Enhancement in $\beta$-Adrenergic Responsiveness of Adenylate Cyclase in Rat Liver after Galactosamine Administration

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ABSTRACT—In rat liver at 48 hours after galactosamine (GalN) treatment, isoproterenol-induced accumulation of cyclic AMP and activation of adenylate cyclase (AC) were significantly enhanced. AC activity stimulated with GppNHp or NaF was also augmented in membranes from GalN-treated rats. However, the MnCl$_2$-stimulated or forskolin-stimulated activity in GalN-treated rats did not differ from the control. These data suggest that the $\beta$-adrenergic responsiveness of AC in rat liver is potentiated, possibly due to the enhanced function of the Gs protein, during the process of regeneration after GalN treatment.

It has been demonstrated that $\beta$-adrenergic receptors play a minor role in the actions of catecholamine in the liver of adult male rat. However, there are certain conditions, such as cholestasis (1), immaturity (2), treatment with carcinogens (3), primary culturing of hepatocytes (4), and liver generation after partial hepatectomy (5), in which $\beta$-adrenergic receptor functions become more important in mediating the actions of catecholamines in adult male rat liver; $\beta$-adrenergic activation of glycogen phosphorylase or adenylate cyclase is much greater. These conditions are generally associated with enhanced proliferation of hepatocytes.

Recently, we have demonstrated that the $\beta$-adrenergic responsiveness of adenylate cyclase is potentiated during the process of liver regeneration after the treatment of rats with carbon tetrachloride (CCl$_4$), and this suggested that the enhanced function of the stimulatory GTP-binding protein (Gs) is involved in the potentiation of the enzyme responsiveness (6). Furthermore, $\beta$-adrenergic stimulation of hepatic DNA synthesis is potentiated during regeneration periods after CCl$_4$ administration, whereas no such enhancement by $\beta$-adrenergic stimulation is observed in the liver of normal control rats (7).

The involvement of $\beta$-adrenergic stimulation in the regulation of DNA synthesis has been shown in liver regeneration after partial hepatectomy (8). In addition, cyclic AMP (8) and cyclic AMP-dependent protein kinase (9) have been implicated in the control of liver regeneration after partial hepatectomy. Thus, it is conceivable that modulation of the hepatic cyclic AMP level could be an important factor for the induction of DNA synthesis in the regenerating liver after the intoxication with a hepatotoxin such as CCl$_4$.

It is generally accepted that CCl$_4$-induced liver injury is attributed to lipid peroxidative degradation of biomembranes that has been triggered by active free-radical metabolites of CCl$_4$, leading to the disintegration of mitochondrial, lysosomal, and cellular membranes, and finally to cell necrosis. On the other hand,
administration of GaIN to rats has been demonstrated to cause a selective trapping of uracil nucleotides, resulting in the inhibition of RNA synthesis and disturbance of the biosynthesis of glycoproteins, leading to deterioration of the cell membranes and thereafter to cell death and necrosis.

To provide additional information on the β-adrenergic responsiveness in liver after hepatotoxin treatment, we have undertaken a similar study using galactosamine (GaIN)-induced hepatitis as a model with a different underlying mechanism (10). In the present study, we examined the ability of adenylate cyclase to respond to β-adrenergic stimulation in the liver after GaIN administration.

Male Wistar rats, weighing 220–260 g and fed a standard laboratory chow ad lib, were used for the experiments. D-Galactosamine hydrochloride (400 mg/kg, body weight) was dissolved in 0.9% NaCl and administered by subcutaneous injection. Normal control rats received the vehicle. In some experiments, CCl4 (1 ml/kg, body weight) in 50% olive oil solution was administered by a stomach tube, and control rats received olive oil alone. At 48 hours after GaIN (or CCl4) administration, a portion of the liver was excised under pentobarbital anesthesia and homogenized with ice-cold medium containing 20 mM HEPES (pH 7.4), 5 mM MgCl2, 0.3 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mM di-thiothreitol (DTT). Crude membranes were prepared from rat liver as described (6, 11) and stored at −80°C until use. Adenylate cyclase activity was assayed as described previously (12) using [α-32P]ATP (800 Ci/mmol, New England Nuclear) in the presence of 0.5 mM 3-isobutyl-1-methylxanthine (IBMX).

