Changes in phospholipid metabolism induced by quinine, 4-aminopyridine and tetraethylammonium in the monocytic cell line THP1

Claudette PELASSY, Nancy CATTAN and Claude AUSSEL*

Unité de Recherches en Immunologie Cellulaire et Moléculaire, INSERM U210, Faculté de Médecine, 06034 Nice Cédex, France

Quinine, 4-aminopyridine and tetraethylammonium, three compounds generally used as effectors of K⁺ channels, strongly modify phospholipid metabolism. In the human monocytic cell line THP1, the three drugs enhanced the incorporation of [³H]serine into phosphatidylserine and that of [³H]inositol into phosphatidylinositol in the absence of significant changes in the uptake of the ³H labels. On the contrary, the biosynthesis of both phosphatidylcholine and phosphatidylethanolamine was strongly inhibited. This inhibition appeared to be mainly due to the inhibition of both [³H]choline and [³H]ethanolamine uptake by the cells, by impairment of choline transport in a competitive mode.

INTRODUCTION

Three compounds, quinine, 4-aminopyridine and tetraethylammonium, generally used as effectors of K⁺ channels, are able to increase phosphatidylserine synthesis in the human T-cell line Jurkat (Aussel *et al.*, 1990a). Changes in phosphatidylserine synthesis occurred in a range of concentrations similar to that used to block K⁺ channels, to inhibit interleukin-2 synthesis or to impair T-cell proliferation induced by lectins or monoclonal antibodies directed against the T-cell receptor complex. Phospholipid synthesis takes part in long-lasting biological events such as cell proliferation. In addition, it is well known that mammalian cells grown in culture have a nutritional requirement for one or more lipid precursors. Thus it was decided to study the effect on phospholipid synthesis of some drugs used to regulate cell proliferation through K⁺-channel blockade. Whether changes in phosphatidylserine induced by K⁺-channel blockers are specific to the Jurkat cell line and whether the synthesis of the other phospholipids is affected by these drugs remained unknown. In the present paper we show that, in the human monocytic cell line THP1, quinine, 4-aminopyridine and tetraethylammonium markedly increase phosphatidylserine and phosphatidylinositol synthesis and strongly decrease phosphatidylcholine and phosphatidylethanolamine synthesis.

MATERIALS AND METHODS

Cells

The monocytic cell line THP1 was cultured in RPMI 1640 (Seromed, Lille, France) supplemented with 5% (v/v) fetal-calf serum, 2 mM-L-glutamine and 1 mM-pyruvate.

Chemicals

Quinine, 4-aminopyridine and tetraethylammonium chloride were purchased from Aldrich Chimie (Strasbourg, France). Hemicholinium-3 was from Sigma Chemical Co. [1-³H]Ethan-1-ol-2-amine hydrochloride (1.85–2.2 GBq/mmol), [methyl-³H]choline chloride (2.8–3.1 GBq/mmol), *myo*-[³H]inositol (2.96–3.34 TBq/mmol) and [³H]serine (0.37–1 TBq/mmol) were purchased from Amersham.

Phospholipid synthesis

THP1 cells (2 × 10⁶) were maintained in 0.5 ml of buffer (pH 7.4) containing 137 mM-NaCl, 2.7 mM-KCl, 1 mM-Na₂HPO₄, 2.5 mM-glucose, 20 mM-Hepes, 0.1% BSA, 1 mM-CaCl₂ and 1 mM-MgCl₂, at 37 °C in the presence of the tritiated precursors for phospholipid synthesis and effectors (see concentrations in the Figure legends). After an incubation period of 2–4 h, the cells were rapidly sedimented in an Eppendorf centrifuge, washed, the supernatants were discarded, and the cell phospholipids were extracted with chloroform/methanol/water (Bligh & Dyer, 1959). This two-step extraction procedure allowed the determination of total ³H-labelled products incorporated into the cells, by measuring radioactivity in a 50 µl sample of the chloroform/methanol extract. Then addition of chloroform and water allowed separation of the organic and aqueous phases. The lipid extracts (organic phases) were analysed by t.l.c. on LK6D plates (Whatman) in the solvent system chloroform/methanol/acetic acid/water (25:15:4:1, by vol.). Authentic phospholipid standards (Sigma Chemical Co., St. Louis, MO, U.S.A.) were run in parallel and detected with iodine vapour. Radioactivity on t.l.c. plates was quantified by using an automatic linear radiochromatography analyser (Berthold) equipped with an 8 mm window.

