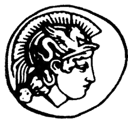

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Aldosterone alters the phospholipid composition of rat colonocytes

L. Mrnka[a], O. Nováková[b], F. Novák[c], E. Tvrzická[d], J. Pácha[a,]*

[a]Institute of Physiology, Czech Academy of Sciences, Vídeňska 1083, 142 20 Prague 4-Krč, Czech Republic
[b]Department of Animal Physiology, Faculty of Sciences, Charles University, Prague, Czech Republic
[c]Department of Biochemistry, Faculty of Sciences, Charles University, Prague, Czech Republic
[d]Fourth Department of Medicine, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Abstract

Previous studies have shown that aldosterone treatment of amphibian epithelial cells results not only in stimulation of Na^+ absorption but also in changes in phospholipid composition which are necessary for the mineralocorticoid action of aldosterone. The present study was designed to investigate the effect of aldosterone on phospholipids of mammalian epithelia. Phospholipid and fatty acid composition was examined in colonic epithelium (mineralocorticoid target tissue) and thymus (non-mineralocorticoid but glucocorticoid target tissue) of rats which had received aldosterone or vehicle by a miniosmotic pump for 7 days. Aldosterone increased the mass of colonic phospholipids relative to cellular proteins with concomitant changes in the percentage distribution of fatty acids, whereas the relative distribution of membrane phospholipds was not changed. Phosphatidylcholine increased the content of polyunsaturated and decreased that of monounsaturated fatty acids, which predominantly reflected the accretion of arachidonic and a decrease in oleic and palmitoleic acids. Within the phosphatidylethanolamine subclass, pretreatment of rats with aldosterone decreased the content of monounsaturated fatty acids (predominantly oleic and palmitoleic acid) and of n-3 fatty acids, and increased the content of saturated fatty acids (palmitic acid). The saturated-to-nonsaturated fatty acid ratio also significantly increased after aldosterone treatment. No changes in thymic phospholipids were seen. The results are consistent with the contention that aldosterone specifically modulates phospholipid concentration and metabolism in mineralocorticoid target tissue. The changes in phospholipid content and its fatty acid composition during the fully developed effect of aldosterone may reflect a physiologically important phenomenon with long-term consequences for membrane structure and function. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Colonocytes; Aldosterone; Thymus; Phospholipids; Fatty acids; Phospholipid metabolism

1. Introduction

Lipid composition is known to play an important role in the regulation of cell function [1]. This effect may be caused directly by physicochemical lipid–protein interactions or indirectly through a phospholipase-dependent signal transduction pathway [2,3]. It is well established that membrane phospholipids serve as a source of products that play a role of second messengers such as inositolphosphates, diacylglycerols, sphingolipids and lysophosphatidic acids [2,4] and free fatty acids [5]. Fatty acids can activate membrane enzymes, channels and transporters by direct interaction with proteins, by altering the interaction with the lipid bilayer or indirectly by metabolic conversion of arachidonic acid to oxygenated metabolites [5]. Very recently, fatty acids have been postulated to modulate corticosteroid-dependent gene expression [6].

Furthermore, data have accumulated that a number of epithelial cell surface functions, including transe-

* Corresponding author. Tel.: +420-2475-2440; fax: +420-2475-2488.
E-mail address: pacha@biomed.cas.cz (J. Pácha).

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pithelial transport and enzyme activities, are modulated by membrane lipids [1]. The generalised physicochemical alterations of the lipid bilayer associated with remodelling of phospholipid composition and their fatty acid content seem to be associated with changes of transport protein functions. Following appropriate stimuli such as dietary lipids or steroids, remodelling of membrane phospholipids leads to changes in transport activities of membrane transporters such as Na^+, K^+-ATPase, the Na^+/H^+-exchanger and the Na^+-glucose transporter [7–10]. Phospholipid metabolism is also believed to play a role in aldosterone regulated electrogenic Na^+ transport via epithelial Na^+ channels. The involvement of mineralocorticoids in phospholipid metabolism was demonstrated in amphibian epithelial cells in which aldosterone produced significant effects on phospholipid remodelling [11–13]. In addition, inhibition of fatty acid metabolism or inhibition of phospholipase A_2 prevented the enhancement of electrogenic amiloride-sensitive Na^+ transport by aldosterone [14,15] and application of phospholipids to the mucosal side decreased the transport capacity of this pathway [16]. These data indicate that one of the possible actions of aldosterone on modification of Na^+ membrane permeability may occur via changes in phospholipid composition. To date, however, information is lacking on the influence of aldosterone on the phospholipids of mammalian mineralocorticoid target epithelia.

