Synthesis of choline phospholipids in neuronal and glial cell cultures by the methylation pathway

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1. INTRODUCTION

The synthesis of choline in the nervous tissue has been a subject of debate (reviews [1,2]). Since the work of [3,4] it was believed that the stepwise methylation of ethanolamine and/or phosphatidylethanolamine in nervous tissue was non-existant or irrelevant until the suggestion that choline might be produced de novo in the rat brain [5–7] through methylation of phosphatidylethanolamine. Methyltransferase activity has been shown in rat brain synaptosomes [8,9], suggesting that nervous tissue may have the necessary machinery for the de novo synthesis of phosphatidylcholine.

The possibility of obtaining cell cultures containing exclusively neurons or glial cells gave us the opportunity to check, in these isolated systems, whether neurons and/or glia can methylate phosphatidylethanolamine to phosphatidylcholine. The results obtained suggest that both cell types have this capacity and that the synthesis of choline phospholipids through the methylation pathway is much higher in glial cells than in neurons.

2. MATERIALS AND METHODS

Cells were obtained from 8-day-old embryo brain (E8) for neurons and 14-day-old embryo brains (E14) for glia using standard procedures [10,11]. The neurons were kept in culture for 8 days (C8) and the glia for 14 days (C14). Both types of cell cultures were incubated with 10 μM [^{3}H]ethanolamine (spec. act. 5 Ci/mmol, Amersham) per dish growth medium. At various time intervals, the cultures were washed with a warm (37°C) 0.147 M NaCl solution. The cells were scraped into 2 ml 0.5 N HCl, homogenized, centrifuged (4000 × g, 15 min) and the pellet extracted with chloroform/methanol (2/1, v/v) as in [12]. The treatment of the cells with 0.5 N HCl hydrolyses the plasmalogens to lysophospholipids, and thus the radioactivity of the lysophosphatidylethanolamine and -choline should mainly represent the incorporation of the label into the plasmalogens. After addition of carriers, phospholipids were separated by bidimensional thin-layer chromatography on silica gel using chloroform–methanol–27% ammonia (65:35:5, by vol.) in the first direction and butanol–acetic acid–water (60:20:20, by vol.) in the second direction. The phospholipids were visualized by iodine vapour, scraped off and counted (after iodine evaporation) in 0.5 ml H_2O and 10 ml Rotiszint 22 (Roth, Karlsruhe) in an Intertechnique SL 30 scintillation spectrometer. Lipid phosphorus was measured as in [13].

3. RESULTS

Both cells incorporated [^{3}H]ethanolamine into ethanolamine phospholipids. In neurons the activity of phosphatidylethanolamine and lysophosphatidylethanolamine (plasmalogens) reached a maximum after 2.5 h incubation, whereas in glial cell the maximum of incorporation was reached only after 15 h (figs.1,2). In neurons, radioactivity, could be detected in monomethyl- and dimethylphosphatidylethanolamine after 15 min incuba-

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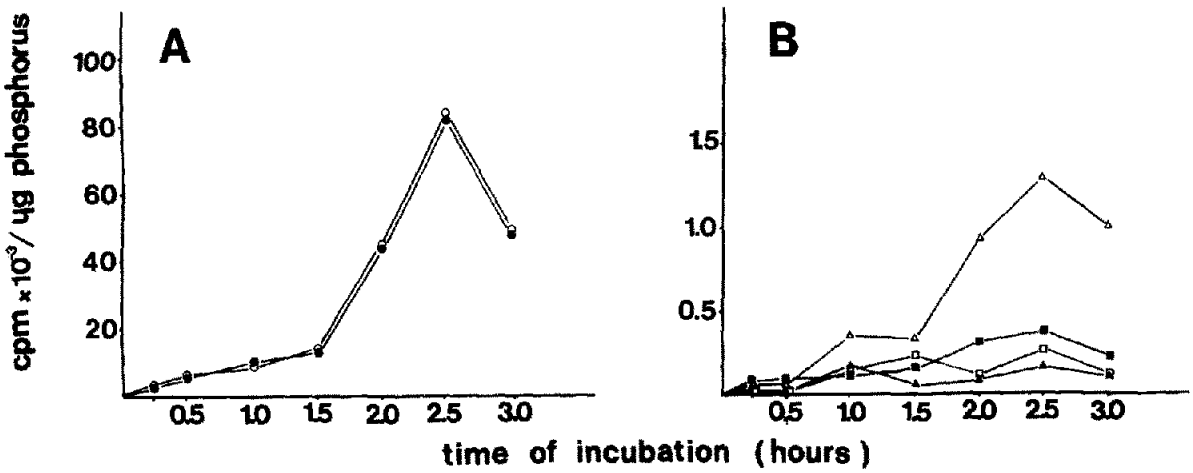

Fig.1. Incorporation of [^{3}H]ethanolamine into phospholipids of neurons, from chicken embryo. Incubation was performed as described in the text. Results are expressed as cpm of each phospholipid/µg total lipid phosphorus. Each point represents the average of 2 expt, each assayed in duplicate. The standard deviation was < 10%. (A) (○—○) phosphatidylethanolamine, (●—●) ethanolamine plasmalogens; (B) (△—△) monomethylphosphatidylethanolamine, (▲—▲) dimethylphosphatidylethanolamine; (□—□) phosphatidylcholine, (■—■) lysophosphatidylcholine.

tion. The activity of monomethylphosphatidylethanolamine increased up to 2.5 h at a much higher rate than in dimethylphosphatidylethanolamine and phosphatidylcholine (fig.1B).

