0.01 mM to 3 mM (KCl was reduced appropriately). At the end of each incubation, the medium was removed and the cells were washed three times with 2 ml of phosphate-buffered saline. More than 99% of medium radioactivity was removed with the medium and the first wash, and $< 0.2\%$ of the medium radioactivity was in the final wash. Cell activity was terminated immediately by adding 2.6 ml of methanol/H_2O (1.6 : 1, v/v).

Phospholipid analysis Total lipids in the cell were extracted by adding 3.4 ml of chloroform and the resulting lower phase was washed twice with 1 ml of chloroform/methanol/0.1 M KCl (3 : 48 : 47, v/v) [9]. Radioactivity was determined on an aliquot of both the upper and lower phases using a Beckman LS7800 liquid scintillation counter. Lower phase lipids were resolved by thin-layer chromatography on Silica gel G plates using the solvent system chloroform/ethanol/water/

triethylamine (4:5:1.4, v/v) [10]. Appropriate standards were used for identification and lipids were visualized by spraying with phosphomolybdate reagent. Spots corresponding to known standards were scraped from the plates and the gel added to vials containing 5 ml of Beckman HP liquid scintillation fluid.

Other methods. Protein was determined using an aliquot of the methanol/H_2O cell suspension according to the method of Lowry et al. [11].

Results

Effect of Na^+ availability on [^{32}P]phosphate incorporation into phospholipid

When expressed relative to the control cultures (145 mM NaCl), removing Na^+ from the medium caused a concentration dependent inhibition of [^{32}P]phosphate uptake and incorporation into phospholipid (Fig. 1). While incorporation of [^{32}P]phosphate into all phospholipids was altered, the rate of change and the maximum suppression varied with phospholipid type. Relative to controls both PE and PC displayed lower incorporatin of [^{32}P]phosphate at higher extracellular Na^+ levels and a greater maximal decrease in a medium without added Na^+ than either PS or PI.

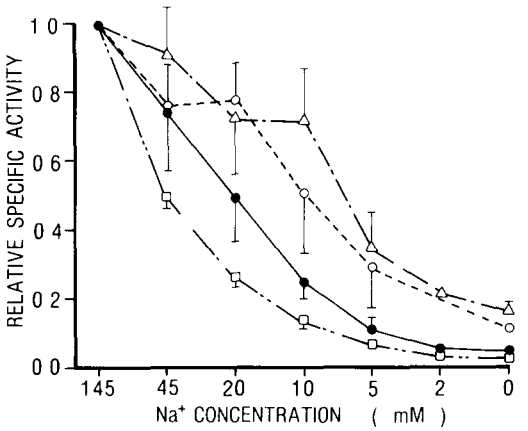

Fig 1 Effect of Na^+ availability on [^{32}P]phosphate incorporation into phospholipids Cultured neuroblastoma cells were pulse-labeled with 8–10 μCi [^{32}P]phosphate (80–100 μCi/μmol)/ml for 1 h in the balanced salt solution containing different concentrations of NaCl Medium osmolarity was maintained by adding choline chloride such that the sum of choline chloride and NaCl equalled 145 mM. Results are expressed relative to the 145 mM NaCl group (relative specific activity) Control values expressed as pmol phosphate incorporated/mg protein were PC, 206 9 ± 60 8; PS, 16 9 ± 6.0, PI, 236 ± 52 2, PE, 383 9 ± 38 5, mean ± S D (n = 6) ●——●, PC, □–· –□, PE, ○– – –○, PS, △– –△, PI

Effect of different monovalent cations and sucrose on [^{32}P]phosphate incorporation into phospholipid

The alternate monovalent cations differed in their capacity to substitute for Na^+ (Table I). Total [^{32}P]phosphate incorporation remained markedly suppressed when KCl, tetraethylammonium chloride, choline chloride or sucrose replaced NaCl in the incubation medium. Li^+ was the most effective substitute as total radioactivity recovered in phospholipid was 50% of the control value compared to 24% for K^+ and 8–10% for the other cations and sucrose. K^+ increased incorporation of [^{32}P]phosphate into PI. Under all conditions, the relative specific activity of PS and PI exceeded that of PC and PE.

