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Low-Salt Diet Alters the Phospholipid Composition of Rat Colonocytes

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Summary

The effect of low-salt diet on phospholipid composition and remodeling was examined in rat colon which represents a mineralocorticoid target tissue. To elucidate this question, male Wistar rats were fed a low-salt diet and drank distilled water (LS, low-salt group) or saline instead of water (HS, high-salt group) for 12 days before the phospholipid concentration and fatty acid composition of isolated colonocytes were examined. The dietary regimens significantly influenced the plasma concentration of aldosterone which was high in LS group and almost zero in HS group. Plasma concentration of corticosterone was unchanged. When expressed in terms of cellular protein content, a significantly higher concentration of phospholipids was found in LS group, with the exception of sphingomyelin (SM) and phosphatidylserine (PS). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) accounted for more than 70 % of total phospholipids in both groups. A comparison of phospholipid distribution in LS and HS groups demonstrated a higher percentage of PE and a small, but significant, decrease of PC and SM in LS group. The percentage of phosphatidylinositol (PI), PS and cardiolipin (CL) were not affected by mineralocorticoid treatment. With respect to the major phospholipids (PE, PC), a higher level of n-6 polyunsaturated fatty acids (PUFA) and lower levels of monounsaturated fatty acids were detected in PC of LS group. The increase of PUFA predominantly reflected an increase in arachidonic acid by 53%. In comparison to the HS group, oleic acid content was decreased in PC and PE isolated from colonocytes of the LS group. Our data indicate that alterations in phospholipid concentration and metabolism can be detected in rats with secondary hyperaldosteronism. The changes in phospholipid concentration and their fatty acid composition during fully developed effect of low dietary Na^+ intake may reflect a physiologically important phenomenon with long-term consequences for membrane structure and function.

Key words

Aldosterone • Fatty acids • Phospholipid metabolism

Introduction

Lipid composition is known to play an important role in the regulation of cell functions including epithelial transport (Yorio and Frazier 1990). This effect may be accomplished indirectly through phospholipase-dependent signal transduction pathways or it may result from several direct, but non-specific, physicochemical

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lipid-protein interactions (Mouritsen and Bloom 1993, Divecha and Irvine 1995). In intestinal epithelium, changes of cell membrane physicochemical state were previously shown to influence the function of a wide variety of membrane-bound proteins such as enzymes and transporters (Brasitus 1983, Dudeja *et al.* 1987a,b). In addition, metabolites of n-6 and n-3 fatty acids such as prostaglandins have been shown to be important regulators of intestinal secretion (Binder *et al.* 1991).

Many investigators have demonstrated that various processes involving dietary changes (Brasitus 1987) or differentiation of enterocytes (Alessandri *et al.* 1993) are associated with remodeling of intestinal phospholipid composition and with changes of fatty acid metabolism. Similarly, several hormones such as glucocorticoids, estrogen or thyroid hormones, have been previously shown to influence the phospholipid composition of different organs including the intestine (Dudeja *et al.* 1987a,b, Brasitus *et al.* 1987, Brasitus and Dudeja 1988, Schwarz *et al.* 1988). The involvement of mineralocorticoids in phospholipid metabolism was demonstrated in amphibian epithelial cells in which aldosterone produced significant effects on phospholipid remodeling (Goodman *et al.* 1971, 1975, Wiesmann *et al.* 1985). In addition, inhibition of fatty acid metabolism prevented the stimulation of electrogenic amiloride-sensitive Na^+ transport by aldosterone (Lien *et al.* 1975), whereas the application of phospholipids to the mucosal side decreased the transport capacity of this pathway (Röpke *et al.* 1997). These data indicate that one of the possible actions of aldosterone on modification of Na^+ membrane permeability in mineralocorticoid target tissues may occur *via* changes in phospholipid composition. At present however, the data are lacking on the influence of aldosterone on the phospholipids of mammalian mineralocorticoid target epithelia.

