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Effects of antiarrhythmic drugs on phospholipid metabolism in Jurkat T cells

The potassium channel blocker, clofilium, specifically increases phosphatidylserine synthesis

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Five antiarrhythmic drugs (bretylium, clofilium, propranolol, *N*-acetylprocainamide and amiodarone) were tested for their ability to modify phospholipid metabolism in Jurkat T lymphocytes. The five drugs, decreased in a dose-dependent mode the biosynthesis of both phosphatidylcholine and phosphatidylethanolamine, this effect was essentially due to impairment of either choline or ethanolamine uptake by the cells. The efficiency of the drugs to inhibit phosphatidylcholine and phosphatidylethanolamine synthesis was in the order: clofilium > amiodarone ≫ propranolol = bretylium ≫ *N*-acetylprocainamide. The IC_{50} varied from 3–5 μM for clofilium to >200 μM for *N*-acetylprocainamide. In contrast, only clofilium, a voltage-gated K^+-channel blocker, was able to increase phosphatidylserine synthesis with an EC_{50} = 50 μM. The effect of clofilium on phosphatidylserine synthesis thus mimics the effect of three other K^+-channel blockers, quinine, 4-aminopyridine and tetraethylammonium, suggesting close relationships between phosphatidylserine synthesis and K^+ channel activity.

Phospholipid; Phosphatidylserine; Potassium channel; Antiarrhythmic drug

1. INTRODUCTION

Cationic amphiphilic drugs, such as antiarrhythmics are known to modify phospholipid metabolism [1]. Amiodarone, for example, has been shown to inhibit choline uptake and phosphatidylcholine (PtdCho) biosynthesis in cardiac myocytes [2]. These metabolic effects of amiodarone raise the question whether the effect on choline uptake could be implicated in some amiodarone's electrophysiologic or toxic effects. As discussed by Rabkin [2], the amiodarone-induced reduction in choline uptake may modulate the outward potassium current in myocytes. On the other hand, we have shown in previous reports [3,4], that in Jurkat T lymphocytes and in the monocytic cell line, THP1, the blockers of voltage-dependent potassium channels such as quinine, 4-aminopyridine and tetraethylammonium strongly modify phospholipid metabolism. Phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) synthesis were inhibited because K^+-channel blockers inhibited both choline and ethanolamine uptake by the cells. An effect very similar to that described for amiodarone [2]. In addition, K^+-channel blockers increased strongly phosphatidylserine (PtdSer) synthesis in Jurkat T cells. Since some antiarrhythmic drugs have been described to block voltage-gated K^+ channels and since Jurkat cells express this type of channel, it was of interest to see whether antiarrhythmic drugs affect phospholipid metabolism in T lymphocytes. The results presented show that the five antiarrhythmic drugs tested decrease PtdCho and PtdEtn synthesis. By contrast, only clofilium, a voltage-dependent K^+-channel blocker, was able to increase in a concentration-dependent mode the biosynthesis of PtdSer.

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2. MATERIALS AND METHODS

2.1. *Cells*

Jurkat D: the human T cell line Jurkat was kindly supplied by Dr. A.M. Schmitt-Verhulst (Centre d'Immunologie, Marseille-Luminy, France). Cells were cloned by limiting dilution and clone D was selected on the basis of its IL-2 production when activated with either PHA, CD3 or CD2 mAbs in the presence of the phorbolester (TPA). Cells were cultured in RPMI 1640 (Seromed, Lille, France) supplemented with 5% fetal calf serum, 2 mM L-glutamine, 1 mM pyruvate.

2.2. *Chemicals*

Amiodarone hydrochloride was purchased from Sigma Chemical Co. (La Verpillère, France). *N*-Acetylprocainamide hydrochloride (NAPA), clofilium tosylate and bretylium tosylate were from RBI (Natick, MA, USA). Propranolol was from Aldrich (Strasbourg, France). [1-^{3}H]Ethan-1-ol-2-amine hydrochloride (1.85–2.2 GBq/mmol), [methyl-^{3}H]choline chloride (2.8–3.1 GBq/mmol) and [^{3}H]serine (0.37–1 TBq/mmol) were purchased from Amersham (Les Ulis, France).

