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Effects of Arsenic on Pyruvate Dehydrogenase Activation

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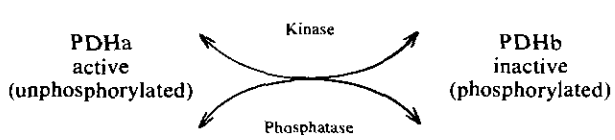
Our studies illuminate a particular site of altered pyruvate utilization by liver mitochondria isolated from arsenic-fed rats. Initially, pyruvate dehydrogenase (PDH) levels were measured before and after *in vitro* activation. The liver homogenates were prepared from male rats given access to deionized drinking water solutions containing 0, 20, 40, and 85 ppm arsenic as sodium arsenate (As^{+5}) for 3 and 6 weeks. After 3 weeks, the effects of arsenic at the highest dose level were pronounced on the basal activity (before activation), with inhibition up to 48% of the control values. The total PDH (after activation) was inhibited by 14, 15, and 28% of the control values at 20, 40, and 85 ppm As^{+5}, respectively. A similar pattern of inhibition of PDH was observed at 6 weeks, although the inhibition was lower at the highest dose. This effect is probably a reflection of mitochondrial regeneration at this time and dose. The inhibition of PDH both before and after activation suggests a direct arsenic effect on pyruvate utilization which does not involve a lipoic acid moiety. Evidence is also presented which indicates an arsenic effect on the regulating kinase and/or phosphatase. The metabolic effects of impaired mitochondrial utilization by pyruvate are also discussed.

Introduction

Arsenic fed to laboratory animals is known to accumulate in the mitochondria which has been related to the swelling of this subcellular organelle in a number of tissues especially the liver (*1*). *In vitro* research has demonstrated that arsenic affects mitochondrial oxidations by causing oligomycin-sensitive stimulation of oxidation in the absence of inorganic phosphate (*2, 3*) and by stimulating the mitochondrial ATPase (*4*). More recently, mitochondrial respiration studies, with liver mitochondria isolated from rats chronically exposed to arsenic, indicated decreased state 3 respiration rates and decreased respiratory control ratios for pyruvate/malate- but not succinate-mediated respiration (*1*).

It has been suggested that the effects of arsenic on mitochondrial pyruvate utilization results from arsenic binding to the lipoic acid and dithiol moieties of the PDH complex (*5*). The initial step in the mitochondrial metabolism of pyruvate, which is catalyzed by this multienzyme complex, involves the formation of acetyl-CoA and the generation of CO_2 and NADH. This complex is composed of three enzymes: pyruvate decarboxylase (pyruvate dehydrogenase), dehydrolipoate transacetylase, and dihydrolipoate dehydrogenase. It is the second two enzymes which involve active dithiol moieties. The first enzyme of the complex is regulated by inactivation and activation reactions, which are controlled by a phosphorylation/dephosphorylation mechanism (*6, 7*). Thus, phosphorylation and the concomitant inactivation of PDH [Eq. (1)] is catalyzed by a MgATP-requiring kinase, and dephosphorylation and concomitant reactivation is catalyzed by a Mg- and Ca-requiring phosphatase (*6-10*). In order to examine whether or not this phosphorylation/dephosphorylation mechanism was a site of action for arsenic, PDH levels, before and after *in vitro* activation, were measured in tissue from arsenic-fed animals.



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Materials and Methods

Chemicals

Cofactors and substrates were obtained from Boehringer-Mannheim, New York. The radiochemical sodium [1- ^{14}C]-pyruvate was obtained from New England Nuclear, Boston.

Animals

The adult male Charles River CD rats used in this experiment were given access to a casein-based purified diet and to deionized drinking water containing 0, 20, 40, and 85 ppm arsenic as sodium arsenate (As^{+5}) for 3 and 6 weeks. The animals were decapitated and hepatic tissue was excised quickly and dropped into liquid nitrogen. The frozen tissue samples were weighed and homogenized in ice cold buffer.

Assay of Pyruvate Dehydrogenase

The PDH activities were determined by the stoichiometric release of radioactive CO_2 from [1-^{14}C]-pyruvate by the method previously described (*11*). This assay method was linear with time and protein concentration within the ranges utilized. The optimal *in vitro* activation conditions for PDH were determined to be exposure to 75*mM* $MgCl_2$ at 37°C for 5 min. Aliquots of each tissue homogenate were assayed before and after Mg activation to determine the basal and total PDH activities, respectively.

Protein Analysis

Total protein concentrations in the homogenates were determined by procedures previously described (*12*).

Results

No mortality occurred among any of the treated animals during the course of the exposure to arsenic. A significant depression of growth rate was observed only among the animals exposed to the 85 ppm As^{+5} dose (1).

The exposure of rats to arsenic for three weeks resulted in a decrease in both the basal and total hepatic PDH activities, as shown in Table 1. The effects on the basal levels of PDH activity as compared to the controls indicated an increasing inhibition with dose level of 20, 23, and 48%. The total PDH levels were also lower, but by a smaller amount, i.e., 14, 15, and 28% (Table 1).

Table 1. Inhibition of rat liver pyruvate dehydrogenase activity after 3 weeks exposure to arsenate in drinking water.