The purpose of this study was to determine whether aldosterone is able to influence the phospholipids in rat colonocytes. The rat colon was chosen because aldosterone stimulates electrogenic amiloride-sensitive Na^+ transport in this intestinal segment [17,18] and this stimulation is accompanied by an enlargement of the plasma membrane surface [19]. For comparison, identical measurements were performed on the rat thymus where only glucocorticoid but not mineralocorticoid receptors are expressed and which represents a glucocorticoid target tissue [20].

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 250–300 g were used in these experiments. Animals were housed in groups of 2–3 animals/cage, fed a standard rat chow (Velaz, Prague, Czech Republic) containing 126 μmol Na^+/g diet and maintained in temperature-controlled animal facilities on a daily photoperiod of 12 h light and 12 h dark. After a one week acclimatization period, rats were divided into two experimental groups. The aldosterone-treated group received aldosterone (60 μg. 100g body wt^{-1}. day^{-1}; dissolved in polyethylene glycol 400) via miniosmotic pumps (Alzet 2001, Alza Corp., Palo Alto, CA, USA) for 7 days before sacrifice. We have previously demonstrated that this dose stimulates colonic electrogenic amiloride-sensitive Na^+ transport to a maximum [17]. The control group received the vehicle only.

Both groups of rats were anaesthetised with ether on the seventh day after implantation of the osmotic minipumps, blood was withdrawn from the abdominal aorta and the colon and thymus were removed. Blood was centrifuged, plasma removed and stored at −70°C. The thymus was immediately frozen in liquid nitrogen and stored at −70°C until further use. The colon was used for isolation of colonocytes immediately.

2.2. Isolation of colonocytes

The rats of both groups were sacrificed on the seventh day of aldosterone infusion and colonocytes were isolated according to the modified method of Roediger and Truelove [21]. Each colon was rinsed thoroughly with 150 mM NaCl containing 1 mM dithiothreitol, everted, distally ligated and then distended by means of a syringe with Ca^{2+}-free bicarbonate buffer (in mM: NaCl, 118; KCl, 4.7; $NaHCO_3$, 24.9; KH_2PO_4, 1.2; $MgSO_4$, 1.2) with 0.25% w/v bovine serum albumin (BSA) and ligated proximally. The distended colonic loop was placed in a plastic flask containing Ca^{2+}-free bicarbonate buffer plus 0.25% BSA and 5 mM EDTA and oxygenated with O_2/CO_2. Incubation of the loops lasted 30 min, after which they were removed and rinsed in a Ca^{2+}-free bicarbonate buffer without EDTA. Colonocytes were disaggregated by manual shaking in TRIS-buffered solution (in mM: NaCl, 20; KCl, 100 $CaCl_2$, 1.25; $MgCl_2$, 1.2; TRIS/HCl, 20.0 glucose, 10 pH 7.4). The suspension was carefully passed through a nylon mesh (nominal pore sizes 75 and 40 μm) and washed by centrifugation twice in a TRIS-buffered solution. Aliquots of cell suspensions were used for the procedures outlined below. The efficacy of colonocyte removal was compared by histological analyses of samples of isolated cells and of colonic tissue sections. Cells were stained with methylene blue and mounted on glass slides. In order to assess the completeness of colonocyte removal, pieces of colonic tissue were fixed immediately after extraction and stained with methylene blue.

2.3. Lactate dehydrogenase, corticosteroid and protein determinations

Colonocyte viability was assessed by the percentage of total lactate dehydrogenase (LDH) released during isolation of the cells. The enzyme activity and its release from colonocytes were measured according to

Malinowska [22]. LDH that had leaked out of the cells was determined in the supernatant of centrifuged samples and total LDH activity was assessed after cell permeabilization of the colonocytes by digitonin.

Plasma concentrations of aldosterone were measured by radioimmunoassay (Immunotech, Prague, Czech Republic) and that of corticosterone by HPLC according to Shimizu et al. [23]. Proteins were determined by the Coomassie blue method [24].

