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1,2-Dimethylhydrazine-induced alterations in colonic plasma membrane fluidity: restriction to the luminal region

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Recently, work in this laboratory has shown that changes in the 'dynamic' component of fluidity, lipid composition and phospholipid methylation activity of distal colonic brush-border membranes could be detected after administration of 1,2-dimethylhydrazine to rats of the Sherman strain for 5–15 weeks, i.e., before the development of colon cancer. The present experiments were therefore conducted to: (1) determine whether similar 'premalignant' biochemical changes could be detected in basolateral membranes of Sherman rats treated with this agent; and (2) clarify the relationship of these membrane changes to the malignant transformation process by examining the effect of 1,2-dimethylhydrazine on these biochemical parameters in colonic antipodal plasma membranes of rats of the Lobund-Wistar strain. This particular strain of rats has previously been shown to be total resistant to the induction of tumors by 1,2-dimethylhydrazine. The results of the present experiments demonstrate that similar biochemical alterations could not be detected in the colonic plasma membranes prepared from either strain of rat treated with 1,2-dimethylhydrazine. These data support the contention that the prior biochemical membrane alterations noted in brush-border membranes of 1,2-dimethylhydrazine-treated animals are, in fact, related to the malignant transformation process and, furthermore, are confined to the luminal surface of distal colonic epithelial cells.

Data supplementary to this article are deposited with, and can be obtained from, Elsevier Science Publishers, B.V., B.B.A. Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to BBA/DD/353/73354/896 (1987) 311. The supplementary information includes: two tables each for Sherman and Lobund-Wistar rats which show the compositional parameters and phospholipid methylation activity values for control and 1,2-dimethylhydrazine-treated plasma membranes.

Abbreviations: 2-AS, DL-2-(9-anthroyl)stearic acid; 12-AS, DL-12-(9-anthroyl)stearic acid.

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Introduction

The relationship between membrane lipid fluidity and the malignant transformation process have received considerable attention in the past years. Several laboratories have described changes in the fluidity of cancer cells (for review see Ref. 1). In this regard, 1,2-dimethylhydrazine is a procarcinogen which has been extensively used to investigate the biological characteristics of colonic tumors [2,3]. In weekly doses of 20 mg/kg of body weight, this agent produces colonic carcinoma in a high percentage of rodents, with a latency period

of approx. 6 months [2,3]. It should be noted, however, that certain strains of rats such as Sprague-Dawley, Wistar and Sherman are highly susceptible, whereas others, such as the Lobund-Wistar strain, are totally resistant to this effect of 1,2-dimethylhydrazine [4–6].

Recently, using this model of experimental colon adenocarcinoma, our laboratory has shown that alterations in the 'dynamic' component of lipid fluidity *, lipid composition and phospholipid methyltransferase activity could be detected in brush-border membranes prepared from distal but not proximal colonocytes of Sherman rats administered 1,2-dimethylhydrazine for 5–15 wk, i.e., prior to the development of colon cancer [7]. While the exact relationship of these alterations to the malignant transformation process in this organ was unclear, based on a number of observations, they did not appear to be due to 1,2-dimethylhydrazine per se or to changes in cellular proliferation [7].

Over the past few years, work in this laboratory has examined the interactions of proteins and lipids in colonic brush-border and basolateral membranes [8–10]. These studies have shown that the colonic antipodal plasma membranes differ in a number of respects, including their lipid fluidity and composition [8–10]. In view of our earlier finding of 1,2-dimethylhydrazine-induced biochemical changes in colonic brush-border membranes, the present studies were conducted to determine whether similar alterations could be detected in: (1) basolateral membranes prepared from colonocytes of Sherman rats treated with 1,2-dimethylhydrazine; and (2) antipodal plasma membranes prepared from colonocytes of 'DMH-resistant' Lobund-Wistar rats administered this agent. The results of these experiments as well as a discussion of their possible significance serve as the basis for the present report.