The purpose of this study was to determine whether high plasma concentration of aldosterone induced by low-salt diet results in remodeling the phospholipids of rat colonocytes. Rat colon was chosen because aldosterone and low-salt diet stimulate electrogenic amiloride-sensitive Na^+ transport in this intestinal segment (Binder *et al.* 1991, Pácha and Pohlová 1995, Pácha *et al.* 1996) and the stimulation is accompanied by enlargement of plasma membrane surface (Vagnerová *et al.* 1997).

Material and Methods

Animals and diets

Male Wistar rats weighing 250-300 g were used in these experiments. Animals were housed in groups of 2-3 animals per 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 one week acclimatization period, rats were allocated to two experimental groups and were fed a low-salt diet (C 1036, Altromin, Lage, Germany) containing 8 μmol Na^+/g diet for 12 days. We have previously demonstrated that this Altromin diet increases plasma concentration of aldosterone and induces electrogenic amiloride-sensitive Na^+ transport in rat colon (Pácha and Pohlová 1995) and epithelial Na^+ channels (Mrnka *et al.* 1999). Two experimental groups with different mineralocorticoid status were employed: 1) low-salt (LS) group which drank distilled water, and 2) high-salt group (HS) which received saline instead of water.

The efficiency of dietary Na^+ intake on hormone level was verified by measuring plasma concentration of aldosterone by radioimmunoassay (Immunotech, Prague, Czech Republic). The plasma level of corticosterone was determined according to Shimizu *et al.* (1983).

Isolation of colonocytes

The rats of both groups were sacrificed on the 12th day of the experimental diet and colonocytes were isolated according to the modified method of Roediger and Truelove (1979). Each colon was rinsed thoroughly with 150 mmol/l NaCl containing 1 mmol/l dithiothreitol, everted and distally ligated. Thereafter they were distended by means of a syringe with Ca^{2+}-free bicarbonate buffer (in mmol/l: NaCl 118; KCl 4.7; $NaHCO_3$ 24.9; KH_2PO_4 1.2; $MgSO_4$ 1.2) containing 0.25 % w/v bovine serum albumin (BSA) and then they were ligated proximally. The distended colonic loop was placed in a plastic flask containing Ca^{2+}-free buffer plus 0.25 % BSA and 5 mmol/l EDTA oxygenated with O_2/CO_2 (95 %/5 %). Incubation of the loops lasted 30 min, after which they were removed and rinsed in Ca^{2+}-free bicarbonate buffer without EDTA. Colonocytes were disaggregated by manual shaking in TRIS-buffered solution (in mmol/l: 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 nylon mesh (nominal pore sizes 75 and 40 μm) and washed by centrifugation twice in TRIS-buffered solution. Aliquots of cell suspensions were used for the procedures outlined below. In order to assess the efficiency of colonocyte removal, pieces of colonic tissue were fixed immediately after the extraction, stained with methylene blue and observed in light microscope.

Lactate dehydrogenase and cellular protein determination

Colonocyte viability and integrity between the experimental groups were assessed by the percentage of total lactate dehydrogenase (LDH) released from the cell

according to Malinowska (1990). LDH that 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. Cellular proteins were determined by the Coomassie blue method (Bradford 1976) in supernatants and cell samples diluted with distilled water.

Phospholipid extraction and analyses

Quantitative extraction of total lipids was carried out following a modified method of Folch *et al.* (1957). The suspension of isolated colonocytes was mixed with eight volumes of chloroform:methanol 2:1 and subsequently homogenized for 1 min (using an all glass homogenizer). Afterwards, a centrifugation step (10 min, 1050 *g* at r_{max}) was performed and the lower phase was withdrawn. The procedure was repeated three times (for the second extraction a mixture chloroform:methanol 7:1 saturated with ammonium hydroxide was employed) and the resulting lipid extracts were collected, mixed 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 (20x20 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 one with chloroform-methanol-acetone-acetic acid-water (70:12.5:17.5:10:4.5) according to a method of Rouser *et al.* (1970). This method resulted in an adequate separation of the following phospholipid (PL) classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE), cardiolipin (CL), phosphatidylinositol (PI) phosphatidyl-serine (PS) and sphingomyelin (SM). The phospholipid spots were visualized by iodine vapour (in the case of fatty acid analysis UV visualization with 2,7 dichlorfluoresceine was used), scraped out and analyzed for phosphorus (Rouser *et al.* 1970). The results were reported either as a concentration (nmol phosphate per mg of cellular protein) or as a percentage of individual lipid class based on total lipid phosphorus.