Effect of Na^+ availability on [^{3}H]arachidonic acid incorporation into phospholipid

Incorporation of [^{3}H]arachidonic acid was relatively insensitive to the Na^+ concentration of the medium (Fig. 2). When neuroblastoma cells were dual-labeled with [^{3}H]arachidonic acid and [^{32}P]phosphate, the pattern of [^{32}P]phosphate incorporation was similar to that of Fig. 1 (data not shown).

Effect of Na^+ availability on [^{14}C]ethanolamine, [^{3}H]choline and [^{32}P]phosphate incorporation into cellular aqueous phase and phospholipids

Uptake and incorporation of [^{14}C]ethanolamine was not influenced by the Na^+ concentration of the medium while [^{3}H]choline transport and utilization for PC synthesis were enhanced (Fig. 3). The latter observation is consistent with reports by McGee [12] that choline uptake is stimulated in neuroblastoma × glioma (NG 108-15) cells by incubation in a Na^+ free medium containing sucrose. In contrast, uptake and incorporation of [^{32}P]phosphate was reduced to approx. 3% of the control value.

Effect of Na^+ and phosphate availability on [^{32}P]phosphate incorporation into phospholipid

Incorporation of phosphate into phospholipid

TABLE I

EFFECT OF ALTERNATE MONOVALENT CATIONS AND SUCROSE ON [^{32}P]PHOSPHATE INCORPORATION INTO PHOSPHOLIPID

Cultured neuroblastoma cells were pulse-labeled with 8–10 μCi [^{32}P]phosphate (80–100 μCi/μmol)/ml for 1 h in the balanced salt solution containing 145 mM of the respective cations (except sucrose, 220 mM) Results are expressed relative to the 145 mM NaCl group Control values expressed as dpm/mg protein were PC, 48 500 ± 8204, PS, 4136 ± 493, PI, 46 663 ± 1828, PE, 151 000 ± 15 056, mean ± range (n = 2)

	% of control			
	PC	PS	PI	PE
NaCl	100	100	100	100
LiCl	28 6±0 2	96 0±25 3	52 8±2 3	21 9±4 4
KCl	2 0±0 1	28 3± 0 1	64 1±0 7	3 5±0 2
TEA Cl [a]	1 5±0 1	18 9± 6 4	23 7±0 9	1 7±0 1
Choline chloride	3 9±0 2	11 6± 3 0	15 2±1 7	1 8±0 1
Sucrose	1 7±0 6	12 5± 4 3	13 7±5 0	1 3±0 5

[a] Tetraethylammonium chloride

was similar in cells incubated in a medium containing 20 mM Na^+ and 0.01 mM phosphate (0.03 ± 0.005 nmol phosphate/mg protein) and one containing no added Na^+ and 0.1 mM phosphate (0.05 ± 0.01 nmol phosphate/mg protein). Despite the quantitative similarity in phosphate

