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Prostaglandin E_2 directly protects isolated rat gastric surface cell membranes against bile salts

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Rat gastric surface cell membranes were prepared and the effect of taurocholic acid assessed by ESR spectroscopy using the 16-doxylstearic acid spin label. Taurocholic acid increased the polar part of the spectra, indicating an augmented amount of spin label molecules with a polar environment. Concomitantly, mobility of the spin label molecule was augmented. The effect of taurocholic acid was completely prevented by the previous addition of prostaglandin E_2. This suggests a direct protective efficiency of prostaglandin E_2 on rat gastric surface cell membranes without the metabolic participation of intact cells.

Introduction

Protection of the gastric mucosa by prostaglandin E_2 against damaging agents, such as acids, NaOH, alcohol, boiling water, nonsteroidal anti-inflammatory drugs and bile acids, in particular taurocholic acid, has been described in several in vivo studies [1–3]. Macroscopic changes, such as lesions and ulcers, were caused by the above-mentioned agents [4,5]. Prostaglandins protect against erosions as well as against a decrease in potential difference [6–9]. Müller-Lissner et al. [10] isolated gastric surface cells and studied the protective effect of the prostaglandin E_2 by the Rubidium efflux method.

The mode of cytoprotection by prostaglandins, however, is still obscure [11,12].

In the present paper, the mechanism of the protective effect of prostaglandin E_2 against bile acid damage of cell membranes is studied by electron spin resonance (ESR) spectroscopy.

Materials

16-Doxylstearic acid was purchased from Aldrich (Steinheim, F.R.G.). Prostaglandin E_2 and taurocholic acid were obtained from Sigma (St. Louis, MO, U.S.A.) in analytical grade. Sucrose was purissimum quality from Merck (Darmstadt, F.R.G.).

Methods

Preparation of rat gastric surface cell membranes

The stomachs of female Wistar rats (body weight about 200 g) were dissected. The mucosa of the gastric antrum and corpus was peeled off. The preparations were controlled by microscope to ensure that predominantly the mucosa was taken. The pieces of tissue were transferred into 2 ml of a 250 mM sucrose solution and were homogenized by Ultra Turrax (Jahnke and Kunkel, Staufen, F.R.G.) for 2×0.1 min; thereafter, the suspension

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was freed of connective tissue using a plastic filter. Subsequently, the membrane suspension was diluted with 4 ml of sucrose solution and kept at room temperature.

The membrane fragments were checked electronmicroscopically for purity (Fig. 1): no whole cells, but occasionally mitochondria as well as nuclei were visible.

Procedure for the ESR measurement

Treatment of the controls. After filtration, 1.5 ml of the membrane suspension in 250 mM sucrose were used for the ESR measurement. Centrifugation was carried out in an Eppendorf 3200 centrifuge for 0.75 min at 22° C. The supernatant was removed and 50 µl of the sucrose solution were added. After vortexing, an aliquot (50 µl of the suspension) was incubated with 1 µl of the 16-doxylstearic acid spin label (616) at a 5 mM concentration for 0.5 min. Thereafter, the spin-labeled membrane suspension was diluted with 1.5 ml of 250 mM sucrose. After centrifugation for 0.75 min in the Eppendorf 3200 centrifuge, the supernatant was removed. Then the membranes were suspended in 50 µl of 250 mM sucrose, and finally measured in the ESR spectrometer (Bruker, Magnet B-E-25).

