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DT-Diaphorase Induction by Lead Acetate in the Liver of Rats

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DT-diaphorase(NAD(P)H:(quinone acceptor)oxidoreductase) is a cytosolic enzyme, which is localized mainly in liver, kidney, and gastrointestinal tract (Emster et al. 1962). Lind et al. (1982) suggested that DT-diaphorase has a role as a cellular control devise against radical formation. Moreover it is considered that DT-diaphorase has an important role in the cellular defense mechanism against cytotoxic and mutagenic compounds (Riley and Workman 1992). This enzyme is induced by aromatic hydrocarbons such as 3-methylcholanthrene (3-MC; Williams et al. 1986) and antioxidants (Benson et al. 1980), and is an interesting enzyme from drug metabolism and toxicological aspects.

In a series of studies, we first reported that the treatment of organotin compounds induces hepatic DT-diaphorase activity accompanied by thymus atrophy in rats (Ariyoshi et al. 1991). Second, the induction ability of hepatic DT-diaphorase of rats treated with lead acetate (PbAc) is high among various metals (Sugiura at al. 1993). Although organotin compounds produce both thymus atrophy and increased DT-diaphorase activity similar to 3-MC, PbAc causes no significant change in thymus weight. These findings suggest that there is a different mechanism involved in the induction of DT-diaphorase with PbAc than with organotin compounds. In the present study, we investigated the induction mechanism of DT-diaphorase by PbAc in the liver of rats.

MATERIALS AND METHODS

NADPH was purchased from Boehringer Mannheim-Yamanouchi Co., Ltd., (Tokyo, Japan). Actinomycin D (AcD) and diltiazem (Dil) were obtained from Sigma Chemical Co., (St. Louis,USA). 2,6 Dichlorophenol indophenol (DCPIP) was obtained from E. Merck A.G. (Darmstadt, Germany). Lead acetate and other chemicals used were of the highest grade quality available from Wako Pure Chemical Industries Ltd., (Osaka, Japan).

Male Wistar rats (200g body weight) were used in this experiment, three to four

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rats per group. Animals were housed in an air conditioned room with a 12/12 hour light and dark cycle and were fed commercial rat chow (CE-2, Clea Ltd, Tokyo, Japan) and water ad libitum. Animals were injected i.p with each chemical dissolved in distilled water at 2 ml/ dose/kg.

DT-diaphorase activity in the liver was estimated at 24 hr after single injection of lead acetate ($Pb(CH_3COO)_2 3H_2O$:PbAc) at the dose of 25, 50 and 100 mg/kg. Subsequently, it was examined at 12, 24, 72 and 120 hr after treatment with the single dose of 100 mg/kg PbAc. To establish whether the induction of DT-diaphorase is attributable to lead as the metal, another lead compound (lead nitrate: $Pb(NO_3)_2$ at 115 mg/kg) and the acetate base of another metal (zinc acetate; $Zn(CH_3COO)_2 2H_2O$: ZnAc at 33 mg/kg) were examined. In preliminary experiment, the dose of both compounds were surveyed at various concentrations for induction of heme oxygenase which is sensitive parameter to metals exposure, as compared with that of PbAc (100 mg/kg).

Since we suspect that DT-diaphorase induction by PbAc is mediated by calcium, animals were treated with Dil (calcium antagonist) at a dose of 4 mg/kg 1 hr before and 12 hr after PbAc treatment. AcD (protein synthesis inhibitor) was also injected at a dose of 0.8 mg/kg 4 hr before and 12 hr after PbAc injection in order to estimate *de novo* protein synthesis. Control rats were treated with saline.

After treatment with chemicals, the animals were sacrificed by decapitation and the liver was homogenized with 4 volumes of 0.25 M sucrose in a Potter-Elvehjem homogenizer with a teflon pestle. Preparation of the 105,000xg soluble fraction (cytosol) was carried out by the procedures previously described by Arizono et al. (1982). DT-diaphorase activity was measured spectrophotometrically by the reduction of DCPIP using procedure of Ernster (1967). Protein concentration was estimated according to the method of Lowry et al. (195 1).

All results are expressed as mean ± S.E. Statistical variations among the experiments were evaluated by unpaired Student's t-test for two-sample comparisons. In each case, P values less than 0.01 were considered significant.

RESULTS AND DISCUSSION

In our previous report, we observed that DT-diaphorase activity is induced 250% by a single treatment of PbAc and 3-fold with repeated treatment compared with control (Sugiura et al. 1993). Although Iannaccone et al. (1976) reported that feeding PbAc induced DT-diaphorase in kidney but not liver, we found that PbAc induces DT-diaphorase activity in both liver and kidney.

The highest level of DT-diaphorase activity 24 hr after treatment was noted at a dose of 100 mg/kg PbAc (Fig.1). DT-diaphorase activity reached a peak after 24 hr and returned to control levels at 72 hr to 120 hr (Fig.2).

