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IN VIVO ^{31}P NUCLEAR MAGNETIC RESONANCE STUDIES OF ARSENITE INDUCED CHANGES IN HEPATIC PHOSPHATE LEVELS

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SUMMARY Hepatic phosphate resonances were evaluated *in vivo* by ^{31}P nuclear magnetic resonance (^{31}P-NMR) following a single intravenous dose of sodium arsenite (10 mg/kg). Acute *in vivo* administration of arsenite rapidly decreased intracellular pools of all ATP phosphate with concomitant increases in inorganic phosphate and phosphomonoesters. In the phosphodiester resonance region, glycerolphosphorylcholine was also increased. The data suggest that liver cannot compensate for the rapid loss of NAD-linked substrate oxidation via other metabolic pathways, such as glycolsis for the production of ATP, and also demonstrate that ^{31}P-NMR spectroscopy can disclose time-dependent metabolic changes of the liver *in vivo*.

INTRODUCTION Phosphorus-31 nuclear magnetic resonance (^{31}P-NMR) has been applied to a variety of biological systems (1-6). A recent *in vivo* ^{31}P-NMR study of rat liver (7) demonstrated some of the advantages of such a non-invasive approach over most other investigations of liver biochemistry and physiology which have employed liver homogenates, suspensions of hepatocytes, liver slices, or isolated perfused liver. A potential limitation of these methods is that removal of the tissue disrupts normal metabolic as well as physiological interactions of the tissue with other organs. In this communication, we report the acute time-dependent effects of arsenite (As^{3+}) on liver energy metabolism *in vivo* as observed by the ^{31}P-NMR using a surface coil (8).

Arsenic is a common and relatively mobile environmental element (9). Numerous *in vivo* studies (10-17) in several species have shown that arsenite is rapidly and extensively accummulated by the liver where it produces cellular injury via inhibition of mitochondrial respiratory function (18). *In vitro* studies (19) have demonstrated energy-dependent

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uptake of arsenite by rat liver mitochondria and a highly selective inhibition of NAD-linked oxidation of pyruvate or α-ketoglutarate (20-23) which is thought to result from complexation of trivalent arsenic with vicinal thiols of the lipoic acid cofactor necessary for oxidation of these substrates (24). The *in vivo* impact of inhibition of mitochondrial respiratory function by arsenite and subsequent effects on tissue ATP and other phosphate levels over time have not been reported. Such data are important in understanding the metabolic ramifications of decreased mitochondrial oxidation of NAD-linked substrates and the role of this phenomenon in arsenic-induced cell injury.

METHODS Male rats (350-450 g) were anesthetized with pentobarbital sodium (45 mg/kg body weight) intraperitoneally. The trachea of the animal was intubated and the right external jugular vein was cannulated for intravenous injection. A section of skin and the muscles lining the abdominal wall, approximately 2 cm in diameter, was excised. A control liver spectrum was observed for five hours from a group of anesthetized rats. A control spectrum was also obtained before the injection of sodium arsenite (Fisher Scientific, Fairlawn, NJ), from which 5 mg/ml was dissolved in 0.9% NaCl immediately prior to injection (10 mg/kg).

An aluminum NMR probe was designed with an inner diameter of 6.7 cm for *in vivo* small animal experiments. Within the probe, a surface coil of two turns (1.8 cm in diameter) was connected to a coaxial cable and was free to be positioned on the animal. The animal was secured in the probe with the area of observation and the surface coil placed close to the homogeneous center of the magnetic field for minimal room temperature shim corrections. The external geometry of the probe was made to fit the widebore (8.9 cm) Oxford superconducting magnet operating at 8.5 tesla. The magnet was interfaced with a Nicolet NT-360 NMR computer system.

In order to corroborate *in vivo* studies, perchloric acid (PCA) extracts of liver were obtained from pulverized liver (4 g) frozen in liquid N_2. The tissue powder was mixed with 8 ml of 12% perchloric acid, transfered to a 30-ml centrifuge tube and centrifuged at 26,000 X g at 4°C for 10 minutes. After centrifugation, the supernatant was removed and immediately adjusted to pH between 7.0 to 7.5 with KOH. The supernatant was centrifuged again at 26,000 X g at 4°C for 10 minutes to remove precipitated $KClO_4$. The final supernatant was passed through a Chelex resin column to remove divalent metal cations. The extracts were lyophilized, later dissolved in deionized distilled water and analyzed by NMR.

