Vol. 92, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 12, 1980 Pages 819-824

MEMBRANE FLUIDITY AND ADENYLATE CYCLASE ACTIVITY IN GENETICALLY OBESE MICE

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Received November 27,1979

SUMMARY. Adipocyte plasma membranes of genetically obese ob/ob mice are more fluid than their lean littermates but the fluidity was normalised in mice maintained at high environmental temperatures. The defective response of adenylate cyclase to isoproterenol was improved after normalisation of membrane fluidity. No major changes in the phospholipid composition of ob/ob membranes were detected.

The fluid-mozaic model is thought to be the best representation of our present knowledge of membrane structure[1]. The proteins within the membrane are highly mobile[2], but their activity and function may be affected by both the lipid microenvironment and the bulk membrane fluidity[3,4]. In the genetically obese ob/ob mouse a wide range of membrane related processes are defective[5], including $[Na^+ + K^+]$-ATPase (E.C.3.6.1.3.) activity[6,7], the hormonal regulation of adenylate cyclase (E.C.4.6.1.1.)[8,9], hormone receptor concentrations[10], and glucose transport[11]. It has not yet been possible to fully explain all these changes on the basis of a single gene defect or as changes secondary to the obese state. However, it has been suggested previously from studies on membrane receptors[12] and on adenylate cyclase regulation[8] that there may be a plasma membrane defect in ob/ob mice. We have investigated the lipid composition and microviscosity of isolated adipocyte plasma membranes prepared from ob/ob mice and have related these to the regulation of adenylate cyclase activity.

METHODS

10-12 week old lean (ob/?) and obese (ob/ob) mice bred in the Department's animal facility were housed either at 24°C or 34°C in a constant light-dark cycle (0700-0700 hrs). Body temperature was measured with a flexible rectal probe attached to a digital thermometer.

Adipocyte plasma membranes were prepared by differential centrifugation followed by discontinuous ficol gradient centrifugation[13] after isolation of adipocytes by the collagenase method[14].

The fluorescent polarisation technique was used to study membrane fluidity[15]. 100 µg membrane protein was suspended in 900 µl 1 mM Tris,

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pH 7.4 at 25°C, 250 mM sucrose to which 1 µl 1 mM DPH in tetrahydrofuran was added. After incubation for 60 min at 25°C, 100 µl of a solution containing 250 mM HEPES, 1 M NaCl, 50 mM KCl, 5 mM $CaCl_2$, pH 7.4 at 20°C were added. After 15 min incubation, the temperature was reduced to 0°C and the fluorescence polarisation measured at 1°C intervals up to 40°C on an Aminco-Bowman Spectrophotofluorimeter. Microviscosity was calculated from the fluorescence polarisation values at 37°C according to the formula proposed by Shinitzky and Barenholz[15].

For compositional analysis, purified membranes were extracted with chloroform:methanol (2:1, v/v). The extracts were applied to thin-layer chromatograms of Silica Gel H containing 3 % (w/w) magnesium hydroxycarbonate and the plates developed in a 2-dimensional system for resolution of all phospholipid classes[16]. Spots were visualised under u.v. light after staining with diphenylhexatriene stain (DPH)[16]. Phosphate was assayed by the method of Fiske and Subbarow[17] after perchloric acid digestion and cholesterol by an enzyme assay[18].

Adenylate cyclase (E.C.4.6.1.1.) activity was assayed in crude membrane preparations prepared as described by Dehaye et al[8]. 15-25 µg of the resuspended 1,500-25,000 g pellet of an adipocyte homogenate was incubated with 0.5 mM ATP, 5 mM $MgCl_2$, 1.0 mM EGTA, 1.0 mM Theophylline, 7.5 mM phosphoenolpyruvate, 30 mM Tris pH 7.4 and 25 U/ml pyruvate kinase (Sigma Chemical Co.) for 10 min at 37°C. After rapidly freezing to stop the reaction, cyclic 3',5'-AMP production was assayed by the adrenal receptor assay of Brown et al[19].