Separation and quantification of fatty acids

Gas chromatography was performed with 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 and Epson LQ 550 printer (Seico Epson Corp., Japan). Chrompack integration software was used for data acquisition and handling. Analyses of fatty acid methylesters 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 total fatty acid content.

Statistical analysis

Results are expressed as means ± SEM. Comparisons between groups were made by Student's t-test. Values of $P<0.05$ were considered to be statistically different. The results concentration of phospholipids and the content of fatty acids in phospholipids were analyzed by two-way ANOVA. The Newman-Keuls multiple range test was used to determine significant differences among individual means.

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 in the experimental groups was assessed by the percentage of total LDH released during incubation of colonocytes at 37 °C for 5 and 30 min, respectively. The percentage of LDH released to the medium was not different between both groups (at 5 min: LS 8±2 %, HS 10±2 %; at 30 min: LS 18±4 %, HS 16±3 %). Plasma concentrations of aldosterone in the animals kept on low-salt diet (786±130 pg/ml, n=9) were very high, i.e. about 200-fold higher than in the HS group (4.0±1.5 pg/ml, n=8). Plasma concentrations of aldosterone in HS group are similar to those found in rats kept on 8 % NaCl diet (31±7 pg/ml). Plasma concentrations of corticosterone was similar in both groups (LS, 61.0±18.3 ng/ml; HS, 51.1±13.6 ng/ml).

To determine the effect of aldosterone on phospholipid composition of rat colonocytes, six major groups of phospholipids were isolated by TLC (PC, PE,

CL, PI, PS and SM). The analyses revealed that phospholipid concentration relative to protein was significantly influenced by the low-salt diet. The phospholipid/cellular protein ratio was 186.5±10.0 nmol P/mg protein (n=9) in LS group and 125.6±20.2 nmol P/mg protein (n=8) in HS group ($P<0.05$). Comparison of the concentration of particular phospholipid species in LS and HS group indicates that, with the exception of SM and PS, the concentrations of all phospholipid species relative to cellular protein were significantly increased in LS animals (Fig. 1).

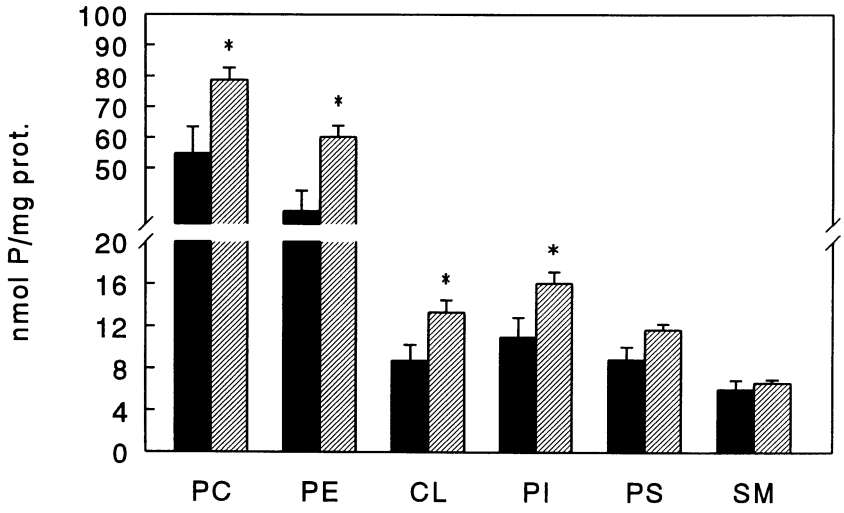

Fig. 1. *Effect of dietary Na^+ intake on colonocyte phospholipid concentration. Solid bars indicate the rats with high Na^+ intake and low concentration of aldosterone, whereas cross-hatched bars represent the animals with low Na^+ intake and high plasma concentration of aldosterone. Phospholipid abbreviations: PC – phosphatidylcholine, PE – phosphatidylethanolamine, CL – cardiolipin, PI – phosphatidylinositol, PS – phosphatidylserine, SM – sphingomyelin. Values represent means ± SEM. *P<0.05*

