BBA Report

BBA 71124

Preparation of fat cell membrane with high sensitivity to lipolytic hormones

M.H. LAUDAT, J. PAIRAULT, P. BAYER, M. MARTIN and Ph. LAUDAT

Unité de Recherches sur le Métabolisme des Lipides I.N.S.E.R.M, Hopital Cochin, 75-Paris 14ème (France)

(Received January 17th, 1972)

SUMMARY

A method for preparation of fat cell membranes ('microsomal fraction') with high sensitivity to lipolytic hormones is described. The adjunction of ATP throughout the whole preparation leads to a higher adenyl cyclase activity than in previously described procedures. Basal activity is enhanced 8 to 12 times with epinephrine, 6 to 8 times with β-1-24 corticotropin, and 12 to 15 times with NaF.

In 1967, Rodbell[1] described the preparation of a membrane fraction, termed 'ghosts', which contains hormone-sensitive adenylate cyclase activity. Recently McKeel and Jarett[2] and Avruch and Wallach[3] described methods for preparing highly purified plasma membrane preparations. McKeel and Jarett showed that the adenylate cyclase activity and its response to F^- was enriched in this fraction. However, the response of the enzyme system to hormones was markedly reduced compared to crude membrane fractions. Avruch and Wallach did not assay their purified plasma membrane preparations for adenylate cyclase activity.

In this paper we will show that adenylate cyclase activity is present in a microsomal fraction prepared according to Avruch and Wallach[3] and that the response of this enzyme to hormones is greatly enhanced when the membranes are isolated in the presence of ATP.

Male Wistar strain rats (160–200 g) or normal mice from Bar Harbor strain (20–25 g), allowed free access to food and water, were sacrified by cervical dislocation.

The halves of epididymal fat pads were pooled (18 g adipose tissue). Isolated fat cells prepared according to Rodbell[4], were washed, first with Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin, then with 20 ml slightly chilled (10°) Medium I (0.25 M sucrose, 3 mM ATP, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4).

All subsequent steps of the fractionation process were carried out at 4°. After centrifugation, washed cells resuspended in 20 ml Medium I were disrupted by six aspirations through a Swinny filter holder fitted with a stainless steel photo etched support as described by Avruch and Wallach[3]. After breakage, the suspension was immediately centrifuged at 20 000 × *g* for 10 min in a refrigerated Sorvall RC 2-B centrifuge SS-34 rotor. The floating fat cake and the infranatant (containing the major part of endoplasmic reticulum) were removed; the resulting pellet was gently redispersed in 12 ml Medium I by two cycles of aspiration and ejection through the Swinny filter. The redispersed pellet was then layered on a 24.5-ml linear sucrose gradient (27.6–54.1%, w/w) containing 1 mM EDTA, 1 mM ATP, 5 mM Tris-HCl, pH 7.4). The gradient was centrifuged at 24 000 rev./min for 60 min in a Beckman L3 centrifuge SW 25.1 Rotor. Three zones containing particulate material were seen in the sucrose gradient. Nuclear ($\rho \sim 1.2$) and mitochondrial ($\rho \sim$ 1.70–1.75) zones were discarded. The upper 'microsomal' zone ($\rho \sim$ 1.13–1.14) immediately under the meniscus was aspirated through the tube bottom and diluted with 6 vol. of Medium II (1 mM EDTA, 1 mM Tris-HCl, pH 7.4) and centrifuged at 30 000 × *g* for 10 min. The resulting pellet was resuspended in Medium II and diluted to obtain a 1 mg/ml protein concentration (method of Lowry *et al.*[5]). This 'microsomal' fraction is named plasma membrane fraction by McKeel and Jarett[2]. 10 g of adipose tissue constantly yielded 1.5–2 mg membrane protein. The membrane may be stored in liquid nitrogen for several weeks without loss of activity. The following enzymatic assays were performed on this preparation:

(1) 5'-Nucleotidase activity was measured as described by Avruch and Wallach[3]. The 'microsomal' fraction contained 5'-nucleotidase, the specific activity of which was 4 to 9 times superior to that in the 20 000 × *g* sediment (Table I).

TABLE I

5'-NUCLEOTIDASE ACTIVITY OF RAT AND MOUSE FAT CELL 'MICROSOMAL' FRACTION

Results are expressed in nmoles P_i liberated per min per mg protein. Numbers in parentheses indicate number of experiments. Values represent the mean ± S.E.

