KINETIC AND ELECTROPHORETIC ABNORMALITY OF CYCLIC AMP PHOSPHODIESTERASE IN GENETICALLY OBESE MOUSE ADIPOCYTES

Jean-Claude KAPLAN°, Anne-Lise PICHARD°
Marie-Hélène LAUDAT[X] and Philippe LAUDAT[X].

°Institut de Pathologie Moléculaire
[X]Unité de Recherches sur le Métabolisme des Lipides
Faculté de Médecine Cochin
75014 - Paris, France

Received February 26, 1973

Summary - In the soluble fraction of adipocytes from Bar Harbor ob/ob mice, phosphodiesterase exhibits abnormal kinetics for cyclic AMP as compared to adipocyte extracts from normal littermates : a 10 fold increase of the high Km enzyme and a 2 fold increase of the corresponding Vmax. Upon starch gel electrophoresis an abnormal pattern was seen. These abnormalities were found regardless the nutritional status of the animals. There was no kinetic abnormality with cyclic GMP.

INTRODUCTION - In the course of our investigations on cyclic AMP mediated effect of hormones on the adipose tissue of Bar Harbor obese hyperglycemic mice (ob/ob) (1-3), we have studied the possible involvment of the $_{c}$AMP degrading enzyme, phosphodiesterase.

MATERIALS AND METHODS - Obese hyperglycemic mice (ob/ob Bar Harbor strain) and their non obese littermates used as normal controls, 7-9 weeks of age, were obtained from "Centre d'Elevage du C.N.R.S." (45 - Orléans-la-Source). Animals were fed ad libitum (unless stated otherwise) on mouse chow. Adipocytes from epididymal and ovarian fat pad were isolated by the method of Rodbell (4). We have used for this study the 20 000 g infranatant obtained in the course of preparation of fat cell membranes according to a previously reported method (5). This fraction represents the cytosol and part of the endoplasmic reticulum in a medium made of 0.25 M sucrose, 10 mM Tris HCl,

pH 7.4, 3 mM ATP and 1 mM EDTA. Cyclic AMP phsophodiesterase was assayed after dialysis against a 50 mM Tris HCl buffer pH 7.4 containing 2 mM Mg SO_4. This step permitted to remove ATP and EDTA which are known inhibitors of phosphodiesterase (6). The extracts contained from 1.2 to 1.8 mg protein/ml. The phosphodiesterase was assayed at 37° C using 8-(^{3}H) labeled $_c$AMP or $_c$GMP as a substrate according to the method described by Thompson and Appleman (7). The enzyme activity was expressed as nanomoles of $_c$AMP hydrolized per minute and mg protein. Kinetic data were obtained from Lineweaver and Burk plots with nine different concentrations of $_c$AMP ranging from 0.2 µM to 0.5 mM. Starch gel electrophoresis with specific staining for phosphodiesterase was performed with the method of Monn and Christiansen (8), slightly modified (9). Prior to electrophoresis the samples were dialyzed and concentrated under vacuum pressure up to approximately 25 mg protein/ml for the ob/ob adipocyte extracts and 50 mg protein/ml for the normal mouse adipocyte extracts.

RESULTS - Kinetic studies using cyclic AMP : Normal mouse adipocyte extracts exhibited two apparent Km's for $_c$AMP : a high Km (4 to 5.10^{-4}M) and a low Km (5 to 7.10^{-6}M) with respective Vmax of 5.5 to 7.5 and 0.16 to 0.20 nanomoles/mg protein/mn (table I). In obese ob/ob the high Km was repeatedly found increased by one order of magnitude (2.10^{-3}M) and the corresponding Vmax multiplied by two (12-16 nanomoles/mg protein/mn), whereas the low Km and the corresponding Vmax remained unchanged (table I). In order to determine whether this kinetic abnormality was specific or artefactual, we have carried out kinetic measurements in adipocyte extracts from ob/ob mice in different nutritional conditions : (a) ob/ob mice having returned to normal weight

TABLE I

Kinetic values of phosphodiesterase using $_{c}$AMP as a substrate

(Vmax expressed in nanomoles / mg prot./mn)