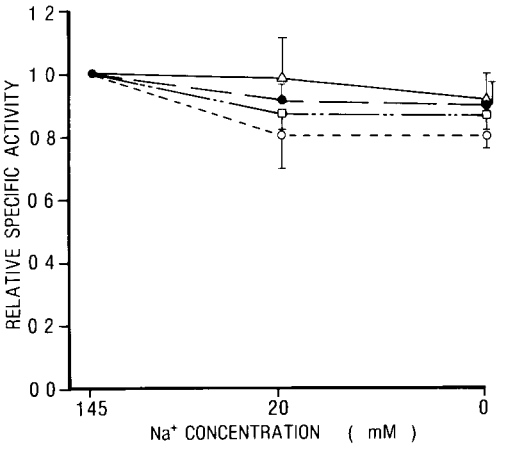

Fig 2 Effect of Na^+ availability on [^{3}H]arachidonic acid incorporation into phospholipids Cultured neuroblastoma cells were pulse-labeled with 2 μmol [^{3}H]arachidonic acid (0 25 μCi/μmol) for 1 h in the balanced salt solution containing different concentrations of NaCl Medium osmolarity was maintained by adding choline chloride such that the sum of choline chloride and NaCl equalled 145 mM Results are mean ± range (n = 2) expressed relative to the 145 mM NaCl group (relative specific activity) Control values expressed as dpm/mg protein were PC, 22244, PS, 1177, PI, 13135, PE, 17039 Replicate experiments gave similar results ●– –●, PC, □– –□, PE, ○– – –○, PS; △———△, PI

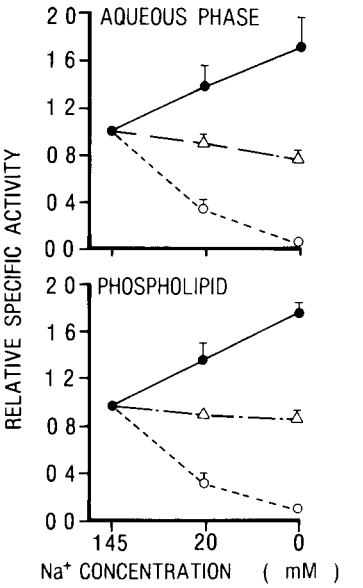

Fig 3 Effect of Na^+ availability on [^{3}H]choline, [^{14}C]ethanolamine and [^{32}P]phosphate incorporation into cellular aqueous phase and phospholipid Cultured neuroblastoma cells were pulse-labeled with 100 nmol [^{3}H]choline (7 5 μCi/μmol) or [^{14}C]ethanolamine (7 5 μCi/μmol) and 8–10 μCi [^{32}P]phosphate (80–100 μCi/μmol)/ml for 1 h in the balanced salt solution containing different concentrations of NaCl. Medium osmolarity was maintained by adding sucrose Results are expressed relative to the 145 mM NaCl group (relative specific activity) Control values expressed as dpm/mg protein were (aqueous phase followed by phospholipid) choline, 108 776 ± 3191, 11 334 ± 452, ethanolamine, 52 582 ± 2629, 31 219 ± 398, phosphate 11 361 373 ± 264 238, 146 298 ± 11 673, mean ± range (n = 2) Replicate experiments gave similar results ●———●, [^{3}H]choline, △– –△, [^{14}C]ethanolamine, ○– – –○, [^{32}P]phosphate

uptake by the cells under the two experimental conditions, the relative distribution of radioactivity recovered in each phospholipid class differed markedly. When the Na^+ content of the medium was reduced (at a constant (0.1 mM) phosphate concentration) [^{32}P]phosphate incorporation into PE and PC was suppressed while uptake into PI was enhanced (Fig. 4A). This response was most apparent when the medium contained less than 20 mM Na^+. In the converse situation, when the phosphate content of the medium was depleted at a constant Na^+ concentration (20 mM), the relative distribution of radioactivity in the phospholipid classes was unchanged (Fig. 4B).

