

ORIGINAL ARTICLE

Stress-Mediated Alteration in Membrane Fluidity Regulates Calcium ATPase Activity in Plasma Membrane of Duodenal Enterocytes of Oophorectomized Rats

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Abstract: In a hypogonadal rat model, calcium ATPase (Ca²⁺-ATPase) activity and membrane fluidity were examined in plasma membrane of duodenal enterocytes under two different stress conditions developed by chemical change (lipid saturation) and physical change (temperature). Data generated show that, under both these stress conditions, calcium ATPase activity was decreased. Membrane fluidity (phase transition temperature) study by fluorescence polarization measurement indicated that, compared to corresponding control enterocyte membranes, differences were seen in the phase transition temperature (T_c) of the oophorectomized rats' enterocyte membrane under both the conditions of stress, which suggested a decrease in membrane fluidity under both the experimental situations. It is proposed that the degree of alteration in calcium ATPase activity in oophorectomized rats' duodenal enterocyte plasma membrane under two different stress conditions was nearly alike, which was influenced by changes in membrane fluidity induced by chemical change (lipid saturation) or physical change (temperature).

Key words: Plasma membrane; Ca²⁺-ATPase; membrane fluidity, high-lipid diet; cold stress

Introduction

Biological membranes are not rigid structures. It is the process of molecular motion within the membrane that is referred to as "fluidity" and it is this process which imparts viscous properties to the hydrophobic portion of the membrane [1]. The term membrane fluidity refers to the physical state of fatty acyl chains comprising the membrane bilayer structure. It is affected by various physical and chemical factors. Components directly affecting the physical properties of the acyl chains are the unsaturation and chain length, but the behaviour of the acyl chains is also influenced by other components of the membrane such as cholesterol proteins and phospholipids and an increase in unsaturation has often been taken to imply increased membrane fluidity [2]. Surgical manipulation like oophorectomy has been reported to cause a rise in serum cholesterol level [3-4], which also may influence the behaviour of acyl chains. Membrane fluidity is also dependent on temperature. The fluidity is low, when the membrane is in the gel state at low temperature, and it is high, when temperature increases. A reversible transition from gel to liquid crystalline state occurs at a characteristic temperature known as a phase transition temperature (T_c)

above which fluidity increases dramatically [5-6]. It has been demonstrated that lipid composition and functional activities of the biological membranes can be modified by feeding diets varying in fatty acid composition [2, 7-10]. Furthermore, reports indicate that fatty acid composition of intestinal brush border, basolateral and microsomal membranes are altered by dietary variation of saturated or polyunsaturated triacylglycerols [11-13]. Indeed, polyunsaturated fatty acids play an important role in regulating the physicochemical properties of intestinal epithelial membrane [14]. Many important cell functions, like transport process are affected by modulation of the activities of the membrane enzymes through changing fatty acyl unsaturation. A significant proportion of this unsaturated fatty acyl moiety in mammalian cell membrane is required to maintain a fluid state for the proper activity of a number of membrane proteins, such as $\text{Na}^+\text{-K}^+\text{-ATPase}$, $\text{Ca}^{2+}\text{-ATPase}$ and $\text{Mg}^{2+}\text{-ATPase}$ [2, 15]. It is also well known that the activity of intestinal alkaline phosphatase (AP) varies directly with the fluidity of different biological membranes [16-17]. The involvement of AP and $\text{Ca}^{2+}\text{-ATPase}$ in calcium absorption has been proposed by many researchers, because the activities of these enzymes correlate with the degree of calcium absorption in different parts of the intestinal tract under different circumstances [18-19]. This confirmed earlier suggestions that both enzymes are expression of the same molecule [20-22]. Additionally, an earlier study from this laboratory on oophorectomized rats revealed that both AP and $\text{Ca}^{2+}\text{-ATPase}$ show similar pattern of response [23]. In the present study, $\text{Ca}^{2+}\text{-ATPase}$ activity and membrane fluidity were examined in plasma membrane of duodenal enterocytes of oophorectomized rats under two different stress conditions : high-fat diet and cold-swim. The idea was to assess regulation of $\text{Ca}^{2+}\text{-ATPase}$ activity in changed situations (stress and oophorectomy) when composition and functional activities of the biological membrane expectedly was modified.

Materials and Methods

Chemicals: ATP (disodium salt), β -mercaptoethanol, EDTA, imidazole, DPH, all were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other analytical grade chemicals were purchased from E. Merck (India).