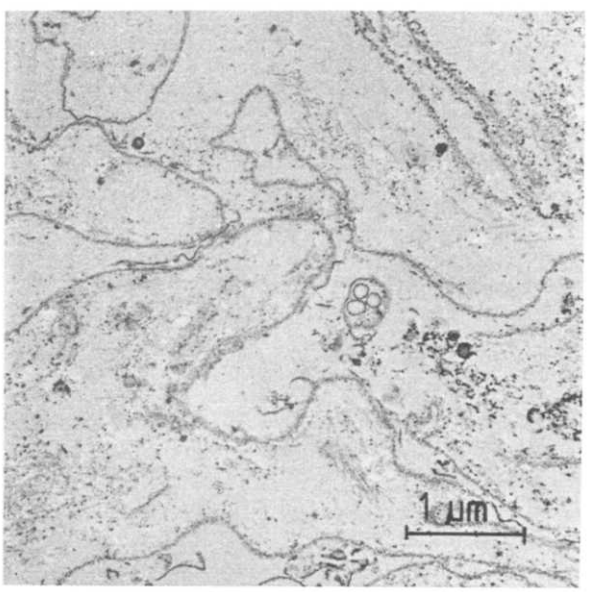

Fig. 1. Gastric surface cell membranes prepared in 250 mM sucrose solution. The magnification is ×15000.

Treatment with taurocholic acid as damaging agent. 50 µl taurocholic acid (200 mM in 250 mM sucrose) were added to 50 µl of suspension after labeling with spin label 616 and incubated at 22° C for 5 min. Then the procedure of washing with 1.5 ml of sucrose was followed as described above.

Treatment with prostaglandin E_2. The protective efficiency of 25 nmol prostaglandin E_2 was investigated after labeling the membranes with spin label 616. 50 µl prostaglandin E_2 (500 µM in 250 mM sucrose) were added and incubated for 10 min, after addition of the spin label. Then taurocholic acid was added and incubated for 5 min. Subsequently, the washing procedure described above was followed.

Analysis of the spectra

As shown in Fig. 2, the spin label 16-doxylstearic acid partitions between more polar and more hydrophobic regions of the membrane.

In order to determine the polarity of the spin label's environment, the coupling constant A_N was measured. Moreover, h_{-1H}/h_{-1P} ratios provide a sensitive measure of a relative partitioning of the *N*-oxyl group between environments of different polarities [13]. The type of spectra obtained, however, did not allow the measurements of $\bar{A}_{\parallel}$ (Fig. 2). Instead of calculating order parameters, therefore, we have indicated values of $\bar{A}_{\perp}$. These are given in Table II. We are confident that these values provide a means of approximating a shift of membrane fluidities in the hydrophobic core [14].

Results

In taurocholic acid-treated rat gastric surface cell membranes ESR spectra of spin label 616 reveal a dramatic increase of the polar part of the high field line (Fig. 2). The increase of polar environment of spin label 616 is also evident from the values of the coupling constants A_N. A distinct increase of A_N value in membranes after taurocholic acid treatment is observed (Table I). In addition, a shift of the apolar part of the high field line of the spectra due to preincubation with taurocholic acid is noted in Table II.

