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EFFECT OF PYRIDOXAL 5′-PHOSPHATE AND VALPROIC ACID ON PHOSPHOLIPID SYNTHESIS IN NEUROBLASTOMA NA

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Abstract—Phospholipid metabolism in neuroblastoma cells in monolayer culture after acute exposure to pyridoxal phosphate (PLP) has been studied. (a) A strong depression of the rate of biosynthesis of cellular phospholipids from labeled choline and ethanolamine, is demonstrated in neuroblastoma cells grown in culture media containing PLP. (b) Valproic acid reverses the effect of PLP on ethanolamine and choline incorporation into cell lipid. Other anticonvulsants (clonazepam, diazepam, carbamazepine, diphenylhydantoin and ethosuximide) have little or no effect on reversing the inhibition of lipid synthesis produced by PLP. (c) PLP decreases the cellular uptake of choline. This effect might be responsible for the decreased lipid synthesis and is partially reversed by valproic acid. (d) The energy charge of the cell is not affected by either PLP or valproic acid, but it is diminished by the two compounds together. (e) The degradation of choline lipids is decreased by PLP and valproic acid. The hydrolysis of phosphocholine and the outflow of choline from cultured cells is also affected by the drugs. Variations of ethanolamine and choline transport should not be due to any effects of PLP or valproic acid on the lipid phase of the membranes since these molecules have no effect on the permeability of liposomes. (f) It is concluded that ethanolamine and choline lipid metabolism in cultured neuroblastoma cells is influenced by PLP and/or valproic acid, probably through a mechanism involving the transport of precursors across the membrane, although other mechanisms cannot be ruled out.

Pyridoxal 5′-phosphate (PLP), the active form of vitamin B_6, is involved as a cofactor in a number of reactions of amino acid metabolism. The participation of this molecule in amino acid decarboxylation reactions is crucial for the synthesis of neurotransmitters, such as GABA [1, 2]. The participation of PLP-dependent enzymes in GABA metabolism should be considered among the possible explanations for the convulsions elicited either by a deficiency or by an excess of PLP [2–4]. The intraventricular administration of PLP has indeed been used to produce tonic-clonic convulsions in the rat [5].

Arienti *et al.* [6] have reported that, after the intraventricular injection of radioactive glycerol, the labeling ratio neutral lipid/phospholipid increased in the brains of rats in which convulsions have been elicited by the administration of PLP. Although this finding indicated a possible implication of PLP dosing on membrane lipid metabolism in the brain of convulsing rats, no explanation of the molecular mechanism(s) underlying this phenomenon was possible, due to the complexity of the biological system under study.

Since the properties of membranes are very important for nerve excitability and conduction, we thought interesting to study this "lipid effect" in some more detail using a system *in vitro*, through which the situation *in vivo* could be modeled.

In the present work, the ability of extracellular PLP to control the incorporation of choline and ethanolamine into nerve cell lipid is examined, using cultured neuroblastoma cells as model.

MATERIALS AND METHODS

Materials. [1-^{3}H]ethanolamine hydrochloride (specific radioactivity of 19,500 Ci/mole) and [*methyl*-^{3}H]choline chloride (specific radioactivity of 15,000 Ci/mole) were a product of Amersham International, (Buckinghamshire, U.K.). 1,2-Diacyl-*sn*-glycero-3-phospho-[U-^{14}C]serine (specific radioactivity of 170 Ci/mole) was synthesized according to Orlando *et al.* [7]. Phosphate buffered saline (PBS), foetal calf serum and Dulbecco's modified MEM were obtained from Gibco Bio-Cult (Paisley, U.K.). Pyridoxal 5′-phosphate was purchased from Fluka (Buchs, Switzerland). Valproic acid (2-propylpentanoic acid), clonazepam, diazepam, carbamazepine and 5,5-diphenylhydantoin were from Sigma Chemical Co. (St Louis, MO). Ethosuximide was obtained from Supelco Inc. (Bellefonte, PA). Other chemicals were produced by Carlo Erba (Milano, Italy). The neuroblastoma NA cell line (a HGDRT subclone of the N2A sympathetic neuroblastoma line) [8] was kindly provided by Dr R. Revoltella, Institute of Cell Biology, University of Rome, Italy.

