CATECHOLAMINE STIMULATION OF ETHANOL OXIDATION BY ISOLATED RAT HEPATOCYTES

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1. Introduction

Catecholamine stimulation of hepatic gluconeogenesis [1–3] and glycogenolysis [3] in the rat is mediated by α-adrenergic receptors without the participation of cAMP. An involvement of Ca^{2+} in response to activation of the α-adrenergic receptor is widely recognized [4]. Catecholamines could elicit a rise in cytosolic $[Ca^{2+}]$ [5–8].

Ca^{2+} must be present in the suspending medium to enable catecholamines to enhance gluconeogenesis from reduced substrates that enter the pathway at triose phosphate (i.e., glycerol or sorbitol) [9,10]. This is not the case for their oxidized counterparts, dihydroxyacetone or fructose, from which gluconeogenesis can be stimulated by catecholamines even in the absence of medium Ca^{2+}. With the reduced substrates in the presence of medium Ca^{2+}, catecholamines caused the oxidation of cytosolic NADH and the reduction of mitochondrial NAD [10]. Furthermore, it was noted that aminooxyacetate, a transaminase inhibitor that blocks the malate–aspartate shuttle, cannot prevent catecholamine stimulation of gluconeogenesis from the reduced triose substrates. We therefore suggested the involvement of the glycerophosphate shuttle in removing excess reducing equivalents when catecholamines are present [9].

Here, the transfer of reducing equivalents (NADH) from the cytosol to the mitochondria was studied by examining ethanol oxidation by fasted hepatocytes. The results provide further evidence for the participation of the glycerophosphate shuttle in catecholamine action.

2. Materials and methods

2.1. *Preparation and incubation of hepatocytes*

The preparation was essentially that in [11] except that immediately upon cannulation of the liver, Ca^{2+}-free Krebs-Henseleit buffer [12] was passed through the liver and flow continued until the liver was excised. After connecting the liver to the perfusion apparatus, a further 50 ml Krebs-Henseleit buffer (at 37°C) was flushed through; then 50 mg collagenase was added to the remaining 100 ml perfusion fluid. Viability of hepatocytes used was 85–95% (as judged by trypan blue exclusion).

Cells were incubated in rubber-stoppered 25 ml flasks in a total volume of 2 ml, containing Krebs-Henseleit medium, 2.5% bovine serum albumin (charcoal treated as in [13] and dialyzed) and 2.4 mM $CaCl_2$, or no added Ca^{2+} where indicated. Dry weights were determined by weighing dried aliquots of the hepatocyte suspension and the values converted to wet weights by multiplying by the conversion factor 3.7 [14].

2.2. *Measurement of ethanol oxidation*

Ethanol oxidation by hepatocytes was determined as in [15]. Ethanol was initially 2.5 mM; and ethanol oxidation was linear with time for up to 40 min, depending on cell concentration and additions to the medium. The incubations were terminated after 30 min by the addition of perchloric acid (final conc. 1.5%). Shorter incubation periods (15 min) were used when pyruvate or lactate were added, but the results were independent of cell concentration over a range of 30–60 mg wet wt hepatocytes. Controls incubated without hepatocytes or with cells plus perchlorate

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Table 1
Catecholamine stimulation of ethanol oxidation by isolated rat hepatocytes

Addition (mM)	Rate of ethanol oxidation	
	Control	+ 10 μM Epinephrine
None	0.76 ± 0.03	1.02 ± 0.04[b]
AOA[a] (0.05)	0.45 ± 0.01	0.61 ± 0.03[b]
Lactate (10)	1.58 ± 0.14	1.81 ± 0.17[b]
Lactate + AOA	0.62 ± 0.02	0.87 ± 0.05[b]
Pyruvate (10)	2.77 ± 0.15	2.74 ± 0.16
Medium Ca^{2+} omitted	0.50 ± 0.03	0.51 ± 0.04
Propranolol (0.1)	0.74 ± 0.04	0.97 ± 0.07[b]
Prazosin (5×10^{-4})	0.76 ± 0.04	0.76 ± 0.05
Phenylephrine (0.05)	1.08 ± 0.04[c]	
Angiotensin (0.01)	1.05 ± 0.11[c]	
Angiotensin + prazosin	1.04 ± 0.09[c]	
A23187 (0.01)	1.08 ± 0.08[c]	

[a] AOA, aminooxyacetate
[b] $p < 0.05$ *vs* corresponding control without epinephrine
[c] $p < 0.05$ *vs* control with no addition

Rates of ethanol oxidation are in μmol . min^{-1} . g wet wt^{-1} and expressed as mean ± SEM. Results of 4 separate preparations are presented. For details of the incubation procedure see section 2

from the beginning of the incubation displayed no loss of ethanol.