RESULTS AND DISCUSSION

The 'break' point in the Arrhenius plot of fluorescence polarisation, P, of the diphenylhexatriene (DPH)-labelled ob/ob mice plasma membranes decreased from 25.1 to 17.5°C (Fig.1). 'Break' points detected either by enzymatic or physical techniques are closely associated with lipid phase separations occuring within the membrane[20,21] and the temperatures at which these occur are related to membrane composition[15]. The microviscosity of membranes from ob/ob mice, calculated from the fluorescence polarisation values, is significantly reduced at physiological temperatures (1.90 v 2.35 poise at 37°C) (Table 1). The change in Arrhenius 'break' point and the reduced microviscosity of membranes from ob/ob mice indicate that these membranes may contain components in a more fluid state.

The body temperature of ob/ob mice is reduced, as a result it has been hypothesised, of defective thermogenesis[5,22]. As animals are known to regulate either their membrane composition[23] or the phospholipid bilayer assymetry[24] according to their body temperature, it was of interest to measure membrane microviscosity in ob/ob mice with a normal body temperature. After increasing the housing temperature of ob/ob mice from 25°C to 34°C for 7 days, their body temperatures were normalised (Table 1). Adipocyte membranes from these mice showed a normalisation of the Arrhenius 'break'

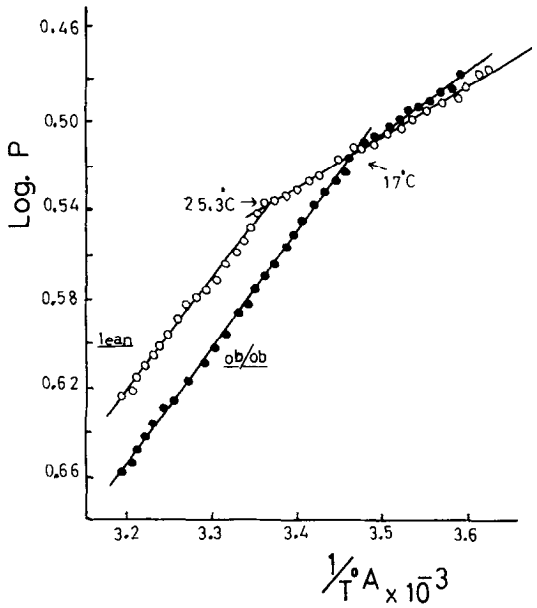

Fig.1. Arrhenius plot of fluorescence polarisation P, against temperature of adipocyte plasma membranes labelled with diphenylhexatriene (DPH).

temperature of 1,6-DPH fluorescent polarisation and a correction of the microviscosity at 37°C back to lean values (Table 1), suggesting that the compositional or structural anomaly in ob/ob membranes had been rectified. Neither total cholesterol nor total phospholipid content of ob/ob plasma membrane is significantly altered (Table 2), nor is there any change in the ratio of sphingomyelins (SM) to phosphatidylcholines (PC). A lower PC:SM ratio of natural phospholipids is normally associated with a decrease in fluidity[15]. It is possible that the decrease in microviscosity of ob/ob

Table 1. Arrhenius break points and microviscosity of DPH-labelled adipocyte plasma membranes from lean and obese ob/ob mice housed at 25°C and 34°C.

Housing Temp (°C)		Body Temp (°C)	Arrhenius Break Point (°C)	Microviscosity (poise) at 37°C
25°C	Lean	36.5 ± 0.5	25.1 ± 0.3	2.35 ± 0.01
	ob/ob	34.6 ± 0.6**	17.6 ± 0.7***	1.90 ± 0.04***
34°C	Lean	36.6 ± 0.4	23.6 ± 0.5	2.55 ± 0.12
	ob/ob	37.0 ± 0.2	23.5 ± 1.2	2.48 ± 0.17

Values are the mean ± S.E.M. for experiments.

** $p < 0.01$, *** $p < 0.001$ compared to Lean values.

Table 2. Lipid composition of adipocyte plasma membranes from lean and obese ob/ob mice.