Hepatic content of cyclic AMP was determined as described previously (6, 11). Briefly, under pentobarbital anesthesia, rats were injected subcutaneously with isoproterenol (250 μg/kg, body weight) or 0.9% NaCl (as basal). Twenty minutes after injection of isoproterenol, a portion of the liver was excised and rapidly frozen in a liquid nitrogen-cooled clamp. Tissue contents of cyclic AMP were measured by radioimmunoassay (13) using a commercial assay kit (Yamasa, Choshi, Japan).

ADP-ribosylation of crude liver membranes with cholera toxin and [α-32P]NAD (25 Ci/mmol, New England Nuclear) was carried out as described previously (6). Samples were electrophoresed in 10% SDS polyacrylamide gels and analyzed by autoradiography. After autoradiography, the radioactivity in each band was determined by excision of the band in the dried gel corresponding to the radiolabeled band on the autoradiograph and counting in a Beckman LS5801 liquid scintillation counter.

Protein was determined by the Coomassie Blue binding method (14) with bovine serum albumin as a standard. Data were analyzed for statistical significance using a two-tailed Student’s t-test.

Injection of GaIN into rats has been known to induce liver damage in which the histologic and biochemical changes observed resemble those in human viral hepatitis (10), with a panlobular focal cell death of liver parenchyma and a prominent inflammatory infiltrate. These dysfunctions of the liver are followed by proliferation of the surviving cells, and the highest rate of regeneration of tissue is observed at 36 to 72 hours after the administration of GaIN (15), similar to what was observed in the liver after CCl4 treatment (11). Light microscopic examination of liver tissue at 48 hr after the administration of hepatotoxins revealed centrilobular necrosis in CCl4-treated rats and clusters of inflammatory cells throughout the lobules and in perportal regions in GaIN-treated rats (data not shown).

As shown in Fig. 1, the hepatic accumulation of cyclic AMP in response to isoproterenol in normal control rats was only marginal, consistent with previous reports (1, 4, 5). On the other hand, in rats at 48 hours after GaIN, isoproterenol-induced accumulation of hepatic cyclic AMP was significantly higher than that in control rats (Fig. 1). Prior treatment of rats with propranolol, a β-adrenergic antagonist, to isoproterenol abolished increases of hepatic
cyclic AMP level induced by isoproterenol (Fig. 1), indicating that hepatic accumulation of cyclic AMP elicited by isoproterenol was mediated via the β-receptors.

As have been shown in our recent report (6), isoproterenol-induced or GppNHp-induced activation of adenylate cyclase was significantly greater in membranes from rats at 48 hours after CCL4 administration compared with those in control rats (Fig. 2A), indicating that the enhanced function of the Gα protein possibly participates in the potentiation of the β-adrenergic responsiveness of adenylate cyclase in rat liver during the process of regeneration after CCL4 treatment.

In accordance with the results in Fig. 1, in crude membranes from rats at 48 hours after GalN administration, the activity of adenylate cyclase stimulated by isoproterenol (10^-5 M) was significantly enhanced, in contrast to that in the control (Fig. 2B). Much less activation of the enzyme was produced by isoproterenol at the same concentration in membranes from control rats (Fig. 2B).

![Fig. 1. Effect of isoproterenol on the hepatic cyclic AMP level in control and galactosamine (GalN)-treated rats. Isoproterenol (ISP, 250 μg/kg body weight) or 0.9% NaCl (as basal) was subcutaneously injected into rats at 48 hours after GalN administration. Propranolol (Prop, 5 mg/kg body weight) was injected 30 min before the administration of isoproterenol. Data are shown as means ± S.E. of four to seven separate experiments. **: P < 0.01, when compared with the isoproterenol-induced accumulation of cyclic AMP in the control. Control, GalN.](image1)