As^{5+}, ppm	Pyruvate dehydrogenase, % inhibition[a]	
	Basal	Total
0	0	0
20	20.0	13.5
40	22.5	15.3
85	47.5	27.6

[a]Basal and total refer to the enzyme activity before and after *in vitro* activation.

The PDH activity expressed as nmoles of CO_2 liberated (pyruvate utilized) per minute per mg of protein is shown in Table 2. The derived data in Table 2 illustrate another effect of arsenic. There was a decrease in the basal/total ratio with increasing arsenic dose level. The difference between the total and basal was relatively constant with increasing arsenic dose level. The ratio of the difference to the basal level went up markedly with increasing dose level, indicating the relative ease of activation increases with increasing dose level.

The effects after 6 weeks of arsenic exposure were inhibition of basal and total PDH activity. However, as shown in Table 3, this inhibition was not observed at the highest dose level.

Table 2. Rat liver pyruvate dehydrogenase activity after 3 weeks exposure to arsenate in drinking water.[a]

As^{5+}, ppm	Pyruvate dehydrogenase, nmole/min-mg		Activation, % (basal/total)	Difference (total − basal)	Difference/basal
	Basal	Total			
0	2.84 ± 0.28	5.03 ± 0.87	60.1 ± 12.3	2.19 ± 0.99	0.82 ± 0.38
20	2.27 ± 0.45	4.34 ± 0.61	51.5 ± 7.7	2.07 ± 0.47	1.34 ± 0.40
40	2.20 ± 0.34	4.26 ± 0.66	49.9 ± 8.2	2.19 ± 0.45	1.39 ± 0.38
85	1.49 ± 0.30	3.64 ± 0.54	42.4 ± 5.9	2.15 ± 0.41	1.80 ± 0.43

[a]Values are the means ± SEM for nine separate animals. Assays were performed in triplicate.

Table 3. Inhibition of rat liver pyruvate dehydrogenase activity after 6 weeks exposure to arsenate in drinking water.

As^{+5}, ppm	Pyruvate dehydrogenase, % inhibition	
	Basal	Total
0	0	0
20	24.7	40.0
40	33.0	43.0
85	2.3	9.9

Discussion

This investigation illustrates a dose-related effect of arsenic on PDH activity after three weeks of exposure. The effect is characterized by a gradual decrease in basal PDH activity, a gradual decrease in total PDH activity and an increase in relative ease in activation with increase in dose level. This last effect may be indicative of a stimulation of the phosphatase, an inhibition of the kinase and/or an effect directly on the pyruvate decarboxylase. Although inhibition of both basal and total PDH activity was observed after 6 weeks exposure at the lower doses, at the highest dose there was not marked inhibition. This pattern of inhibition may be indicative of mitochondrial regeneration at this highest dose after six weeks.

The reaction involving the phosphatase and the kinase are illustrated by Eq. (2).

$$E_1 + E_k \cdot X \rightarrow E_1 \cdot X + E_k$$
$$E_1 + E_p \cdot X \leftarrow E_1 \cdot X + E_p \qquad (2)$$

Here E_1 denotes PDH, E_k is kinase, E_p is phosphatase, and X denotes P_i or As_i. The active E_1 (PDH) is phosphorylated by $E_k \cdot X$. The inactive $E_1 \cdot X$ complex that forms is reactivated by E_p, which removes the phosphate group to form E_1 and $E_p \cdot X$. Thus, at some time, each of the three enzymes must bind to a phosphate moiety. By analogy, arsenate may bind to any or all of these enzymes and thus interfere with the PDH activity directly or the regulation of PDH activity.

The possible metabolic effects of this inhibition of PDH activity are illustrated in Figure 1. If PDH activity is substantially inhibited, there will be a decrease in acetyl-CoA formation which leads to a decrease in carbon flow through the tricarboxylic acid cycle. This decreased flow leads to a decrease in NADH generated for ATP formation via the electron-transport chain. Also, there will be a decrease in the citrate available to leave the mitochondria to supply acetyl-CoA for fatty acid synthesis. In turn, the pyruvate not utilized by the mitochondria will probably spill back into the cytosol and be converted to lactate.

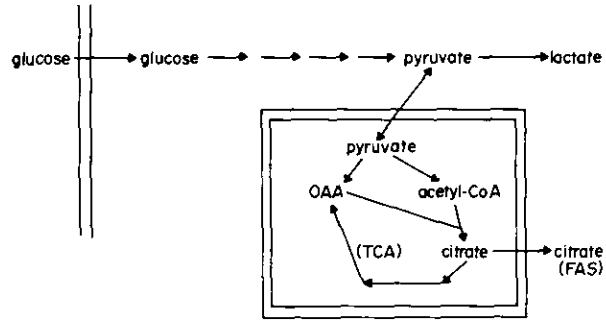

FIGURE 1. Schematic illustration of the flow of glucose metabolites into the mitochondria. TCA = tricarboxylic acid cycle, FAS = fatty acid synthesis.

Thus, the observed effects on mitochondrial metabolism of pyruvate would support previous *in vitro* mitochondrial studies which indicate poor utilization of pyruvate/malate compared to succinate. Also, the probable decrease in availability of citrate for the synthesis of fatty acids would in turn result in less storage triglycerides.

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