RESULTS

Effect of quinine, 4-aminopyridine and tetraethylammonium on phospholipid synthesis

Phospholipid synthesis in THP1 cells was monitored by measuring the incorporation of [³H]choline, [³H]ethanolamine, [³H]inositol or [³H]serine into the corresponding phospholipid, in the absence or presence of quinine, 4-aminopyridine or tetraethylammonium. A concentration-dependent inhibition of phosphatidylcholine (Fig. 1a) and phosphatidylethanolamine (Fig. 1b) synthesis was observed. The IC₅₀ (concn. giving 50% inhibition) for inhibition of phosphatidylcholine synthesis was 101 ± 13 µM, 3.75 ± 1.25 mM and 9.98 ± 0.5 mM for quinine, 4-aminopyridine and tetraethylammonium respectively. Identical results were obtained for the inhibition of phosphatidylethanolamine synthesis by these three drugs. Concomitantly, net

Abbreviation used: IC₅₀, concn. giving 50% inhibition.

* To whom correspondence should be addressed.

Fig. 1. Phospholipid synthesis in THP1 cells

Cells were incubated at 37 °C for 3 h in the presence of 4 μ Ci of [3 H]choline (a), 4 μ Ci of [3 H]ethanolamine (b), 40 μ Ci of [3 H]serine (c) or 40 μ Ci of [3 H]inositol (d) and various concentrations of quinine ($\square$), 4-aminopyridine ($\blacksquare$) or tetraethylammonium ($\circ$). When tetraethylammonium was used, iso-osmoticity of the medium was maintained by replacing NaCl by an equivalent amount of tetraethylammonium. After lipid extraction as described in the Materials and method section, phospholipids were analysed by t.l.c. Radioactivity was measured by using a linear radiochromatography analyser. Results are expressed as percentages of values for untreated controls; each point represents the mean of three experiments. Basal values of radioactivity were: 1181 $\pm$ 67, 5950 $\pm$ 122, 227 $\pm$ 35 and 355 $\pm$ 28 c.p.m. (per 2×10^6 cells) for phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine respectively.

increases in the incorporation of [3 H]serine and [3 H]inositol into phosphatidylserine (Fig. 1c) and phosphatidylinositol (Fig. 1d) were observed.

Relationships between polar head-group incorporation and phospholipid synthesis

In order to verify whether the changes observed in phospholipid metabolism are due to eventual modifications of the uptake of the different phospholipid polar head groups, we have measured the total incorporation of the four tritiated polar head groups by the cells and plotted this value versus the amount of phospholipid synthesized. As shown in Fig. 2, a linear relationship was found between the total uptake of [3 H]choline or [3 H]ethanolamine and the amount of phosphatidylcholine or phosphatidylethanolamine synthesized. In contrast, both phosphatidylserine synthesis and phosphatidylinositol synthesis were found to be largely independent of the uptake of the corresponding polar head group, i.e. [3 H]inositol and [3 H]serine respectively, by the cells. Similar results were obtained when cells were treated with quinine (Fig. 2a), 4-aminopyridine (Fig. 2b) or tetraethylammonium (Fig. 2c).

Quinine, 4-aminopyridine and tetraethylammonium inhibit the uptake of choline and ethanolamine

The above data strongly suggested that both [3 H]choline and [3 H]ethanolamine uptakes were affected by the three drugs used. We have thus measured the total incorporation of both [3 H]choline and [3 H]ethanolamine by the cells as a function of the concentration of quinine, 4-aminopyridine and tetraethylammonium.

A concentration-dependent inhibition of [3 H]choline (Fig. 3a) and [3 H]ethanolamine (Fig. 3b) uptake was found. The IC_{50} values for the inhibition of both choline and ethanolamine uptakes were 79.7 $\pm$ 11 μ M, 2.5 $\pm$ 1.2 mM and 10.7 $\pm$ 1.3 mM for

quinine, 4-aminopyridine and tetraethylammonium respectively. On the contrary, the uptake of both [3 H]serine and [3 H]inositol was not affected by the drugs (results not shown).