Cell cultures. Neuroblastoma NA cells were cul-

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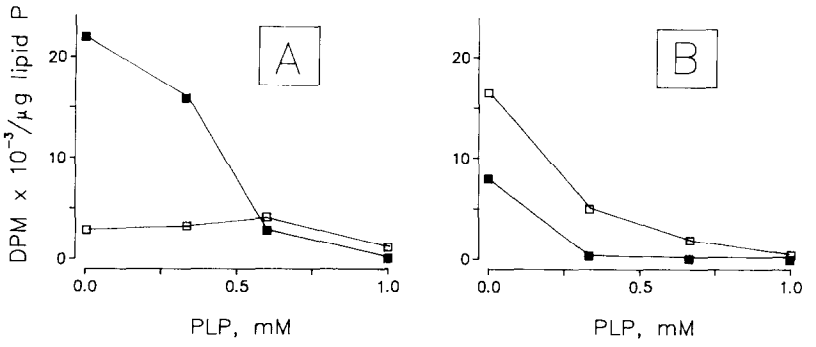

Fig. 1. Effect on PLP on the incorporation of labeled ethanolamine and choline. Neuroblastoma cell cultures were preincubated 30 min with PBS containing PLP, then the medium was removed and cells incubated 1 hr with fresh medium containing labeled ethanolamine (A) or labeled choline (B). The radioactivity of water-soluble compounds (open symbols) and lipid (solid symbols) was measured. Experimental points are the average of three separate determinations. SD was less than 10%.

Table 1. The effect of the preincubation with PLP and antiepileptic drugs on the incorporation of labeled choline and ethanolamine into the phospholipids of NA neuroblastoma cells

Preincubation	Lipid radioactivity* Phosphatidylethanolamine	Phosphatidylcholine
Controls	100	100
0.6 mM PLP	14 ± 3	5 ± 2
0.6 mM PLP + 3 mM VAL	120 ± 9	25 ± 6
0.6 mM PLP + 3 μM CLO	9 ± 3	2 ± 1
0.6 mM PLP + 0.1 mM DIA	18 ± 5	2 ± 1
0.6 mM PLP + 0.15 mM CAR	7 ± 2	1 ± 1
0.6 mM PLP + 0.5 mM DPH	29 ± 6	2 ± 1
0.6 mM PLP + 2.5 mM ETS	5 ± 3	0

Sets of three separated flasks containing neuroblastoma cells were preincubated for 30 min in PBS + the drugs at the indicated concentrations. After the removal of the medium, cells were incubated 1 hr with PBS containing the labeled precursors (choline or ethanolamine).

PLP = pyridoxal 5′-phosphate; VAL = valproic acid; CLO = clonazepam; DIA = diazepam; CAR = carbamazepine; DPH = diphenylhydantoin; ETS = ethosuximide.

* The incorporation of the labeled precursor into lipids is expressed as percent of control (preincubated in the absence of any drugs) ± SD. The radioactivity of controls was 2.0×10^4 DPM of phosphatidylethanolamine/μg total lipid phosphorus and 7.5×10^2 DPM of phosphatidylcholine/μg total lipid phosphorus.

tured at 37° in 25 cm^2 Falcon flasks with Dulbecco's modified MEM, supplemented with 10% heat-inactivated foetal calf serum, 100 mg of streptomycin/l and 10,000 I.U. of penicillin/l. The medium was equilibrated with 95% air and 5% CO_2. Confluent cultures were used throughout the work.

