Catecholamine control of enzymes involved in isocitrate oxidation of rat liver mitochondria

Vladimir I. Kulinsky, Ludmila V. Trufanova and Alexei E. Medvedev

Department of Biochemistry of Krasnoyarsk Medical Institute, 660022 Krasnoyarsk, USSR

Received 13 August 1984

Treatment of rats or liver homogenates with catecholamines (isoproterenol or noradrenaline) increased activities of both NAD^+-dependent isocitrate dehydrogenase and $NAD(P)^+$-transhydrogenase (in the direction of hydrogen transfer $NADPH \rightarrow NAD^+$) with no change in $NADP^+$-dependent isocitrate dehydrogenase. These effects were realized via β-adrenoceptors. Cyclic AMP mimicked the catecholamine action on incubation with liver homogenate. The effects of catecholamines and cyclic AMP were not additive.

Catecholamine *Adrenoceptor* *Cyclic AMP* *Mitochondria* *$NAD(P)^+$-dependent isocitrate dehydrogenase* *$NAD(P)^+$-transhydrogenase*

1. INTRODUCTION

During the last decade the existence of catecholamine control of liver mitochondrial function [1–8], including the stimulation of mouse-liver NAD^+-dependent isocitrate dehydrogenase [2], was firmly recognized. However, problems related to the point(s) and mechanism of catecholamine action on mitochondrial metabolism are far from solved. It has been accepted and well documented that isocitrate oxidation can proceed within mitochondria in two pathways: (i) via NAD^+-dependent isocitrate dehydrogenase (EC 1.1.1.41); (ii) via $NADP^+$-dependent isocitrate dehydrogenase (EC 1.1.1.42) coupled with $NAD(P)^+$-transhydrogenase (EC 1.6.1.1):

$$NADPH + NAD^+ \rightleftharpoons NADP^+ + NADH$$ [9–10].

We found that catecholamine controls both pathways of isocitrate oxidation in rat liver mitochondria.

2. MATERIALS AND METHODS

Male Wistar rats (150–200 g) were anesthetised with sodium thiopental (100 mg/kg body wt intraperitoneally). Phenylephrine (10 mg/kg) or isoproterenol (2.3 mg/kg) was injected subcutaneously 15 and 6 min, respectively, before killing. Noradrenaline (1 μM) was incubated with 10% (w/v) liver homogenate for 10 min at 30°C in the presence of 1 mM theophylline and 1 mM ascorbate. Cyclic AMP (1 μM) was incubated under similar conditions but without ascorbate. Thereafter homogenates were cooled in ice and mitochondria were prepared according to [11] in 0.25 M sucrose, 1 mM EDTA, 10 mM Tris–HCl buffer (pH 7.4).

The final mitochondrial pellet was lysed by the addition of 1% Triton X-100 and, after 20 min storage in ice, centrifuged at 18 000 × g for 20 min. The supernatant was used for the measurement of enzymes activities: NAD^+- and $NADP^+$-dependent isocitrate dehydrogenases [12,13] and $NAD(P)^+$-transhydrogenase [14]. Protein was determined by a modified Lowry method [15]. The activities of the enzymes were expressed as nmol NAD(P)H/min per mg protein.

3. RESULTS

Administration of α_1-adrenoceptor agonist phenylephrine increased neither the activities of

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

NAD^+- and $NADP^+$-dependent isocitrate dehydrogenases nor that of $NAD(P)^+$-transhydrogenase ($P > 0.3$–0.6, not shown). Treatment of animals with the β-adrenergic agonist isoproterenol leads to marked stimulation of NAD^+-dependent isocitrate dehydrogenase and $NAD(P)^+$-transhydrogenase without any change in the activity of $NADP^+$-dependent isocitrate dehydrogenase (table 1).

The increased activities of both NAD^+-dependent isocitrate dehydrogenase and $NAD(P)^+$-transhydrogenase were also observed in vitro after the incubation of noradrenaline with liver homogenate (table 2). It should be noted that stimulation of these enzymes in vivo and in vitro by catecholamines occurred to the same extent. Pretreatment of liver homogenate with the β-adrenoceptor antagonist propranolol but not with the α-adrenoceptor antagonist phentolamine completely prevented noradrenaline action on $NAD(P)^+$-transhydrogenase (table 3). Similar results were obtained for the catecholamine stimulation of NAD^+-dependent isocitrate dehydrogenase (not shown).