![Fig. 2. Adenylate cyclase activity in crude liver membranes from control, galactosamine (GalN)-treated, and CCL4-treated rats. The concentrations of agents used were as follows: isoproterenol (ISP), 10^-5 M; GppNHp, 10^-5 M; NaF, 10^-2 M; MnCl2, 2 × 10^-2 M. The basal activity (pmol/mg protein/10 min) in A was 40.5 ± 2.4 and 45.2 ± 1.3 in crude membranes from control and CCL4-treated rats, respectively. The basal activity (pmol/mg protein/10 min) in B and C was 45.4 ± 8.2 and 50.9 ± 3.9 in crude membranes from control and GalN-treated rats, respectively. Isoproterenol-stimulated adenylate cyclase activity was assayed in the presence of 10^-5 M GppNHp, and they are expressed as the increments induced by isoproterenol over the activity with GppNHp (10^-5 M) alone. The activity (pmol/mg protein/10 min) with GppNHp (10^-5 M) was 94.5 ± 6.2 and 114.6 ± 2.6 in crude membranes from control and GalN-treated rats, respectively. Results are shown as the mean ± S.E. of four to six separate experiments. *, **: P < 0.05, P < 0.01, compared with the corresponding control value. Control, CCL4, GalN.](image2)
GppNHp, a hydrolysis-resistant guanine nucleotide analog, can regulate adenylate cyclase through GTP-binding proteins. GppNHp effectively activated adenylate cyclase in membranes from control rats (Fig. 2B). Furthermore, the adenylate cyclase response to GppNHp was significantly greater in membranes at 48 hours compared with membranes from the control rats (Fig. 2B). The activation of the enzyme in response to NaF was also potentiated in membranes from rats at 48 hours (Fig. 2C). However, there was no significant differences in MnCl2-stimulated (Fig. 2B) or forskolin (10^-4 M)-stimulated adenylate cyclase activity (control, 887.4 ± 24.3 pmol/mg protein/10 min; GaIN-treated rats, 897.2 ± 21.2 pmol/mg protein/10 min) in membranes from control rats and those from GaIN-treated rats, indicating that the ability of the catalytic unit of adenylate cyclase did not change at 48 hours after GaIN administration.

Hepatic accumulation of cyclic AMP in response to β-adrenergic stimulation was significantly potentiated during liver regeneration after GaIN treatment (Fig. 1). This enhanced accumulation of hepatic cyclic AMP is likely to result from the potentiation of β-adrenergic responsiveness of adenylate cyclase, since the β-adrenergic stimulation of the enzyme activity measured in the presence of the phosphodiesterase inhibitor IBMX was also significantly augmented (Fig. 2B).

GppNHp-stimulated adenylate cyclase activity was potentiated in liver membranes at 48 hours after GaIN treatment (Fig. 2B), suggesting the function of the Gs protein in regulating adenylate cyclase activity is enhanced at 48 hours. Supporting this, activation of the enzyme with NaF, which is known to activate the Gs protein, was found to be greater in membranes at 48 hours compared with membranes from the control (Fig. 2C). It is likely, therefore, that the enhanced function of the Gs protein is possibly involved in the potentiation of adenylate cyclase responsiveness to β-adrenergic stimulation at 48 hours after GaIN treatment. These data are in agreement with our previous study on the liver after CCl4 treatment, and they suggest that alteration in the Gs protein function is an important factor for regulating the β-adrenergic responsiveness of adenylate cyclase in the liver after the intoxication of the liver with hepatotoxin.

With respect to the alteration of the amount of Gs protein, no appreciable differences were observed in cholera toxin-catalyzed ADP-ribosylation of proteins with Mr. 42 kDa and 50 kDa in crude liver membranes from control and GaIN-treated rats (the amounts of 32P radioactivity incorporated into the 42- and 50-kDa proteins were 482 ± 39 cpm and 208 ± 20 cpm in control rats and 495 ± 34 cpm and 220 ± 31 cpm in GaIN-treated rats, respectively), similar to the findings in the liver after CCl4 administration (6). Further studies are needed to elucidate the precise mechanism(s) for the enhancement in the Gs protein function in the liver during the regeneration periods after the treatment of a hepatotoxin such as GaIN and CCl4.

An increase in β-receptor number has accompanied most of the cases in which β-adrenergic responsiveness of adenylate cyclase is enhanced (1, 2). The possibility that an increase in the β-receptor number is one of the mechanisms responsible for enhanced β-adrenergic responsiveness of the enzyme observed in liver membranes from GaIN-treated rats cannot be excluded, although there was no increase in the number of β-receptors in liver membranes from CCl4-treated rats (6).

Both the stimulatory effect of catecholamine on adenylate cyclase of rat liver and the β-adrenergic effect on hepatic DNA synthesis are mediated via the β2-receptor subtype (7). Thus, the data presented here, taken together with our previous findings, suggest that the enhancement of β-adrenergic responsiveness, probably due to the increased function of the Gs protein, may have physiological significance in vivo, providing favorable conditions for regeneration and repair of the liver after hepatotoxin intoxication.
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