Effect of PLP on phospholipid synthesis. The effect of PLP on the synthesis of phosphatidylcholine and phosphatidylethanolamine was evaluated treating neuroblastoma cells with PLP-containing solutions before incubating them with radioactive choline or ethanolamine. Cultures were washed twice with PBS to remove medium and floating cells; 5 ml of PBS containing up to 1 mM PLP were then added to each flask. After 30 min incubation at 37°, the medium was removed and cells were washed three times with 10 ml of PBS each time. PBS (2 ml) containing 400 nCi of [1-^{3}H]ethanolamine (specific radioactivity of 19,500 Ci/mole) or 500 nCi of [*methyl*-^{3}H]choline (specific radioactivity of 15,000 Ci/mole) was then added to each flask. After 1 hr of incubation at 37°, the medium was removed and the flasks were washed with cold PBS. The cells were disrupted by adding 3 ml of methanol to each flask and subsequently scraped off. The methanolic suspensions were then transferred to test tubes and chloroform was added to a chloroform/methanol ratio of 2:1 (v/v). Lipid was extracted according to Folch *et al.* [9]. The upper phases were used to analyze water-soluble lipid precursors, whereas the chloroform phase was concentrated *in vacuo* for lipid analysis. Phospholipid classes were separated by TLC on silica gel G plates (Carlo Erba) using chloroform/methanol/water (65:25:4, v/v/v) as developing mixture. The spots corresponding to phosphatidylcholine and phosphatidylethanolamine were scraped off from the plate and collected into a scintillation vial. Methanol/water (1 ml; 1:1, v/v) was added to each vial and then radioactivity determined using Emulsifier scintillator (Packard Instrument Co. Inc., Des Plaines, IL). Water-soluble compounds were analyzed by ion exchange high performance liquid chromatography on Aminex A-14 (Bio-Rad, Richmond, CA), using a Perkin–Elmer Liquid Chromatograph, according to Floridi *et al.* [10]. Eluates (1 ml fractions) were collected into

Table 2. Label distribution among choline containing compounds after the incubation of neuroblastoma cells with PLP or valproic acid

Preincubation	Choline	Phosphocholine	Phosphatidylcholine
Control	4046 ± 360	8959 ± 510	805 ± 150
0.6 mM PLP	1620 ± 290	274 ± 97	43 ± 25
3 mM VAL	4035 ± 320	8905 ± 620	810 ± 105
0.6 mM PLP + 3 mM VAL	3201 ± 315	3725 ± 405	204 ± 70

Sets of three separated flasks containing neuroblastoma cells were preincubated for 30 min in PBS with the indicated drugs. After the removal of the medium, they were incubated 1 hr with 2 ml of PBS containing 500 nCi of labeled choline. The radioactivity incorporated into choline compounds is expressed as DPM/μg of total lipid phosphorus. Each value represents the mean (± SD) of three separate experiments. PLP = pyridoxal 5'-phosphate; VAL = valproic acid. Only traces of labeled CDP-choline were present.

scintillation vials and the radioactivity of choline, ethanolamine and of the corresponding phosphoric esters and CDP-derivatives was measured by liquid scintillation using Instagel (Packard Instrument Co. Inc.).

Effect of anticonvulsants on phospholipid synthesis. Cell cultures were washed with PBS as described above, and incubated with PBS solutions containing 0.6 mM PLP to which one of the following anticonvulsants were added (3 mM valproic acid, 3 μM clonazepam, 0.1 mM diazepam, 0.15 mM carbamazepine, 0.25 mM diphenylhydantoin or 2.5 mM ethosuximide). The medium was removed 30 min later, cell monolayers were washed with PBS and supplemented with 2 ml of PBS containing [*methyl*-^{3}H]chloline (500 nCi, specific radioactivity of 15,000 Ci/mole) or [1-^{3}H]ethanolamine (300 nCi, specific radioactivity of 19,500 Ci/mole). After 1 hr of incubation at 37°, the medium was removed and the cells were washed with PBS and disrupted with methanol. Lipids were extracted and their radioactivity measured as described above.

The effect of valproic acid on phospholipid synthesis was studied in more detail. Cell cultures were washed with PBS and incubated with PBS containing 0.6 mM PLP and up to 3 mM valproic acid. Control cultures were incubated with PBS containing valproic acid, but no PLP. The medium was removed 30 min later and the cells washed with fresh PBS. PBS (2 ml) supplemented with [*methyl*-^{3}H]choline (500 nCi, specific radioactivity of 15,000 Ci/mole) or [1-^{3}H]ethanolamine (400 nCi, specific radioactivity of 19,500 Ci/mole) was added to each flask. The medium was removed after 1 hr of incubation at 37°, and the cells were washed with PBS. Lipid and water-soluble compounds were extracted and analyzed as described above.