Table 1. Relative content of phospholipids extracted from rat colonocytes

PL	HS group	LS group
PC	43.8±0.5	42.3±0.3*
PE	28.1±1.2	32.2±0.7*
CL	6.9±0.2	7.1±0.4
PI	8.8±0.4	8.6±0.4
PS	7.3±0.4	6.3±0.2
SM	5.1±0.4	3.6±0.2*

*Values represent means ± SEM of 8 animals in each group and are expressed as percentage of total phospholipids. LS group – rats kept on low dietary Na^+ intake, HS group – rats kept on high dietary Na^+ intake. Phospholipid abbreviations: PC – phosphatidylcholine, PE – phosphatidylethanolamine, CL – cardiolipin, PI –phosphatidylinositol, PS - phosphatidylserine, and SM – sphingomyelin. * Significantly different from HS group (P<0.05).*

The relative distribution of phospholipid species in colonocytes is shown in Table 1. In general, PC and PE, which were the predominant phospholipid species in both experimental groups, accounted for more than 70 % of all phospholipids. The low-salt diet significantly decreased the percentage of PC and SM, whereas stimulated the percentage of PE. These data indicate that a chronic decrease in Na^+ intake is associated with a small remodeling of phospholipid distribution.

The fatty acid distribution in the two main species of phospholipids is shown in Table 2. The major saturated fatty acids were 16:0 and 18:0, the sum of which represented over 20 % of total fatty acids in PE and 35 % in PC. The major unsaturated fatty acids were 18:1n-9 (24-28 % in PE and 32-36 % in PC), 18:2n-6 (6 % in PE and 9 % in PC) and 20:4n-6 (24-29 % in PE and 6-9 % in PC). A comparison of the fatty acid levels in PC and PE revealed that colonocytes from Na^+-depleted rats possess higher level of n-6 polyunsaturated fatty acids and lower percentage of monounsaturated fatty acids. The percentage of arachidonate (20:4n-6) was substantially increased,

whereas that of oleate (18:1n-9) was decreased. The percentage of the sum of poly- and monounsaturated fatty acids was not significantly changed in PE, although the percentage of oleic acid was significantly decreased in the LS group. The percentage of saturated and monounsaturated fatty acids was significantly higher in PC than in PE, whereas the percentage of n-6 and n-3 polyunsaturated fatty acids was significantly higher in PE than in PC (two-way ANOVA $P<0.01$).

Table 2. Fatty acid composition in phosphatidylethanolamine (PE) and phosphatidylcholine (PC) extracted from rat colonocytes.