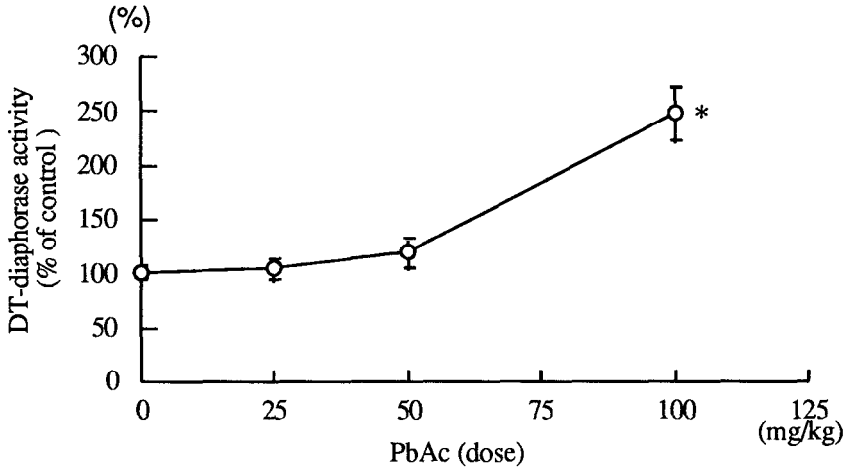

Figure 1. Dose effects on DT-diaphorase activity in rat liver 24 hr after a single dose of PbAc was administered. Each value represents % of control and the mean ± S.E. of 4-8 rats. Significantly different from corresponding mean of control, *P<0.01. Control value: 281 ± 34 nmol DCPIP reduced /mg protein/min.

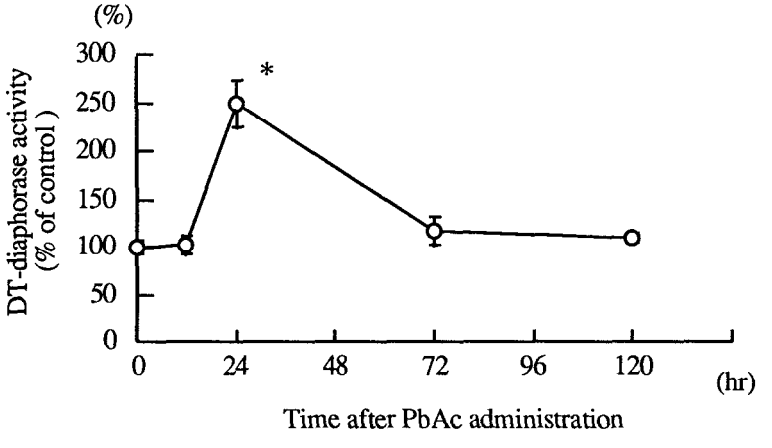

Figure 2. Time course changes in DT-diaphorase activity in rat liver after PbAc (100 mg/kg) administration. Each value represents % of control and the mean ± SE. of 4-8 rats. Significantly different from corresponding mean of control, *P<O.Ol. Control value: 281 ± 34 nmol DCPIP reduced /mg protein/min.

Heme oxygenase and metallothionein are known to be sensitive parameters, which respond to metal exposure (Arizono et al. 1991). In general, the physiological response to treatments with metals are devided into two groups. Cadmium, zinc and mercury can bind to metallothionein in vivo, where repeated treatment with these metals decreases heme oxygenase activity as compared with a single treatment. Metals such as lead, cobalt and nickel have been identified as metals which do not have stable replacement ability to zinc in metallothionein. After repeated treatment with these metals, both heme oxygenase and metallothionein increased several fold higher than that observed from a single treatment. Since no relationship between single and repeated treatment of PbAc was found in DT-diaphorase activity by Sugiura et al. (1993), the induction mechanism of DT-diaphorase by PbAc is thought to be different from that of heme oxygenase or metallothionein. We observed that lead nitrate induced DT-diaphorase (210% to control) similar to PbAc, however ZnAc did not increase activity (Fig.3). Consequently, the induction of DT-diaphorase by PbAc can be attributed to the metal lead.

Many cellular responses to stimuli are wholly or partly mediated by changes in the distribution of calcium (Pounds 1990). The cytotoxicity of several compounds are partly due to alteration of calcium ion homeostasis, resulting in cell death. Ohira and Ariyoshi (1978) reported that the administration of PbAc causes an alteration in calcium distribution. The induction of hepatic DT-diaphorase by PbAc decreased significantly with concominant administration of Dil (calcium antagonist) or AcD (protein synthesis inhibitor), respectiveIy (Fig.4). Therefore, DT-diaphorase induction by PbAc treatment may occur *de novo* protein synthesis mediated with calcium. On the other hand, calcium treatment did not cause the enhancement of DT-diaphorase activity (data not shown). The result suggests that DT-diaphorase induction could be initiate the susceptible change of cellular calcium which associate with the alteration of physiological condition.

