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25-Hydroxycholesterol promotes myelin basic protein-induced leakage of phospholipid vesicles

Raquel F. Epand and Richard M. Epand

Department of Biochemistry, McMaster University, Health Sciences Centre, Hamilton (Canada)

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Among several cholesterol oxidation products, 25-hydroxycholesterol is particularly potent in enhancing basic protein-induced carboxyfluorescence leakage from liposomes. Both myelin basic protein and poly(L-arginine) are effective at submicromolar concentrations in stimulating this vesicle rupture. Leakage is greatly augmented by the presence of only 1% of the 25-hydroxycholesterol. The possible relevance of these findings to myelin pathology is discussed.

Introduction

Myelin has a particularly slow rate of metabolic turnover [1] which may allow degradation products to accumulate to a greater extent than occurs in more metabolically active tissues. With regard to the lipid components of myelin, the degradation products are largely the result of oxidation and hydrolysis. The lipid hydrolysis product, lysophosphatidylcholine, promotes myelin basic protein-dependent membrane fusion [2]. In addition, lipid peroxidation is thought to have particular importance in altering the physical properties of the myelin membrane [3,4]. One of the products of lipid peroxidation, malondialdehyde, increases with aging [3] and in Alzheimer's disease [4]. Aliphatic aldehydes are also produced by lipid peroxidation and they markedly enhance myelin basic protein-induced aggregation and fusion of liposomes [5]. Superoxide dismutase and glutathione peroxidase protect tissues against lipid peroxidation. The activity of superoxide dismutase decreases with age [6]. In addition, decreased glutathione peroxidase activity is associated with multiple sclerosis [7–9].

Cholesterol is another lipid component that is susceptible to oxidation [10]. Cholesterol is particularly prevalent in myelin. Cholesterol oxidation occurs in the presence of oxidized phospholipids [11]. Among the cholesterol oxidation products, 25-hydroxycholesterol is particularly cytotoxic [12,13]. It may play a role in spinal cord injury. There is a 61% increase in the tissue levels of 25-hydroxycholesterol following spinal cord compression injury [14]. Accumulation of lipid oxidation products can be detected as early as 1–5 min after injury. Thus, lipid oxidation occurs prior to tissue necrosis. Agents which scavenge free radicals and prevent peroxidative reactions also prevent or reduce post-traumatic tissue loss and paraplegia [14]. Thus, 25-hydroxycholesterol may play a role, together with myelin basic protein, in

Abbreviations: poly(L-Arg), poly(L-arginine) (M_r 40000); PC, egg phosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

Correspondence: R.M. Epand, Department of Biochemistry, McMaster University, Health Sciences Centre, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5.

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inducing damage in the spinal cord and in brain white matter. The effect of 25-hydroxycholesterol on myelin basic protein-induced vesicle leakage in model systems is the subject of this study.

Materials

Myelin basic protein was isolated from human brain as previously described [15]. The following were obtained from commercial sources: poly(L-arginine), $M_r \approx 40\,000$, from Sigma Chemical Company, St. Louis, MO; egg phosphatidylcholine (PC) and dioleoylphosphatidylethanolamine (DOPE) from Avanti Polar Lipids, Inc., Birmingham, AL; cholesterol, from Nucheck, Elysian, MN; 5-cholesten-3β, 25α-diol (25α-hydroxycholesterol) from Steraloids, Inc., Wilton, NH; 5(6)-carboxyfluorescein, from Eastman Kodak Company, Rochester, NY.

The purity of cholesterol and the 25α-hydroxy cholesterol was checked by thin-layer chromatography on a silica plate with two solvent systems: (1) methanol/chloroform/glacial acetic acid (100 : 5 : 1, v/v) and (2) cyclohexane/diethyl ether/glacial acetic acid (100 : 99 : 1, v/v). Only one spot was observed for both compounds upon development of the plates with iodine vapours.

Methods

Sample preparation

Solutions of phospholipids, cholesterol and 25αhydroxycholesterol were made in chloroform/methanol (2 : 1, v/v). The solvent was then evaporated with a stream of N_2 so as to deposit the lipid on the walls of a glass test-tube as a film. The last traces of solvent were then removed in a vacuum desiccator equipped with a liquid N_2 trap.

Vesicle separation

Films containing 2.5 mg each of PC and DOPE, as well as varying amounts of cholesterol and 25α-hydroxycholesterol were suspended in 0.5 ml of 0.25 M carboxyfluorescein in 0.01 M Hepes buffer, (pH 7.4) containing 0.1 M sodium chloride and 0.002% sodium azide. The suspension was sonicated for 1 h at room temperature, in a bath-type sonicator under an Argon atmosphere. The sample was then passed through a Sephadex G-50 column (approx. 15 × 2 cm) eluted with the Hepes buffer (pH 7.4). The vesicles entrapping carboxyfluorescein came out in the void volume and were easily identified by their yellow color. The column was washed between each sample with 0.1 M potassium phosphate (pH 12) and then re-equilibrated with the Hepes buffer. The vesicles were kept in an ice bath to minimize vesicle disruption.

Fluorescence measurements

To 2 ml Hepes buffer, pH 7.4 in the cuvette, 10 μl of carboxyfluorescein-entrapped vesicles were added and the zero-time fluorescence was measured for about a minute. Vesicle leakage was induced by adding 10 μl of a myelin basic protein or a poly(L-Arg) solution in Hepes buffer (pH 7.4) at a concentration of approx. 1 mg/ml. This corresponds to a lipid-to-protein ratio of about 100 : 1 for myelin basic protein, accounting for dilution of the lipid on the column. The time course of the increase in fluorescence signal was followed for about 10 min. Most of the protein or polypeptide-induced leakage occurred during the first minute. A plateau was reached after 6 or 7 min. The excitation wavelength was 490 nm and the emission was 520 nm, 100% leakage was produced by addition of 100 μl, 5% Triton.