	Phosphatidylethanolamine		Phosphatidylcholine	
Fatty acids	**HS group**	**LS group**	**HS group**	**LS group**
14:0	0.29±0.03	0.58±0.17	1.0±0.1	1.1±0.4
16:0	6.5±0.2	8.8±2.2	22.2±0.3	20.6±2.6
16:1	1.3±0.02	1.4±0.3	2.5±0.2	2.2±0.3
18:0	13.7±0.2	13.9±0.5	12.5±0.6	12.7±1.0
18:1n-9	28.5±0.7	24.1±1.3*	35.8±0.7	32.0±0.7*
18:1n-7	2.5±0.1	2.8±0.5	5.8±0.3	6.2±0.2
18:2n-6	6.2±0.2	6.5±0.2	8.2±0.2	9.0±0.4
18:3n-6	0.14±0.04	0.20±0.03	0.15±0.01	0.21±0.01*
18:3n-3	0.20±0.03	0.29±0.08	0.09±0.02	0.12±0.02
20:0	0.44±0.02	0.50±0.03	0.50±0.04	0.64±0.08
20:1	0.62±0.05	0.52±0.02	0.71±0.06	0.60±0.05
20:2	3.9±0.1	2.7±0.3*	0.51±0.08	0.49±0.05
20:3n-6	3.0±0.2	3.2±0.1	2.0±0.1	2.5±0.3
20:4n-6	24.4±0.9	27.8±3.1	6.0±0.3	9.1±1.0*
20:5n-3	2.7±0.2	2.0±0.3	0.76±0.07	0.83±0.18
22:4n-6	0.97±0.08	1.1±0.1	0.22±0.03	0.35±0.06
22:5n-3	0.68±0.03	0.50±0.07	0.18±0.02	0.35±0.15
22:6n-3	4.0±0.2	3.1±0.5	0.86±0.06	1.0±0.3
ΣSAT	20.9±0.3	23.8±2.1	36.2±0.7	35.1±2.2
Σn-6	34.7±0.8	38.9±3.1	16.6±0.4	21.2±1.4*
Σn-3	7.6±0.04	5.9±0.9	1.9±0.1	2.3±0.6
ΣMUFA	32.9±0.7	28.8±2.0	44.8±0.5	40.9± 0.6*

*Values represent means ± SEM and are expressed as molar percentage of total fatty acids (8 animals in both groups). SAT – saturated fatty acids, n-6 – polyunsaturated fatty acids (n-6), n-3 – polyunsaturated fatty acids (n-3), MUFA – monounsaturated fatty acids, LS group – rats kept on low dietary Na^+ intake; HS group – rats kept on high dietary Na^+ intake. * Significantly different from HS group ($P<0.05$).*

Discussion

This study demonstrates that a drastic reduction of Na^+ intake exerts a significant effect on phospholipid metabolism and on physicochemical properties of colonocyte membranes. It can be suggested that aldosterone or other factors such as angiotensin II may be involved in the action of the low-salt diet on colonic phospholipids. Indeed, angiotensin II stimulates the biosynthesis of phosphatidylcholine (Hatch *et al.* 1997)

and generation of diacylglycerol (Patel *et al.* 1991). Similarly, phospholipids have been reported to be modified by various steroid hormones such as glucocorticoids, estrogens and 1,25-dihydroxyvitamin D_3 in a number of different tissues (Murray *et al.* 1979, Rooney 1985, Brasitus *et al.* 1987, Shionome *et al.* 1992, Marra and de Alaniz 1995). Aldosterone has been identified as a hormone which changes phospholipid turnover and composition in amphibian toad bladder (Goodman *et al.* 1971, 1975, Lien *et al.* 1975) but not in mammalian salivary duct, which is also a mineralocorticoid target tissue (Bercier and Frazier 1993). In the toad bladder, aldosterone increases the phospholipid deacylation and reacylation cycle, elevates the percentage of long-chain polyunsaturated fatty acids and stimulates phospholipid methylation (Goodman *et al.* 1971, 1975, Lien *et al.* 1975, Wiesmann *et al.* 1985).

A general feature of aldosterone action is that the qualitative effects of this hormone may depend on the time of exposure to the hormone (Rossier and Palmer 1992). The "early" phase (generally spanning several hours) and "late" (around 24 hours) or "very late" phase (several days) can be distinguished biophysically, biochemically and pharmacologically. The "very late" phase is also characterized by morphological changes of the cells. All of the above mentioned studies of aldosterone on phospholipids were performed only during "early" phase, i.e. in the tissue exposed to aldosterone only for several hours, while observations during "late" and "very late" phase were not reported. Our present study indicates for the first time that chronically reduced Na^+ intake and/or increased plasma concentration of aldosterone induce changes in the concentration and composition of membrane phospholipids. In the present study, low-salt diet produced an increase in plasma aldosterone concentration and in total phospholipid concentration of rat colonocytes (nmol P_i/mg cellular protein). It also altered percentage distribution of phospholipid species. It would be tentative to suggest that the increased phospholipid concentration of colonocytes might reflect the increased membrane surfaces of colonocytes exposed chronically to aldosterone during the "very late" phase (Kashagarian *et al.* 1980, Vagnerová *et al.* 1997). The mechanism(s) responsible for these changes are still unclear. As far as we know, there are no data indicating an aldosterone effect on the enzymes of phospholipid biosynthesis. Earlier studies have demonstrated glucocorticoid effect on the rate of lung phosphatidylcholine biosynthesis as well as on the activity of phosphocholine cytidylyltransferase (Rooney 1985) and the effects of angiotensin II on PI-specific phospholipase C, PC-specific phospholipase C and phospholipase D (Barnett et *al.* 1995).