These differences clearly indicate the disin-

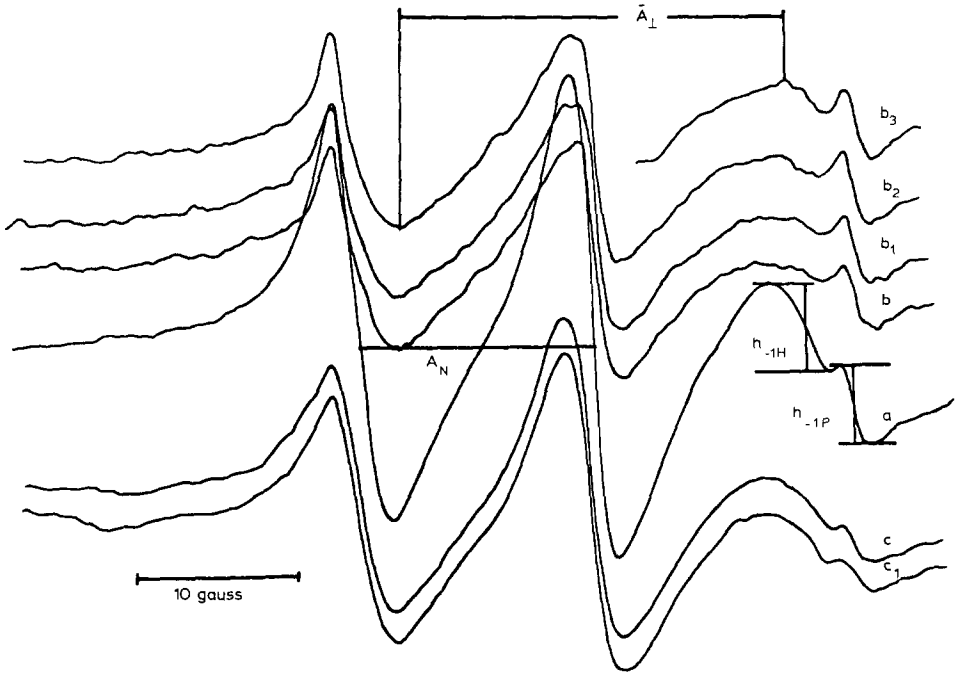

Fig. 2. ESR spectra of spin label 616 in rat gastric surface cell membranes. Consecutive spectra were taken at 2.5-min intervals. (a) Control, (b) spin labeled membranes in the presence of taurocholic acid (100 mM) 5 min after addition of taurocholic acid the membranes were washed with sucrose medium and the pellets were resuspended (see Methods) with 50 μl of sucrose medium. b_1, spectrum obtained 2.5 min after spectrum 6; b_2, spectrum obtained 5.0 min after spectrum b; b_3, spectrum obtained 7.5 min after spectrum b; c, membranes in the presence of 50 μl prostaglandin E_2 (500 μM). Subsequently, 50 μl taurocholic acid (200 mM) were added. After incubation for 5 min and washing (see above under b, and also in Methods) the membranes were resuspended with 50 μl of fresh sucrose medium. Consecutively, spectra were taken at 2.5-min intervals.

tegration of the mucosa cell membranes due to the addition of bile salts, and higher polarity of spin label environment following this damage, as shown schematically in Fig. 4.

We have further investigated whether or not prostaglandin E_2 may preserve membrane integrity. As can be seen in Fig. 2 and Tables I and II, the previous addition of prostaglandin E_2 preserves the original spectral values.

TABLE I

VALUES OF COUPLING CONSTANTS A_N FOR SPIN LABEL 616 IN GASTRIC SURFACE CELL MEMBRANES

The results were statistically assessed using a paired t-test. Significant differences are found between control and taurocholic acid ($P < 0.01$). Amount of membranes, 1.5 ml. 1.0 μl of spin label (5 mM) was used.

Condition	A_N (G)	n
Controls	14.30 ± 0.22	7
+ Taurocholic acid	14.79 ± 0.36	7
+ Prostaglandin E_2, + taurocholic acid	14.30 ± 0.16	5

TABLE II

VALUES OF $\bar{A}_\perp$ FOR SPIN LABEL 616 IN GASTRIC SURFACE CELL MEMBRANES

The results were statistically assessed using a paired t-test. Significant differences are found between control and taurocholic acid ($P < 0.01$), and between control and taurocholic acid in sucrose, no membranes ($P < 0.01$). Amount of membranes, 1.5 ml. 1.0 μl spin label (5 mM) was used.

Condition	$\bar{A}_\perp$ (G)	n
Controls	22.5 ± 0.26	12
+ Taurocholic acid	25.7 ± 0.33	6
+ Prostaglandin E_2, + taurocholic acid	22.5 ± 0.21	5
Taurocholic acid in in sucrose, no membranes	27.2 ± 0.19	6

TABLE III

VALUES OF COUPLING CONSTANTS A_N FOR SPIN LABEL 616 IN DIFFERENT AMOUNTS OF GASTRIC SURFACE CELL MEMBRANES

1.0 μl of spin label (5 mM) was used.

