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EFFECTS OF PROCHLORPERAZINE ON THE FUNCTION OF INTEGRAL MEMBRANE PROTEINS

ANDREW DANNENBERG and DAVID ZAKIM*

Division of Digestive Diseases, Department of Medicine, Cornell University Medical College, New York, NY 10021, U.S.A.

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Abstract—We have studied the effects of prochlorperazine on the activities of UDP-glucuronosyltransferase and glucose-6-phosphatase (glucose-6-P'ase) in rat liver microsomes. The activity of UDP-glucuronosyltransferase was increased in a graded fashion by addition of prochlorperazine. Maximal stimulation occurred at 1 mg prochlorperazine to 2 mg microsomal protein, which resulted in a 6-fold increase in activity. However, with smaller concentrations of drug, there was a time-dependent increase in the activity of UDP-glucuronosyltransferase. Sensitivity of UDP-glucuronosyltransferase to activation by UDP-*N*-acetylglucosamine was lost after treatment of microsomes with prochlorperazine. These results indicate that prochlorperazine causes a profound reorganization of the interactions between lipids and enzyme since the activity and allosteric properties of UDP-glucuronosyltransferase are known to depend on interactions with lipids in a gel phase. Glucose-6-P'ase also was activated in a graded fashion by prochlorperazine; 1 mg of drug/2 mg microsomal protein resulted in a 60% increase in activity. The temperature-dependent instability of glucose-6-P'ase was increased by treatment of microsomes with prochlorperazine and could be prevented only partially by substrate. We conclude that prochlorperazine disrupts the structural organization between lipids and proteins in microsomal membranes, altering thereby the activity and regulation of at least two different integral membrane proteins.

There is a large body of evidence to suggest that the therapeutic effects of phenothiazines are due in part to the modulation of membrane function [1–3]. Reduced binding of dopamine to its membrane receptor, for example, is believed to be the basis for the antipsychotic effect of phenothiazines [4]. Experiments *in vivo* and *in vitro* have demonstrated that phenothiazines alter membrane structure [5, 6]; and phenothiazines, when added to pure lipid systems, alter the thermotropic properties of bilayers [7]. Studies of the effects of phenothiazines on the properties of membrane-bound enzymes for which function depends on the lipid matrix interacting with the enzyme are limited, however. Microsomal UDP-glucuronosyltransferase (EC 2.4.1.17) is an attractive system for such studies because this enzyme has been purified and its function in reconstituted systems has been shown to depend on the physical state of the phospholipid environment in which it is embedded [8–14]. In addition, the activity of UDP-glucuronosyltransferase in microsomes can be manipulated by treatment of microsomes with lipid-soluble compounds [15, 16]. Microsomal glucose-6-phosphatase (glucose-6-P'ase) has many properties in common with UDP-glucuronosyltransferase, in terms of the effects of lipid-soluble agents on enzyme activity [17–20]. Although this enzyme has not been purified and the relationship between function and interactions with the lipid regions of membranes is less certain than for the UDP-glucuronosyltransferase, we also have examined the effects of prochlorperazine on the activity of glucose-6-P'ase in microsomes.

* Address correspondence to: Dr. David Zakim, Division of Digestive Diseases, Department of Medicine, Cornell University Medical College, 1300 York Ave., New York, NY 10021.

MATERIALS AND METHODS

Materials. Prochlorperazine edisylate, triflupromazine, chlorpromazine, UDP-glucuronic acid, UDP-*N*-acetylglucosamine, *p*-nitrophenol and glucose-6-phosphate were purchased from Sigma. All other chemicals used were the best available commercial grades. Microsomes were prepared from fresh Wistar rat liver, as previously described [21]. The GT_{2P} type of UDP-glucuronosyltransferase was purified by the method of Hochman and Zakim [11].

Assay of UDP-glucuronosyltransferase activity. Microsomes were added to fractions containing 60 mM 1,4-piperazinediethanesulfonic acid (Pipes) buffer, pH 6.1, and a range of prochlorperazine concentrations (0 to 2.3 mM) at 0°. The protein concentration in these mixtures was 1.3 mg/ml. Immediately after mixing, an aliquot of 0.13 mg of microsomal protein was removed and added to an assay mixture containing 0.05 mM *p*-nitrophenol, 1.0 mM UDP-glucuronic acid, 1.0 mM $MgCl_2$ and 50 mM Tris–HCl, pH 7.5, in a total volume of 0.5 ml. Aliquots of 0.1 ml were removed from the assay tubes at 5-min intervals and added to 1.0 ml of 2% (w/v) trichloroacetic acid to stop the reaction. After the protein was removed by centrifugation, 40 μl of 10 M NaOH was added to the supernatant fraction, and the optical density was measured at 400 nm. Blank assays contained all components except UDP-glucuronic acid. Assays were at 37° and reflected true initial rates of activity.