Materials and Methods

Materials. *S*-Adenosyl-L-[*methyl*-^{3}H]methionine (5.0–15.0 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Fatty acids, methyl esters, gas-liquid chromatography columns and lipid standards were all purchased from Applied Science Corp. (State College, PA) and/or Supelco (Bellefonte, PA). 1,6-Diphenyl-1,3,5-hexatriene, DL-2-(9-anthroyl)stearic acid and DL-12-(9-anthroyl) stearic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI) or Molecular Probes Inc. (Junction City, OR). All other materials were obtained from either Fisher Chemical Co (Fairlawn, NJ) or Sigma Chemical Co., (St. Louis, MO) unless otherwise indicated.

Isolation of colonic epithelial cell plasma membranes. Male rats of the Sherman or Lobund-Wistar strain weighing 75–100 g were given weekly subcutaneous injections of diluent or 1,2-dimethylhydrazine dihydrochloride (Sigma Chemical Co., St. Louis, MO) at a dose of 20 mg/kg body weight for 5, 10 and 15 wk or 5 wk, respectively, as previously described.

The animals were maintained on a pelleted diet (CAMM Maintenance Rodent Diet, CAMM Research Institute, Inc., Wayne, NJ) with water and food ad libitum. At specified times (see above), control and 1,2-dimethylhydrazine-treated rats were fasted for 18 h with water ad libitum, the animals were killed and colons removed, and epithelial cells were harvested from proximal and distal segments as previously described [7–10]. The cells from each segment were then pooled separately and used to isolate basolateral [8] and/or brush-border [10] membranes as described. The purity of each basolateral and brush-border membrane preparation was assessed using the markers sodium-potassium-dependent adenosine triphosphatase ($(Na^+ + K^+)$-ATPase) and alkaline phosphatase, respectively; specific activity ratios ((purified membranes)/(original homogenates)) ranged from 15 to 18 for $(Na^+ + K^+)$-ATPase and from 12 to 15 for alkaline phosphatase and were similar for diluent and 1,2-di-

* The term 'lipid fluidity' as applied to anisotropic bilayer membranes is used to denote the relative motional freedom of the lipid molecules or substituents thereof. A more detailed description of the sense in which the term is used is given in Ref. 19. Briefly, as evaluated by steady-state fluorescence polarization of lipid fluorophores, fluidity is assessed via the parameters of the modified Perrin relationship described in Materials and Methods. An increase in fluidity corresponds to a decrease in either the correlation time, T_c, or the hindered anisotropy, r_∞, of the fluorophore. Hence, the term combines the concepts of the 'dynamic and static (lipid order) components' of fluidity.

methylhydrazine-treated preparations. In agreement with earlier studies [8,10], each of these plasma membrane preparations was found to be minimally contaminated with intracellular membranes. Protein was estimated by the method of Lowry et al. [11]. Liposomes were prepared from the extracted lipids of each membrane as previously described [12].

Fluorescence polarization studies. Steady-state fluorescence polarization studies were performed with a Perkin-Elmer 650-40 Spectrofluorometer (Perkin-Elmer Corp., Norwalk, CT) adapted for fluorescence polarization using the lipid-soluble fluorophores 1,6-diphenyl-1,3,5-hexatriene, 2-AS and 12-AS as previously described [13,14]. Fluorescence emission intensities recorded parallel and perpendicular to the excitation plane of each probe could be taken sequentially with this instrument and the anisotropy values calculated, or the anisotropy values could be obtained directly from the instrument. These measurements were found to differ by 2% or less. The content of each fluorophore in the preparations was estimated fluorometrically as described [15] and the final ratios of probe/lipid ranged from 0.001 to 0.002. Corrections for light scattering were also routinely performed as described [14].