Animals: Female Wistar rats weighing 120-150 g were used for this study. Upon arrival, they were acclimatized for 7 days in an environmentally controlled animal laboratory with 12 h light and dark schedule and free access to drinking water. They were provided with a control laboratory diet [24] composed of carbohydrates 71% (equal parts of arrowroot starch and sucrose), protein 18% (casein), fat 7% (groundnut oil), salt mixture 4% [25] and vitamins were supplied according to Chatterjee *et al.*, [26]. After 7 days of acclimatization, animals were randomly divided into five groups consisting of six rats in each group: (A) sham-operated control, (B) bilaterally oophorectomized, (C) bilaterally oophorectomized + sunflower oil enriched diet, (D) bilaterally oophorectomized + groundnut oil enriched diet, and (E) bilaterally oophorectomized + coconut oil enriched diet. Under light ether anaesthesia, bilateral oophorectomies were performed dorsolaterally in the

animals of group B, C, D and E. Animals of group A were subjected to sham-operation. During the next 7 days of recuperation, all the animals were maintained on that same control laboratory diet. The animals of group C, D, and E, after recovery, were supplied with a high-lipid diet [27] for 15 days, while the animals of group A and group B were provided with control laboratory diet. The composition of high-lipid diet was 48% carbohydrate (equal volume of arrowroot starch and sucrose) and 30% fat (sunflower oil for group C, groundnut oil for group D and coconut oil for group E). Remaining composition of the diet was same as described for control laboratory diet [24-26]. To overcome the impact of any altered food intake, control (group A) was pair-fed with experimental groups B, C, D and E. The percentage composition of fatty acids [28] of the edible oils used in the present investigation are sunflower oil [16:1(trace), 18:1 (14), 18:2(73), other polyunsaturated acids (1)]; groundnut oil [14:0 (trace), 4-16(9), 18:0(3), 16:1(trace), 18:1(65), 18:2(17) and other polyunsaturated acids (trace)]; coconut oil [4-12(63), 4-16(9), 14:0 (18), 18:0(2), 18:1(8), 18:2(2.0)].

In an identical manner, animals were reared, grouped and prepared for second part of the study. They were randomly divided into five groups consisting of six rats in each group: (A) sham-operated control, (B) bilaterally oophorectomized, (C) bilaterally oophorectomized + stress induced (15°C), (D) bilaterally oophorectomized + stress induced (8°C), and (E) bilaterally oophorectomized + stress induced (4°C). Details of surgical oophorectomy procedure, post-operative diets etc. all were identical as first part of the study, except recuperation period in these animals were allowed for 15 days. After the recovery period was over, the animals of group C, D and E were exposed to cold-swim stress according to Shu *et al.*, [29]. In brief, the animals of group C, D and E were subjected to cold stress at different intensities, i.e., 15°C, 8°C, and 4°C respectively and were forced to swim for 5 minutes everyday for 7 consecutive days.

Collection of Intestine and isolation and preparation of membranes: After the experimental period was over, animals of all groups were sacrificed. The abdomen was opened and the whole intestine was quickly removed. The duodenum portion was separated and homogenized in a glass homogenizer and spun for 10 minutes at 1,500 rpm in a cold buffer containing 25mM imidazole, 0.25 M sucrose, 1 mM EDTA and 1 mM β -ME (pH7.5). The suspension was then centrifuged at 12,000 rpm (10,000 g) for 10 min at 4°C. The supernatant was then collected and centrifuged at 100,000 g for 1 hr. The pellets (plasma membrane) were resuspended in the above buffer and assayed for protein and enzyme activity.

Protein Assay: The protein was assayed following the method of Lowry *et al.*, [30] using bovine serum albumin as standard.

Enzyme Assay: Ca^{2+} -ATPase in the isolated plasma membrane fraction was assayed as described previously [31]. The assay mixture in a final volume of 1 ml (pH 8.5) contained, 25mM imidazole in 25mM sucrose, 0.5 mM EDTA, 1 mM β -ME, 3mM CaCl_2 , 4 mM ATP and 10 μg of membrane protein [32]. The Ca^{2+} -ATPase activity was determined against a blank that contained all the ingredients except membrane protein. After a 30 min incubation at 37°C, the reaction was terminated by addition

of 0.2ml of 30% ice cold TCA and the liberated free inorganic phosphate was determined by following the method of Sen *et al.*, [33].