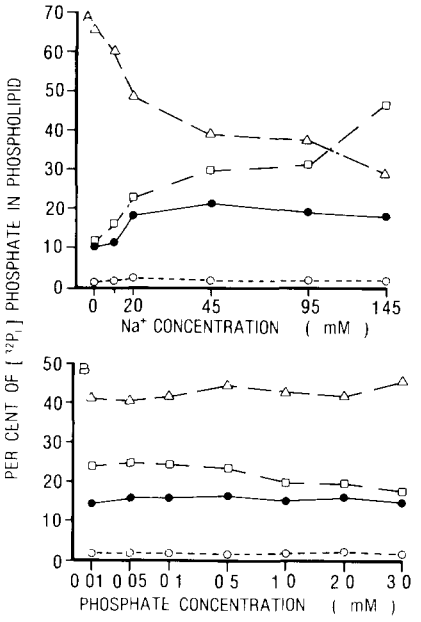

Fig 4 Effect of Na^+ and phosphate availability on the relative incorporation of [^{32}P]phosphate into phospholipids Cultured neuroblastoma cells were pulse-labeled with 8–10 μCi [^{32}P]phosphate (80–100 μCi/μmol)/ml for 1 h in the balanced salt solution In panel A, the NaCl concentration was varied from 0 to 145 mM while the phosphate concentration was held constant at 0 1 mM (as KH_2PO_4) In panel B, the NaCl concentration was held constant at 20 mM while the phosphate concentration was varied from 0 01 mM to 3.0 mM (KH_2PO_4 replaced the appropriate amount of KCl) In each case, medium osmolarity was maintained by adding choline chloride such that the sum of choline chloride and NaCl equalled 145 mM Values are expressed as the mean ($n = 2$) with variability less than 2% Replicate experiments gave similar results. ●———●, PC, □– –□, PE, ○– – –○, PS, △– –△, PI

Effect of sequential incubation in either high Na^+ or Na^+-free media on [^{32}P]phosphate incorporation into cellular aqueous phase and phospholipid

Both the total uptake of [^{32}P]phosphate and the pattern of incorporation into phospholipid varied with the sequence of the incubation conditions (Fig. 5). When cells were incubated in a medium without added Na^+ for 2 h, [^{32}P]phosphate uptake into the aqueous phase was reduced to 2% of the control value. The relative specific activity of phospholipids isolated from these cultures was similar to that of cells incubated for 1 h (Fig. 1). That is, incorporation of [^{32}P]phosphate into PE, PC, PS and PI was 2.0 ± 0.4%, 5.2 ± 0.1%, 11.3 ± 1.8% and 12.4 ± 0.9%, respectively, of the control value. Changing cells initially incubated in the Na^+-free medium to one containing 145 mM Na^+ reversed the inhibitory effects of Na^+ depletion on phospholipid metabolism. Uptake of [^{32}P]phos-

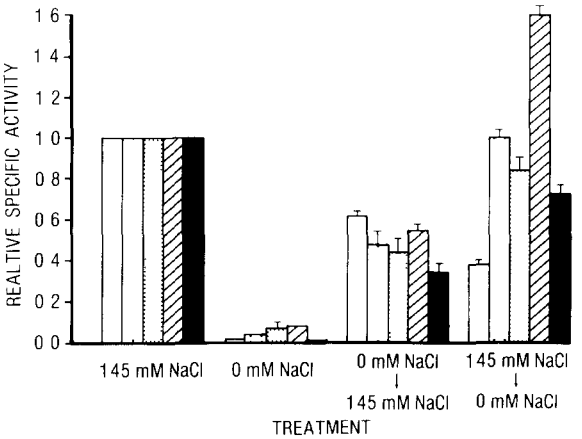

Fig 5 Effect of sequential incubation in either high Na^+ or Na^+ free media on [^{32}P]phosphate incorporation into phospholipid Cultured neuroblastoma cells were pulse-labeled with 8–10 μCi [^{32}P]phosphate (80–100 μCi/μmol)/ml in the balanced salt solution containing 0 mM or 145 mM NaCl for 2 h or for 1 h in one medium followed by 1 h in the alternate medium Medium osmolarity was maintained by adding choline chloride such that the sum of choline chloride and NaCl equalled 145 mM Results were expressed relative to the 145 mM NaCl group (relative specific activity) Control values expressed as dpm/mg protein were aqueous phase 11927000 ± 109000, PC, 51168 ± 3077, PS, 3386 ± 593, PI, 53918 ± 1557, PE, 78148 ± 3117, mean ± range ($n = 2$) Replicate experiments gave similar results In order aqueous phase, open bars, PC, bars with large dots, PS, bars with small dots, PI, cross-hatched bars; PE, solid bars