Detailed analysis of fatty acids in the major phospholipid species (PC and PE) has demonstrated a significant remodeling of the fatty acid content in PE and especially in the PC fraction, after Na^+ depletion. Remodeling was characterized by an increased fraction of arachidonate and a decreased fraction of oleate which is in agreement with results of previous studies performed on toad urinary bladder (Goodman *et al.* 1971, 1975). Toad urinary bladder treated with aldosterone for several hours increased the weight percentage of several long-chain polyunsaturated fatty acids. Furthermore, aldosterone enhanced the elongation and desaturation of oleate and also influenced the phospholipase activity. It would therefore appear reasonable to suggest that aldosterone-induced changes in the enzyme activities involved in fatty acid desaturation, elongation, synthesis or deacylation-reacylation shuttle may, at least in part, be responsible for the changes of fatty acid composition in rat phospholipids. In this regards, aldosterone-induced alterations of Δ-5, Δ-6 and Δ-9 desaturase activities in the liver have been demonstrated (Mara and de Alaniz, 1990, 1995, de Alaniz and Marra 1992). In addition, Chen and Nilsson (1993) demonstrated high activities of Δ-6 and Δ-5 desaturase-elongase in human colonocyte CaCo-2 cell line. At present, there are no data available on rat colonic intestinal desaturase or on the effect of corticosteroid hormones to make comparisons. Aldosterone or angiotensin II could also influence the activity or regulation of enzymes required to liberate fatty acids such as phospholipase A_2 (PLA_2). Increased specific activities of tissue-free fatty acids and the fall of phospholipid fatty acid activities observed after aldosterone treatment in toad bladder prelabeled with [^{14}C]acetate (Goodman *et al.* 1975) or in heart myocytes after angiotensin II (Lokuta *et al.* 1994) are consistent with a stimulation of endogenous phospholipase activities. However, the effect of aldosterone on 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 ileum (Lilja *et al.* 1994). Such inhibition does not seem to be universal, because no significant changes of jejunal PLA_2 activity were found after dexamethasone administration (Brasitus *et al.* 1987).

Regardless of the exact mechanisms involved, it is obvious that decreased dietary Na^+ intake produces in colonic epithelium not only the changes in Na^+ transport capacity, but also phospholipid remodeling and alterations in phospholipid concentration. This in turn, might 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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References

ALESSANDRI J-M, JOANNIC J-L, DURAND GA: Polyunsaturated fatty acids as differentiation markers of rat jejunal epithelial cells: a modeling approach. *J Nutr Biochem* **4:** 97-104, 1993.

BARNETT RL, RUFFINI R, RAMSAMMY L, PASMANTIER R, FRIEDLAENDER MM, NORD EP: cGMP antagonizes angiotensin-mediated phosphatidylcholine hydrolysis and C kinase activation in mesangial cells. *Am J Physiol* **268:** C376-C381, 1995.

BERCIER JG, FRAZIER LW: The effect of aldosterone on phospholipid and phosphoinositide metabolism in rat submandibular gland. *Arch Oral Biol* **38:** 393-398, 1993.

BINDER HJ, SANDLE GI, RAJENDRAN VM: Colonic fluid and electrolyte transport in health and disease. In: *The Large Intestine: Physiology, Pathophysiology and Disease.* SF PHILLIPS, JH PEMBERTON, RG SHORTER (eds), Raven Press, New York, 1991, pp 141-168.