Fluorescence polarization was expressed as the fluorescence anisotropy, r. The results were assessed according to the modified Perrin relationship [16,17]:

$$r = r_\infty + (r_0 - r_\infty)[T_c/(T_c + T_f)]$$

where r is the fluorescence anisotropy, r_0 is the maximal limiting anisotropy, taken as 0.365 for diphenylhexatriene [18] and 0.285 for the 2-AS and 12-AS [19], r_∞ is the limiting hindered anisotropy, T_c is the correlation time and T_f is the mean lifetime of the excited-state. The lifetime, T_f, was estimated by total fluorescence intensity as previously described [14]. No changes in the excited-state lifetimes were demonstrated using each probe in each preparation. Values of r_∞ for diphenylhexatriene were calculated from r values as previously described by Van Blitterswijk et al. [20].

Composition studies. Lipids were extracted from plasma membrane by the method of Folch et al. [21]. Cholesterol [22] and phospholipids [23] were measured as previously described. The phospholipid composition of the extracts was further analyzed by thin-layer chromatography according to the method of Katz et al. [24]. Fatty acids of the total lipid extracts were derivatized and the fatty acid methyl esters were analyzed according to the methods of Gartner and Vahouny [25] on a Hewlett-Packard 5790A gas-liquid chromatograph equipped with a flame ionization detector as described previously [25].

Phospholipid methylation activity. The methylation of phospholipids was measured by incorporation of the [^{3}H]methyl groups from *S*-adenosyl-L-[*methyl*-^{3}H]methionine (5.0–15.0 Ci/mmol) into phospholipids as previously described [7,26]. The products of phospholipid methylation were identified as described [7,26]. The chemical identity of the methylated products was further established by two-dimensional chromatography [27] and by hydrolysis of the phospholipids and identification of their free bases as described by Schneider and Vance [28]. Additionally, both labeled and unlabeled *S*-adenosyl-L-methionine were routinely purified by ion-exchange before use [29]. All enzymatic reactions were performed under maximal velocity (V_{max}) conditions and were linear with respect to time and protein.

Histological studies. At 5, 10 and 15 week, 1-cm proximal and distal colonic segments from each control and 1,2-dimethylhydrazine-treated animal killed were immediately fixed in 4% paraformaldehyde. Fixed specimens were then embedded in parafin for light microscopic examination and stained with hematoxylin and eosin [30].

Statistical methods. All results are expressed as mean values ± S.E. Paired or unpaired t-tests were used for all statistical analysis. $P < 0.05$ was considered significant.

Results

Light microscopic studies

In agreement with our earlier published observations [7], despite extensive sampling of the proximal and distal segments of colon in control and 1,2-dimethylhydrazine-treated animals, no evidence of severe atypia, carcinoma in situ, or microscopic adenocarcinomas were evident at any

time period examined by light microscopy (not shown). Inflammation was also found to be absent or minimal in each colonic segment at these times [7].

Fluorescence polarization studies

The 'static' and 'dynamic' components of lipid fluidity of basolateral membranes and their liposomes prepared from proximal and distal colonocytes of control and 1,2-dimethylhydrazine-treated Sherman rats at 5, 10 and 15 wk were assessed by steady-state fluorescence polarization techniques, utilizing r_∞ values of diphenylhexatriene and r values of 2-AS and 12-AS, respectively. The results of the membrane studies are summarized in Table I. As can be seen from this table, in contrast to our earlier findings in distal colonic brush-border membranes [7], both components of lipid fluidity separate of proximal and distal control and 1,2-dimethylhydrazine-treated basolateral membranes were similar at each time point using the three fluorophores. Liposomes prepared from each of these membranes also demonstrated similar fluorescence polarization values at each period examined using these probes (not shown).

As shown in Table II, after 5 wk administration of 1,2-dimethylhydrazine, both components of fluidity of control and treated antipodal membranes of Lobund-Wistar rats were also found to be similar using these fluorophores.

