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Sphingosine and unsaturated fatty acids modulate the base exchange enzyme activities of rat brain membranes

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The base exchange enzymes catalyze the incorporation of L-serine, ethanolamine and choline into their corresponding phospholipids. The L-serine base enzyme activity was increased 120% by 0.1 mM sphingosine. There was a modest increase of the ethanolamine base exchange enzyme activity but the choline base exchange enzyme activity was unaffected. Na-arachadonate, Na-oleate and Na-linolenate at 0.2 mM concentration increased the activity of the L-serine and ethanolamine base exchange enzymes but inhibited the choline base exchange enzyme activity. A model is proposed suggesting that modulations of the L-serine base exchange enzyme may participate in the regulation of the calcium phospholipid-dependent protein kinase C.

Sphingosine; Arachadonic acid; Oleic acid; Serine base exchange enzymes; Phosphatidylserine; Base exchange enzymes; Metabolic regulation

1. INTRODUCTION

The base exchange enzymes catalyze the Ca^{2+}-dependent, energy-independent incorporation of either L-serine or ethanolamine, or choline into their corresponding phospholipid. This does not result in the net synthesis of new phospholipid but merely a remodeling of the amino alcohol portion of a pre-existing molecule [1,2]. However, the serine base exchange enzyme is believed the principal, if not exclusive, mechanism available for phosphatidylserine production in mammalian tissues [3].

Although these activities are ubiquitous in nature, they have not been assigned any physiological function, aside for phosphatidylserine production in mammalian tissues. However, there have been isolated and unrelated reports of modulation of these activities in response to particular experimental circumstances. The most convincing experimental evidence obtained with intact cells or organs is revealed by either increased or decreased incorporation of L-serine into its corresponding phospholipid. More commonly there have been in vitro observations on the ability of specific compounds to effect the measured activity of these individual base exchange enzymes. The calmodulin antagonists, such as trifluoperazine, stimulate the three base exchange activities present in rat brain microsomes [4], rabbit platelet membranes [5], human platelet membranes [6] and stimulate the incorporation of labeled serine into phosphatidylserine with intact rabbit platelets [7]. The phorbol ester 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) in vivo treatment of HL60 cells inhibited by 30% the incorporation of radioactive serine into phosphatidylserine [8]. Activation of Jurkat T cells with anti CD_3 or anti TCR antibodies or PHA decreased the incorporation of radioactive serine into phosphatidylserine [9].

TPA treatment of human neuroblastoma cell line L-A-N-2 increases the incorporation of radioactive choline [10] as well as radioactive ethanolamine and L-serine into their corresponding phospholipids (in preparation). This suggested that the base exchange enzyme activities may be responsible for the observed increased precursor incorporation into phospholipid, since this is responsible for phosphatidylserine formation. To investigate this possibility we examined the effect of several biologically active free fatty acids, 1,2-diacylglyceride and sphingosine on the ability of a rat brain membrane fraction enriched in the base exchange enzymes to incorporate either [^{14}C]ethanolamine or [^{14}C]serine or [^{14}C]choline into their corresponding phospholipids.

2. MATERIALS AND METHODS

The preparation of the rat brain membranes enriched in the base exchange enzymes and the assay procedure were as previously described [11], except that the Azolectin microdispersion was omitted from the incubations. The reaction mixture contained from 150 μg to 190 μg particulate protein, 5 mM $CaCl_2$, 50 mM HEPES pH 8.0, either 0.48 mM [^{14}C]ethanolamine ($0.5–1.0 \times 10^4$ dpm/nmol) or 0.38 μM [^{14}C]L-serine ($2–3 \times 10^4$ dpm/nmol) or 0.38 mM [^{14}C]choline ($2–3 \times 10^4$ dpm/nmol) in a total incubation volume of 0.24 ml at 37°C for 20 min. The incorporation into lipids was carried out as previously described [11]. The activities varied with individual membrane prepara-

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tions from 8 to 20 nmol/mg protein for the choline base exchange enzyme activity, from 52 to 95 for the ethanolamine base exchange enzyme activity and from 9 to 28 for the L-serine base exchange activity.

The radioactive compounds were purchased from NEN, Boston, MA; the various lipids from Seredary, London, Ontario. The data presented are from a representative experiment that had been repeated on at least three separate occassions with freshly prepared membranes varying somewhat in their absolute activities.

3. RESULTS

Phorbol esters when presented to cell cultures provokes the liberation of arachadonate, and presumably other unsaturated fatty acids esterified at the C-2 position of phospholipids, through phospholipase A_2 activation [12].