	Lean	ob/ob
Cholesterol (μmoles/mg prot.)	0.42*	0.43
Phospholipid (μmoles/mg prot.)	1.20	1.20
Cholesterol : phospholipid	0.35	0.36
Phospholipid composition (% total)		
Phosphatidyl inositol	7.4	7.1
Phosphatidyl ethanolamine	28.0	28.0
Phosphatidyl choline	41.0	40.0
Sphingomyelins	17.0	17.0
Phosphatidyl serine	6.6	7.9

*Values represent the mean of duplicate determinations

membranes may reflect an increased occurrence of unsaturated fatty acid in the phospholipids, a possibility that is currently being investigated. It is unlikely to reflect a change in the assymetric distribution of phospholipid across the bilayer as similar fluidity changes were observed in extracted phospholipid vesicles as in the intact membranes (unpublished observations).

The response of ob/ob mouse adipose tissue adenylate cyclase to β-agonists has been shown to be severely impaired in a number of reports[8,9]. This lack of response to isoproterenol was confirmed in the present experiments in animals housed at normal temperatures (25°C) (Fig.2) when basal activity was only 35 % of that in lean mice and minimal stimulation was apparent at 10^{-6} - 10^{-5}M isoproterenol, although a marked increase in activity was seen at higher concentrations. After housing the obese mice at 34°C for 7 days to normalise gross membrane fluidity, the adipose tissue adenylate cyclase showed comparable dose-response curve to that of lean mice although basal activity remained low. No significant changes in the NaF-stimulated adenylate cyclase activity were apparent after housing at 34°C, the activity in ob/ob mice remaining between 60-70 % of that in lean mice.

The defect in the regulation of adenylate cyclase in ob/ob mice has been attributed to both a 30 % reduction in the number of β-receptors[25], and to a defect in the interaction between the hormone receptor and catalytic subunit of the enzyme[8,9]. Using a ^{3}H-dihydroalprenolol ligand rather than the ^{3}H-Norepinephrine used in the previous study[25], we have recently observed a 70 % fall in the number of β-receptors in ob/ob adipocytes (Poat, Hyslop & York, unpublished observations). Although we do not yet know if β-receptor number increases when membrane fluidity is

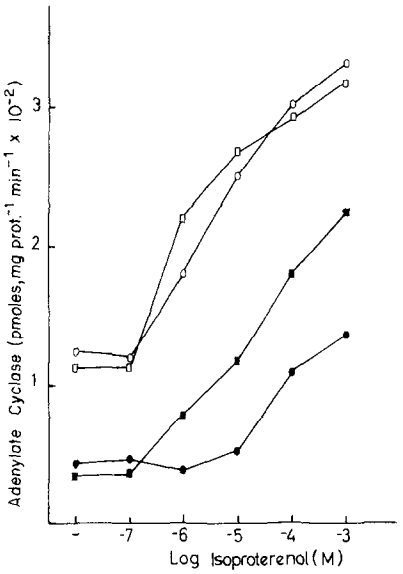

Fig. 2. Adenylate cyclase activity in crude membrane preparations from adipose tissue of lean (o,□) and obese (●,■) ob/ob mice housed at 25°C (o,●) and 34°C (□,■). Each point represents the mean of duplicate observations in a single experiment. Repeat experiments gave very similar results.

rectified, there is some evidence that the number of insulin receptors and β-receptors is inversely proportional to membrane fluidity[26,27]. In other reports it has been shown that although basal adenylate cyclase activity is insensitive to changes in membrane fluidity, the activity is affected when the enzyme is coupled to the glucagon or the β-receptor[4,20,27,28]. Phospholipids are thought to have an important role in this coupling mechanism[29]. Indeed, it has recently been reported that hepatic plasma membranes fluidised by enrichment with either dimyristoyl- or dioleyl-phosphatidylcholine show a loss of adenylate cyclase response to isoproterenol[27]. Our results are consistent with these observations and suggest that the altered lipid environment of the hormone receptor may be responsible for the impaired regulation of adenylate cyclase in ob/ob mice housed at 25°C.

The increase in membrane fluidity of ob/ob mice may not be related directly to their gene-defect since it is corrected by normalising body temperature. However, since body temperature is low from 10 days of age, the work suggests that many of the metabolic defects which have been reported in these mice[5-12], could be related in part at least to altered membrane characteristics. The results also suggest the need for further study of membrane composition in such disease states as diabetes, obesity and the hyperlipoproteinemias.

ACKNOWLEDGMENT

This work was supported in part by a project grant from the British Diabetic Association.

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