Condition	A_N (G)	Amounts of membranes (ml)	n
Controls	15.19	1.0	3
Controls	14.66	1.5	3
Controls	14.42	2.0	2
+ Taurocholic acid	15.33	1.5	1
+ Prostaglandin E_2, + taurocholic acid	14.33	1.5	1

Different amounts of gastric surface cell membranes have been subjected to experimentation as shown in Table III. The A_N values underline the reliability of the observed spectral changes by taurocholic acid and prostaglandin E_2 + taurocholic acid, even with different amounts of membranes.

Discussion

Previous studies on bile acid damage and the protective mechanism of prostaglandins have been devoted almost entirely to changes in whole stomach cells or intact stomach tissue [3,15].

A differentiation between direct or indirect protective action by prostaglandins is very difficult in such systems. For this reason, we have carried out investigations on isolated stomach cell membranes; any indirect or metabolic action of prostaglandins is abolished in this system.

Spin label 616 mainly probes the hydrophobic membrane interior [14]. Under conditions of bile acid-induced damage a significant increase in the polar environment of spin label molecules is de-

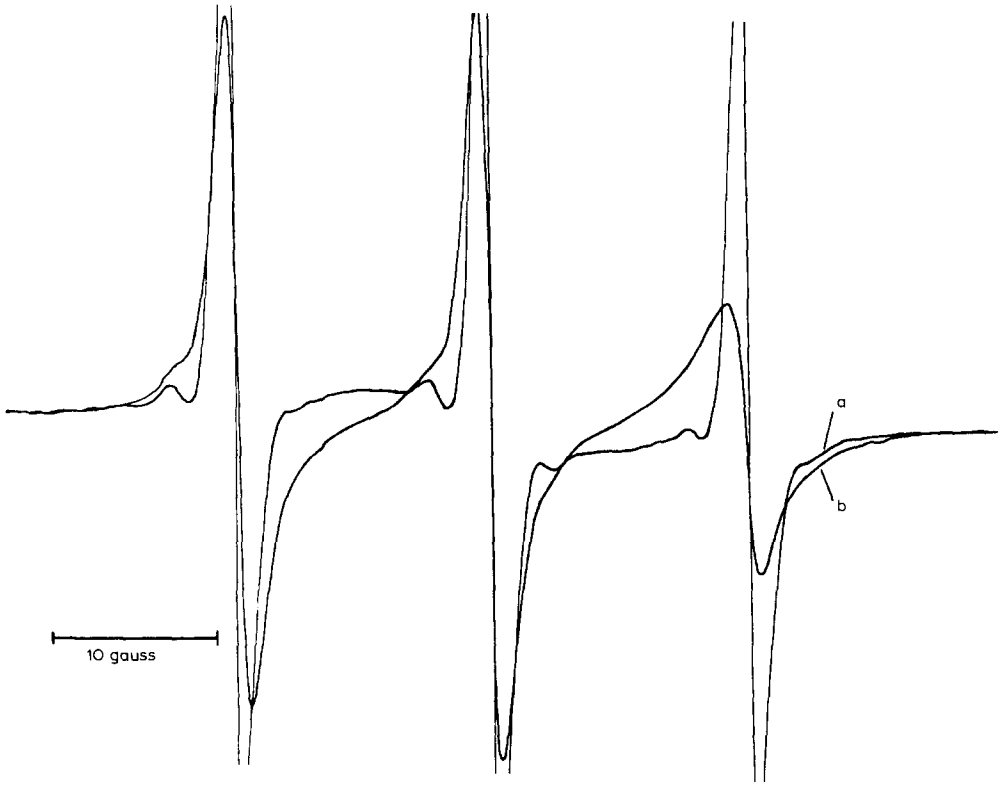

Fig. 3. ESR spectra of spin label 616 in sucrose medium a, 0.5 μl spin label in 50 μl sucrose; b, 0.5 μl spin label + 0.5 mg taurocholic acid in 50 μl sucrose.

tected. However, this effect cannot be simply ascribed to signal gained from the polar medium, because before taking the spectra, excess aqueous spin label as well as taurocholic acid in the medium were removed by washing.