phate into the aqueous phase was approx. 60% of the control value while incorporation into phospholipid was 50% of that exhibited by cultures incubated continuously in the high Na^+ medium for 2 h. When cells were changed from the 145 mM Na^+ medium to one containing no added Na^+, [^{32}P]phosphate recovered in the aqueous phase was 40% of the control value. In addition, although total radioactivity recovered in phospholipid was similar to that of control cultures, there was a specific enrichment of [^{32}P]phosphate in PI and suppressed incorporation into PE.

Effect of ouabain and amiloride on [^{32}P]phosphate incorporation into cellular aqueous phase and phospholipid

Including ouabain, to reduce ($Na^+ + K^+$)-ATPase activity, and amiloride, to inhibit the Na^+/H^+ antiport, did not appreciably alter the effect of Na^+ on phosphate transport and incorporation into phospholipid (Fig. 6). The relative specific activity of [^{32}P]phosphate incorporated into PC, PE, PS and PI was similar to that described in Fig. 1 (data not shown).

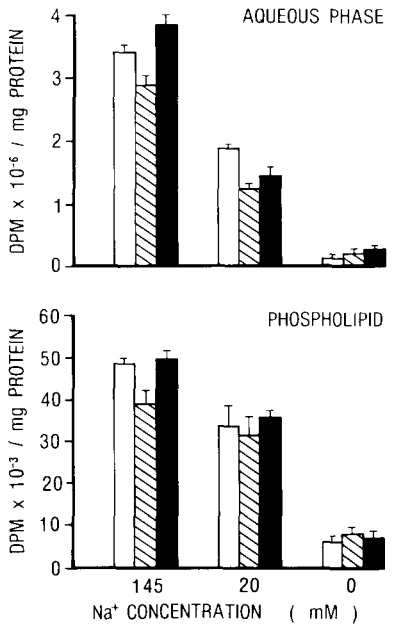

Fig 6 Effect of ouabain and amiloride on [^{32}P]phosphate incorporation into cellular aqueous phase and phospholipid Cultured neuroblastoma cells were pulse-labeled with 8–10 μCi [^{32}P]phosphate (80–100 μCi/μmol)/ml for 1 h in the balanced salt solution containing different concentrations of NaCl, and ouabain (5 mM) or amiloride (1 mM) Medium osmolarity was maintained by adding choline chloride and NaCl such that the sum of choline chloride and NaCl equalled 145 mM Values are expressed as the mean ± range ($n = 2$) Replicate experiments gave similar results Control, open bars, ouabain, cross-hatched bars, amiloride, solid bars

Discussion

Maintenance of the continuous turnover of membrane phospholipid components is dependent on a steady supply of biosynthetic precursors This study demonstrated a direct relationship between the Na^+ concentration of the medium and phosphate transport and utilization for phospholipid synthesis. Moreover, a medium without added Na^+ decreased incorporation of [^{32}P]phosphate into PE and PC to a greater extent than PI or PS.

Phosphate is utilized for phospholipid synthesis in several ways. Fatty acids undergo an ATP-dependent activation to the respective acyl-CoA thioesters prior to esterification to either diacylglycerol or phosphatidic acid; the ATP phosphate in this step does not form part of the phospholipid molecule. For PC and PE, phosphate is incorporated at the choline or ethanolamine kinase step, and this phosphate group is eventually incorporated into the final phospholipid molecule. For PI, phosphate is incorporated into glycolytic precursors prior to formation of the triose phosphates. An alternative pathway, direct phosphorylation of glycerol by a kinase cannot be entirely excluded but seems unlikely to be a major route in our experiments since neuroblastoma cells incorporate less than 0.01% of labeled glycerol into phospolipids [13].