Results

A number of oxidized forms of cholesterol were tested to determine their effect on myelin basic protein-induced vesicle leakage. Cholesterol oxidation products were added to vesicles composed of DOPE, egg PC, cholesterol and oxidized cholesterol. No carboxyfluorescein leakage was observed prior to the addition of myelin basic protein, with any of the vesicles used. Vesicle leakage was initiated by the addition of myelin basic protein. Neither 7α-hydroxycholesterol, 20α-hydroxycholesterol, 7β-hydroxycholesterol, cholestane-3β,5α,6β-triol nor 7-ketocholesterol caused an increase in carboxyfluorescein leakage from these vesicles compared with vesicles not containing cholesterol oxidation products. Only 25-hydroxycholesterol markedly increased vesicle leakage (by about 30%) when present in the vesicles.

The modulation of myelin basic protein-in-

duced vesicle rupture by 25-hydroxycholesterol was further investigated. Poly(L-Arg) also had the ability to cause an increase in carboxyfluorescein leakage by about 25% in excess of that produced by myelin basic protein. The results of the myelin basic protein and poly(L-Arg)-induced fusion are summarized in Table I. Only low concentrations of myelin basic protein (between about 10^{-9} and 10^{-7} M) and 25-hydroxycholesterol are required to cause increased carboxyfluorescein leakage. The minimum quantity of 25-hydroxycholesterol required for this effect was difficult to determine. At low mol fractions of 25-hydroxycholesterol, the leakiness of the vesicles was dependent on the state of the Sephadex column used for gel filtration of the vesicles. If carboxyfluorescein-entrapped vesicles with low oxysterol concentration were passed through a fresh Sephadex column or through a Sephadex column previously treated with vesicles not containing oxysterol, then the leakage from vesicles was significantly lower than if they were passed through a column that had been previously exposed to a high oxysterol-containing vesicle preparation. These results suggest that some oxysterol binds to the column and can be transferred to later specimens. The lowest concentration of 25-hydroxycholesterol which reproducibly gave a significant increase in leakage was 1% of total lipid in the vesicle but the true lower limit may be even less because of apparent binding of the oxysterol to the Sephadex column.

In contrast to myelin basic protein and poly(L-Arg), poly(L-lysine) had little effect on vesicle rupture. Other proteins, like ribonuclease and bovine serum albumin had no effect at all. Thus, there is no specific requirement for myelin basic protein, since another polycationic peptide, poly(L-Arg), also induces vesicle rupture. However, there is some specificity in the phenomenon, since the cationic poly(L-lysine) or ribonuclease do not induce carboxyfluorescence leakage. Myelin basic protein and poly(L-Arg) produced no leakage of vesicles at concentrations below 10^{-9} M protein using a lipid concentration of about 20 μM.

TABLE I

CARBOXYFLUORESCEIN LEAKAGE FROM LIPOSOMES WITH MYELIN BASIC PROTEIN OR POLY(L-Arg): EFFECT OF LIPID COMPOSITION

The lipid ratios are PC/dioleylPE/cholesterol/25α-hydroxycholesterol. The percentages of total carboxyfluorescein leaked out of vesicles 8 min after the addition of 340 nM myelin basic protein or 125 nM poly(L-Arg) at 25 °C are shown. Values are precise to $\pm$15% of the value using different vesicle preparations. Each number represents an average of two to four independent determinations. n.d., not determined.

Lipid ratio	Leakage (%)	
	myelin basic protein	poly(L-Arg)
1:1:1:0	21	31
1:1:1.6:0	35	43
1:1:1.4:0.04	47	n.d.
1:1:1.1:0.35	50	n.d.
1:1:1.1:0.6	69	82
1:1:1.1:0.7	62	94

Discussion

Cholesterol promotes aggregation of liposomes in the presence of myelin basic protein [5]. Cholesterol also makes these vesicles more susceptible to fusion in the presence of aliphatic aldehydes and alkanes. It has been suggested that these two phenomenon may be related to the roles of these lipids in myelin [16]. Cholesterol would stabilize a compact myelin structure, while lipid oxidation products such as aliphatic aldehydes and alkanes would break down the permeability barrier of the membrane by inducing fusion between successive layers of myelin.

Oxysterols are also important products of lipid oxidation. Several oxysterols have been shown to perturb membrane properties [10]. In particular, 25-hydroxycholesterol causes packing defects in model membranes which lead to increased calcium [17] and glucose [18] permeability. 25-Hydroxycholesterol has also been shown to be potent in inducing arterial endothelial damage [19] and inhibiting the calcium channel in red blood cells [20]. This oxysterol also accumulates after spinal cord injury [14]. Demel and coworkers [18] demonstrated the increase in flux of permeant glucose with 25-hydroxycholesterol and they showed from monolayer studies that 25-hydroxycholesterol, in contrast to cholesterol or several other oxysterols, does not exhibit a condensing effect but rather acts as a spacer molecule. Since both hydroxyl

groups of 25-hydroxycholesterol cannot be simultaneously exposed on a bilayer membrane surface, this oxysterol may be particularly perturbing to membrane structure. In the present work, we demonstrate that 25-hydroxycholesterol can induce rupture of membranes, allowing passage of the impermanent anion, carboxyfluorescein. This membrane destabilization is not observed in the absence of myelin basic protein. It therefore has particular relevance for myelin pathology. Accumulation of 25-hydroxycholesterol in the myelin basic protein-containing myelin membrane can lead to breakdown of the myelin permeability barrier.

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