THE RELATIONSHIPS BETWEEN THE PHOSPHOLIPID POOL AND THE BASE-EXCHANGE REACTION IN THE Ca^{2+}-STIMULATED INCORPORATION OF ETHANOLAMINE INTO BRAIN MICROSOMAL PHOSPHOLIPIDS

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Received 11 November 1974

1. Introduction

It has been shown in recent years that a Ca^{2+}-dependent base-exchange system occurs in purified brain microsomes which converts in vitro, at the expenses of membrane-bound phospholipids, labelled free ethanolamine, serine and choline into the corresponding phosphoglycerides [1–4]. Little information has been given, however, about the type of phospholipid which participates in the reaction at the nerve membrane level [2]. The experiments described here are aimed therefore at an examination of the type and degree of exchange that takes place in vitro at the brain microsomal level between endogenous phospholipid and choline, ethanolamine or L-serine, when the microsomal membranes are prelabelled in vitro in their PE either with 1,2-[^{14}C]ethanolamine by the base-exchange reaction [1,2] or with radioactive CDPE by de novo synthesis of PE (see [5]). It is shown that different degrees of displacement of the lipid-bound labelled ethanolamine are obtained by the exchange in vitro dependent on the mechanism of prelabelling the PE-containing microsomes. The result points to the existence in brain of at least two biochemically different pools of microsomal PE.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; CDPE, cytidine-5′-disphosphate-ethanolamine; S.A., specific activity; TLC, thin-layer chromatography; EPG, ethanolamine phosphoglycerides.

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2. Experimental

Brain microsomes from male Sprague-Dawley rats (120–150 g) were prepared and purified, as described elsewhere [2]. Two distinct types of incubation were then performed with the separated microsomes :

Incubation (a) : incorporation by base-exchange

The incubation mixture (0.5 ml) contained 2.5 mM $CaCl_2$, 40 mM *N*-2-hydroxyethylpiperazine–*N*-2-ethanesulfonic acid buffer, pH 8.1, 0.32 M sucrose–2 mM dithiothreitol, 1–2 mg of microsomal protein and 0.2 mM 1,2-[^{14}C]ethanolamine (S.A. of 1.79 nCi/nmol). After 15 min of shaking at 37°C, the incubation mixture was diluted 15-fold by adding a cold solution of 0.32 M sucrose-2 mM β-mercaptoethanol and the microsomes pelleted at 44 000 rpm for 60 min at 4°C. The pellet was well freed from its supernatant, the walls of the incubation test-tubes carefully drained with filter paper, and the microsomes immediately used for a subsequent incubation, as explained later. Less than 1 nCi/mg protein was left by this procedure as non-incorporated [^{14}C]ethanolamine in the microsomal pellet, and the corresponding values were always considered for each experiment in the final calculation. Control experiments (control a) were included in this set in which the microsomes were incubated for the base-exchange reaction with similar amounts of unlabelled ethanolamine, in place of the labelled base, but were otherwise treated identically.

Incubation (b) : de novo synthesis of PE

Incubation was carried out with 1–2 mg of microsomal protein and 0.4 mM labelled CDPE (S.A. of 3.13 nCi/nmol) in a final vol. of 0.5 ml at 40°C for 40 min, as described elsewhere [5]. After incubation, the mix-

ture was freed from any unreacted labelled CDPE, as reported under incubation (a). Control b, comparable with control a, was also included.

After incubation (a) or (b) the re-isolated microsomes were re-incubated under the standard conditions for base-exchange (see incubation a) but in the absence of isotope and in the presence of either unlabelled ethanolamine (2 mM), choline (4.5 mM) or L-serine (2 mM), to study the displacement of the lipid-bound base, due to exchange. Experiments were performed in absence of added cold base to study the displacement by water alone.

Re-incubations of the unlabelled microsomes (controls a and b) were also performed but in the presence of labelled ethanolamine, choline or L-serine at concentrations similar to those reported above.