Each spectrum was accumulated for 1024 pulses (40,000 for extracts) at a resonance frequency of 146.15 MHz, with a pulse length of 40 microseconds and a pulse interval of 0.5 seconds (1 second for extracts). The acquisition time for each *in vivo* spectrum was 12.02 minutes and a 12 Hz exponential apodization function for resolution enhancement was applied to the data. The percentage of total phosphates was computed from peak areas of the transformed data by an integration program. All resonance peaks were referenced to α-ATP which was chosen as 7.91 ppm, and was insensitive to changes in pH and metal ion concentrations.

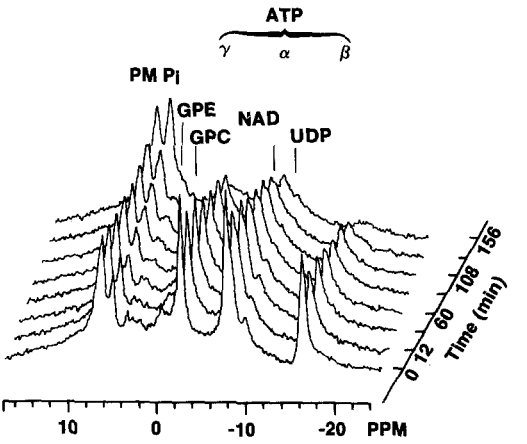

Figure 1. ^{31}P-NMR spectra obtained from rat liver in vivo showing time-dependent changes in phosphorus resonances. The first spectrum is a control prior to the injection. The subsequent ones were obtained following an i.v. dose of sodium arsenite (10mg/kg).

RESULTS AND DISCUSSION A control spectrum obtained prior to each intravenous injection of sodium arsenite was comparable to control spectra obtained from livers of anesthetized rats during five hours of observation. No detectable changes for all phosphate levels were observed with time in the control liver. In vivo time-dependent effects of a single intravenous dose of sodium arsenite on phosphate levels in liver were compared with control (Fig. 1) and demonstrated a progressive decrease in α-, β-, and γ-ATP resonances with concomitant increases in phosphomonoesters (PM) and inorganic phosphate (P_i) levels. Comparing the peaks in Fig. 1 to those obtained from a PCA extract of liver (Fig. 2), shows that the main peaks in the PM region are phosphocholine (PCh) and adenosinemonophosphate (AMP). Since other resonances also exist in the PM region, in vivo increases in PM cannot be explicitly ascribed to any one compound. In the phospodiester (PDE) region, the predominant peaks mainly consist of glycerolphosphoryl-choline (GPC) and glycerolphosphorylethanolamine (GPE). Both GPC and GPE change with time following arsenite injection as is shown in both extract and in vivo spectra. The variation of PDEs may be related in part to changes in mitochondrial membrane structure, similar to that previously shown in studies of mitochondrial ultrastructural biogenesis following

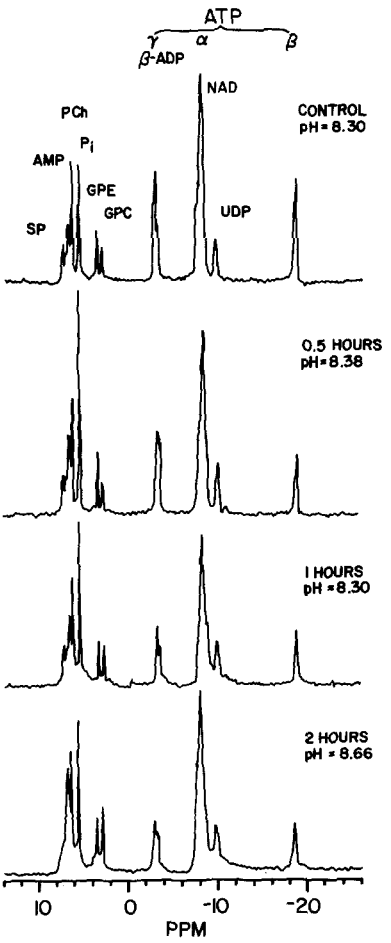

Figure 2. ^{31}P-NMR spectra obtained form PCA extracts of livers excised from rats at 0.5, 1.0 and 2.0 hours following an i.v. dose of sodium arsenite (10 mg/kg). A control spectrum is shown in the top panel.