Assay of glucose-6-P'ase activity. Microsomes were added to fractions containing 60 mM Pipes, pH 6.1, and a range of prochlorperazine concentrations (0 to 2.3 mM) in a total volume of 0.5 ml at 0°. The concentration of protein in these mixtures was 1.3 mg/ml. Immediately after mixing, a portion of microsomal protein was added to an assay mixture containing 5.0 mM glucose-6-phosphate and 60 mM Pipes, pH 6.1, at 37°. The protein concentration in the assay portion of each experiment was 0.4 mg/ml. Aliquots of 0.2 ml were removed from the assay tubes 10 min later and added to equal volumes of 10% (w/v) trichloroacetic acid. The precipitated protein was removed by centrifugation. Phosphate was measured by the method of Sumner [22].

RESULTS

Effect of prochlorperazine on the activity of UDP-glucuronosyltransferase in microsomes. The effect of prochlorperazine on the activity of UDP-glucuronosyltransferase is shown in Fig. 1. There was a graded increase in enzyme activity with increasing amounts of prochlorperazine. Additions of drug in excess of 0.5 mg/mg microsomal protein did not increase the enzyme activity beyond that shown in the figure. In addition, the kinetics changed from non-Michaelis–Menten to Michaelis–Menten in the presence of prochlorperazine. This is reflected by the data in Fig. 2. This type of change in the kinetic pattern displayed by the enzyme is typical for activators of UDP-glucuronosyltransferase such as treatment of microsomes with detergents or phospholipase A_2 [23–25].

The activity of UDP-glucuronosyltransferase in microsomes is only one property that is sensitive to lipid-soluble agents [23–26]. The sensitivity of the enzyme to activation by UDP-*N*-acetylglucosamine also can be manipulated by adding surface active compounds to microsomes [23, 26]. The effect of prochlorperazine on the sensitivity of the enzyme to activation by UDP-*N*-acetylglucosamine is shown in Fig. 3. There was an 11-fold activation of UDP-glucuronosyltransferase by 4 mM UDP-*N*-acetylglucosamine for enzyme in intact, untreated microsomes, but no concentration of this effector activated enzyme in microsomes treated previously with prochlorperazine. In fact, relatively high concentrations of UDP-*N*-acetylglucosamine inhibited the prochlorperazine-treated enzyme. The extent of activation by prochlorperazine in the experiments in Fig.

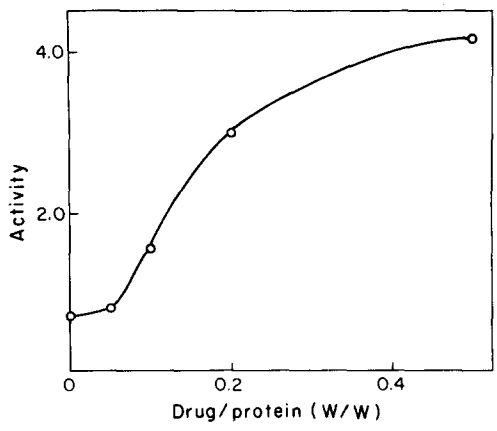

Fig. 1. Effect of prochlorperazine on the activity of UDP-glucuronosyltransferase in rat liver microsomes. Microsomes were treated at 0° with prochlorperazine at the indicated ratios of drug to microsomal protein. Enzyme activity was measured at 37° using concentrations of *p*-nitrophenol and UDP-glucuronic acid of 0.05 and 1.0 mM respectively. Activities are expressed as nanomoles of substrate metabolized per milligram of protein per minute.

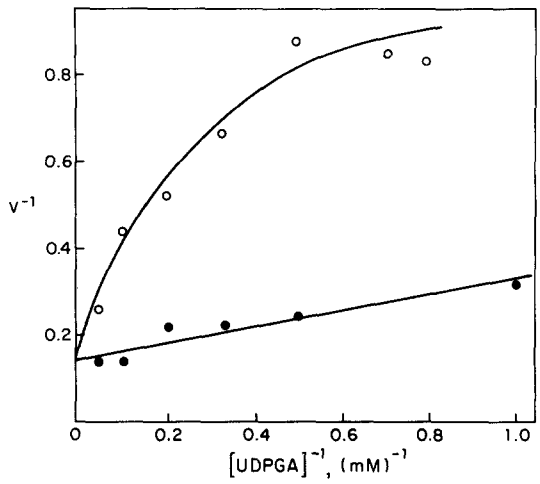

Fig. 2. Rate of glucuronidation of *p*-nitrophenol as a function of the concentration of UDP-glucuronic acid. Data are plotted in double-reciprocal form. The reaction mixture contained 0.05 mM *p*-nitrophenol and concentrations of UDP-glucuronic acid which varied from 1.0 to 25.0 mM. Key: (○) untreated microsomes, and (●) microsomes treated with prochlorperazine at a drug/protein (w/w) ratio of 1/2. Activities are expressed as in Fig. 1.