2.3. *Phospholipid synthesis*

Jurkat cells (2×10^6) were maintained in 0.5 ml of a buffer (pH 7.4) containing 137 mM NaCl, 2.7 mM KCl, 1 mM Na_2HPO_4, 2.5 mM glucose, 20 mM HEPES, 0.1% bovine serum albumin, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, at 37°C in the presence of the tritiated precursors for phospholipid synthesis and effectors (see concentrations in the figure legends). After a 3 h incubation period the cells were rapidly sedimented and washed in an Eppendorf centrifuge, the supernatants were discarded and the cell phospholipids were extracted using chloroform/methanol/water according to Bligh and Dyer [5]. This two-step extraction procedure allowed the determination of total [^{3}H]products incorporated into the cells, by measuring radioactivity on a 50 μl sample of the chloroform/methanol extract. For this purpose, the 50 μl sample was mixed with 3 ml of Picofluor-30 (Packard, Rungis, France) and radioactivity was determined by scintillation with a Kontron βmatic counter. Then the addition of chloroform and water allowed the separation of the organic and aqueous phases. The lipid extracts (organic phases) were analyzed by thin-layer chromatography on LK6D plates (Whatman) in a solvent system composed of chloroform/methanol/acetic acid/water (75:45:12:3). Authentic phospholipids standards (Sigma Chemicals, St. Louis, MO) were run in parallel and detected with iodide vapors. Radioactivity on TLC plates was quantified by using an automatic linear radiochromatography analyzer (Berthold) equipped with a 8 mm window.

3. RESULTS

3.1. *Choline uptake and PtdCho synthesis*

Measurements of total [^{3}H]choline uptake and of [^{3}H]choline incorporation into PtdCho in Jurkat T cells in the presence of various amounts of either amiodarone, NAPA, clofilium, propranolol or bretylium are depicted in Fig. 1. The five drugs induced a concentration-dependent decrease of [^{3}H]choline uptake by the cells and consequently induced a dose-dependent decrease of PtdCho synthesis as measured by the incorporation of [^{3}H]choline into this phospholipid.

3.2. *Ethanolamine uptake and PtdEtn synthesis*

Since in our previous reports [3,4] we have shown that K^+-channel blockers had similar effects on both choline- and ethanolamine-containing phospholipids, we have also studied the effect of the five above-mentioned drugs on [^{3}H]ethanolamine uptake and [^{3}H]ethanolamine incorporation into PtdEtn. As shown in Fig. 2, all the drugs studied decreased both [^{3}H]ethanolamine uptake and PtdEtn synthesis. The IC_{50} (Table I) indicated that the drugs studied inhibited both the uptake of the phospholipid polar head groups and phospholipid synthesis in the following order: clofilium > amiodarone ≫ propranolol = bretylium ≫ NAPA.

3.3. *PtdSer synthesis*

Previous results [3,4] indicated that K^+-channel blockers such as quinine, 4-aminopyridine and tetraethylammonium were able to increase PtdSer synthesis in absence of significant changes in the uptake of [^{3}H]serine into the cells. In order to verify the specificity of this unexpected result, we measured PtdSer synthesis in the presence of either amiodarone, NAPA, clofilium, propranolol and bretylium. As shown in Fig. 3, only clofilium was able to increase PtdSer synthesis in a dose-dependent mode with an EC_{50} = 50 μM. The 4 other drugs, amiodarone, NAPA, propranolol and bretylium, tested at concentrations up to 200 μM, had no significant effect on either [^{3}H]serine uptake or PtdSer synthesis (Fig. 3). In the above experiments PtdSer synthesis was measured after a 3 h incubation period; it was thus of interest to study the kinetic of PtdSer synthesis in control and K^+-channel blocker-treated cells. As shown in Fig. 4, PtdSer synthesis was measured from 0 to 60 min, and clofilium at 100 μM rapidly increased PtdSer

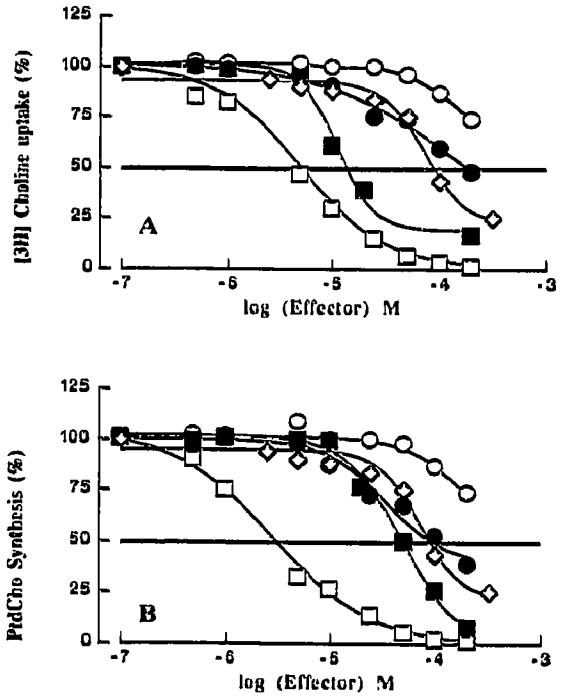

Fig. 1. Effect of antiarrhythmic drugs on [^{3}H]choline uptake (A) and phosphatidylcholine synthesis (B) in Jurkat T lymphocytes. Cells were incubated in the presence of 4 μCi [^{3}H]choline for 2 h then treated as described in section 2. Results are expressed as % versus untreated cells. (□) Clofilium; (■) amiodarone; (●) bretylium; (○) propranolol; and (◇) *N*-acetylprocainamide.