Source of adipose tissue extract	"high Km"	"low Km"
Controls (normal littermates) (5 exp)	Km = 4-5.10^{-4}M Vmax = 5.5-7.5	Km = 5-7.10^{-6}M Vmax = 0.16-0.2
ob/ob obese (3 exp)	Km = 2.10^{-3}M Vmax = 12-16	Km = 2-5.10^{-6}M Vmax = 0.06-0.15
ob/ob reduced weight (1 exp)	Km = 2.10^{-3}M Vmax = 12	Km = 2.10^{-6}M Vmax = 0.08
ob/ob fasting (1 exp)	Km = 2.10^{-3}M Vmax = 17	Km = 3.10^{-6}M Vmax = 0.14
ob/ob young "pre-obese" (1 exp)	Km = 3.10^{-3}M Vmax = 16	Km = 7.10^{-6}M Vmax = 0.15

after a two week period of restricted diet (2.5 g chow a day) ; (b) ob/ob after a 24 hour fast ; (c) "pre-obese" ob/ob mice sacrified at an early stage (4 weeks) when the obesity is not yet apparent while the hyperinsulinism is already present. In these three groups the same abnormal kinetic pattern was also found viz. an increase of the high Km and the corresponding Vmax (table I).

When $_{c}$GMP was used as a substrate in the phosphodiesterase reaction, only one apparent Km for $_{c}$GMP was found in normal mouse adipocyte extracts (Km : 2 mM and Vmax : 10 nanomoles/mn/mg protein). In the different groups of ob/ob mice the observed kinetic values for $_{c}$GMP were identical to those found in normal mice.

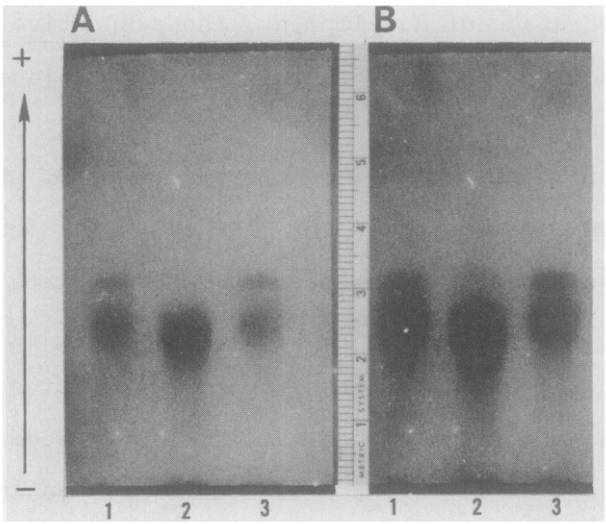

Fig. 1 - Starch gel electrophoresis (pH 7.7) of soluble phosphodiesterase from adipocytes of obese hyperglycemic (ob/ob) mice and their control non-obese (thin) littermates. The enzyme was visualized according to the method of Monn and Christiansen (8) in which the 5'-AMP formed is linked to NADH oxidation through adenylate kinase, pyruvate kinase and lactate dehydrogenase. The dark bands represent zones where fluorescing NADH has been oxidized into non-fluorescing NAD^+. No bands were seen if $_cAMP$ was omitted in the reaction mixture.
Channel 1 and 3 : ob/ob mouse adipocyte extracts (protein concentration : 26 mg/ml). Channel 2 : adipocyte extracts from control mice (protein concentration : 45 mg/ml). Photographs taken under longwave UV light on 3000 ASA Polaroid film through a yellow filter, after incubation for 10 minutes (A) and 40 minutes (B).

Electrophoresis : Recent investigations have shown the existence of multiple molecular forms of phosphodiesterase distinguishable by electrophoresis followed by specific staining (8-12). We have therefore submitted the extracts of the above mentioned groups to this technique. The pattern was found to be different in ob/ob mice as compared to their normal littermates. As shown on fig. 1, in normal adipocyte extracts the phosphodiesterase appears essentially as a major band, with a second more anodic band only visible if the protein concentration of the sample submitted to electrophoresis is increased and the incubation time prolonged. In contrast, in ob/ob mice, whatever their state

of nutrition or stage of development, the respective intensity of the two fractions was modified with considerable enhancement of the anodic band (fig. 1).

DISCUSSION - Our findings showing the existence of phosphodiesterase with two different Km's for $_{c}$AMP in normal mouse adipocytes are in agreement with previously reported data obtained in other species (13-16).