Determination of the cellular energy charge. The cellular levels of adenine nucleotides were measured according to Floridi *et al.* [11]. After the removal of culture medium and washing with PBS, cells were incubated 30 min at 37° with PBS containing either 0.6 mM PLP or 3 mM valproic acid or 0.6 mM PLP plus 3 mM valproic acid. Controls were made omitting the drugs. The medium was successively removed and cell monolayers were washed with PBS. Methanol/water (3 ml; 1:1, v/v) was added to each flask and cellular material was scraped off with a rubber spatula. The suspensions were sonicated briefly using a 100 W MSE instrument equipped with a microtip and centrifuged 20 min at 100,000 *g*. The supernatant was dried *in vacuo* and the pellet dissolved in 200 μl of 0.1 M 2-methyl-2-amino-1-propanol + 0.1 M NaCl, adjusted to pH 9.90 with 3 M HCl. The analysis of the nucleotides was performed as described by Floridi *et al.* [11].

Effect of PLP and valproic acid on phospholipid degradation. Membrane phosphatidylcholine was labeled incubating cell cultures with 2 ml of complete culture medium supplemented with 300 nCi/ml of [*methyl*-^{3}H]choline (specific radioactivity in culture medium of 10,400 Ci/mol). The medium was removed after 5 hr of incubation at 37° and cells were washed with PBS. PBS (5 ml) containing either 0.6 mM PLP or 3 mM valproic acid or 0.6 mM PLP + 3 mM valproic acid was added to cell monolayers and the cultures were incubated at 37° for 30 min. Controls were made omitting the drugs. The medium was subsequently recovered (extracellular medium), cell monolayers were washed with fresh PBS and 2 ml of methanol added to each flask. Chloroform was then added to the methanolic suspensions to a final chloroform:methanol ratio of 2:1 (v/v). Water-soluble (intracellular fraction) and lipid extracts were obtained according to Folch *et al.* [9]. Phosphatidylcholine was isolated by TLC and its radioactivity counted as described above. The radioactivity of choline, phosphocholine and CDP-choline was determined on aliquots of the extracellular medium and on the intracellular aqueous fraction after separation by HPLC using Aminex A-14 ion exchange resin, as described above.

Effect of PLP and valproic acid on the permeability of lipid bilayers. The possibility that the incubation of cells with PLP and/or valproic acid modifies the permeability of the lipid phase of cell membranes has been taken into consideration. Small unilamellar vesicles were prepared according to the following procedure. Ox brain phosphatidylcholine (20 μmol), ox brain phosphatidylethanolamine (6 μmol), cholesterol (6 μmol) and 1,2-diacyl-*sn*-glycero-3-phospho-[U-^{14}C]serine (250 nCi, specific radioactivity of 170 Ci/mol) were dissolved in chloroform/methanol (2:1, v/v) and the solution dried under a gentle N_2 flux. The lipid material was then suspended in 0.15 M NaCl (1.2 ml) containing either 100 μCi of [*methyl*-^{3}H]choline (specific radioactivity of 15,000 Ci/mol) or 100 μCi of [6,6'(n)-^{3}H] sucrose (specific radioactivity of 6,000 Ci/mol) or 100 μCi of tritiated inulin (specific radioactivity of 1,980 Ci/

mol). The suspensions were stirred for 2 hr, and sonicated to clearness. Liposomes were isolated from non-encapsulated labeled compounds by gel filtration on a Sephadex G-50 column (2 × 30 cm). Aliquots (50 μl) of this suspension were used to determine the $^3H/^{14}C$ ratio. Aliquots of the same suspensions (0.5 ml) were supplemented with PLP in 0.15 M NaCl to 0.6 mM final concentration or with valproic acid in 0.15 M NaCl to final concentrations ranging from 1 to 3 mM. Other aliquots of the same suspensions were supplemented with both PLP and valproic acid at final concentrations of 0.6 mM and 3 mM, respectively. The final volume of the mixtures was 0.6 ml. Controls were made omitting both drugs. The suspensions were incubated at 37° for 30 min and then loaded on Sephadex G-50 columns (1 × 20 cm) equilibrated with 0.15 M NaCl. Liposomes were eluted with 0.15 M NaCl and counted for the $^3H/^{14}C$ isotope ratio.

RESULTS

Effect of PLP on phospholipid synthesis

The incorporation of radioactive amino alcohols into lipid was strongly hindered by a pre-treatment of cells with PLP. Indeed, 0.3 mM PLP was sufficient to reduce the incorporation of choline by more than 90% (Fig. 1B). The effect of PLP treatment was dramatic also when ethanolamine was the labeled precursor, although, in this case, larger doses of PLP were required to inhibit by more than 90% the formation of radioactive ethanolamine lipids (Fig. 1A).