Cyclic AMP incubation with liver homogenate mimicked the effect of the β-adrenergic agonists on the activities of both enzymes (table 4). Cyclic AMP action was highly specific: other adenine

Table 1

Effect of isoproterenol administration to rat on the activities of enzymes involved in isocitrate oxidation of liver mitochondria

Series	NAD^+-dependent isocitrate	$NADP^+$-dependent dehydrogenase	$NAD(P)^+$-transhydrogenase
Control	11.4 ± 0.9 ($n = 10$)	68.3 ± 5.4 ($n = 5$)	12.3 ± 0.9 ($n = 11$)
Isoproterenol	14.6 ± 0.7 ($n = 10$)	76.0 ± 7.1 ($n = 5$)	17.2 ± 1.1 ($n = 11$)
P	< 0.01	> 0.6	< 0.001

Experimental conditions are described in section 2. Concentration of substrate for each reaction: (a) for NAD^+-dependent isocitrate dehydrogenase - 2 mM NAD^+, 4 mM isocitrate, 1 mM $MnSO_4$; (b) for $NADP^+$-dependent isocitrate dehydrogenase - 0.5 mM $NADP^+$, 0.5 mM isocitrate, 8 mM $MgCl_2$; (c) for $NAD(P)^+$-transhydrogenase - 1 mM NAD^+, 0.25 mM NADPH and regenerating system: 2 mM isocitrate, 300 munits $NADP^+$-dependent isocitrate dehydrogenase, 3 mM $MgCl_2$. In all experiments 1 mM KCN was used. Series were compared using Student's t-test

Table 2

Influence of incubation of noradrenaline with liver homogenate on the activities of isocitrate dehydrogenases and $NAD(P)^+$-transhydrogenase of rat liver mitochondria

Series	NAD^+-dependent isocitrate	$NADP^+$-dependent dehydrogenase	$NAD(P)^+$-transhydrogenase
Control	10.8 ± 0.8 ($n = 8$)	70.1 ± 4.3 ($n = 5$)	13.1 ± 1.4 ($n = 6$)
Noradrenaline	13.3 ± 1.2 ($n = 8$)	69.6 ± 1.9 ($n = 5$)	20.2 ± 0.7 ($n = 6$)
P	< 0.01	> 0.9	< 0.001

All experimental conditions are described in section 2 and table 1, except that $MnSO_4$ was substituted by $MgCl_2$ (8 mM) in the assay medium for NAD^+-dependent isocitrate dehydrogenase

Table 3

Effects of adrenoceptor antagonists on the stimulatory action of noradrenaline on $NAD(P)^+$-transhydrogenase activity

Series	− Phentolamine	+ Phentolamine (10 μM)	− Propranolol	+ Propranolol (10 μM)
Control	8.2 ± 0.6	7.6 ± 0.9	10.3 ± 1.0	10.7 ± 1.2
Noradrenaline	12.3 ± 0.8	11.5 ± 1.1	16.5 ± 1.1	10.9 ± 0.7
P	< 0.01	< 0.02	< 0.01	> 0.8

The adrenoceptor antagonists were added to the homogenate 10 min before noradrenaline. In each series $n = 7$–8

Table 4

Effect of the incubation of cyclic AMP with liver homogenate on the activities of mitochondrial isocitrate dehydrogenase and $NAD(P)^+$-transhydrogenase

Series	NAD^+-dependent isocitrate	$NADP^+$-dependent dehydrogenase	$NAD(P)^+$-transhydrogenase
Control	11.8 ± 0.8 ($n = 11$)	88.8 ± 6.2 ($n = 10$)	14.4 ± 1.7 ($n = 6$)
Cyclic AMP	14.6 ± 1.1	91.8 ± 6.9	20.9 ± 1.9
P	< 0.01	> 0.6	< 0.001

Incubation conditions of cyclic AMP with liver homogenate are described in section 2

nucleotides (5′-AMP, ADP, ATP, 2′,3′-AMP) and adenosine at the same concentration (1 μM) stimulated neither NAD^+-dependent isocitrate dehydrogenase ($P > 0.7$) nor $NAD(P)^+$-transhydrogenase ($P > 0.2$–0.7). The effects of noradrenaline and cyclic AMP were not additive when both were applied to liver homogenate ($P > 0.3$–0.9).