Prochaska and Talalay (1988) speculated that inducers of DT-diaphorase can be classified into two groups; a monofunctional inducer or a bifunctional inducer. The monofunctional inducers (antioxidants) activate phase II drug metabolizing enzymes such as UDP-glucuronyl transferase, selectively. The bifunctional inducers (planer aromatic compounds) activate both phase I enzymes, which is related to cytochrome P450 1A subfamily, and phase II enzymes via the cytosolic Ah receptor. It is well known that inducers of the cytochrome P450 1A subfamily such as 3-MC and β-naphthoflavone are also good inducers of DT-diaphorase, accompanied by thymus atrophy. These bifunctional inducers involved in the activation of xenobiotic response element in promoter site which is located upstream of DNA, and mediated phase I and phase II enzymes gene expression. The activation of phase II enzymes by monofunctional inducers such as phenolic antioxidants may be mediated antioxidant responsive element in promoter site of DNA and related with gene transcription (Favreau and Pickett 1991). The cytosolic free calcium ion is taken up into cellular organelles such as the endoplasmic reticulum and mitochondria. The importance of cellular calcium ion homeostasis is

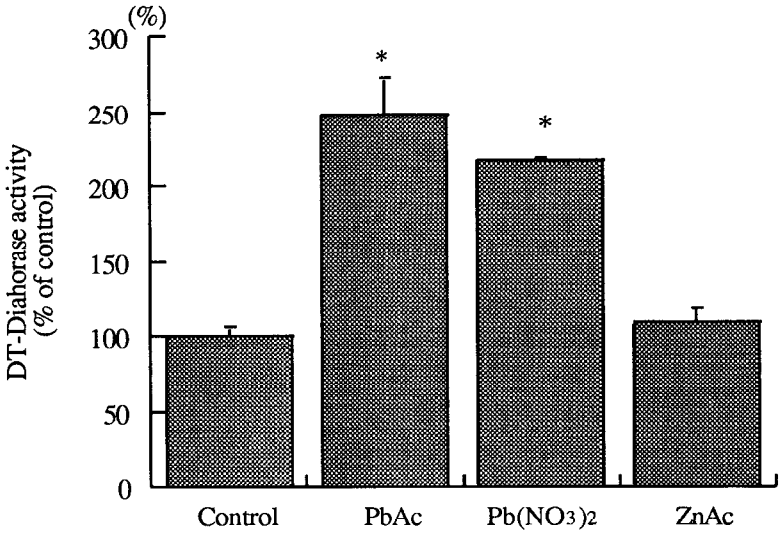

Figure 3. Effects on DT-diaphorase activity by the single treatment with PbAc (100 mg/kg), $Pb(NO_3)_2$ (115 mg/kg) and ZnAc (33 mg/kg). Each value represents % of control and the mean ± S.E. of 4-8 rats. Significantly different from corresponding mean of control, *P<0.01.

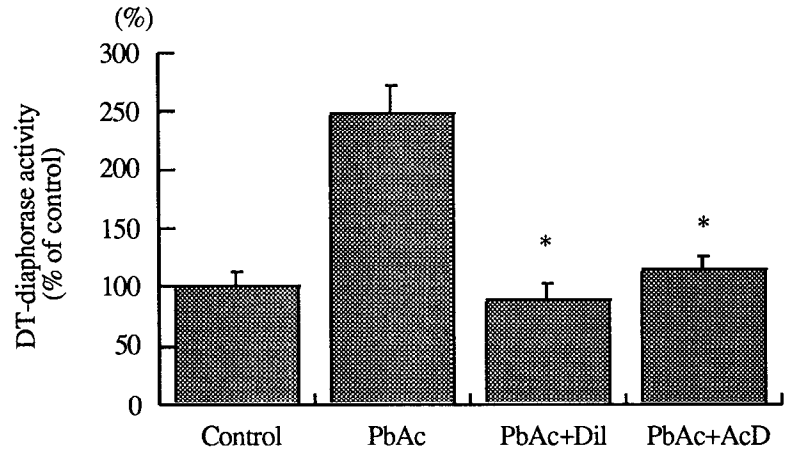

Figure 4. Effects of diltiazem (Dil) and actinomycin D (AcD) on DT-diaphorase activity induced by PbAc. Dil (4 mg/kg) injected 1 hr before and 12 hr after and AcD (0.8 mg/kg) injected 4 hr before and 12 hr after PbAc (100 mgkg) treatment, respectively. Each value represents % of control and the mean ± SE. of 4-8 rats. *:P<0.01, as compared to PbAc alone.

illustrated by the fact that an increased cytosolic calcium level is a frequent and early phenomenon in cell injury. If higher cellular calcium levels stimulate activation of antioxidant responsive elements, PbAc might be classified as a monofunctional inducer of DT-diaphorase.

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