2.4. Phospholipid extraction and analyses

Quantitative extraction of total lipids was carried out by a modified method of Folch et al. [25]. The suspension of isolated colonocytes was mixed with eight volumes of chloroform:methanol (2:1) and subsequently homogenized for 1 min in an all-glass homogenizer. The lower phase was withdrawn after centrifugation (10 min, 1050 × g at r_{max}). This procedure was repeated twice more; a mixture of chloroform:methanol (7:1) saturated with ammonium hydroxide added to the sample and the resulting lipid extracts were collected, mixed well with 0.9% sodium chloride and centrifuged (15 min, 1050 × g at r_{max}). The lower lipid containing phase was taken away and evaporated in a stream of nitrogen.

Lipid samples were separated by two-dimensional thin-layer chromatography. Silica Gel H (Merck, Darmstadt, Germany) as a slurry of 22.5 g in 62 ml of water containing 2.5 g of Magnon (Merck, Darmstadt, Germany) was spread with a 0.25 cm fixed spreader (Desaga, Germany) on glass plates (20 × 20 cm) and activated (1 h, 110°C). After application of lipid samples, the plates were developed in the first dimension with chloroform–methanol–water–28% ammonium hydroxide (70:25:4:1) and in the second with chloroform–methanol–acetone–acetic acid–water (70:12.5:17.5:10:4.5) according to a modified method of Rouser et al. [26]. This method resulted in adequate separation of the following phospholipid classes: phosphatidylcholine, phosphatidylethanolamine, cardiolipin, phosphatidylinositol, phosphatidylserine and sphingomyelin. The phospholipid spots were visualised by iodine vapour (in the case of fatty acid analysis UV visualisation with 2,7-dichlorfluorescein was used), scraped and analysed for phosphorus [26]. The results were expressed either as a concentration (nmol phosphate per mg of cellular protein or g wet weight) or as a percentage of individual lipid class based on total lipid phosphorus.

2.5. Separation and quantification of fatty acids

Gas chromatography was performed on a Chrompack Model 9000 gas chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a capillary split/splitless injector and flame ionization detector. The chromatograph was interfaced with an IBM PS/2 Model 30 computer. Chrompack integration software was used for data acquisition and handling. Analyses of fatty acid methyl esters were performed on a fused silica capillary column (25 m, 0.25 mm I.D.) coated with chemically bonded CP-WAX 52 CB stationary phase (Chrompack). The oven temperature was programmed from 150°C to 230°C at 2°C/min and then kept isothermal for 10 min. The injector and detector temperature was 250°C. Hydrogen carrier gas was maintained at a head pressure of 80 kPa, with a split ratio of 1:20. Quantification of fatty acids was expressed as molar percentage of the total fatty acid content.

2.6. Statistical analysis

Data are presented as the mean ± SEM. When appropriate, the data were analysed by Hoteling's or Student's test to compare the set of phospholipids and fatty acids of aldosterone-treated and vehicle-treated group. Values of $P < 0.05$ were considered statistically different.

3. Results

Our cell suspension contained a mixture of single colonocytes and clumps of epithelial cells. Contamination by intestinal flora, muscle or connective tissue structures was negligible. The colonocyte viability between the aldosterone-treated (ALDO) and control (CTRL) groups was assessed by the percentage of total LDH released during incubation of colonocytes at 37°C for 5 and 30 min. The percentage of LDH released into the medium did not differ in both groups (time 5 min: ALDO 10 ± 3%, CTRL, 9 ± 2%; time 30 min: ALDO, 15 ± 3%, CTRL, 18 ± 3%). Plasma con-

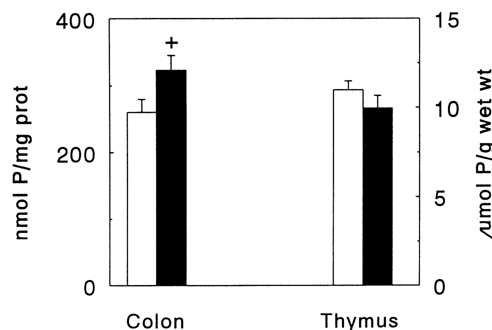

Fig. 1. A comparison of mean values of phospholipid mass in colonocytes and thymus of aldosterone treated (black columns) and control rats (white columns). Results are presented as the mean ± SEM of six animals in each group. + $P < 0.05$.

centrations of aldosterone were high in the ALDO-group (5060 ± 530 pg/ml) and much lower in the CTRL-group (120 ± 27 pg/ml). Plasma concentrations of corticosterone were similar in both groups (ALDO, 46 ± 9 ng/ml; CTRL, 58 ± 11 ng/ml).