After 3,6 and 9 min or re-incubation, the samples were inactivated by heating at 100°C for 3 min. Samples were also treated soon after the microsomal addition (zero time) and used for calculation of experimental data. After removal in the cold of the supernatant and three washes with cold H_2O of the precipitate, lipid labelling was determined, as reported elsewhere [6]. The aqueous phase together with washings were brought to pH 8.5–9.0 and successively chromatographed on AG 4 X 100 resin columns (25 X 1 cm) by eluting with 40 ml of H_2O and then with 0.1 M formic acid. The ethanolamine-containing fractions were lyophilized, dissolved in 10 ml H_2O and aliquots taken for radioactivity measurements [7].

Separation and determination of EPG and estimations of protein and phospholipid-P were carried out, as described elsewhere [6].

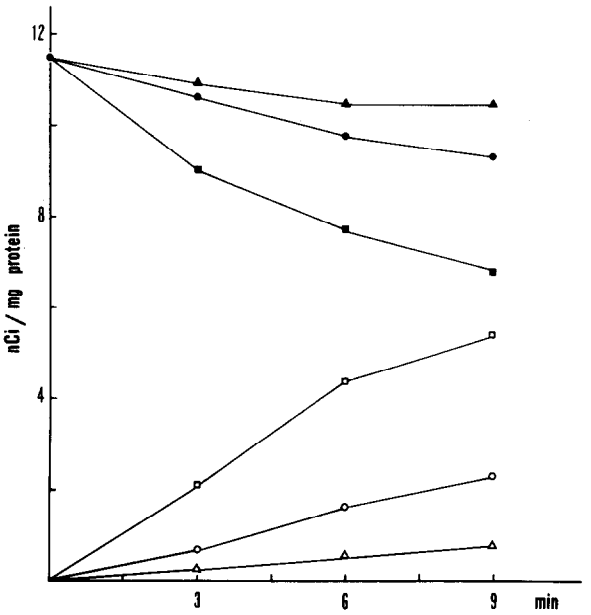

Fig. 1. The Ca^{2+}-stimulated displacement of 1,2-[^{14}C]ethanolamine from microsomal labelled PE by the addition of cold choline (▲–▲–▲), (△–△–△), L-serine (●–●–●), (○–○–○) or ethanolamine (■–■–■), (□–□–□) in the re-incubation medium (incubations and analytical methods were performed as described under Experimental). On the top (black symbols): loss of radioactivity of microsomal PE expressed as nCi/mg protein. On the bottom (white symbols) : release of 1,2-[^{14}C] ethanolamine into aqueous phase.

3. Results and discussion

Fig.1 shows that ethanolamine, L-serine and choline all displace, in the presence of Ca^{2+} ions, the ethanolamine moiety of microsomal PE, though at different rates. The released ethanolamine was almost quantitatively estimated in the aqueous phase. The data have been corrected for the release of lipid radioactivity due to water alone, which was however always negligible for the time intervals examined.

Fig.1 shows that 41.1%, 20% and 9.5% of the labelled PE was displaced after 9 min of reincubation in the exchange reaction with ethanolamine, L-serine and choline, respectively (average of three experiments). Now, by re-incubating under similar experimental conditions the brain microsomes with labelled bases (see control a of Experimental), in place of the cold compounds, only 3.71, 1.80 and 1.37 nmol/mg microsomal protein/9 min of ethanolamine, L-serine and choline respectively were found to be incorporated. These data indicate therefore, together with those on percentages of displacement, that the total available pool of PE for all types of exchange in these experiments was of about 9 nmol (13 nmol in the exchange with choline), which is only a small amount (5–6%) of the total PE (150 nmol) present in 1 mg of brain microsomal protein [8].