BRADFORD MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:** 248-254, 1976.

BRASITUS TA: Lipid dynamics and protein-lipid interactions in rat colonic epithelial cell basolateral membranes. *Biochim Biophys Acta* **728:** 20-30, 1983.

BRASITUS TA: Modulation of intestinal transport processes by dietary cholesterol and triacylglycerols. *J Pediatr Gastroenterol Nutr* **6:** 657-662, 1987.

BRASITUS TA, DUDEJA PK: Effect of hypothyroidism on the lipid composition and fluidity of rat colonic apical plasma membranes. *Biochim Biophys Acta* **939:** 189-196, 1988.

BRASITUS TA, DUDEJA PK, DAHIYA R, HALLINE A: Dexamethasone-induced alterations in lipid composition and fluidity of rat proximal-small-intestinal brush-border membranes. *Biochem J* **248:** 455-461, 1987.

CHEN Q, NILSSON A: Desaturation and chain elongation of n-3 and n-6 polyunsaturated fatty acids in the human CaCo-2 cell line. *Biochim Biophys Acta* **1166:** 193-201, 1993.

DE ALANIZ M.J, MARRA CA: Glucocorticoid and mineralocorticoid hormones depress liver Δ 5 desaturase activity through different mechanisms. *Lipids* **27:** 599-604, 1992.

DIVECHA A, IRVINE RF: Phospholipid signalling. *Cell* **80:** 269-278, 1995.

DUDEJA PK, FOSTER ES, BRASITUS TA: Modulation of rat distal colonic brush-border membrane Na^+-H^+ exchange by dexamethasone: role of lipid fluidity. *Biochim Biophys Acta* **905:** 485-493, 1987a.

DUDEJA PK, FOSTER ES, DAHIYA R, BRASITUS TL: Modulation of Na^+-H^+ exchange by ethinyl estradiol in rat colonic brush-border membrane vesicles. *Biochim Biophys Acta* **899:** 222-228, 1987b.

FOLCH J, LEES M, SLOANE-STANLEY GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226:** 497-509, 1957.

GOODMAN DBP, ALLEN JE, RASMUSSEN H: Studies on the mechanism of action of aldosterone: hormone-induced changes in lipid metabolism. *Biochemistry* **10:** 3825-3831, 1971.

GOODMAN DBP, WONG M, RASMUSSEN H: Aldosterone-induced membrane phospholipid fatty acid metabolism in the toad urinary bladder. *Biochemistry* **14:** 2803-2809, 1975.

HATCH GM, LEE D, MAN RY, KROGER EA, CHOY PC: On the mechanism of the losartan-mediated inhibition of phosphatidylcholine biosynthesis in H9c2 cells. *Biochim Biophys Acta* **1347:** 183-190, 1997.

KASHAGARIAN M, TAYLOR CR, BINDER HJ, HAYSLETT JP: Amplification of cell membrane surface in potassium adaptation. *Lab Invest* **42:** 581-588, 1980.

LIEN EL, GOODMAN DBP, RASMUSSEN H: Effects of an acetyl-coenzyme A carboxylase inhibitor and a sodium-sparing diuretic on aldosterone-stimulated sodium transport, lipid synthesis, and phospholipid fatty acid composition in the toad urinary bladder. *Biochemistry* **14:** 2749-2754, 1975.

LILJA I, DIMBERG J, SJODAHL R, TAGESSON C, GUSTAFSON-SVARD C: Effect of endotoxin and dexamethasone on group I and II phospholipase A_2 in rat ileum and stomach. *Gut* **35:** 40-45, 1994.

LOKUTA AJ, COOPER C, GAA ST, WANG HE, ROGERS TB: Angiotensin II stimulates the release of phospholipid–derived second messenger through multiple receptor subtypes in heart cells. *J Biol Chem* **269:** 4832-4838, 1994.

MALINOWSKA DH: Permeabilizing parietal cells. *Methods Enzymol* **192:** 108-124, 1990.