To determine the effect of aldosterone on phospholipid composition of rat colonocytes, six major groups of phospholipids were isolated by TLC: phosphatidylcholine, phosphatidylethanolamine, cardiolipin, phosphatidylinositol, phosphatidylserine and sphingomyelin. The total colonic phospholipid mass relative to protein was significantly higher in colonocytes of aldosterone-treated rats than in control animals (Fig. 1). Thymic phospholipids were not changed. The percentage levels of particular phospholipid species remained unaffected by aldosterone both in colonocytes and the thymus (Table 1). The predominant phospholipids in both tissues were phosphatidylcholine and phosphatidylethanolamine that accounted for approximately 75% of total phospholipids. All other phospholipids were present in lesser amounts (from 2% to 9%).

The fatty acid distribution in phosphatidylcholine and phosphatidylethanolamine is shown in Tables 2 and 3. The major fatty acids in both phospholipid classes were palmitic (16:0), stearic (18:0), oleic (18:1n-9), vaccenic (18:1n-7), linoleic (18:2n-6) and arachidonic acids (20:4n-9), though the content of fatty acids not only differed between colonocytes and the thymus but also between phosphatidylcholine and phosphatidylethanolamine. Stearic and arachidonic acids accounted for the dominant saturated and unsaturated fatty acids in phosphatidylethanolamine, whereas palmitic and oleic acids predominated in phosphatidylcholine. Aldosterone modulated the fatty acid profile in colonocytes but was without any effect on fatty acid composition in the thymus. In phosphatidylcholine (Table 2), aldosterone produced a significant increase in the level of arachidonic (+35%) and palmitic acids (+9%) and a decrease in stearic (−9%) and monounsaturated fatty acids, particularly in oleic (−9%) and palmitoleic acids (16:1n-9; −19%). The proportion of the sum of n-6 and n-3 polyunsaturated fatty acids was not significantly changed even though an insignificant increase of n-6 fatty acids was obvious. In case of the phosphatidylethanolamine fraction, aldosterone significantly decreased the level of oleic (−12%) and palmitoleic acids (−17%) but did not alter the level of arachidonic acid. Fatty acids of the n-3 family were significantly lower (−27%) whereas saturated fatty acids were enhanced, particularly due to palmitic acid (+22%). The saturated-to-unsaturated fatty acid ratio increased after aldosterone treatment (ALDO: 0.325 ± 0.009; CTRL: 0.271 ± 0.004, $P < 0.05$) in phosphatidylethanolamine but was without any significant effect in phosphatidylcholine (ALDO: 0.582 ± 0.014; CTRL: 0.541 ± 0.002).

Table 1
Relative percentage of phospholipids extracted from rat colonocytes and thymus after aldosterone administration to rats[a]

PL	Colonocytes		Thymus	
	Aldosterone	Control	Aldosterone	Control
PC	43.2 ± 0.3	43.9 ± 0.4	54.3 ± 0.6	54.1 ± 0.8
PE	32.0 ± 0.9	31.8 ± 0.8	27.1 ± 0.7	27.2 ± 1.2
CL	6.4 ± 0.4	6.7 ± 0.6	2.2 ± 0.3	2.5 ± 0.1
PI	9.1 ± 0.5	8.9 ± 0.4	7.3 ± 0.6	6.7 ± 0.2
PS	5.8 ± 0.6	5.4 ± 0.5	7.3 ± 0.5	8.4 ± 0.2
SM	3.4 ± 0.4	3.3 ± 0.3	1.8 ± 0.2	1.7 ± 0.2

[a] Values represent means ± SEM of six animals in each group expressed as percentage of total phospholipids. The rats received aldosterone or vehicle by miniosmotic pumps for 7 days. PL, phospholipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin; PS, phosphatidylserine; PI, phosphatidylinositol, and SM, sphingomyelin.