In ob/ob mice our results indicate that quantitative and qualitative abnormalities of the phosphodiesterase system occur in the soluble fraction of isolated fat cells from genetically obese mice. Although the significance of these findings remains unclear at the present time, the data reported here suggest for the first time that phosphodiesterase might be implied in the pathogenesis of obesity. The fact that the kinetic and electrophoretic abnormalities of phosphodiesterase persist in fasting mice, as well as mice which have recovered a normal weight after a restricted diet indicates that they are not mere artefacts directly or indirectly connected to overweight or food intake excess. Moreover it is significant that an identical kinetic abnormality was found in ob/ob mice at the age of 4 weeks, when the animals weight is still not different from that of the normal littermates and when the blood insulin level is already increased (17). It is therefore concluded that the phosphodiesterase abnormality is an early phenomenon preceeding the onset of clinical obesity. It might be related to the hyperinsulinism, although the question of the effect of insulin upon phosphodiesterase is still controversial (13, 18, 19). It should be emphasized that the kinetic alterations we observed in the ob/ob adipocytes are entirely different from those found by Loten and Sneyd in normal rat adipocytes incubated in the presence

of insulin (19). The phosphodiesterase abnormality in ob/ob adipocytes may as well represent the primary genetic defect or be more likely the consequence of a still unknown underlying phenomenon. Any conclusion as to whether the primary cause involves a specific regulatory system activating (20) or inhibiting phosphodiesterase (21), or a more general process with pleiotropic effect on the adipocyte is premature. In this respect noteworthy is the fact that the abnormalities found in the ob/ob mice involve both membrane systems - insulin and catechol receptors, adenylcyclase (1-3) -, and a cytosolic enzyme, phosphodiesterase. This points to an abnormality of cellular differentiation of the adipocyte.

ACKNOWLEDGMENTS

This study was supported by the Institut National de la Santé et de la Recherche Médicale and by the Centre National de la Recherche Scientifique.

REFERENCES

1. Laudat, M.H., Combret, Y. and Laudat, Ph., C.R. Acad. Sci., 276, 407 (1973).
2. Freychet, P., Laudat, M.H., Rosselin, G.,Kahn, C.R., Gorden, Ph. and Roth, J., FEBS Letters, 25, 339 (1972).
3. Laudat, M.H., Pairault, J. and Laudat, Ph. (in preparation).
4. Rodbell, M., J. Biol. Chem., 239, 375 (1964).
5. Laudat, M.H., Pairault, J., Bayer, P., Martin, M. and Laudat, Ph., Biochim. Biophys. Acta, 255, 1005 (1972).
6. Cheung, W.Y., Adv. Biochem. Psychopharmacol., 3, 51 (1970).
7. Thompson, W.J. and Appleman, M.M., Biochemistry, 10, 311 (1971).
8. Monn, E. and Christiansen, R.O., Science, 173, 540 (1971).
9. Pichard, A.L., Hanoune, J. and Kaplan, J.C., submitted for publication.
10. Monn, E., Desautel, M. and Christiansen, R.O., Endocrinology, 91, 716 (1972).
11. Goren, E.M., Hirsch, A.H. and Rosen, O.M., Anal. Biochem., 43, 156 (1971).
12. Pichard, A.L., Hanoune, J. and Kaplan, J.C., Biochim. Biophys. Acta, 279, 217 (1972).
13. Hepp, K.D., Menahan, L.A., Wieland, O. and Williams, R.H., Biochim. Biophys. Acta, 184, 554 (1969).
14. Murad, F., Manganiello, V. and Vaughan, M., J. Biol. Chem., 245, 3352 (1970).
15. Solomon, S.S., J. Lab. Clin. Med., 79, 598 (1972).

16. Klotz, U.,Berndt, S. and Stock, K., Life Sci., 11, 7 (1972).
17. Chlouverakis, C., Dade, E.F. and Batt, R.A., Metabolism, 19, 687 (1970).
18. Blecher, M., Merlino, N.S. and Ro'ane, J.T., J. Biol. Chem., 243, 3973 (1968).
19. Loten, E.G. and Sneyd, J.G.T., Biochem. J., 120, 187 (1970).
20. Cheung, W.Y., J. Biol. Chem., 246, 2859 (1971).
21. Miki, N. and Yoshida, H., Biochim. Biophys. Acta, 268, 166 (1972).