3.1. *Effect of fatty acids*

Arachadonate is an activator of protein kinases α and γ [13], and arachadonate, or oleate, or linolenate or linoleate will activate a protein kinase C of human neutrophils [14]. The results of incubating these rat brain membranes in the presence of varying concentrations of sodium oleate revealed that the choline base exchange enzyme activity was inhibited in a dose-dependent manner. In contrast the serine and ethanolamine base exchange enzyme activity was stimulated at intermediate, 0.1–0.5 mM, concentrations of sodium oleate (Fig. 1, right panel). Identical results were obtained with the same concentrations of sodium linolenate. Sodium arachadonate at concentrations above 0.5 mM inhibited both the L-serine and ethanolamine base exchange enzyme activities but at 0.1–0.25 mM concentrations, the serine base exchange activity was doubled and the ethanolamine base exchange enzyme activities increased by 65%. However, the choline base exchange enzyme activity was inhibited in a dose dependent manner by all concentrations of arachadonate employed (Fig. 1, left panel). Na palmitate was without any effect on these activities.

3.2. *Effect of sphingosine*

The free sphingosine content present in freshly isolated human neutrophils varies from 13 to 101 pmol/10^7 cells and was increased upon incubation at 37°C. This increase in free sphingosine was prevented by TPA and arachadonic acid [15]. Sphingosine inhibits protein kinase C [16], TRH binding to pituitary cells [17], TPA activation of Na^+/H^+ exchange [18], the NGF stimulated neurite outgrowth of PC-12 cells [19], phosphatidic acid phosphatase [20a,b] and the lipogenic effect of insulin and hGH on adipocytes [21]. Sphingosine may act as a positive regulator of cell growth [22] and is an activator of an 80 kDa diacylglycerol kinase of pig thymus, but an inhibitor of an 150 kDa form of this enzyme [23]. The rat brain membrane preparation was incubated with varying concentrations of sphingosine to determine if this compound could modulate the activity of the base exchange enzymes. There was a doubling of the L-serine base exchange enzyme activity at 0.2 mM sphingosine and only a 25% increase of the ethanolamine base exchange enzyme at 0.1 mM sphingosine.

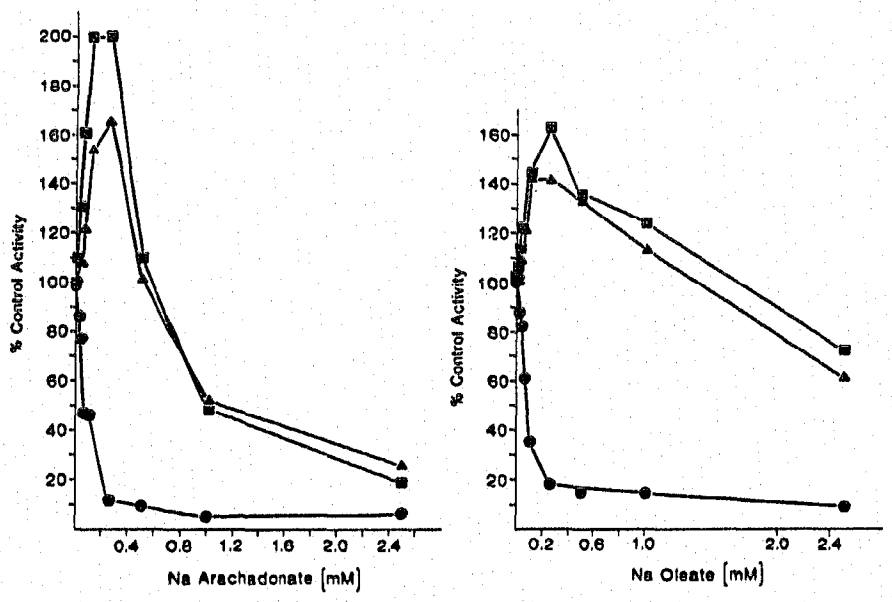

Fig. 1. Right. The effect of varying sodium oleate concentrations upon the individual base exchange enzyme activities of rat brain membranes enriched in the endoplasmic reticullum. The L-serine base exchange enzyme activity is shown as squares, that of the choline base exchange enzyme as circles and that of the ethanolamine base exchange enzyme as triangles. Left. The effects of varying sodium arachadonate concentrations upon the individual base exchange enzyme activities of rat brain membranes enriched in the endoplasmic reticulum. The L-serine base exchange activity is shown as squares, that of the choline base exchange enzyme as circles and that of the ethanolamine base exchange enzyme as triangles.