The preincubation with PLP also produced a strong decrease of the radioactivity of intracellular water-soluble choline-containing compounds (choline, phosphocholine and CDP-choline), whereas the radioactivity of the corresponding ethanolamine compounds was not affected by the treatment. This difference is probably due to the fact that the medium contains 28 μM choline but no ethanolamine.

Since the sum of lipid and water-soluble radioactivity (Fig. 1) represents the total radioactivity in the cells, it can be inferred that the pretreatment with PLP had a dramatic effect on the uptake of radioactive amino alcohols supplied in the culture medium.

Effect of anticonvulsants on phospholipid labeling

The strong decrease of choline and ethanolamine incorporation into lipid caused by the pretreatment with PLP was reversed by valproic acid. This effect was very well evident for ethanolamine and less pronounced for choline. However, this capacity was not shared by other anticonvulsants, although the concentrations used in this work were generally higher than those reported as "therapeutic" [12] (Table 1).

The cells preincubated with 0.6 mM PLP + 3 mM valproic acid exhibited a 5-fold and 8.5-fold increase in the rate of choline and ethanolamine incorporation into lipid, respectively, in comparison with the cells preincubated with 0.6 mM PLP alone.

The preincubation with valproic acid alone did not influence significantly the utilization of radioactive choline and ethanolamine (Fig. 2, solid symbols). On the contrary, the inhibitory effect of PLP on the uptake of choline and ethanolamine was remarkably affected by valproic acid. In fact, 1 mM valproic acid in the preincubation medium reversed completely the inhibition of PE synthesis produced by 0.6 mM PLP (Fig. 2A). At higher valproate concentrations, the synthesis of phosphatidylethanolamine in the presence of 0.6 mM PLP was even more efficient than that of the control.

The effect of valproic acid on the uptake of choline and on its conversion into phosphatidylcholine was less noticeable (Fig 2C/D). In fact, after the preincubation with a medium containing 0.6 mM PLP and 3 mM valproic acid, the amount of phosphatidylcholine synthesized from labeled choline was only 25% of that of the controls. The labeling of the water-soluble choline-containing compounds was also affected by the incubation with valproic acid. In fact, in the presence of 0.6 mM PLP, the addition of 3 mM valproate produced a 4-fold increase of the water-soluble radioactivity in cells (Fig. 2D).

Label distribution among the water-soluble precursors of phosphatidylcholine

The distribution of label between choline and phosphocholine, after 1 hr incubation with [*methyl*-^3H]choline is reported in Table 2. Only traces of CDP-choline were found in control cells, whereas phosphocholine was the most heavily labeled water-soluble compound. Preincubation of cells with 0.6 mM PLP produced a dramatic decrease of phosphocholine labeling, whereas the treatment with valproic acid was ineffective. The inhibitory effect of PLP was reversed partially by the addition of 3 mM valproic acid.

Cellular energy charge

The cellular level of adenine nucleotides was determined after the treatment of cells with PLP and/or valproic acid, to examine a possible correlation between the modified phospholipid labeling and the energy charge of the cells. Experimental data (Table 3) show that no significant changes of cellular energy charge was produced by PLP and valproic acid, whereas ATP depletion occurred in cells treated with PBS containing both 3 mM valproate and 0.6 mM PLP.

Degradation of phospholipids

The effect of PLP and valproic acid on the degradation of phosphatidylcholine was studied using cells whose phosphatidylcholine had been previously labeled incubating the cultures with radioactive choline. Both valproic acid and PLP partially protected phosphatidylcholine from degradation (Table 4). After 30 min of incubation of prelabeled cells in unsupplemented PBS, only 33% of total radioactivity was found in the lipid, as a consequence of phosphatidylcholine degradation to water-soluble products. About 50% of total radioactivity flowed from the cell into the culture medium as free choline.