Lipid composition studies

Prior studies in model bilayers and natural membranes have correlated a high lipid fluidity with low molar ratios of cholesterol/phospholipid and sphingomyelin/phosphatidylcholine [18,31–33]. A low ratio of protein/lipid (w/w) [12] as well as less saturated or shorter fatty acyl chains in phospholipids [33,34] have also been correlated with a higher membrane fluidity. Despite finding no differences in the fluidity of 1,2-dimethylhydrazine-treated and control plasma membranes of Sherman and Lobund-Wistar rats, it was of interest to examine these parameters in view of the possibility that simultaneous alterations in several of these parameter may serve to 'offset' each other, thereby, maintaining a constant fluidity [35,36]. This latter phenomenon has been termed 'homeoviscous adaptation' [35,36] and several examples of this have recently been shown to occur in various membranes [9,35,36]. No differences, however, in any of these compositional parameters could be detected between control and 1,2-di-

TABLE I

FLUORESCENCE POLARIZATION STUDIES OF BASOLATERAL MEMBRANES OF PROXIMAL AND DISTAL COLONOCYTES USING DPH, 2-AS AND 12-AS AFTER 5, 10 AND 15 WEEKS OF ADMINISTRATION OF 1,2-DIMETHYLHYDRAZINE OR DILUENT TO SHERMAN RATS

Values represent means ± S.E. of number of preparations (N) is examined. Abbreviations: P, proximal; D, distal; BLM, basolateral membranes; DMH, 1,2-dimethylhydrazine.

Duration of treatment	Preparations	(N)	Limiting anisotropy, r_∞, at 25°C	2-AS anisotropy, r, at 25°C	12-AS anisotropy, r, at 25°C
5	control (P) BLM	6	0.232 ± 0.006	0.153 ± 0.004	0.093 ± 0.005
	DMH (P) BLM	6	0.229 ± 0.003	0.167 ± 0.010	0.102 ± 0.008
	control (D)BLM	6	0.229 ± 0.003	0.169 ± 0.008	0.104 ± 0.007
	DMH (D)BLM	6	0.229 ± 0.003	0.167 ± 0.003	0.102 ± 0.006
10	control (P) BLM	5	0.210 ± 0.004	0.161 ± 0.006	0.099 ± 0.003
	DMH (P) BLM	5	0.221 ± 0.012	0.165 ± 0.003	0.106 ± 0.004
	control (D)BLM	5	0.216 ± 0.004	0.161 ± 0.007	0.099 ± 0.006
	DMH (D)BLM	5	0.213 ± 0.007	0.163 ± 0.003	0.100 ± 0.003
15	control (P) BLM	4	0.204 ± 0.006	0.168 ± 0.005	0.102 ± 0.003
	DMH (P) BLM	4	0.208 ± 0.002	0.163 ± 0.006	0.106 ± 0.004
	control (D)BLM	3	0.217 ± 0.004	0.164 ± 0.004	0.105 ± 0.005
	DMH (D)BLM	3	0.224 ± 0.004	0.163 ± 0.003	0.107 ± 0.004

TABLE II

FLUORESCENCE POLARIZATION STUDIES OF ANTIPODAL PLASMA MEMBRANES PREPARED FROM PROXIMAL AND DISTAL COLOCYTES AFTER 5 WEEKS OF ADMINISTRATION OF 1,2-DIMETHYLHYDRAZINE OR DILUENT TO LOBUND-WISTAR RATS

Values represent means ± S.E. of number of preparations (N) examined. Abbreviations: P, proximal; D, distal; BBM, brush-border membranes; BLM, basolateral membranes; DMH, 1,2-dimethylhydrazine.