Fluorescence polarization measurement on membrane associated DPH: Twenty five micro litre membrane protein (1mg/ml) was taken in 3ml of 25mM imidazole buffer (pH 7.5) and 5µl of 2mM 1,6-diphenyl-1,3,5-hexatriene (DPH) solution in tetrahydrofuran was added to it. DPH is a probe that measures the fluidity state of the membrane hydrocarbon regions [34]. The buffer with these additions was thoroughly mixed for 20min and kept at room temperature for 30min in the dark. The changes in polarization with temperature were measured in a Perkin-Elmer fluorescence spectrophotometer (Model : MPF-44B) [excitation wavelength 360 nm and emission wavelength 428 nm]. Temperature was maintained in a circulating bath equipped with an external flow circuit. The scattering effect due to the protein alone was subtracted from the membrane incubated with DPH. Fluorescence polarization P was calculated from the following relation described elsewhere [35].

$$P = \frac{I_{VV} - I_{VH}}{I_{VV} + I_{VH}}$$

Where, I_{VV} and I_{VH} respectively are the intensities of the vertical and horizontal polarized light. When P is plotted against $1/T$ ($^{\circ}K$), a straight line is expected if the probe environment maintains the same structural conformation. Any deviation from linearity indicates phase transition or any other structural change.

Statistical Analysis: Values were expressed as mean \pm SE. Significance was determined by two tail Student's *t*-test using Stats Direct Statistical software (StatsDirect Ltd., UK, Version 2.6.5). Differences were considered significant if $p < 0.05$.

Results

Effect of high-lipid diet on calcium ATP-ase activity: Calcium ATP-ase activity in the plasma membrane of duodenal enterocytes of oophorectomized rats supplemented with different edible oils has been depicted in Table 1. Results indicate that, compared to sham-operated control (group A), Ca^{2+} -ATP-ase activity in the plasma membrane of duodenal enterocytes of oophorectomized rats (Group B) was significantly ($p < 0.05$) reduced (20.17%). When these oophorectomized rats (Group B) were supplemented with high-lipid diet (30%), containing groundnut oil (Group D) or coconut oil (Group E), such decrease in calcium ATPase activity was more pronounced (Group D : 35.29%, $p < 0.05$ and Group E: 41.69%, $p < 0.01$). However, in sunflower supplemented rats (Group C), this well-defined response in calcium ATPase activity was absent (Group C: 25.71%, $p > 0.05$).

Table-1:Ca²⁺ATPase activity of isolated duodenal enterocyte plasma membrane of rats under different experimental conditions

Parameters	Experimental Groups				
	Control (Group A)	Oophorec tomized (Group B)	Oophorec tomized + sunflower oil (Group C)	Oophorec tomized+ groundnut oil supplemented (Group D)	Oophorec tomized + coconut oil (Group E)
Ca ²⁺ ATPase activity (μ mole/mg protein/hr)	5.95 \pm 0.38	4.75 \pm 0.27*	4.42 \pm 0.24 ^{NS}	3.85 \pm 0.23*	3.47 \pm 0.25**

Values are mean \pm SE (n=6). : *denotes p<0.05, **denotes p<0.01 and ^{NS} denotes not significant (p>0.05) based on Student's *t*-test. Comparisons were made between Gr. A vs. Gr. B, Gr. B vs. Gr. C, Gr. B vs. Gr. D, and Gr. B vs. Gr. E.

Effect of cold stress on calcium ATPase activity: Calcium ATP-ase activity in the plasma membrane of duodenal enterocytes of oophorectomized rats subjected to cold-swim stress (15°C, 8°C and 4°C) has been depicted in Table 2. Results indicate that, compared to sham-operated control (Group A), Ca²⁺-ATPase activity in the plasma membrane of duodenal enterocytes of oophorectomized rats (Group B) was significantly (p<0.05) reduced (20.17%). When these oophorectomized rats (Group B) were subjected to cold swim at 8°C (Group D) or 4°C (Group E), such decrease in calcium ATPase activity was more pronounced (Group D: 33.11%, p<0.05 and Group E: 35.13%, p<0.01). However, such well-defined response of calcium ATP-ase was absent when rats were subjected to cold swim at 15°C (Group C: 23.53%, p>0.05).

Table-2: Ca²⁺ATPase activity of isolated duodenal enterocyte plasma membrane of rats under different experimental conditions

Parameters	Experimental Groups				
	Control (Group A)	Oophorec tomized (Group B)	Oophorec tomized + stress (15°C) (Group C)	Oophorec tomized + stress (8°C) (Group D)	Oophorec tomized + stress (4°C) (Group E)
Ca ²⁺ ATPase activity (μ mole/mg protein/hr)	5.95 \pm 0.38	4.75 \pm 0.27*	4.55 \pm 0.19 ^{NS}	3.98 \pm 0.18*	3.86 \pm 0.14**

Values are mean \pm SE (n=6). *denotes p<0.05, **denotes p<0.01 and ^{NS} denotes not significant (p>0.05) based on Student's *t*-test. Comparisons were made between Gr. A vs. Gr. B, Gr. B vs. Gr. C, Gr. B vs. Gr. D, and Gr. B vs. Gr. E.