Decreased incorporation of [^{32}P]phosphate by cells incubated in a medium without added Na^+ was not due to inhibition of phospholipid synthesis. The incorporation of [^{3}H]arachidonic acid into phospholipid and of [^{14}C]ethanolamine into PE was normal, while incorporation of [^{3}H]choline into PC was actually enhanced. Accordingly, of the steps in phospholipid synthesis that we measured, only [^{32}P]phosphate incorporation was affected. Since utilization of fatty acid, ethanolamine and choline was either normal or increased, this suggests that the total mass of phosphate, as

opposed to [^{32}P]phosphate, incorporated into phospholipid was not altered. Thus, the effect of Na^+ was not generalized to all intracellular pools of phosphate-containing precursors utilized for phospholipid synthesis, but was quite specific to those containing [^{32}P]phosphate.

The incorporation of [^{32}P]phosphate into the individual phospholipids was acutely and differentially sensitive to the Na^+ concentration of the medium. The most marked effect was noted when the medium contained less than 20 mM Na^+. Since the normal intracellular Na^+ level is maintained at approx. 20 mM by ($Na^+ + K^+$)-ATPase, this extracellular Na^+ concentration may represent the point at which the electroneutral transport of phosphate coupled with Na^+ is maximally inhibited. Li^+ partially replaced Na^+. Cellular transport of Li^+ is similar to Na^+ as this cation is an effective substrate for the voltage-sensitive Na^+ channel [14], for the Na^+/H^+ antiport [15], and for ($Na^+ + K^+$)-ATPase [16]. Enhanced radiolabeling of phospholipid when Li^+ replaced Na^+ in the incubation medium probably reflects a substitution of Li^+ for Na^+ in the electroneutral transport of phosphate. Although Li^+ increased the amount of [^{32}P]phosphate in phospholipid, the relative specific activity of PS and PI was still greater than PE or PC. Further, differential labeling of phospholipids was not observed when phosphate uptake was decreased to an equivalent extent by incubating cells in a low phosphate medium containing Na^+. Overall, this suggests that the effect of Na^+ on [^{32}P]phosphate utilization for phospholipid synthesis was highly specific.

Of the [^{32}P]phosphate utilized for phospholipid synthesis at lower Na^+ concentrations, relatively more (as compared to controls) was incorporated into PI through phosphatidic acid than into PE and PC by phosphorylation of ethanolamine and choline, respectively. The relative specific activity of PS consistently approximated that of PI. In other cell systems, PS is derived from PE by base exchange of ethanolamine with serine. If PE were the only source of PS, incorporation of [^{32}P]phosphate would be expected to parallel that of the parent molecule. This was not the case however, suggesting the possibility of alternate pathways for PS synthesis in neuroblastoma cells. For example, while not substantiated by experimental evidence, the data in this study are consistent with PS being derived from PI by base exchange of inositol with serine.

The driving force for the Na^+ gradient, and hence for Na^+-dependent phosphate uptake, is provided by ($Na^+ + K^+$)-ATPase. A possible role for this enzyme was investigated by incubating cells in the presence of ouabain We reasoned that the inhibition of [^{32}P]phosphate uptake caused by Na^+-depletion might be mimicked in the 145 mM Na^+ medium by including ouabain. In support of this possibility, Abdel-Latif [5] noted a linear decrease in [^{32}P]phosphate incorporation into PC in the rabbit iris smooth muscle preparation when incubated in the presence of ouabain and 125 mM NaCl. Incorporation was decreased to 35% of the control value suggesting that 65% of the ($Na^+ + K^+$)-ATPase is ouabain sensitive. Our in vitro estimates also indicate a 60% inhibition of ($Na^+ + K^+$)-ATPase by ouabain in neuroblastoma cells (unpublished observations). Thus, the inability of ouabain to influence [^{32}P]phosphate transport in the present study is difficult to explain. Possibly the in vitro estimates of the ouabain sensitivity of ($Na^+ + K^+$)-ATPase do not directly reflect the actual in vivo capacity. Alternatively, in contrast to the iris smooth muscle, residual enzyme activity may be sufficient to maintain secondary active transport of phosphate in neuroblastoma cells.