This inhomogeneous potentiality of the microsomal PE (5–6% of the total pool) to be displaced by cold bases can be proved by another way. If we assume that the PE pool of the brain microsomes was labelled

homogeneously before reincubation, we may then calculate according to Bjerve [9] the reaction rates of exchange between PE and cold bases by dividing the decrease in PE radioactivity (fig.1) by the S.A. of PE at the start of the reincubation, which was found to be 0.0572 nCi/nmol. Accordingly, the reaction rates of ethanolamine, L-serine and choline exchanges were calculated to be 131, 50 and 34 nmol/mg protein/9 min, which contrasts sharply with the corresponding maximal levels of incorporation of 3.71, 1.80 and 1.37 nmol/mg protein/9 min, found in the present work (control a). The values of maximal incorporation of the three labelled bases by exchange result therefore to be only about 4% of those calculated by the degree of the displacement, and this value is similar to that of 5–6% calculated before on the basis of the pool of the exchanging PE molecules at the membrane level. It must be mentioned that the base concentration used in all the displacement reactions or incorporation experiments was saturating (see Experimental), i.e. well above the K_m values, at the pH and Ca^{2+} concentration used, which are 8×10^{-5} M, 4×10^{-4} M and 5.88×10^{-4} M for ethanolamine, L-serine and choline, respectively [4].

Experiments have been subsequently performed by carrying out the base-exchange reaction between unlabelled bases and microsomal membranes which were previously labelled in their PE moiety through the CDPE-mediated pathway, in place of the base-exchange reaction.

Table 1 shows that L-serine and choline do not displace the ethanolamine from PE and that ethanolamine itself displaces poorly, contrary to the findings of fig.1. If from previous discussion we assume that the exchanging molecules of PE represent only 5–6% of the microsomal PE content, then the active radioactive pool in table 1 is only of 0.62 nCi (5% of 12.51 nCi). This signifies that the displacement of 0.34 nCi of ethanolamine after 6 min of preincubation (table 1) represents 50% of exchange, which is not very different from the 33.3% reported in fig.1 at the same period of observation. This finding again demonstrates that only a small part of the total PE pool of brain microsomal membranes is available to the exchange enzyme, and that therefore the high degree of base displacement due to the exchange enzyme is no longer observed when the PE molecules are labelled by the Kennedy's pathway, in place of the exchange reaction.

Table 1
The Ca^{2+}-stimulated displacement of rat brain microsomal phosphatidylethanolamine synthesized in vitro by the CDPE-mediated pathway

Addition	nCi of lipid-bound ethanolamine displaced at the following time intervals of re-incubation (min)[a]		
	3	6	9
Choline	0	0	0
L-Serine	0.12	0	0
Ethanolamine	0.08	0.34	0.56

Experiments were performed with brain microsomes by carrying out incubation (b) and re-incubating the particles under conditions of base-exchange, as reported under Experimental, in the presence of either cold choline, L-serine or ethanolamine.

[a] nCi of prelabelled phosphatidylethanolamine at zero time: 12.51 per mg of protein. Correction has been made for release of radioactivity by water alone. Data reported in nCi/mg protein.

Experiments have been finally performed by reincubating under condition of 'base-exchange' brain microsomes which were prelabelled in vivo with ethanolamine [10] in their PE molecules and subsequently isolated after 15 hr from treatment. Table 2 indicates

Table 2
The Ca^{2+}-stimulated displacement of rat brain microsomal phosphatidylethanolamine synthesized from administered ethanolamine in vivo

Addition	nCi of lipid-bound ethanolamine displaced at the following time intervals of re-incubation (min)[a]		
	3	6	9
Choline	0	0.13	0.13
L-Serine	0	0.12	0.37
Ethanolamine	0.10	0.39	0.80

Rats (100–120 g) were injected intracerebrally (10) with 4.5 μCi of labelled ethanolamine (S.A. of 225 μCi/μmol) and microsomes prepared as described in the text after 15 hr from treatment. Experiments of re-incubation performed as reported in the text and in table 1.

[a] nCi of pre-labelled phosphatidylethanolamine at zero time: 15.97 mg of protein. Correction has been made for release of radioactivity by water alone. Data reported in nCi/mg protein.

that the results are similar to those of table 1. These last data indicate that the PE of brain microsomes must have been labelled in vivo mainly through CDPE-mediated pathway, rather than by exchange reactions, which agrees with previous findings obtained with liver tissue [11].

Acknowledgement

This work was supported by a grant from the Consiglio Nazionale delle Ricerche, Rome (contract n.74/00259.04/115.3381 and contribute n.74/00177.04/457444).

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