To the opposite, in glial cells, the radioactivity in the three phospholipids could be detected only after 1 h incubation (fig.2B). The radioactivity increased thereafter during 15 h for monomethyl-

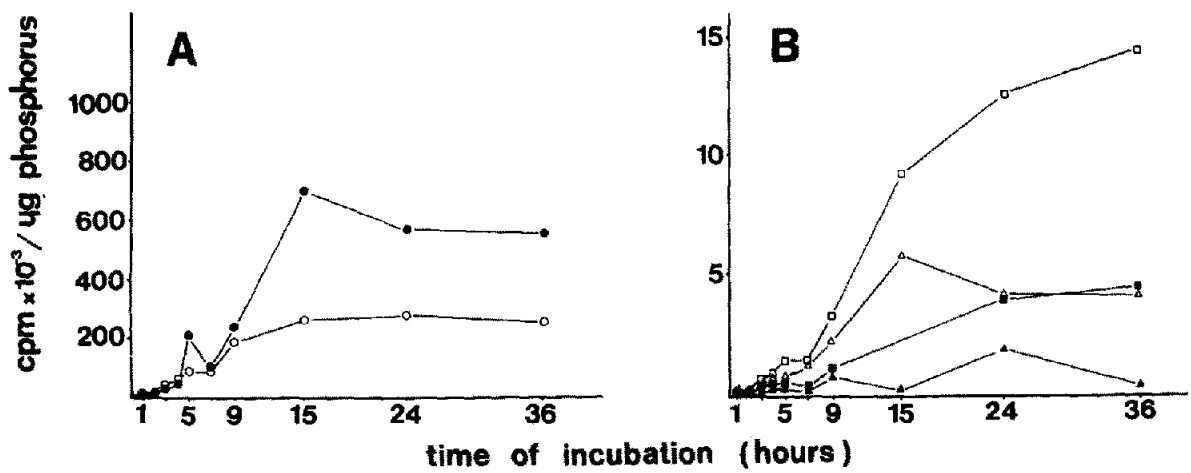

Fig.2. Incorporation of [^{3}H]ethanolamine into phospholipids of glial cells from chicken embryo. Incubation was performed as described in the text. Results are expressed as cpm of each phospholipid/µg total lipid phosphorus. Each point represents the average of 2 expt, each assayed in duplicate. The standard deviation was < 10%. (A) (○—○) phosphatidylethanolamine, (●—●) ethanolamine plasmalogens; (B) (△—△) monomethylphosphatidylethanolamine, (▲—▲) dimethylphosphatidylethanolamine; (□—□) phosphatidylcholine, (■—■) lysophosphatidylcholine.

phosphatidylethanolamine and up to 36 h for phosphatidylcholine. The activity of dimethylphosphatidylethanolamine remained very low and reached a plateau after 9 h incubation. Moreover, after 3 h incubation the activity of phosphatidylcholine increased at a much higher rate than in monomethyl and dimethylphosphatidylethanolamine. Between 3–36 h incubation the activity of phosphatidylcholine is higher when compared to monomethyl- and dimethylphosphatidylethanolamine. It should also be noted that the radioactivity of the phosphatidylcholine is ~2–3-times higher in glial cells than in neurons.

4. DISCUSSION

This work indicates that neurons and glial cells from chick embryo hemispheres in culture are able to synthesize phosphatidylcholine by stepwise methylation of ethanolamine and/or ethanolamine phospholipids. The occurrence of radioactive monomethyl- and dimethylphosphatidylethanolamine suggests that at least part of the synthesis of phosphatidylcholine in neurons and glial cells is catalyzed by methylation of the aminophospholipids. However, recent experiments also showed that both cells incubated with radioactive ethanolamine contain radioactive labelled phosphorylcholine (not shown), the amount being much higher in neurons than in glial cells after short incubation times, suggesting that either free ethanolamine or phosphorylethanolamine may be methylated in both cells. The results indicate that ethanolamine may be metabolized differently in neurons and glial cells. The higher amount of phosphatidylcholine synthesis in glial cells compared to neurons after long incubation times and the fact that choline of glial cells may be utilized by neurons during K^+ depolarization [14] suggest that glial cells may be a storage site (réservoir) of choline for neurons. The implication of such a mechanism in the synthesis of acetylcholine is under investigation.

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