2.3. *Animals and chemicals*

Male Sprague-Dawley rats (200–300 g), fasted 48 h prior to hepatocyte isolation, were used throughout. Collagenase (CSL II) was from Worthington, propranolol from Ayerst, aminooxyacetate from Upjohn, and prazosin was a gift from Pfizer. All other chemicals were from Sigma.

3. Results

The rate of ethanol oxidation by hepatocytes from fasted rats is a reflection of shuttle activities transferring reducing equivalents from NADH in the cytosol to the mitochondrial matrix [16]. Table 1 shows that ethanol oxidation was stimulated by epinephrine and that this stimulation persisted when the malate–aspartate shuttle was blocked by aminooxyacetate or accelerated by the addition of lactate. Addition of lactate plus aminooxyacetate resulted in an intermediate rate of ethanol oxidation* which was also stimulated by epinephrine. In the presence of pyruvate, which directly reoxidizes cytosolic NADH, ethanol oxidation is shuttle-independent and was accordingly unaffected by epinephrine. Table 1 also demonstrates that epinephrine was ineffective in the absence of medium Ca^{2+}.

The adrenergic specificity of the catecholamine response was demonstrated by showing that the α_1-antagonist prazosin [17] blocked the stimulation of ethanol oxidation but propanolol did not. Furthermore, both phenylephrine (an α-agonist) and angiotensin, which causes an increased cytosolic Ca^{2+} [7], mimicked the epinephrine response. Angiotensin was still effective in the presence of prazosin, eliminating nonspecific inhibition by the α-antagonist. Finally the Ca^{2+} ionophore, A23187, also caused an augmented rate of ethanol oxidation.

4. Discussion

The results of catecholamine stimulation of ethanol oxidation parallel our previous findings with reduced substrates for gluconeogenesis. Catecholamines stimulated both ethanol oxidation and gluconeogenesis from reduced substrates that enter the gluconeogenesis pathway at the level of triose phosphate [9] even in the presence of the malate–aspartate shuttle inhibitor, aminooxyacetate. The elimination of the epinephrine stimulation with prazosin, but not propranolol, and the duplication of the epinephrine response by phenylephrine suggest that in rat liver, catecholamines stimulate ethanol oxidation through α-receptors just as they stimulate gluconeogenesis. The demonstration that ethanol oxidation can be stimulated by epinephrine even in the presence of lactate, which augments the intermediates for the malate–aspartate shuttle [15], provides further proof that the stimulation of reducing equivalent transport is localized to the glycerophosphate shuttle.

The important facet of catecholamine response is the augmentation of cytosolic Ca^{2+}, since angiotensin,

* In hepatocytes metabolizing ethanol, addition of 10 mM lactate causes a >2-fold rise in glutamate concentration [14]. Glutamate can protect glutamate–oxalacetate transaminase against aminooxyacetate inhibition (N. W. Cornell, personal communication). This would explain why the transaminase inhibitor does not suppress ethanol oxidation as completely in the presence of lactate as in its absence

which increases this pool [7], also stimulated ethanol oxidation. In [18], glucose synthesis from glycerol, but not fructose, was stimulated by this hormone. Our results suggest the explanation for the results in [18] is that angiotensin stimulates the glycerophosphate shuttle and removes the excess cytosolic NADH generated when glycerol is the substrate for gluconeogenesis.

That a rise in cytosolic Ca^{2+} is part of the catecholamine stimulation of gluconeogenesis has been often suggested. The mitochondrial glycerophosphate dehydrogenase, situated on the outer face of the inner membrane, has been demonstrated to be stimulated by Ca^{2+} in several tissues including rat liver [19].

Beyond removal of excess reducing equivalents, there must be an alternative site of catecholamine action since gluconeogenesis from both lactate and pyruvate is stimulated by catecholamines. A possible mechanism for activation of phosphoenolpyruvate carboxykinase has been reported [20].

These findings suggest that reducing equivalent transfer can now be assessed in the whole cell, measuring either glycerol gluconeogenesis or ethanol oxidation as a reflection of the increased cytosolic Ca^{2+} triggered by catecholamines.

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