MARRA CA, DE ALANIZ MJ: Mineralocorticoids modify rat liver Δ 6 desaturase activity and other parameters of lipid metabolism. *Biochem Int* **22:** 483-493, 1990.

MARRA CA, DE ALANIZ MJ: Regulatory effect of various steroid hormones on the incorporation and metabolism of [^{14}C]stearate in rat hepatoma cells in culture. *Mol Cell Biochem* **145:** 1-9, 1995.

MOURITSEN OG, BLOOM M: Models of lipid-protein interactions in membranes. *Annu Rev Biophys Biomol Struct* **22:** 145-171, 1993.

MRNKA L, POHLOVÁ I, PÁCHA J: Permissive effect of thyroid hormones on induction of colonic sodium transport by aldosterone. *Physiol Res* **47:** 14P, 1998.

MURRAY DK, RUHMANN-WENNHOLD A, NELSON DH: Dexamethasone effect on the phospholipid content of isolated fat cell ghosts from adrenalectomized rats. *Endocrinology* **105:** 774-777, 1979.

PÁCHA J, POHLOVÁ I: Relationship between dietary Na^+ intake, aldosterone and colonic amiloride-sensitive Na^+ transport. *Br J Nutr* **73:** 633-640, 1995.

PÁCHA J, POHLOVÁ I, ZEMANOVÁ Z: Hypothyroidism affects the expression of electrogenic amiloride-sensitive sodium transport in rat colon. *Gastroenterology* **111:** 1551-1557, 1996.

PATEL JM, SEKHARAM KM, BLOCK ER: Angiotensin receptor-mediated stimulation of diacylglycerol production in pulmonary artery endothelial cells. *Am J Respir Cell Mol Biol* **5:** 321-327, 1991.

ROEDIGER WEW, TRUELOVE SC: Method of preparing isolated colonic epithelial cells (colonocytes) for metabolic studies. *Gut* **20:** 484-488, 1979.

ROONEY SA: The surfactant system and lung phospholipid biochemistry. *Am Rev Respir Dis* **131:** 439-460, 1985.

RŐPKE M, UNMACK MA, WILLUMSEN NJ, FREDERIKSEN O: Comparative aspects of actions of a short-chain phospholipid on epithelial Na^+ channels and tight junction conductance. *Comp Biochem Physiol A* **118:** 211-214, 1997.

ROSSIER B, PALMER LG: Mechanisms of aldosterone action on sodium and potassium transport. In: *The Kidney: Physiology and Pathophysiology*. DW SELDIN, G GIEBISCH (eds), Raven Press, New York, 1992, pp 1373-1409.

ROUSER G, FLEISHNER SF, YAMAMOTO A: Two-dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* **5:** 494-496, 1970.

SCHWARZ SM, BOSTWICK HE, MEDOW MS: Estrogen modulates ileal basolateral membrane lipid dynamics and Na^+-K^+-ATPase activity. *Am J Physiol* **254:** G687-G694, 1988.

SHIMIZU K, AMAGAYA S, OGIHARA Y: Analysis of corticosterone in the serum of mice and rats using high-performance liquid chromatography. *J Chromatogr* **272:** 170-175, 1983.

SHIONOME M, SHINKI T, TAKAHASHI N, HASEGAWA K, SUDA T: 1α,25-dihydroxyvitamin D_3 modulation of lipid metabolism in established bone marrow-derived stromal cells, MC3T3-G2/PA6. *J Cell Biochem* **48:** 424-430, 1992.

VAGNEROVÁ R, KUBÍNOVÁ L, PÁCHA J: Correlation of function and structure in developing rat distal colon. *Cell Tissue Res* **288:** 95-99, 1997.

WIESMANN WP, JOHNSON JP, MIURA GA, CHIANG PK: Aldosterone-stimulated transmethylations are linked to sodium transport. *Am J Physiol* **248:** F43-F47, 1985.

YORIO T, FRAZIER LW: Phospholipids and electrolyte transport. *Proc Soc Exp Biol Med* **195:** 293-303, 1990.

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