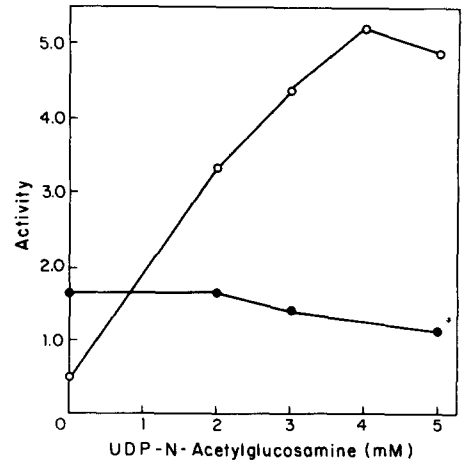

Fig. 3. Effect of UDP-*N*-acetylglucosamine on the activity of UDP-glucuronosyltransferase in rat liver microsomes. Microsomes from rat liver were assayed as described in Materials and Methods at 37° using 0.05 mM *p*-nitrophenol, 1.0 mM UDP-glucuronic acid and the indicated concentration of UDP-*N*-acetylglucosamine. Units of activity are as in Fig. 1. Key: (○) untreated microsomes, and (●) microsomes treated with prochlorperazine at a drug/protein (w/w) ratio of 1/2.

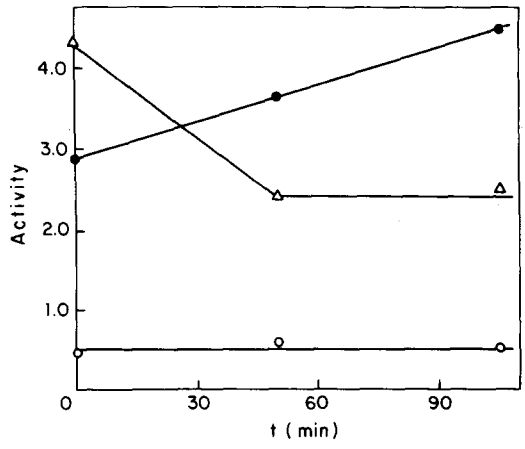

Fig. 4. Effect of prochlorperazine on the activity of UDP-glucuronosyltransferase as a function of duration of treatment. Microsomal UDP-glucuronosyltransferase activity was measured in the presence of 0.05 mM *p*-nitrophenol and 1.0 mM UDP-glucuronic acid after different periods of treatment at 24°. Key: untreated microsomes (○), and microsomes treated with drug at ratios of 1/5 (●) or 1/2 (△). Units of activity are as in Fig. 1.

3 was less than for those in Fig. 1, but this difference does not alter the significance of the data in Fig. 3.

Addition of prochlorperazine to pure UDP-glucuronosyltransferase or to enzyme reconstituted with pure lysolecithin micelles had no effect on the activity of the enzyme (data not shown). This suggests that prochlorperazine altered the function of the enzyme in microsomes via effects on microsomal lipid–protein interactions and not because prochlorperazine interacted directly with the enzyme. Pig liver microsomes and not rat were the source of pure UDP-glucuronosyltransferase. The differences in response of enzyme in rat microsomes versus pure enzyme from pig microsomes could not be attributed, however, to difference between species. Thus, prochlorperazine activated UDP-glucuronosyltransferase in pig microsomes (data not shown).