Effect of high-lipid diet on the fluorescence polarization profile: The phase transition temperature (T_c) of plasma membrane of duodenal enterocytes of control rats as determined by DPH probe was 36.5°C . Compared to control, the phase transition temperature of the plasma membrane of duodenal enterocytes of oophorectomized and different fat supplemented groups of rats respectively were : oophorectomized (Group B; 37.5°C), oophorectomized + sunflower oil (Group C; 38°C), oophorectomized + groundnut oil (Group D; 39.5°C) and oophorectomized + coconut oil (40.5°C) (Figure 1).

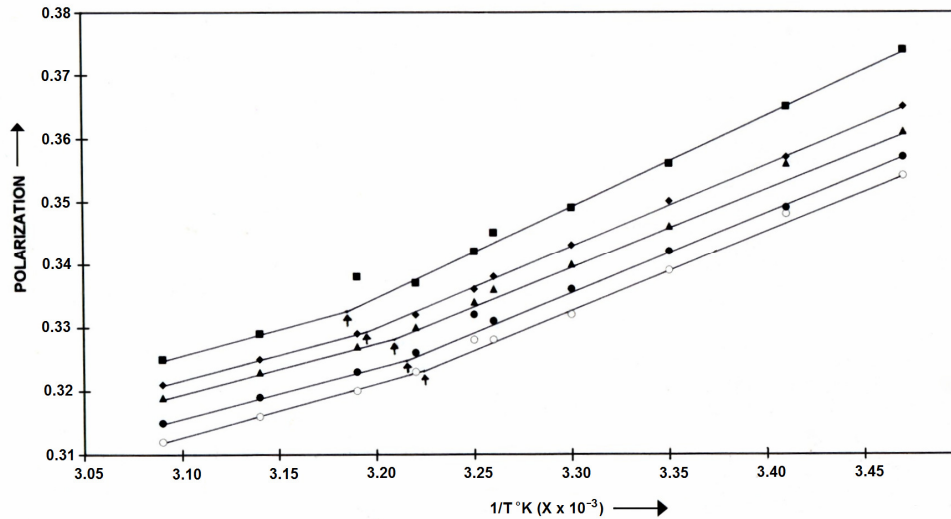


Figure-1: Effect of high-intake of different edible oils on the fluorescence polarization profile of plasma membrane isolated from rat duodenal enterocytes. Data shown are the average of six separate experiments \pm mean deviation (5%).

[○- control, ● - ectomy, ▲ - sunflower oil, ◆ - groundnut oil, ■ - coconut oil]

Effect of cold stress on the fluorescence polarization profile: The phase transition temperature (T_c) of plasma membrane of duodenal enterocytes of control rats as determined by DPH probe was 36.5°C . Compared to control, the phase transition temperature of the plasma membrane of duodenal enterocytes of oophorectomized and different cold-swim subjected groups of rats respectively were: oophorectomized (Group B; 37.5°C), oophorectomized + cold swim (15°C) (Group C; 37°C), oophorectomized + cold swim (8°C) (Group D; 39°C), and oophorectomized + cold swim (4°C) (Group E; 40°C) (Figure 2).

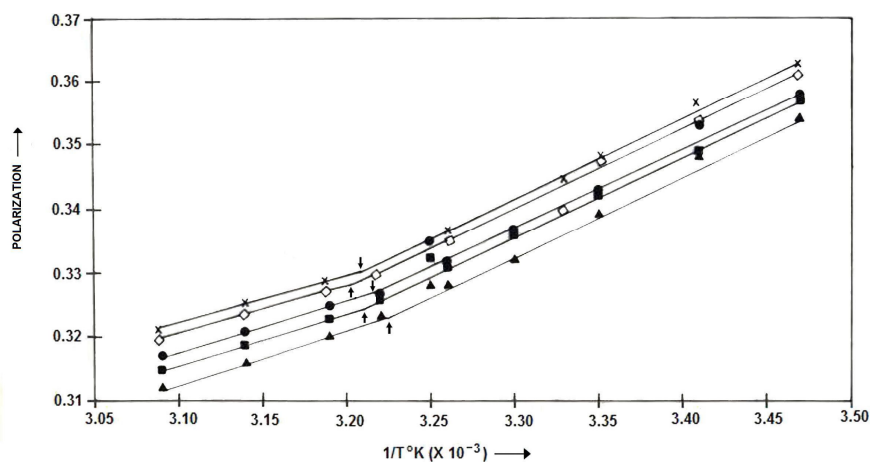


Figure-2: Effect of cold stress on fluorescence polarization profile of plasma membrane isolated from rat duodenal enterocytes. Data shown are the average of six separate experiments \pm mean deviation (5%). [▲ - control, ■ - ectomy, ● - stress (15°C), ◇ - stress (8°C), X - stress (4°C)]