4. Discussion

The experiments performed in the present study were aimed to obtain data regarding the effect of

Table 2
Fatty acid composition in phosphatidylcholine extracted from rat colonocytes and thymus[a]

FA	Colonocytes		Thymus	
	Aldosterone	Controls	Aldosterone	Controls
14:0	1.23 ± 0.11	1.24 ± 0.18	0.90 ± 0.18	0.90 ± 0.07
16:0	27.67 ± 0.61	25.31 ± 0.51[b]	37.41 ± 1.01	37.81 ± 0.47
16:1n-9	2.61 ± 0.13	3.21 ± 0.11[b]	1.89 ± 0.17	1.76 ± 0.09
18:0	7.33 ± 0.17	8.02 ± 0.19[b]	9.01 ± 0.46	9.06 ± 0.20
18:1n-9	27.05 ± 0.75	29.63 ± 0.6[b]	16.29 ± 0.95	16.13 ± 0.74
18:1n-7	6.82 ± 0.28	7.21 ± 0.30	7.69 ± 0.33	7.47 ± 0.23
18:2n-6	11.28 ± 1.43	11.35 ± 1.50	5.20 ± 0.59	5.22 ± 0.51
18:3n-6	0.25 ± 0.04	0.18 ± 0.03	0.07 ± 0.01	0.05 ± 0.01
18:3n-3	0.26 ± 0.03	0.25 ± 0.04	0.15 ± 0.01	0.13 ± 0.02
20:0	0.47 ± 0.07	0.55 ± 0.03	0.09 ± 0.01	0.11 ± 0.01
20:1n-9	0.77 ± 0.07	1.05 ± 0.10[b]	2.58 ± 0.23	2.68 ± 0.13
20:2n-6	1.33 ± 0.28	1.16 ± 0.11	2.00 ± 0.12	1.82 ± 0.10
20:3n-6	2.07 ± 0.13	1.93 ± 0.05	1.21 ± 0.09	1.19 ± 0.05
20:4n-6	9.41 ± 0.43	6.98 ± 0.22[b]	13.59 ± 0.95	13.71 ± 0.52
20:5n-3	0.20 ± 0.02	0.22 ± 0.02	0.09 ± 0.02	0.10 ± 0.01
22:4n-6	0.36 ± 0.03	0.37 ± 0.04	0.94 ± 0.08	0.99 ± 0.06
22:5n-3	0.20 ± 0.04	0.45 ± 0.17	0.32 ± 0.02	0.28 ± 0.03
22:6n-3	0.68 ± 0.09	0.93 ± 0.13	0.56 ± 0.05	0.57 ± 0.08
ΣSAT	36.70 ± 0.1	35.07 ± 0.22	47.40 ± 1.18	47.89 ± 0.49
Σn-6	24.20 ± 0.9	21.97 ± 1.30	23.03 ± 1.48	22.98 ± 0.72
Σn-3	1.33 ± 0.13	1.86 ± 0.32	1.11 ± 0.08	1.09 ± 0.12
ΣMUFA	37.24 ± 0.71	41.10 ± 1.45	28.46 ± 1.50	28.04 ± 0.99

[a] Values represent means ± SEM of six animals and are expressed as molar percentage of total fatty acids.
[b] Significantly different from aldosterone-treated group ($P < 0.05$).

aldosterone on the phospholipid and fatty acid composition of cells that are (colonocytes) or are not (thymocytes) mineralocorticoid-sensitive. To our knowledge, these experiments have not previously been performed in mammals, yet aldosterone influences the metabolism of phospholipids in amphibian mineralocorticoid target epithelia and these metabolic changes are involved in the regulation of Na^+ absorption in amphibian epithelia [11,12,17]. In the toad bladder, aldosterone increases the phospholipid deacylation and reacylation cycle, the weight percentage of long-chain polyunsaturated fatty acids and methylation of phospholipids [11–13,15]. Furthermore, increased phospholipase A_2 activity has also been implicated as the mechanism involved in the effect of aldosterone [12,14]. However, Frazier and Yorio [27] did not find any influence of aldosterone on phospholipid turnover using [^{32}P]-orthophosphate incorporation into phospholipids of the toad bladder. Similarly, the aldosterone effect on phospholipid turnover was absent in the rat salivary duct, which is also a mineralocorticoid target tissue [28].