Table I

Effectors	Cho uptake (μM)	PtdCho (μM)	Etn uptake (μM)	PtdEtn (μM)
Clofilium	5.0	2.9	2.8	3.7
Amiodarone	11.3	34.4	9.7	16.8
Propranolol	100	105	100	90
Bretylium	130	100	111	95
NAPA	>200	>200	>200	>200

IC_{50} for the inhibition of [^{3}H]choline uptake (Cho uptake), phosphatidylcholine synthesis (PtdCho), [^{3}H]ethanolamine uptake (Etn uptake) and phosphatidylethanolamine synthesis (PtdEtn) by antiarrhythmic drugs. Each value represents the mean of duplicate determinations calculated from the curves in Figs. 1 and 2.

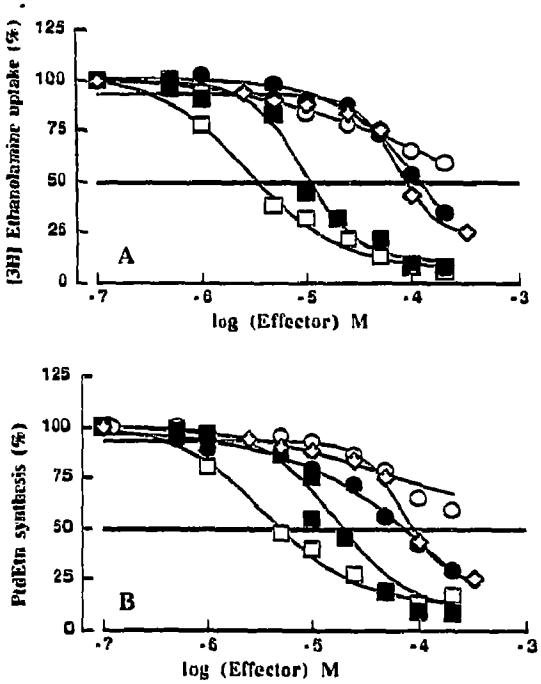

Fig. 2. Effect of antiarrhythmic drugs on [^{3}H]ethanolamine uptake (A) and phosphatidylethanolamine synthesis (B) in Jurkat T cells. Cells were incubated as described in the legend of Fig. 1 but in the presence of 4 μCi [^{3}H]ethanolamine. Results are expressed as % versus untreated cells. (□) Clofilium; (■) amiodarone; (●) bretylium; (○) propranolol; and (◇) *N*-acetylprocainamide.

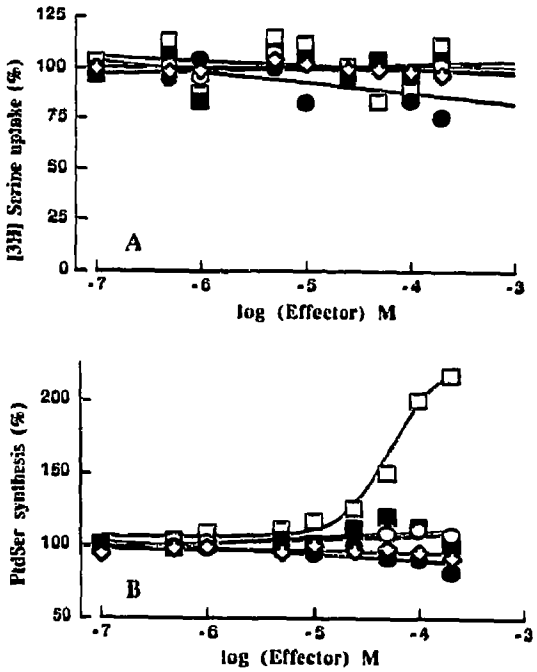

Fig. 3. Effect of antiarrhythmic drugs on [^{3}H]serine uptake and phosphatidylserine synthesis in Jurkat T lymphocytes. Cells were incubated for 2 h in the presence of 4 μCi [^{3}H]serine. Other details as in section 2. (□) Clofilium; (■) amiodarone; (●) bretylium; (○) propranolol; and (◇) *N*-acetylprocainamide.

synthesis. The differences between the points for control and clofilium-treated cells were highly significant ($P < 0.05$, $n = 6$) after 15 min of treatment.