in vivo arsenate exposure (25). The progressive decline of high energy ATP levels indicates that arsenite, which is known to specifically inhibit mitochondrial NAD-linked substrates does indeed produce marked deceases in cellular ATP pools. Similar findings have been recently reported using perfused rat heart (26), but in contrast with their studies, no unsual phosphomonoesters at 3.9 and 4.04 ppm (85% orthophosphoric acid = 0 ppm) were detected in the rat liver.

The data from both *in vivo* and extract experiments have been quantified in Table 1, in which values from present data and published data were

tabulated for comparision. Regardless of the difference in experimental conditions, values of percentage ATP and P_i from our control studies were comparable to others, and percentage of ATP from extracts were consistently lower than in vivo results. The difference of values between extract and in vivo studies is common and comments have been given in (7). The lower values for PDEs which were obtained in vivo were possibly due to the relatively rapid pulsing, which could be saturating part of the PDE resonance signals. However, the data from extracts clearly show that not only do PDEs increase with time, but the relative ratio of GPE/GPC changes approximately from greater than one to equal to one (Table 1). The change in the ratio of GPE/GPC has also been observed in glucagon perfused rat

TABLE 1

Dietary	Species & Methods	NMR Pulse Condirions	%ATP	%P_i	%PDE	GPE/GPC	Ref No.
In vivo Control							
Fed[a]	Male Wistar	81.00 MHz 10.00 usec (30°) 4.00 sec	56.0	8.8	8.5	-	7
Fed	Male Charles River CD	146.15 MHz 40.00 usec 500.00 msec 4.20 sec	52±4[b]	6±2[b]	3±1[c]	1.4±0.2[c]	*
Extract Control							
Fed[a]	Male Wistar	81.00 MHz 10.00 usec 8.00 sec	29.5	9.2	11.5	1.70	7
Fed	Male Charles River CD	145.15 MHz 40.00 usec 1.00 sec	29.5	5.6	5.2[d]	1.70[d]	*
Fed[f]	Female Long Evans	36.00 MHz - (45°) 890.00 msec	26.4	8.9	3.4	1.10	29
Extract Treated							
As^{3+} 30 min	Male Charles River CD	146.15 MHz 40.00 usec 1.00 sec	17.3	10.0	4.9[d]	1.80[d]	*
60 min	same	same	16.7	10.2	5.2[d]	1.10[d]	*
120 min	same	same	15.7	8.3	7.3[d]	1.00[d]	*

* Data from present studies
a Estimated from spectra with a cut and weigh method
b N=6, c N=4, d N=2, f From published table
Others are representative data

livers (27). Rats treated with sodium arsenite (10 mg/kg i.p.), have been shown to produce hyperglycemia (28), suggesting the possibility of glucagon involvement in mediating the observed changes in ATP levels. Further studies are needed to evaluate these suggested metabolic interactions between liver energy metabolism and other organs involved in regulation of carbohydrate metabolism during arsenite intoxication.

Thus, acute in vivo administration of arsenite produced observable decreases in intracellular ATP pools with concomitant increases in P_i, PM and GPC. These data indicate that arsenite, which inhibits mitochondrial NAD-linked substrate oxidation, induces the loss of intracellular ATP despite the fact that glycolysis and succinate oxidations are resistant to this agent (18,20-24). Such findings suggest that the liver apparently cannot compensate for rapid loss of NAD-linked substrate oxidation via other metabolic pathways (e.g. glycolysis) which produce ATP. In addition, the results also show that arsenite not only induces shifts in the intracellular phosphorous pools from ATP to P_i but also to other organic phosphorous species (e.g. GPC and GPE). These investigations also demonstrate that ^{31}P-NMR can be a useful tool for the time-dependent studies of in vivo cellular energy metabolism.

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