Choline transport in THP1 cells

The uptake of [3 H]choline into THP1 cells was linear for 1 h over a large range of choline concentrations (results not shown), allowing the measurement of kinetic parameters of the choline-transport system in these cells. The results obtained, shown in Fig. 4, indicated that the drugs were competitive inhibitors of choline uptake. V_{max} (5 $\pm$ 0.2 μ mol/min per 10^6 cells for controls; $n = 6$) remained quite constant (less than 20 % variation) in the presence of the drugs. In contrast, the apparent K_m (8.5 μ M for control cells) was changed considerably. The values found were 15.4 μ M in the presence of quinine, 27.1 μ M in the presence of 4-aminopyridine and 20 μ M in the presence of tetraethylammonium. Hemicholinium-3, a well-known competitive inhibitor of choline transport (Barker & Mittag, 1975; Ancelin & Vial, 1986; Sheff *et al.*, 1990, 1991), was included at 20 μ M for comparison in several experiments; an apparent K_m of 13.9 μ M was found, with a V_{max} of 4.95 μ mol/min per 10^6 cells.

Changes in phosphatidylserine and phosphatidylinositol synthesis are not correlated with the inhibition of choline incorporation

Since the three drugs quinine, 4-aminopyridine and tetraethylammonium concomitantly inhibited phosphatidylethanolamine and phosphatidylcholine synthesis and enhanced phosphatidylserine and phosphatidylinositol synthesis, we tested whether a well-known competitive inhibitor of choline transport, hemicholinium-3 (Ancelin & Vial, 1986; Sheff *et al.*, 1990) affected serine uptake or phosphatidylserine metabolism. As shown in Fig. 5(a), hemicholinium-3 inhibits both [3 H]choline uptake and phosphatidylcholine synthesis, with an IC_{50} of 20 mM. However, this drug did not significantly modify either the uptake

Fig. 2. Relationships between the uptake of polar head group (total radioactivity incorporated by the cells) and phospholipid synthesis (radioactivity incorporated into phospholipids as determined after extraction and t.l.c.) in THP1 cells treated with various amounts of quinine (a), 4-aminopyridine (b) or tetraethylammonium (c)

The concentrations of the three drugs were as in Fig. 1. Cells were incubated in the presence of [³H]choline (□), [³H]ethanolamine (○), [³H]serine (■) or [³H]inositol (●). Other details were as for Fig. 1 and in the Materials and methods section. The 100% value corresponds to the conditions without drugs.

of [³H]serine or the synthesis of phosphatidylserine. Similar results (Fig. 5b) were obtained concerning [³H]ethanolamine uptake and phosphatidylethanolamine synthesis on the one hand and [³H]inositol uptake and phosphatidylinositol synthesis on the other.

DISCUSSION

Lipid compositional changes are known to regulate cellular metabolism, since altered phospholipid patterns exert a variety of effects on the activity of membrane-bound enzymes and receptors (for review, see Kuo, 1985). On the other hand, changes in the activity of K⁺ channels have been involved both in the regulation of cell metabolism as well as in the regulation of cell proliferation (Gardner, 1990; Gelfand, 1989; Gallin, 1986). For example, in the immune system, the participation of K⁺ channels in the regulation of interleukin-2 synthesis and T-cell proliferation has been obtained from experiments demonstrating that the classical K⁺-channel blockers such as quinine, 4-aminopyridine and tetraethylammonium were able to block T-cell responses at concentrations similar to those generally used

Fig. 3. Effect of quinine (□), 4-aminopyridine (■) and tetraethylammonium (○) on the uptake of [³H]choline (a) and [³H]ethanolamine (b)

Cells were incubated for 2 h in the presence of 4 μ Ci of either [³H]choline or [³H]ethanolamine. Results are expressed as percentages of values for control untreated cells. Radioactivity of control cells was 32494 ± 557 and 10117 ± 252 c.p.m. (per 2×10^6 cells) for ethanolamine and choline respectively.