	Adipose tissue Origin	*5'-nucleotidase specific activity*	*Purification*
Rat (6)	20 000 × *g* pellet	8.3 ± 4	1
	"Microsomal" fraction	33 ± 8.3	4
Mouse (1)	20 000 × *g* pellet	5	1
	"Microsomal" fraction	45	9

(2) Adenyl cyclase activity was assayed using Krishna's method as described by Rodbell[6]

Results of preliminary tests on membrane preparations obtained in presence or absence of ATP are shown in Table II. The addition of ATP increased the activity of adenyl cyclase and the response to epinephrine. As shown in Table III, relative to basal activity,

TABLE II

PROTECTIVE EFFECT OF ATP ON THE ACTIVITY OF ADENYL CYCLASE DURING THE ISOLATION OF RAT FAT CELL MEMBRANES

The following conditions were used for adenyl cyclase assay. 10 μg of membrane protein were incubated for 10 min at 37° in a 60-μl reaction volume containing 1.6 mM [α-^{32}P]ATP, 10 mM theophylline, 5 mM $MgCl_2$, 0.1% bovine serum albumin, 17 mM Tris–HCl (pH 7.4), an ATP regenerating system consisting of 10 mM phosphocreatine and 0.2 mg/ml creatine phosphokinase (35 units/mg). Hormones when added were at indicated concentration. The results are expressed in nmoles cyclic AMP per 10 min per mg protein. Values represent the mean ± S.E.

Procedure of membrane isolation	*Number of experiments*	*Adenyl cyclase activity*	
		Basal	*(−)-Epinephrine ($1.2 \cdot 10^{-4}$ M)*
With ATP*	5	0.8 ± 0.5	7.4 ± 3.8
Without ATP**	3	0.6 ± 0.3	2.9 ± 1.4

*Standard method as described in text.
**ATP omitted during the whole procedure.

TABLE III

EFFECTS OF (−)-EPINEPHRINE, β-1-24 CORTICOTROPIN, F^- ON THE ADENYL CYCLASE ACTIVITY OF RAT AND MOUSE FAT CELL MEMBRANES

Adenyl cyclase assays were performed as described in Table II. The results are expressed in nmoles cyclic AMP per 10 min per mg protein. Numbers in parentheses indicate number of experiments. Values represent the mean ± S.E.

Adipose tissue origin	*Starting material*	*Adenyl cyclase activity*			
		Basal	*(−)-Epinephrine (10^{-4} M)*	*β-1-24 Corticotropin (10^{-5} M)*	*Na F ($5 \cdot 10^{-3}$ M)*
Rat	'Microsomal' fraction	0.8 ± 0.5 (5)	7.4 ± 3.8 (5)	7 ± 3.3 (3)	13.6 ± 5 (3)
Mouse	'Microsomal' fraction	1.9 (1)	14.3 (1)	8.1 (1)	

the enzyme was stimulated from 8 to 12 times by (−)-epinephrine (10^{-4} M), 6 to 9 times by corticotropin (10^{-5} M) and 12 to 15 times by NaF ($5 \cdot 10^{-3}$ M). In other experiments, activity in presence of $5 \cdot 10^{-7}$ M (−)-epinephrine was 2 times that of the basal activity. It is of particular interest to point out that ATP used along the whole preparation protected against loss of both hormonal (Table II) and F^- response as compared with the results of McKeel and Jarett[2].

In conclusion, the preparation of fat cell membranes described here contains an adenyl cyclase system that is very sensitive to lipolytic hormones and which has specific activities far higher than preparations described hitherto. This preparation should be useful for studying the mechanism of hormone action and for isolating the components involved in the regulation of adenyl cyclase activity.

REFERENCES

1 M. Rodbell, *J. Biol. Chem.*, 242 (1967) 5744.
2 D.W. McKeel and L. Jarett, *J. Cell Biol.*, 44 (1970) 417.
3 J. Avruch and D.F.H. Wallach, *Biochim. Biophys. Acta*, 233 (1971) 334.
4 M. Rodbell, *J. Biol. Chem.*, 239 (1964) 375.
5 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
6 M. Rodbell, *J. Biol. Chem.*, 244 (1969) 3468.