Effect of Systemic Anaphylaxis on the Hepatic Drug-Metabolizing System in Rats

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ABSTRACT—Rats were immunized by intraperitoneal injection of ovalbumin emulsified with Freund incomplete adjuvant, and then the effect of an intravenous challenge with ovalbumin on hepatic drug-metabolizing enzyme activities was examined. The cytochrome P-450 content and ethylmorphine N-demethylase, benzphetamine N-demethylase, arylhydrocarbon hydroxylase and 7-ethoxycoumarin O-deethylase activities significantly decreased in rats treated with ovalbumin compared with control groups treated with saline, whereas there was no significant reduction in cytochrome b5, NADPH-cytochrome c reductase and NADH-cytochrome c reductase.

Keywords: Systemic anaphylaxis (rat), Hepatic drug-metabolizing activity

Environmental and physiological stress has been shown to alter the drug-metabolizing capacity of the host (1). Acute anaphylactic shock is a systemic allergic reaction which occurs in an appropriately sensitized individual following the challenge with antigen. When the antigen comes in contact with mast cells fixed with immunoglobulin E (IgE), the mast cells are degranulated, and large amounts of histamine, slow-reacting substance of anaphylaxis (SRS-A), and other chemical mediators are released. These substances are vasoactive, increase permeability, affect membrane stability and cause the release of other mediators. In these cases, various physiological factors can be changed by these substances and especially during systemic anaphylaxis (2). Furthermore, the mediators of the anaphylaxis are cytokines released from activated mononuclear leukocytes (2). It has long been known that interferon or IL-1 causes a decrease in hepatic cytochrome P-450 content (3); and more recently, it has been shown that a recombinant interferon or IL-1 suppresses cytochrome P-450 levels and activities when injected into animals (4–6).

In contrast to the well-studied immunological effect on the intestinal transport of macromolecules and drugs, few studies have been done to investigate the immunological effects on the hepatic drug-metabolizing enzyme system. The present study was designed to investigate the effect of an intravenous challenge with ovalbumin on the hepatic drug-metabolizing activity in rats immunized with ovalbumin.

Male Wistar rats, 150–200 g, were used in these studies, and the immunization schedule was the same as described previously (7). For intraperitoneal immunization, 1 mg of ovalbumin (crystallized and lyophylized, grade III; Sigma Chemical Co., St. Louis, MO, USA) dissolved in 0.25 ml of saline was emulsified with an equal volume of Freund incomplete adjuvant (Nacalai Tesque, Inc., Kyoto), and this was then injected intraperitoneally. Ovalbumin (0.5 mg) dissolved in 0.25 ml of saline was boosted intravenously in rats immunized twice at 10-day intervals thereafter. In a preliminary experiment, ovalbumin was shown to most effectively depress cytochrome P-450 content 24 hr after administration in rats immunized with ovalbumin. Since there was no significant difference in the cytochrome P-450 content between ovalbumin- and saline-treated animals in our preliminary studies, control rats received 0.5 ml Freund incomplete adjuvant emulsified with saline on the same schedule as for the ovalbumin-treated rats. Phenobarbital-pretreated groups received intraperitoneal injection of 80 mg/kg
daily for two days. Ovalbumin was injected immediately at the last phenobarbital or 3-methylcholanthrene treatment.

Animals were killed by decapitation, and the livers were excised, perfused with cold 1.15% KCl and homogenized in 3 volumes of 0.1 M phosphate buffer (pH 7.4) with a Potter Elvehjem glass homogenizer with a Teflon pestle. The homogenates were centrifuged at 9,000 × g for 20 min, and the resulting post-mitochondrial supernatant fraction was centrifuged at 105,000 × g for 1 hr. The microsomal fractions were stored at −80°C until use. The contents of cytochromes P-450 and b5 were determined according to the methods of Omura and Sato (8) and Omura and Takesue (9), respectively. Arylhydrocarbon hydroxylase and 7-ethoxycoumarin O-deethylase activities in microsomes were determined as described by Nebert and Gelboin (10) and Greenlee and Poland (11), respectively. Benzphetamine N-demethylase and ethylmorphine N-demethylase activities were determined in the incubation medium described previously (6), measuring the amount of formaldehyde formed by the method of Nash (12). NADPH-cytochrome c reductase and NADH-cytochrome c reductase were determined as described by Phillips and Langdon (13). Protein concentrations were determined by the method of Bradford (14) with bovine serum albumin as the standard.

Systemic anaphylaxis of rats with ovalbumin led to considerable reduction in body weight and microsomal protein content of the liver, whereas there was no significant reduction in the fresh weight of the liver. However, significant changes were observed in some components of the hepatic drug-metabolizing enzyme activity. Cytochrome P-450 content and the activity of ethylmorphine N-demethylase, benzphetamine N-demethylase, arylhydrocarbon hydroxylase and 7-ethoxycoumarin O-deethylase in hepatic microsomes from systemic anaphylaxis rats were 71%, 59%, 67%, 52% and 59% of those in the microsomes from the control, respectively. The activities of NADPH-cytochrome c reductase and NADH-cytochrome c reductase and cytochrome b5 content did not change significantly (Table 1). The effect of anaphylaxis upon animals undergoing microsomal enzyme induction was also examined in rats. As seen in Fig. 1, phenobarbital treatment (80 mg/kg, i.p., once a day for 2 days) caused a 1.3- to 2.3-fold increase in cytochrome P-450 content and ethylmorphine N-demethylase and benzphetamine N-demethylase activities in both the control and anaphylactic animals. These elevations were not affected with the exception of the cytochrome P-450 content by systemic anaphylaxis.