Time-dependent effects of prochlorperazine on the activity of UDP-glucuronosyltransferase. Data presented elsewhere suggest that the lipid environment of UDP-glucuronosyltransferase in intact microsomes is a domain of relatively high viscosity, i.e. comparable with that for phosphatidylcholines in a gel state [10, 11, 27]. This organization of enzyme and lipids in intact, freshly isolated microsomes appears to be unstable, and deteriorated in a time- and temperature-dependent manner leading to activation of the enzyme and a loss of sensitivity to allosteric activation by UDP-*N*-acetylglucosamine*. These time-dependent effects could be important in the context of the data in Figs 1, 2 and 3. This idea is substantiated by the data in Fig. 4. Shown here are instantaneous activations of enzyme by prochlorperazine (time = 0 at concentrations of 0.2 and 0.5 mg per mg of microsomal protein). There was an initial 5-fold activation by the former concentration of drug and an 8-fold activation by the latter. If microsomes treated with prochlorperazine were allowed to stand at 24°, however, there was a further time-dependent activation in microsomes treated with 0.2 mg prochlorperazine/mg microsomal protein. UDP-Glucuronosyltransferase in microsomes treated with 0.5 mg prochlorperazine was unstable at 24°; activity at 50 min was less than at 0 time. The activity of UDP-glucuronosyltransferase in untreated microsomes changed little, however, over 90 min at 24°. In other words, prochlorperazine accelerated the spontaneous rate of activation of UDP-glucuronosyltransferase. This result suggests that the long-term effects of drug on membrane function might not be predictable from examining only the instantaneous effects of relatively small amounts of drug on the function being studied.

Effect of prochlorperazine on the activity of glucose-6-P'ase in microsomes. Glucose-6-P'ase activity was increased immediately in a graded fashion by the addition of prochlorperazine (Fig. 5). Maximum activation of 60% was achieved when 1 mg prochlorperazine was added to 2 mg of microsomal protein. The activity of glucose-6-P'ase in untreated microsomes was stable at 24° for a period of at least 90 min. Treatment of microsomes with prochlorperazine destabilized glucose-6-P'ase, however (Fig. 5), and the rate of prochlorperazine-induced inactivation increased with increasing concentrations of drug and temperature. Loss of activity was prevented only partially by substrate.

Effects of trifluprομazine and chlorpromazine on the activities of UDP-glucuronosyltransferase and glucose-6-P'ase in microsomes. The effects of trifluprομazine and chlorpromazine, two other lipid-soluble phenothiazines, were assessed. The agents were tested using the same methodology used for prochlorperazine. Trifluprομazine and chlorpromazine caused activation of both enzymes (data not shown). As was the case of prochlorperazine,

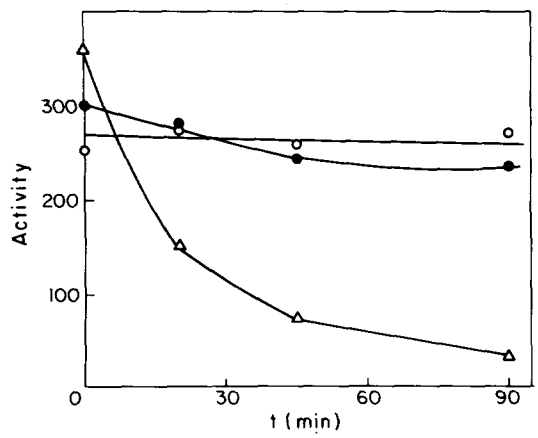

Fig. 5. Effect of prochlorperazine on the activity of glucose-6-phosphatase as a function of duration of treatment. Glucose-6-phosphatase activity in rat liver microsomes was measured in the presence of 5.0 mM glucose-6-phosphate after different periods of treatment at 24°. The assay temperature was 37°. Activity is expressed as nanomoles of glucose-6-phosphate metabolized per milligram of protein per minute. Key: untreated microsomes (○) and microsomes treated with ratios of prochlorperazine/microsomal protein of 1/5 (●) or 1/2 (△).

* A. Dannenberg, Y. Hochman and D. Zakim, manuscript in preparation.

additions of these drugs in excess of 0.5 mg/mg microsomal protein did not further increase activity. These drugs were similar to prochlorperazine in another respect. Both activated glucuronosyltransferase to a much greater extent than glucose-6-P'ase.

DISCUSSION

The data presented above indicate that phenothiazines induce a transition in membrane structure and concomitant changes in the function of at least two integral membrane proteins. We believe that this is an important observation for the following reasons. Drugs that modulate the activity of membrane-bound enzymes may be useful in disease states such as neonatal jaundice and glucose-6-P'ase deficiency, type B. It may be possible to activate the "defective" enzymes in these conditions which appear to express less than their maximal potential activties *in situ* [28, 29]. Furthermore, understanding the complications of phenothiazine use, such as cholestatic jaundice or tardive dyskinesia, may depend on understanding how these agents alter membranes. Finally, the series of experiments reported above demonstrates the importance of considering time-dependent effects of a drug on membrane structure and protein function. For example, based on the activation profile at time zero, prochlorperazine might seem to be a potentially useful agent in patients with deficiency of glucose-6-P'ase. However, if one considers the time-dependent effects, it is obvious that the enzyme in prochlorperazine-treated microsomes is highly unstable.

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