Fig. 4. Lineweaver-Burk plot of kinetic data for control (□), 100 μ M-quinine (○) and 5 mM-4-aminopyridine (■)-treated cells

The uptake of [³H]choline was measured for 20 min in the presence of various concentrations (2–50 μ M) of unlabelled choline. The lines drawn are those derived by non-linear least-squares regression analysis; derived parameter values are given in the text. Units of v are μ mol/min per 10^6 cells ($\times 100$).

to block the K⁺ channel when studied by electrophysiological techniques (Grissmer & Cahalan, 1989; Cahalan *et al.*, 1985). Recently we showed (Aussel *et al.*, 1990a) that, in the Jurkat T-cell line, quinine, 4-aminopyridine and tetraethylammonium were able, with similar concentration/response curves, to inhibit K⁺ channels, to inhibit interleukin-2 synthesis and to enhance phosphatidylserine synthesis. To see whether these effects of K⁺-channel blockers are related or not to a particular cell line, i.e. Jurkat, we have undertaken a study of phospholipid metabolism in the THP1 cell line. In the present paper, we provide evidence that the classical K⁺-channel blockers, i.e. quinine, 4-amino-

Fig. 5. Effect of hemicholinium-3 on phospholipid polar head-group incorporation and phospholipid synthesis

Cells were incubated in the presence of either [^3H]choline or [^3H]serine (a) or either [^3H]ethanolamine or [^3H]inositol (b). In (a), ○ and ● represent the total incorporation of [^3H]choline and phosphatidylcholine synthesis respectively, and □ and ■ represent [^3H]serine incorporation and phosphatidylserine synthesis respectively. In (b), ○ represents [^3H]ethanolamine incorporation, ● phosphatidylethanolamine synthesis, □ [^3H]inositol incorporation and ■ phosphatidylinositol synthesis. Experimental details are given in the Materials and methods section.

pyridine and tetraethylammonium, markedly change phospholipid metabolism in THP1 cells. The three drugs clearly inhibit both phosphatidylcholine and phosphatidylethanolamine synthesis (Fig. 1). The mechanism of this inhibition involves a decreased uptake of the polar head group into the cells (Figs. 3 and 4), since the drugs act as competitive inhibitors of choline transport. On the contrary, synthesis of both phosphatidylserine and phosphatidylinositol was increased without noticeable changes in the uptake of either serine or *myo*-inositol into the cells. Use of hemicholinium-3, a drug that is known as a competitive inhibitor of choline transport in various cell types (Ancelin & Vial, 1986; Sheff *et al.*, 1990), unambiguously demonstrated that inhibition of both choline uptake and phosphatidylcholine synthesis or ethanolamine uptake and ethanolamine synthesis remained without effect on the synthesis of the other phospholipids, phosphatidylserine and phosphatidylinositol. This indicates that the pathways leading to the synthesis

of the different phospholipid classes are not directly correlated. In addition, and in contrast with our previous results obtained in Jurkat cells (Aussel *et al.*, 1990b), no incorporation of [^3H]serine into phosphatidylethanolamine (through decarboxylation of phosphatidylserine) nor into phosphatidylcholine (through the transmethylation of phosphatidylethanolamine) could be demonstrated in THP1 cells.

Changes in phosphatidylserine and/or phosphatidylinositol synthesis induced by quinine, 4-aminopyridine and tetraethylammonium may represent either a side effect of these drugs or an intermediary step between the blockade of K^+ channels and long-lasting biological responses such as protein synthesis and cell proliferation. Further work is necessary to elucidate the exact mechanism of regulation of phospholipid metabolism, and especially the regulation of the base-exchange enzyme system (that leads to the biosynthesis of phosphatidylserine), by these drugs and the eventual participation of K^+ channels in this regulation. Interestingly, a recent paper (Smejtek *et al.*, 1990) demonstrated that the potencies of aminopyridines in blocking K^+ channels correlate with the association constants of the drugs with phosphatidylserine membranes. The possibility that aminopyridines act at the intracellular face of the K^+ channel and that this binding domain has characteristics similar to that of the phosphatidylserine head group (Molgo *et al.*, 1985) emphasize the relationships between K^+ channels and phosphatidylserine.

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