Intracellular pH is regulated in part by the Na^+/H^+ antiport. Inhibiting proton exchange by incubating cells in a low Na^+ medium decreases intracellular pH [17,18]. This might contribute to the differential labeling of phospholipids since both choline and ethanolamine kinase exhibit an alkaline pH optimum [19]. If intracellular pH were a factor, incorporation of [^{32}P]phosphate into phospholipid would be similar in cells incubated in the 145 mM Na^+ medium with amiloride, an inhibitor of the Na^+/H^+ antiport, and a medium without added Na^+. However, uptake and incorporation of [^{32}P]phosphate were not altered by amiloride over the range of Na^+ levels tested suggesting that intracellular pH was not a major factor.

As two ATP-requiring processes that donate [γ-^{32}P]phosphate to phospholipid, namely, phosphorylation of choline and ethanolamine on the one hand, and phosphatidic acid synthesis on the

other, were affected so differently, the ATP pools available to these pathways must have different specific activities. Thus, the differential labeling of phospholipid may be related to a functional compartmentation of the ATP pools utilized for phospholipid synthesis. Alternative explanations such as base exchange and differences in metabolic turnover rate seem less likely. While base exchange could contribute to decrease incorporation of [^{32}P]phosphate relative to choline or ethanolamine, one would expect that fatty acid incorporation would also be reduced, particularly if the entire backbone were being conserved. Further, base exchange is not a major pathway of PC synthesis nor has Na^+-sensitivity been reported [19,20]. The marked specificity of Na^+ argues against the possibility that differential labeling of phospholipids is due to variations in metabolic turnover rate during nonequilibrium conditions. For example, when [^{32}P]phosphate incorporation was suppressed to a similar extent by incubating cells for 1 h in media depleted in either Na^+ or phosphate, only the case of limited Na^+ availability caused differential labeling of phospholipids. In addition, when the ATP pools were prelabeled with [^{32}P]phosphate by incubating cells in a high Na^+ medium, subsequent incubation in a medium without added Na^+ caused a specific enrichment of [^{32}P]phosphate in PI and suppressed incorporation into PE. This partitioning occurred even though the total amount of radioactivity recovered in phospholipid was comparable to that of control cultures incubated continuously in the high Na^+ medium.

While differences in ATP pools within cells are intuitively plausible, direct proof is difficult to obtain and, to our knowledge, this is the first suggestion of such compartmentation that is based on evidence from lipid biosynthetic pathways. Further, the observation that these pools become functionally differentiated as the Na^+ concentration is varied has important implications for our understanding of intracellular regulation.

In conclusion, we have evaluated the effect of Na^+ depletion on phospholipid metabolism in cultured neuroblastoma cells. During short term incubations, removing Na^+ from the medium inhibited [^{32}P]phosphate, but not fatty acid, choline or ethanolamine, incorporation into phospholipid. Of the [^{32}P]phosphate incorporated, there appeared to be preferential compartmentation into an ATP pool utilized for PI synthesis with relatively less incorporation into ATP pools used in the synthesis of phosphocholine and phosphoethanolamine, precursors for PC and PE, respectively. This differential labeling pattern observed when Na^+ was removed from the incubation medium suggests that ATP pools available to phospholipid biosynthetic processes may be heterogeneous and distinct. The significance of intracellular compartmentation of ATP pools for control of phospholipid metabolism remains to be determined.

Acknowledgements

This work was supported by a Program Grant (PG-16), Fellowship (N.T.G.), and Career Investigator Award (M.W.S.) from the Medical Research Council of Canada. The skilled technical assistance of Mr. R. Zwicker is gratefully acknowledged.

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