A general problem concerning aldosterone is that its qualitative effects may depend on the duration of exposure to this hormone [29]. The "early" phase that usually lasts for several hours and the "late" (around 24 h) or "very late" phase (several days) can be distinguished biophysically, biochemically and pharmacologically; the "very late" phase is also characterised by morphological changes of the cells. All the abovementioned studies of aldosterone on phospholipids were exclusively performed during the "early" phase, i.e. when the tissue was only exposed to aldosterone for a few hours. Observations during the "late" and "very late" phases have not been studied. Our study indicates for the first time that chronically increased plasma concentrations of aldosterone are able to induce tissue-specific changes in phospholipid composition. This effect of aldosterone depends on the mineralocorticoid sensitivity of the tissue. While speculative, it would be reasonable to suggest that the increased phospholipid mass in colonocytes reflects an increased membrane surface of colonocytes exposed chronically to aldosterone during the "very late" phase [19,30].

The detailed analysis of fatty acids in major phospholipid species has demonstrated a significant remodelling of the fatty acid content in phosphatidylethanolamine and phosphatidylcholine after aldosterone application. This remodelling was characterised by an increased content of arachidonic acid and a reduced content of oleic and palmitoleic acids in phosphatidylcholine. Similarly, fatty acid analysis of phosphatidylethanolamine demonstrated a significantly lower content of oleic and palmitoleic acids and of n-3 fatty acids and an enhanced content of saturated fatty acids. These findings are in agreement with results of previous studies performed on the toad urinary bladder [11,12]. The weight percentage of several long-chain polyunsaturated fatty acids increased in the toad urinary bladder treated with aldosterone for several hours. Furthermore, aldosterone enhanced the elongation and desaturation of oleic acid and influenced phospholipase activity [14]. It seems reasonable to suggest that the aldosterone-induced changes in enzyme activities involved in fatty acid desaturation, elongation, synthesis or the deacylation–reacylation shuttle may, at least in part, be responsible for the changes in fatty acid composition of phospholipids. It has been shown recently that serum- and glucocorticoid-regulated kinase (sgk) of the Ser/Thr protein kinase family is an aldosterone-induced gene that is involved in induction of aldosterone-dependent effects [31,32]. It is tempting to speculate that sgk could phosphorylate and thereby regulate proteins such as enzymes, mediating modifications of phospholipid metabolism. In this connection previous studies have detected alterations of liver Δ-5, 6 and 9 desaturase activities induced by aldosterone [33–35]. At present, there are no data available on rat colonic intestinal desaturase and the effect of corticosteroid hormones to enable a comparison even though high activities of Δ-6 and Δ-5 desaturase-elongase have

Table 3
Fatty acid composition in phosphatidylethanolamine extracted from rat colonocytes and thymus[a]