After 30 min of incubation in a medium containing 0.6 mM PLP, the degradation of phosphatidylcholine was reduced and a dramatic change in the distribution of water-soluble choline compounds was

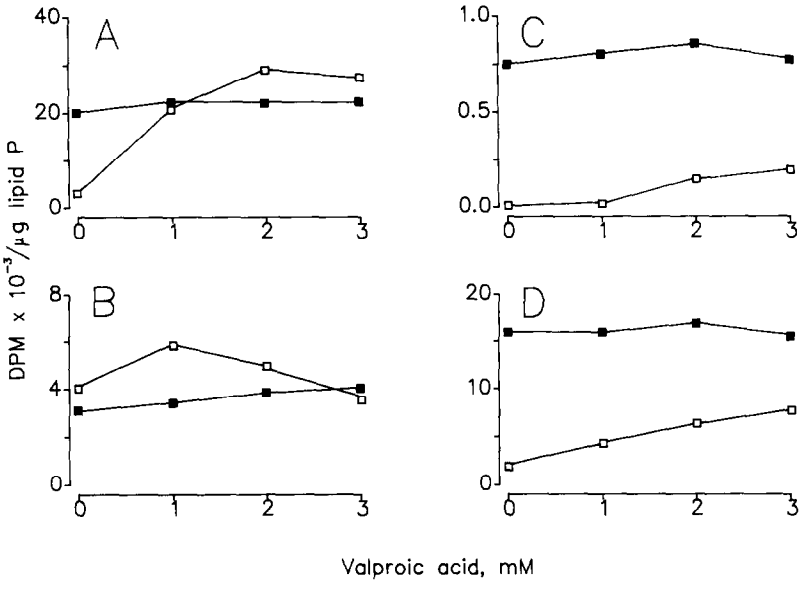

Fig. 2. Effect of valproic acid on lipid synthesis. Neuroblastoma cell cultures were incubated 30 min with PBS containing valproic acid (solid symbols) or 0.6 mM PLP + valproic acid (open symbols). The medium was removed and the cells incubated 1 hr with fresh medium containing labeled ethanolamine or choline. The radioactivity of phosphatidylethanolamine (A), ethanolamine-containing water-soluble compounds (B), phosphatidylcholine (C) and choline-containing water-soluble compounds (D) was measured. Experimental points are the average of three separate determinations. SD was less than 10%.

Table 3. Energy charge of cells after the incubation with PLP and valproic acid

Treatment	Energy charge First experiment	Second experiment
Control	0.88	0.85
0.6 mM PLP	0.89	0.84
3 mM VAL	0.85	0.84
3 mM VAL + 0.6 mM PLP	0.52	0.50

Neuroblastoma cells were incubated 30 min in PBS with the indicated drugs. After the removal of the medium, nucleotides were extracted, separated by HPLC and determined as described in Materials and Methods. The energy charge was calculated as ([ATP] + ½ [ADP])/([ATP] + [ADP] + [AMP]). PLP = pyridoxal 5'-phosphate; VAL = valproic acid.

observed. In fact, only a small portion of water-soluble radioactivity was present in the extracellular medium, the rest being retained in the cell. The molecules which accumulated in cells were phosphocholine and CDP-choline. A smaller accumulation of phosphocholine and CDP-choline was observed after the incubation of prelabeled cells with 3 mM valproic acid in PBS. After incubation of prelabeled cells with PBS containing 3 mM valproic acid and 0.6 mM PLP, the flow of labeled choline from the cells to the medium was very limited, as it was after the incubation with PLP alone. In any tested conditions, choline was the only labeled compound present in the extracellular medium.

Effect of PLP and valproic acid on the permeability of lipid bilayers

PLP and valproic acid did not produce leakage of liposomes, as demonstrated by the retention of the original isotope ratio, following incubation of ^{14}C-labeled liposomes containing tritiated inulin. The $^{3}H/^{14}C$ isotope ratio, in fact, was 5.4 both after incubation of liposomes with 0.6 mM PLP and/or 3 mM valproic acid in 0.15 M NaCl and after incubation with 0.15 M NaCl.

When ^{14}C-liposomes containing ^{3}H-choline or ^{3}H-sucrose were incubated with 0.6 mM PLP and/or 3 mM valproic acid, no significant changes of the original isotope ratio were detected. These results indicate that the permeability of liposomes was not affected by treatments with PLP and valproic acid, in the concentration ranges used throughout this work.

DISCUSSION

Convulsive seizures are known to produce changes on brain lipid metabolism [13]. An increase of the cerebral levels of diacylglycerol and free fatty acids occurs during seizures [13, 14] and the synthesis of brain phospholipids has been shown to be influenced by the intracerebral injection of several convulsants, among which PLP [6, 15].