Probe	Preparation	(N)	Anisotropy, r	Limiting hindered anisotropy, r_∞, at 25°C	Order parameter, S, at 25°C
DPH	control (P) BBM	3	0.217 ± 0.004	0.189 ± 0.004	0.720 ± 0.007
	DMH (P) BBM	3	0.213 ± 0.005	0.184 ± 0.005	0.710 ± 0.008
	control (D)BBM	3	0.244 ± 0.005	0.225 ± 0.006	0.785 ± 0.009
	DMH (D)BBM	3	0.242 ± 0.005	0.223 ± 0.005	0.782 ± 0.008
	control (P) BLM	4	0.202 ± 0.003	0.167 ± 0.004	0.676 ± 0.007
	DMH (P) BLM	4	0.205 ± 0.002	0.173 ± 0.003	0.688 ± 0.006
	control (D)BLM	4	0.211 ± 0.002	0.181 ± 0.003	0.704 ± 0.005
	DMH (D)BLM	4	0.211 ± 0.002	0.181 ± 0.002	0.704 ± 0.005
2-AS	control (P) BBM	3	0.167 ± 0.002	–	–
	DMH (P) BBM	3	0.169 ± 0.003	–	–
	control (P) BBM	3	0.167 ± 0.004	–	–
	DMH (P) BBM	3	0.171 ± 0.002	–	–
	control (D)BLM	4	0.149 ± 0.003	–	–
	DMH (D)BLM	4	0.149 ± 0.002	–	–
	control (D)BLM	4	0.147 ± 0.004	–	–
	DMH (D)BLM	4	0.146 ± 0.001	–	–
12-AS	control (P) BBM	3	0.118 ± 0.002	–	–
	DMH (P) BBM	3	0.123 ± 0.003	–	–
	control (P) BBM	3	0.116 ± 0.003	–	–
	DMH (P) BBM	3	0.123 ± 0.003	–	–
	control (D)BLM	4	0.101 ± 0.002	–	–
	DMH (D)BLM	4	0.100 ± 0.003	–	–
	control (D)BLM	4	0.104 ± 0.002	–	–
	DMH (D)BLM	4	0.103 ± 0.002	–	–

methylhydrazine-treated plasma membranes prepared from colonocytes of Sherman or Lobund-Wistar rats at the various time points examined (not shown: consult BBA Data Deposition as quoted on the first page of this article).

Phospholipid methylation studies

No differences in the specific activities of the phospholipid methyltransferase were also seen between control and DMH-treated plasma membranes prepared from colonocytes of Sherman or Lobund-Wistar rats at the various time points examined (not shown: BBA Data Deposition).

Discussion

Previous studies in our laboratory have demonstrated that alterations in the 'dynamic' component of fluidity, lipid composition, and phospholipid methylation activity of brush-border membranes could be detected in the preneoplastic colons of Sherman rats, after the administration of 1,2-dimethylhydrazine [7]. Furthermore, these changes varied with the duration of treatment (5–15 wk) and were restricted to preparations prepared from distal colonocytes [7]. The latter observation is particularly interesting in view of the marked predilection for the development of neoplasia in this segment of the colon in rodents administered this agent [2].

In contrast to these earlier findings, however, the present experiments failed to detect similar alterations in any of these biochemical parameters in colonic basolateral membranes of Sherman rats

or colonic antipodal plasma membranes of Lobund-Wistar rats treated with 1,2-dimethylhydrazine. These results deserve further comment. First, taken together with our prior findings [7], the present data suggest that biochemical alterations exist in the surface membranes of distal colonic epithelial cells prepared from 1,2-dimethylhydrazine-treated animals but are confined to the luminal region of these cells. In this regard, recent studies by our laboratory have demonstrated that changes in the fluidity of colonic brush-border membranes can influence water permeability [37] and neutral sodium transport [37]. While speculative, the 1,2-dimethylhydrazine-induced alterations in fluidity of these plasma membranes might, therefore, be associated with changes in the permeability of as yet unknown factor(s) from the colonic lumen into epithelial cells, thereby leading to the development of colon cancer. Additional experiments, however, will be necessary to clarify this issue.

Second, since Lobund-Wistar rats are totally resistant to the tumor-inducing effects of 1,2-dimethylhydrazine [4–6], the present results strongly support the contention that the prior changes noted in the fluidity and phospholipid methyltransferase activity of distal colonic brush-border membranes, after administration of 1,2-dimethylhydrazine for 5 wk, are related to the malignant transformation process itself rather than to any effect of the drug per se. Further studies in this animal model of colonic adenocarcinoma should elucidate the mechanism(s) responsible for these membrane alterations as well as clarify their functional significance.

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