EFFECTS OF REDUCING AND OXIDIZING AGENTS ON THE ADENYLATE CYCLASE ACTIVITY IN ADIPOCYTE PLASMA MEMBRANES

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1. Introduction

A well established and widely accepted concept is the activation of adenylate cyclase as a consequence of the interference of hormones with the plasma membrane. The concomittant increase of cyclic AMP, the second messenger, results in an increase of metabolic activity of the cell. Little is however known about the metabolic control of this activation.

Some agents of possible physiological significance on the adenylate cyclase activity has been studied. For example Ca^{++} [1], guanine nucleotides [2,3] and adenosine [4] have been demonstrated to have an effect on the enzyme activity, and may be of importance for its physiological regulation in the cell.

In the present paper we want to stress the importance of the oxidation–reduction state as a possible regulator of the adenylate cyclase activity and demonstrate the inhibitory effect of reduced nicotine amide adenine dinucleotide on the adenylate cyclase activity in isolated adipocyte plasma membranes.

2. Materials and methods

Fat cell membranes were prepared from epididymal fat from rats weighing 150–200 g as described by Avruch and Wallach [5] as modified by Harwood et al. [6] but without addition of mercaptoethanol. Adenylate cyclase activity was assayed according to Salomon et al. [7]. The incubations were run for 10 min and the incubation medium contained 25 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.1 mM ATP labelled with [^{32}P]ATP 10 mM theophylline and an ATP-regenerating system consisting of 25 mM creatinine phosphate and 1 mg/ml creatine kinase. The incubation was initiated by the addition of the membrane preparation in a volume of 10 μl to each incubation tube, the final volume being 50 μl. Protein was determined according to Lowry et al. [8]. Nucleotides and other finechemicals were from Sigma Co, St Louis, USA, isotopes from New England Nuclear GmbH, Langen, Germany, noradrenaline bitartrate was from ASTRA AB, Södertälje, Sweden, and 1-24 ACTH from Ciba, Basel, Switzerland. Other reagents used were of analytical grade.

3. Results and discussion

In table 1 is shown the effect of NADH on adenylate cyclase activity. As seen in the table, basal as well as hormone and fluoride-stimulated activity is inhibited in a concentration-dependent fashion. NAD added to the incubation in 10 mM concentration (table 1) or higher does not affect the enzyme activity, which

Table 1
Effect of NADH and NAD on basal and hormone activated adenylate cyclase activity

	cAMP, (nmoles/mg prot/10 min)		
		Noradrenaline (3 μM)	ACTH (1-24) (0.3 μM)
–	2.3	12.6	6.2
NADH, 1 mM	1.8	9.1	3.7
NADH, 3 mM	1.3	4.9	2.9
NADH, 10 mM	0.9	3.5	1.3
NAD, 10 mM	1.0	12.8	5.3

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Table 2
Effect of NADPH on adenylate cyclase activity in adipocyte plasma membranes

NADPH (mM)	cAMP, (nmoles/mg prot/10 min) Basal	Noradrenaline (30 μM)	ACTH (1-24) (0.3 μM)	F^- (100 mM)
	1.22	4.64	1.68	1.98
1	1.17	4.45	1.82	2.10
3	1.12	4.36	1.61	2.08
10	1.30	5.71	1.72	2.22

stresses the fact that the reduced state of the nucleotide is of importance for the effect. On the other hand NADPH has no such inhibitory effect as seen in table 2. On the contrary NADPH tends to stimulate the cyclase. NADH inhibition is independent of the concentration of hormone added as is seen in table 3. Increasing concentrations of noradrenaline at a constant concentration of NADH gives the same relative inhibition, independent of the hormone concentration.

If ferricyanide is added to an incubation mixture as an oxidizing agent the fluoride stimulated activity shows a pronounced stimulation (table 4). The inhibitory effect of ferricyanide on the ACTH stimulation can be related to the inactivation of ACTH by oxidizing agents [9].

Atebrine, which interferes with the NADH dehydrogenase flavin in mitochondria [10], inhibits the basal adenylate cyclase activity as well as hormone stimulated activity but has no effect on the fluoride stimulated activity (table 5). Azide, which has recently been introduced as a stimulant of adenylate cyclase [11],

Table 3
Effect of varying noradrenaline concentrations on the NADH inhibition of adenylate cyclase activity

Noradrenaline (μM)	cAMP, nmoles/mg prot/10 min –	NADH (5 mM)	Percent inhibition
0	4.4	2.0	65
1	9.1	3.3	65
3	10.0	5.0	50
10	13.5	5.8	57
30	15.5	6.1	61

Table 4
Effect of NADH and ferricyanide on adenylate cyclase activity

Additions	cAMP, nmoles/mg prot/10 min –	ACTH (1-24) (0.3 μM)	F^- (10 mM)
–	1.8	3.5	7.0
NADH, 3 mM	1.4	2.2	5.2
NADH, 10 mM	0.7	1.2	3.4
Potassium ferricyanide 10 mM	1.2	1.9	10.2

gives rise to an activity, which is inhibited by atebrine (table 5). This is a further evidence for a difference in stimulation mechanism between azide and fluoride.

The finding that the adenylate cyclase is sensitive to the redox state of the environment, opens a new line of investigation on the control mechanism of hormone activation. The finding that NADH but not NADPH or NAD inhibits the system makes it likely that the effect is specific and enzyme-mediated and not an unspecific redox artefact. Although the NADH concentration used might seem to be high, the concentration of NADH + NAD which has been observed in the cytosol is in the same milimolar range [12]. If the inhibition would be due to, e.g. a lipid peroxidation one would rather expect the NADPH to be inhibitory. As a control responsive to the metabolic state of the cell mediation by NADH seems to be ideal. Some biological applications are obvious. An example is the high degree of NAD-reduction in neoplastic cells, noted by O. Warburg [13] and lately shown to be present in virally transformed cells [14],

Table 5
Effect of atebrine on adenylate cyclase activity

Atebrine (mM)	cAMP (nmoles/mg prot/10 min)	Noradrenaline (30 μM)	Fluoride (10 mM)	Azide (100 mM)
–	4.5	9.2	15.1	13.5
1	2.2	4.6	14.6	9.0
3	0.4	1.5	12.5	3.2

the reason for the low activity [15] of adenylate cyclase of such cells? In such a case the rapid proliferation of cells, which seems to be related to a low cyclic AMP level, might as Warburg postulated, be initially due to an increased NADH/NAD ratio.

It is also tempting to speculate about the nature of the sensor for the NADH. The finding that ferric ions tend to stimulate cyclase activity and inhibition of cyclase by atebrine might indicate, that the sensor might be a redox enzyme system. NADH dehydrogenase is known in plasma membranes and preliminary studies indicate hormone response in these dehydrogenases which may relate to the control of the cyclase (F. L. Crane and H. Löw, in preparation).

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