Discussion

The dynamic nature of biological membranes is important for its function and is dependent on membrane constituents. Activities of membrane bound enzymes also depend on the state of fluidity of the membranes. In the present study, polarization profile of duodenal enterocyte plasma membrane of oophorectomized rats were examined following high-lipid diet supplementation with various kind of saturation and unsaturation characteristics and cold-swim stress of different intensities (15°C, 8°C and 4°C). The measurement of polarization with the fluorescence probe DPH in the plasma membranes characterizes the rotational motion of the probe molecule in the hydrophobic environment of the membranes [36-37]. When polarization (P) is plotted against 1/T, a straight line is expected if the probe environment maintains the same structural conformation. Any deviation from linearity indicates a phase transition. An increase in the phase transition temperature (T_c) of the membrane from control indicates that the membrane becomes more rigid. In the present study, surgical manipulation like oophorectomy showed an increase in phase transition temperature of enterocyte plasma membrane, suggesting that oophorectomy possibly had an influence in changing the phase transition temperature of the duodenal enterocyte plasma membrane (Figure 1 and 2), by increasing serum cholesterol level and behaviour of acyl chains [3- 4]. When oophorectomized rats were supplemented with dietary fats of variable saturation and unsaturation characteristics, more noticeable increase in phase transition temperature was observed (Figure 1 and 2). Further analysis of results of polarization studies revealed that control membrane was most fluid than oophorectomized and other fat supplemented groups, corroborating well with an earlier report that phase transition temperature varies with the ratio of saturated and unsaturated fatty acids present in the oils, which is almost negligible in sunflower oil compared to groundnut (0.146)

and coconut oil (9.58) [28]. This interpretation finds support from earlier reports that phase transition temperature decreases with the degree of the unsaturation [38-41]. Analyses of the results of second part of this study revealed that control membrane was most fluid than oophorectomy and other cold-swim experimental groups. This speculation earns its support from earlier suggestions that membrane is less fluid at low transition temperature when the membrane is in the gel state and is higher with rise in transition temperature [5- 6]. Thus, decrease in membrane fluidity was evident from our results of phase transition temperature of plasma membrane of duodenal enterocytes of oophorectomized rats fed with high-lipid diet or exposed to different degrees of cold-swim. Many important cell functions, like transport processes are affected by modulation of the activities of the membrane bound enzymes through changing of fatty acyl unsaturation. Such unsaturation has been reported to be induced in the membranes of cells when subjected to dietary fat supplementation. A significant proportion of this unsaturated fatty acyl moiety in mammalian cell membranes are required to maintain a "fluid" state for the proper activity of a number of membrane bound enzymes, such as $\text{Na}^+\text{-K}^+$ ATPase, Ca^{2+} -ATPase and Mg^{2+} ATPase [2]. To ascertain the interrelationship between fluidity of membrane and Ca^{2+} -ATPase activity under two different stress conditions, namely lipid saturation and temperature, we measured membrane bound Ca^{2+} -ATPase activity in both experimental conditions. In the present study, Ca^{2+} -ATPase activity was significantly decreased in bilaterally oophorectomized rats (Group B) as well as in rats fed with high lipid diets containing groundnut and coconut oil (Group D and E) (Table 1), suggesting that supplementation of fatty acids through these oils possibly modified membrane acylation and fluidity, and, thus, reduced Ca^{2+} -ATPase activity. Identical results of Ca^{2+} -ATPase activity were obtained with cold swim-stress at 8°C and 4°C, indicating that such physical change (temperature variation) possibly had an influence on membrane transition temperature and its fluidity (Figure 2), which was instrumental in decreasing Ca^{2+} -ATPase activity (Table 2). This is quite in agreement with an earlier observation that activity of intestinal alkaline phosphatase (AP) may be affected by an alteration of fluidity of biological membranes [16].

Conclusions

Chemical change (lipid saturation) or physical change (temperature) induced alteration in membrane fluidity can influence Ca^{2+} -ATPase activity in oophorectomized rats. Further detailed study is required to elucidate the mechanism of Ca^{2+} -ATPase regulation under these experimental conditions.

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