FA	Colonocytes		Thymus	
	Aldosterone	Controls	Aldosterone	Controls
14:0	0.40 ± 0.04	0.43 ± 0.07	0.23 ± 0.05	0.20 ± 0.04
16:0	8.50 ± 0.35	6.97 ± 0.22[b]	6.95 ± 0.80	6.97 ± 0.66
16:1n-9	1.38 ± 0.05	1.67 ± 0.11[b]	1.04 ± 0.11	0.85 ± 0.12
18:0	14.56 ± 0.54	13.34 ± 0.41	19.26 ± 0.66	19.97 ± 0.18
18:1n-9	20.29 ± 0.49	23.04 ± 0.91[b]	10.65 ± 0.57	11.18 ± 0.84
18:1n-7	3.54 ± 0.22	3.37 ± 0.16	3.55 ± 0.25	3.59 ± 0.19
18:2n-6	7.30 ± 0.99	6.69 ± 0.58	2.12 ± 0.40	2.40 ± 0.40
18:3n-6	0.16 ± 0.02	0.16 ± 0.03	0.07 ± 0.03	0.05 ± 0.01
18:3n-3	0.31 ± 0.06	0.34 ± 0.03	0.24 ± 0.06	0.14 ± 0.01
20:0	0.64 ± 0.03	0.54 ± 0.02[b]	0.09 ± 0.01	0.12 ± 0.02
20:1n-9	0.58 ± 0.04	0.84 ± 0.05[b]	1.30 ± 0.10	1.25 ± 0.08
20:2n-6	1.96 ± 0.10	2.35 ± 0.09[b]	3.62 ± 0.61	2.14 ± 0.15
20:3n-6	2.93 ± 0.14	2.83 ± 0.15	0.89 ± 0.10	0.91 ± 0.07
20:4n-6	32.00 ± 1.24	30.65 ± 0.86	41.74 ± 1.59	42.53 ± 1.85
20:5n-3	0.87 ± 0.03	1.14 ± 0.11	0.22 ± 0.03	0.35 ± 0.08
22:4n-6	1.58 ± 0.07	2.07 ± 0.14[b]	4.59 ± 0.18	4.32 ± 0.29
22:5n-3	0.43 ± 0.05	0.74 ± 0.07[b]	1.26 ± 0.14	0.88 ± 0.15
22:6n-3	2.38 ± 0.16	3.00 ± 0.31	2.18 ± 0.20	2.15 ± 0.07
ΣSAT	24.11 ± 0.62	21.24 ± 0.31[b]	26.52 ± 0.87	27.27 ± 0.71
Σn-6	45.92 ± 1.23	44.98 ± 1.18	53.04 ± 1.10	52.35 ± 1.67
Σn-3	3.99 ± 0.14	5.50 ± 0.35[b]	3.89 ± 0.31	3.52 ± 0.09
ΣMUFA	25.98 ± 0.88	28.93 ± 1.08	16.54 ± 0.90	16.87 ± 1.10

[a] Values represent means ± SEM of 6 animals and are expressed as molar percentage of total fatty acids.
[b] Significantly different from aldosterone-treated group ($P < 0.05$).

been demonstrated in the human colonocyte CaCo-2 cell line [36]. The decreased oleate content in both major phospholipids after aldosterone treatment might be related to a lower activity of colonic Δ-9 desaturase. Since the ratios of dihomo-γ-linolenic (20:3n-6) to arachidonic acid and linoleic to γ-linolenic acid (18:3n-6) were not influenced by aldosterone in a similar way, these results argue against a steroid action on Δ-6 and Δ-5 desaturase activities. Aldosterone could also influence the activity or regulation of enzymes required to liberate fatty acids such as phospholipase A_2(PLA_2). Increased specific radioactivities of tissue-free fatty acids and the fall of phospholipid fatty acid radioactivities observed after aldosterone treatment in the toad bladder are consistent with enhanced endogenous phospholipase activities [12]. However, the effect of aldosterone on mammalian PLA_2 has not been identified. A significant decrease in the activity and expression at the PLA_2 mRNA level was observed after dexamethasone treatment in various tissues, including the ileum [37]. Such inhibition does not seem to be universal, because no significant changes of jejunal PLA_2 activity were found after dexamethasone administration [8]. It is also conceivable that the effect of aldosterone may be indirect and that the changes in colonocyte phospholipids may be primarily a response to elevated cell Na^+ uptake during stimulated Na^+ absorption. This mechanism was proposed for the regulation of cell membrane surface in renal collecting duct [38].

The significant shift in fatty acid composition with a subsequent increase in saturated-to-unsaturated fatty acid ratio would theoretically reduce the fluidity and/or membrane–protein interactions which could influence cellular responses to a variety of stimuli. For example, Brasitus and co-workers [7,10] reported that physico-chemical changes of membrane phospholipids are associated with significant alterations of transport proteins. We therefore cannot exclude the possibility that changes of phospholipid matrix are involved in the regulation of Na^+ absorption controlled by aldosterone. Relevant in this connection is the recent study of Röpke et al. [16] who have shown that changes in the phospholipid apical domain modulates Na^+ absorption in amphibian and mammalian epithelia. Nevertheless, further studies will be necessary to address this issue.

Regardless of the exact mechanisms involved, it is obvious that aldosterone produces phospholipid remodelling and alterations in phospholipid mass in the colonic epithelium. This in turn, may change the lipid fluidity and influence a number of enzymatic and transport processes in the plasma membranes. Further studies will be required to elucidate the mechanisms involved in the production of the phospholipid alterations and to shed more light on the possible relationship between phospholipid metabolism and regulation of Na^+ transport by aldosterone in mammalian epithelia.

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