Similarly, 3-methylcholanthrene treatment (40 mg/kg, i.p., once a day for 2 days) caused a 1.5- to 2-fold increase in cytochrome P-450 and 7-ethoxycoumarin O-deethylase in both the control and anaphylactic animals (data not shown).

Acute and specific pathophysiologic changes occur in both circulatory and cell functions in the host during systemic anaphylaxis (1). Reduction of body weight and microsomal protein content in the liver suggest that the impairment of drug-metabolizing enzyme activity in systemic anaphylaxis may be at least partially due to systemic toxicity. The adverse effects of systemic anaphylaxis on

| Parameters | Control | Model rats |
|------------|---------|------------|
| Body weight, g | 215.9 ± 2.71 | 193.9 ± 6.19 (89) |
| Liver weight, g | 6.9 ± 0.20 | 6.8 ± 0.35 (98) |
| Protein, mg/g liver | 11.5 ± 0.11 | 9.5 ± 0.52 (82) |
| Cytochrome P-450 | 0.47 ± 0.072 | 0.33 ± 0.018 (70) |
| Cytochrome b5 | 0.11 ± 0.011 | 0.09 ± 0.009 (81) |
| NADPH-cytochrome c reductase | 0.19 ± 0.002 | 0.15 ± 0.025 (78) |
| NADH-cytochrome c reductase | 4.99 ± 0.782 | 4.30 ± 0.658 (86) |
| Ethylmorphine N-demethylase | 308.2 ± 15.58 | 183.0 ± 18.36 (59) |
| Benzphetamine N-demethylase | 276.5 ± 19.20 | 186.6 ± 28.58 (67) |
| Arylhydrocarbon hydroxylase | 2.39 ± 0.445 | 1.26 ± 0.461 (52) |
| 7-Ethoxycoumarin O-deethylase | 0.65 ± 0.127 | 0.39 ± 0.046 (60) |

Each group consisted of 5 rats. Ovalbumin was administered intravenously to rats immunized with ovalbumin, as described in Materials and Methods. Ovalbumin-immunized rats administered saline instead of ovalbumin served as the control. Parameters are expressed as the mean ± S.E. of five observations from separate animals. Percent of the control value are indicated in parentheses. Each value was analyzed by Student's t-test; a P < 0.05, with respect to the control. a nmol/mg protein, b unit/min/mg protein, c nmol/20 min/mg protein, d nmol/10 min/mg protein, e nmol/15 min/mg protein.
the hepatic mixed-function oxidase (MFO) system seem to be specific since other associated components, viz. NADPH-cytochrome c reductase, NADH-cytochrome c reductase and cytochrome b₅, were not altered significantly during this shock. Since no cytochrome P-420, an inactive form of cytochrome P-450, was found in this experiment, it is suggested that the decrease of cytochrome P-450 content is not due to a denaturation of cytochrome P-450 to cytochrome P-420, but anabolism or catabolism of cytochrome P-450 might be affected by systemic anaphylaxis. Specific suppression of the hepatic MFO oxidase system during systemic anaphylaxis may be due to specific alterations in the synthesis, function or catalysis of cytochrome P-450.

Furthermore, alterations in hepatic drug-metabolizing enzymes after the administration of interferon inducers or cytokine inducers such as endotoxin are well documented in experimental animals (3). Some investigators reported that endotoxin inhibited the activity of hepatic mitochondrial δ-aminolevulinic acid synthetase and increased the heme oxygenase activity (15). The results suggest that systemic anaphylaxis may occur through the same phenomena in hepatocytes.

Similar induction patterns of cytochrome P-450 and associated activities by phenobarbital treatment in both control and anaphylactic groups of animals indicated that the complete sequence of events involved in the synthesis of the components was preserved during this anaphylaxis. Hence, the loss in cytochrome P-450 and associated activities may be attributed to a decrease of cytochrome P-450 synthesis rather than accelerated degradation of this hemoprotein. The molecular mechanisms of this impairment of the hepatic drug-metabolizing enzyme activity during systemic anaphylaxis are not understood clearly and, hence, require further investigation. Impairment of the MFO system by systemic anaphylaxis may affect the efficacy and pharmacological properties of drugs as well as the metabolism of endogenous substances with possible physiological, pharmacological and toxicological consequences.

The results presented in this communication establish impairment of the hepatic microsomal drug-metabolizing enzyme activity during systemic anaphylaxis of rats with ovalbumin. The decrease of cytochrome P-450 synthesis appears to be a possible cause of impairment of the drug-metabolizing enzyme activity.

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