The observed shifts of the apolar part of the third line of the spectra indicated by $A_\perp$ values as well as the increase in the coupling constant A_N after addition of bile acid point into the same direction (Tables I and II).

We conclude that bile acids increase the polarity in the hydrophobic domain of the membrane [10] and enhancement of fluidity [16] can be explained by these results.

Taurocholic acid forms micelles in aqueous solution. The interaction of spin label 616 with such micelles is shown in Fig. 4. The shift of the apolar part of the third line into direction of the micellar peak of taurocholic acid (Figs. 2, 3 and 4 and Table II) may indicate the existence of micellar aggregates of bile acids.

In freshly prepared membranes, prostaglandin E_2 completely prevents the observed spectral changes due to taurocholic acid. This result demonstrates a thorough protection of isolated membranes by prostaglandin E_2. This is due to a direct action of prostaglandin E_2 on the membrane. What could the molecular mechanism of prostaglandin E_2 be in this system? To our knowledge, there is no indication for a specific protective effect of prostaglandin E_2 on rat gastric mucosa. A molecular mechanism of a more general nature could probably explain the protective efficiency of prostaglandin E_2 towards other damaging influences as, for example, of boiling water, ethyl alcohol and nonsteroidal anti-inflammatory drugs [1,17,18]. Some of these agents are known for their protein-denaturating effect. By increasing molecular motion, heat interferes with hydrogen bonds. Denaturation by ethanol is probably brought about by perturbation of hydrophobic interactions. Polar and hydrophobic interactions both serve as stabilizing factors in membrane lipids. In order to explain the efficiency of prostaglandin E_2 on rat gastric surface cell membranes, we presently favour the following working hypothesis: The prostaglandin E_2 possibly serves to maintain the distances between membrane phospholipid molecules. We speculate that prostaglandin E_2, by polar interaction, stabilizes the polar phospholipid head groups, and hence prevents the detrimental influence of the bile acids.

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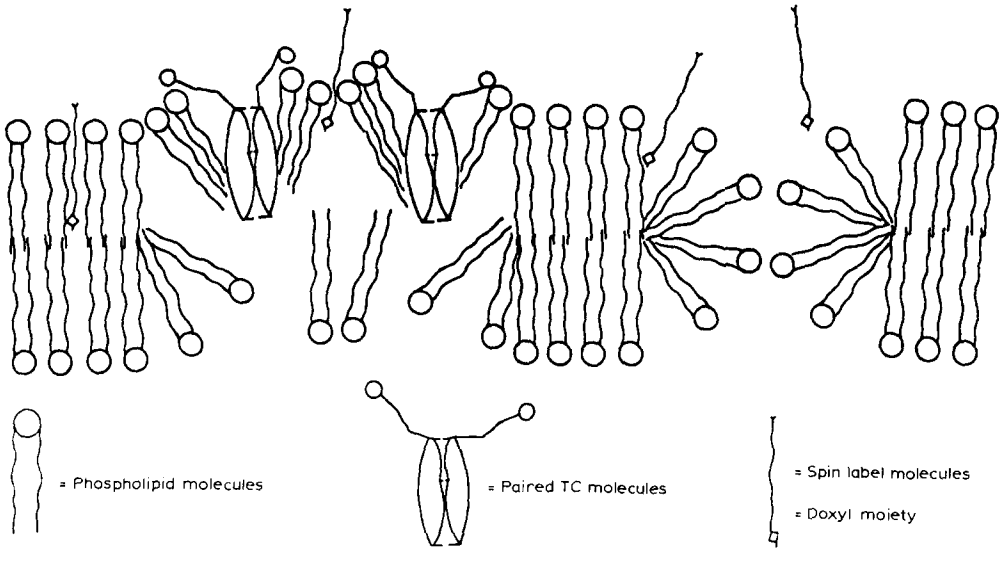

Fig. 4. Scheme of a probable interruption of a membrane bilayer by taurocholic acid (TC).

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