Summarizing the above results we may conclude that the stimulation of both enzyme activities by catecholamines occurs via a β-adrenergic cyclic AMP-dependent mechanism.

4. DISCUSSION

The altered activities of NAD^+-dependent isocitrate dehydrogenase and $NAD(P)^+$-transhydrogenase after catecholamine injection or its incubation with liver homogenate were observed in Triton X-100 mitochondrial extracts under saturating concentrations of substrates. This implies that the hormone stimulation of these enzymes is probably connected with changes in the catalytic properties themselves. Authors in [16] came to the same conclusion when they found stimulation of succinate dehydrogenase in mitochondrial extracts after treating a rat with glucagon.

The participation of $NADP^+$-dependent isocitrate dehydrogenase in the Krebs cycle is determined by $NAD(P)^+$-transhydrogenase [9,10]. Thus, there is evidence that catecholamines act only on the activities of the rate-limiting enzymes of both pathways of isocitrate oxidation: i.e., NAD^+-dependent isocitrate dehydrogenase and $NAD(P)^+$-transhydrogenase. However, the importance of $NAD(P)^+$-transhydrogenase (in the direction $NADPH \rightarrow NAD^+$) is also connected with the ability of this enzyme to function as an energy generator [17–19]. Therefore, it seems reasonable to propose that not only the Krebs cycle enzymes but also coupling processes in mitochondria may be involved in the 'sphere' of catecholamine control.

REFERENCES

[1] Garrison, J.C. and Haynes, R.C. (1975) J. Biol. Chem. 250, 2769–2777.
[2] Kulinsky, V.I. and Trufanova, L.V. (1975) Dokl. Akad. Sci. SSSR 224, 1439–1441.
[3] Titheradge, M.A. and Coore, H.G. (1976) FEBS Lett. 71, 73–78.
[4] Kulinsky, V.I. and Vorobieva, L.M. (1977) Bull. Exp. Biol. Med. (Moscow) 84, 675–677.
[5] Taylor, W.M., Prpić, V., Exton, J.H. and Bygrave, F. (1980) Biochem. J. 188, 443–450.
[6] Kulinsky, V.I. (1981) in: 6th Joint Symp. Biochem. Soc. GDR and USSR, Regulation in Metabolism and Bioenergetics, p. 64, Tallin.
[7] Crompton, M.N., Kessar, P. and Al-Nasser, J. (1983) Biochem. J. 216, 333–342.
[8] Hernandez-Satomayor, S.M.T. and Garcia-Sainz, J.A. (1984) FEBS Lett. 166, 385–388.
[9] Nicholls, D.G. and Garland, P.D. (1969) Biochem. J. 114, 215–226.
[10] Smith, C.M. and Plaut, G.W.E. (1979) Eur. J. Biochem. 97, 283–295.
[11] Pedersen, P.L., Greenwalt, J.W., Reynafarje, B., Hullihen, J., Decker, C.L., Soper, J.W. and Bustamente, P. (1978) Methods Cell Biol. 20, 411–481.
[12] Goebell, H. and Klingenberg, M. (1964) Biochem. Z. 340, 441–464.
[13] Plaut, G.W.E. and Aogaichi, T. (1968) J. Biol. Chem. 243, 5572–5583.
[14] Rydström, J. (1979) Methods Enzymol. 55 F, 261–275.
[15] Ohnishi, T. and Barr, J. (1978) Anal. Biochem. 86, 193–200.
[16] Siess, E.A. and Wieland, O.H. (1978) FEBS Lett. 93, 301–306.
[17] Rydström, J. (1977) Biochim. Biophys. Acta 463, 155–184.
[18] Van de Stadt, R.J., Nieuwenhuis, F.J.R.M. and Van Dam, K. (1971) Biochim. Biophys. Acta 234, 173–176.
[19] Dontsov, A.E., Grinius, L.L., Jasaitis, A.A., Severina, I.I. and Skulachev, V.P. (1972) J. Bioenerg. 3, 277–303.