3.4. *Metabolism of PtdSer into PtdEtn by decarboxylation*

In Jurkat cells [6], a portion of PtdSer is converted into PtdEtn by decarboxylation; we have thus studied this pathway in the presence of 100 μM of the different drugs. The results obtained indicated that clofilium, at this concentration, strongly raised PtdSer synthesis (5593 ± 161 cpm vs. 2622 ± 97 cpm for controls), while bretylium, amiodarone, propranolol and NAPA caused less than 15% change. Concomitantly, the incorporation of [^{3}H]serine into PtdEtn resulting from decarboxylation of ^{3}H-labelled PtdSer was measured and appeared non-significantly changed in the presence of clofilium (326 ± 17 cpm vs. 297 ± 27 cpm for untreated cells). The four other drugs were also without significant effect (less than 10% change).

4. DISCUSSION

Antiarrhythmic drugs such as amiodarone or propranolol belong to the family of cationic amphiphilic drugs. Although these drugs have been found extremely effective in the treatment of various cardiac arrhythmias, one limiting factor to their use is their toxicity. Alteration of phospholipid metabolism by cationic amphiphilic drugs has been recently reviewed [1]. Recent results obtained by Rabkin [2] have shown that amiodarone inhibits choline uptake and as a consequence PtdCho synthesis in cultured chick cardiac myocytes. In the present report, we confirm the results obtained by Rabkin on the effect of amiodarone on PtdCho synthesis and we extend these results to other antiarrhythmic drugs and to PtdEtn synthesis in different cells. Indeed, we have tested the effect of five antiarrhythmic drugs on PtdCho, PtdEtn and PtdSer metabolism in the Jurkat T cell line. The results obtained clearly indicate that all antiarrhythmic drugs tested decreased PtdCho and PtdEtn synthesis in a dose-dependent mode. This effect appears mainly due to a decreased uptake of the tritiated phospholipid polar head groups by the cells. Structural relationships between choline/ethanolamine and the antiarrhythmic drugs tested (all the drugs tested contain an ammonium group) might be the cause for the decreased uptake of choline and ethanolamine by the cells.

Results obtained when studying phosphatidylserine synthesis show that only clofilium is able to stimulate the synthesis of this phospholipid. Similar results have been previously observed [3,4] in cells treated with well-known K^+-channel blockers such as quinine, quinidine, 4-aminopyridine and tetraethylammonium. Since clofilium has been previously shown to block voltage-gated

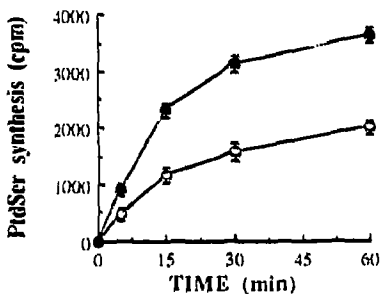

Fig. 4. Kinetics of clofilium-induced phosphatidylserine synthesis in Jurkat T cells. Results are expressed in cpm of [^{3}H]serine incorporated into phosphatidylserine measured after thin-layer chromatography of phospholipid extracts. Each point is the mean ± SD of two experiments each done in triplicate. (○) Control cells; and (●) clofilium-treated cells.

K^+-channel [7] and since this type of channel has been demonstrated to be the major K^+-channel expressed by T lymphocytes [8–10], including Jurkat T cells [3], it could be hypothesized that clofilium blocks K^+-channel in T cells. The mechanism of K^+-channel-blocker-induced PtdSer synthesis remains unknown. In T cells, as in other cells, PtdSer is synthesized by an exchange of polar head group with preexisting phospholipids such as PtdCho and PtdEtn [11]. The base exchange enzyme system is activated by Ca^{2+} ions and is energy independent [12,13]. A change in Ca^{2+} induced by K^+-channel blockers in Jurkat cells appears unlikely to explain the enhanced synthesis of PtdSer since we have shown (unpublished results) that K^+-channel blockers including quinine, quinidine and clofilium had no effect on intracellular Ca^{2+} concentration and does not affect changes in $[Ca^{2+}]_i$ induced by the classical T cell activators such as phytohaemagglutinin and monoclonal antibodies directed against the T cell receptor complex such as CD3. On the other hand, the stimulation of PtdSer synthesis is not due to a consequence of the inhibition of either PtdCho or PtdEtn synthesis since the other antiarrhythmic drugs have no effect on PtdSer and since we have previously shown that inhibitors of choline transport such as hemicholinium-3, decamethonium and dodecyltrimethyl ammonium strongly decreased PtdCho and PtdEtn synthesis but had no effect on PtdSer [4]. The strong and rapid changes observed in phospholipid metabolism may shed a new light on some side-effects of antiarrhythmic drugs but further work is necessary to elucidate the mechanism leading to activation of PtdSer synthesis by K^+-channel blockers. In addition, complex relationships between K^+-channel activity and PtdSer might exist since it has been shown that the ability of aminopyridines to block K^+-channels correlates with their potencies to bind to PtdSer membranes [14]. On the other hand, it has been shown [15] that the binding domain of aminopyridines at the intracellular face of the K^+-channel has characteristics similar to that of the PtdSer head group.

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