In the present study, neuroblastoma cells have been used as a model for studying the changes produced by PLP and by some anticonvulsant drugs on phospholipid metabolism. In fact this model would permit a precise concentration of drugs in the extracellular medium and a correct evaluation of labeled compounds entering and leaving the cell to be obtained.

The results reported in this paper indicate that the preincubation of cells with a PLP-containing medium produces a concentration-dependent decrease of eth-

Table 4. Label distribution among choline-containing compounds after 30 min of incubation with PLP and valproic acid of prelabeled neuroblastoma cells

Treatment	Lipids	Water-soluble compounds Cytosol			Medium
	Phosphatidylcholine	Choline	Phosphocholine	CDP-choline	Choline
Control 1	82.5 ± 2.3	5.2 ± 1.1	11.3 ± 2.1	traces	0
Control 2	33.0 ± 0.4	1.0 ± 0.2	14.7 ± 4.1	1.1 ± 0.1	49.1 ± 3.1
0.6 mM PLP	48.0 ± 0.2	1.5 ± 0.2	38.0 ± 1.2	4.9 ± 0.6	6.9 ± 1.0
3 mM VAL	40.6 ± 3.1	0.9 ± 0.1	28.2 ± 2.2	1.9 ± 0.2	28.4 ± 1.6
0.6 mM PLP + 3 mM VAL	40.5 ± 1.6	1.9 ± 0.3	49.7 ± 2.5	2.5 ± 0.2	5.6 ± 0.9

Three separated flasks of neuroblastoma cells were incubated 5 hr in Dulbecco's MEM supplemented with 300 nCi/ml of [*methyl*-^{3}H]choline. The medium was then removed, cell monolayers washed and supplemented with PBS containing PLP and/or valproic acid. After 30 min of incubation at 37°, the medium was removed and collected and the cells harvested in methanol. Choline-containing compounds were then separated and counted for radioactivity.

Control 1: After the incubation with labeled choline, the medium was removed, the cells washed and harvested immediately.

Control 2: After the incubation with labeled choline, the cells were incubated with fresh PBS to which no drugs had been added.

Data are expressed as percent of total radioactivity (80,591 ± 1023 DPM/μg total lipid phosphorus) and represent the mean ± SD of three experiments. PLP = pyridoxal 5'-phosphate; VAL = valproic acid.

anolamine and choline incorporation into lipid, which is not due to changes of the energy charge of the cells. The decreased incorporation of choline into lipid seems to originate from a decreased uptake of the free base and to its slower conversion into phosphoric ester. Since PLP does not diffuse easily across cell membranes [16], it is supposed to react only with the primary amino groups of proteins and phospholipids on the outer surface of the cell, although effects due to increased concentrations of PLP inside the cells cannot be ruled out. As a consequence, the depression of phosphatidylcholine synthesis, which is carried out by enzymes located in the cytosol and in the endoplasmic reticulum membranes, should be the consequence of changes at the plasma membrane level. In particular, the inactivation of specific carriers by PLP could explain the concentration-dependent decrease of the uptake of choline by the cultured cells.

The ability of synthesizing phospholipid was completely or partially restored when cells were preincubated with PBS containing both PLP and valproic acid, although the energy charge of the cells was strongly reduced, in these conditions. An explanation for this could be that valproic acid increases the permeability of plasma membranes to choline. Since the permeability of the lipid bilayers to choline and sucrose is not affected by PLP and valproic acid, as demonstrated by using artificial lipid membranes, the decreased uptake of choline can be ascribed to non-lipid components of membranes.

PLP and valproic acid appear to affect the degradation of cellular phosphatidylcholine. In fact, the labeled lipid is converted into its degradation products at a slower rate when cells are incubated with a medium containing PLP or valproate. The increased stability of phosphatidylcholine seems to be due to the accumulation of phosphocholine and CDP-choline in the cytoplasm of cells treated with PLP or valproic acid.

This paper demonstrates that PLP supplemented in the culture medium of neuroblastoma cells affects the metabolism of membrane lipids. Valproic acid reverses the effects produced by PLP on the biosynthetic pathways, but not on the catabolism of phosphatidylcholine. At present, no convincing explanations can be given for these experimental data. However, the correspondence existing between the physiological effects produced by PLP and valproic acid on the animal and the biochemical changes described in this paper suggest that a similar mechanism might underlay both phenomena.

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