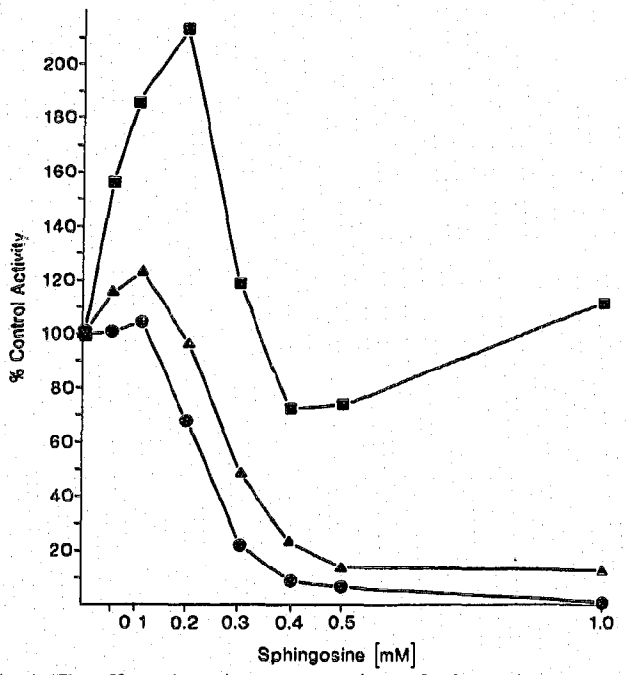

Fig. 2. The effect of varying concentrations of sphingosine upon the individual base exchange enzyme activities of rat brain membranes enriched in the endoplasmic reticulum. The L-serine base exchange activity is shown as squares, that of the choline base exchange enzyme as circles and that of the ethanolamine base exchange enzyme as triangles.

Higher concentrations of sphingosine completely inhibited the choline base exchange enzyme, inhibited the ethanolamine base exchange enzyme by 85% and the L-serine base exchange enzyme by about 30% (Fig. 2).

3.3. *Effect of diacylglycerol and nucleotides*

1,2-Diolein from 0.05 to 5 mM did not effect either of the three base exchange enzyme activities. The presence of 10 mM GTP reduced the choline base exchange enzyme activity by 30%, that of the ethanolamine base exchange enzyme activity by 25% and that of the serine base exchange enzyme activity by 47%. However, the presence of ATP, CTP, GTP, UTP, ADP, cAMP, GTPγS, cGMP, or GDP at the same concentration did not effect these three enzyme activities.

4. DISCUSSION

Two separate enzymes possessing L-serine base exchange activity have been partially purified and characterized. The preferential phospholipid acceptor for both is phosphatidylethanolamine and there are similar optimums for pH and calcium. The characteristic feature distinguishing these two enzymes is that one utilizes only L-serine [24] as a substrate and for the other enzyme both ethanolamine and L-serine are substrates [25]. The existence of two distinct serine base exchange enzymes may explain the twofold stimulation of the L-serine base exchange enzyme activity by sphingosine and only a 25% increase of the ethanolamine base exchange enzyme activity. Sphingosine may be a preferential activator of the L-serine base exchange enzyme while arachadonate and oleate stimulate the ethanolamine-serine base exchange enzyme activity.

The concentration of arachadonate and oleate in rat brain is 5 and 25 μM, respectively. Experimentally induced hypoxia elevates arachadonate to 170 μM and the oleate to 75 μM presumably by phospholipase A_2 activation [26]. 1,2-Dioctinoate but not TPA [27] markedly increased the free sphingosine content of GH_3 cells. The free sphingosine concentrations of murine tissue varied from 10 to 30 μM [28]. Rat liver plasma membranes are reported to have 7 μM free sphingosine which increased several fold during in vitro incubations [29].

The model

The ability to modulate the base exchange enzyme activities suggests that the cellular responses, including protein kinase activation, of some agonists may involve the L-serine base exchange enzyme activity. Stimulation of this enzyme would result in increased phosphatidylserine production and it is this phospholipid that is an effective protein kinase C activator.

Stimulation of phospholipase A_2 activity would cause a significant increase in protein kinase C-related intracellular processes since the unsaturated free fatty acids released would directly activate the protein kinases α and γ. Also, the oleic acid released has been demonstrated as the most effective activator of mammalian phospholipase D [2] and this would lead to diacylglycerol formation [30]. The diacylglycerol would also provoke sphingolipid breakdown elevating the free sphingosine content which would stimulate the L-serine base exchange enzyme. The oleic and arachadonate released would also stimulate the ethanolamine L-serine base exchange enzyme activity also contributing to the increased phosphatidylserine production. Phosphatidylserine and diacylglycerol would be expected to stimulate the Ca^{2+}-phospholipid-dependent protein kinase C activity [13].

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