canine[2] and rat[21] parietal cells and between gastrin and histamine in dog and rabbit cell preparations[2,22]. Whether the different mechanisms of action – elevation of endogenous cAMP (histamine) and adenylate cyclase independent transmitter systems (gastrin and carbachol) – are involved in these events is not known. Anyway, according to this view and the concept of hexoprenaline as a partial histamine H_2-receptor agonist, potentiation of ^{14}C-AP uptake due to carbachol is conceivable. The cell preparations used consist of 20–30% of parietal cells. Therefore, we cannot exclude the possibility that hexoprenaline releases gastrin or histamine from endocrine nonparietal cells. The release of gastrin by adrenaline[23], as well as that of histamine by some adrenergic agents, has been reported[20]. In some of our experiments (data not shown), we used cells prepared from the whole rat stomach, including the antrum. In these preparations far more gastrin containing cells are available, but the results were similar. Therefore, it seems reasonable to suspect that release of gastrin does not contribute to potentiation between hexoprenaline and carbachol; however, more studies with enriched fractions of different pools of gastric mucosal cells are necessary to elucidate the mechanisms involved in the action of hexoprenaline on gastric cells. It may be concluded that β-adrenoceptor stimulation functionally modulates gastric acid secretion.

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Changes in hepatic glycosaminoglycans following endotoxin administration

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Summary. Incorporation of [35]S-sulfate into hepatic glycosaminoglycans (GAGs) is affected by intravenous administration of endotoxin. There are significant increases in total labeled glycosaminoglycans and in the percentage of labeled dermatan sulfate 72 h post-endotoxin.

Following intravenous administration of endotoxin, the classical systemic manifestations include an increase in vascular resistance[1,2] and capillary permeability as well as alterations in pulmonary ventilation and fever[3–5]. In addition, endotoxin has been shown to cause necrosis of liver tissue and hemorrhage with the infiltration of leukocytes[6,7]. Although the liver is the primary organ responsible for the detoxification and excretion of endotoxin, hepatocellular damage with decreased bile production occurs following endotoxin administration. Changes in the rate of synthesis and distribution of GAGs have been observed in different organs in response to tissue damage[8–13]. In carbon tetrachloride-induced liver disease, there is an increase in the synthesis of all GAGs and an increase in the relative amount of dermatan sulfate[9,14]. Alterations in the normal distribution of GAGs have also been noted in various forms of hepatic neoplasms[15,16]. It is the purpose of this study to investigate the effect of intravenous endotoxin administration on GAGs in the liver.

Materials and methods. Female Sprague-Dawley rats weighing 190–200 g and fed an unrestricted commercial diet were used throughout. Rats were injected i.v. with 0.5 ml of an endotoxin suspension consisting of 1 mg *E. coli* lipopolysaccharide (Sigma Chemical Co., St. Louis, MO) per 1 ml sterile 0.9% NaCl. They were labeled by i.p. injection of a total dose of 400 µCi [35]S-sulfate (New England Nuclear, Boston, MA), given in 2 parts: 0, 12, 48, and 72 h following endotoxin administration and again 12 h later. This procedure has been used to label glycosaminoglycans and measure their turnover rates both in vivo and in vitro[8,13,17].

Rats were sacrified by i.p. injection of chloral hydrate 12 h

Incorporation of ^{35}S in GAGs in the livers of rats following i.v. administration of endotoxin

Hours post endotoxin	Number of rats	Total counts/ mg liver	Relative ^{35}S incorporation (%) Heparin/ Heparan sulfate	Chondroitin 6-sulfate	Chondroitin 4-sulfate	Dermatan sulfate
0	6	182.9 ± 77.9	81.1 ± 6.62	5.1 ± 2.50	6.3 ± 1.92	7.4 ± 4.6
12	5	194.6 ± 42.0	78.8 ± 6.51	5.4 ± 1.57	6.3 ± 1.00	9.3 ± 4.38
48	3	329.9 ± 74.9	73.7 ± 3.69	6.6 ± 1.14	7.4 ± 0.15	13.6 ± 0.14
72	5	366.8 ± 72.2*	70.9 ± 5.5*	7.5 ± 2.71	7.4 ± 2.25	14.2 ± 1.86*

*, $p < 0.05$ (72 h vs 0 h).

after the last injection of [35]S-sulfate. The livers were resected and dissected free of systemic and portal vasculature. The hepatic parenchyma was washed in 0.9% NaCl, homogenized, dialyzed free of salts, lyophilized, and delipidated in 3 changes of acetone. Each sample was then digested with papain (Sigma Chemical Co., St. Louis, MO), activated with 5 mM cysteine HCl and 5 mM EDTA, in 0.1 N sodium acetate buffer, pH 5.5, at 57 °C. The digests were then precipitated with 10% TCA at 4 °C, the supernatants were dialyzed free of TCA and small polypeptides, and 3 mg of carrier chondroitin sulfate (Sigma Chemical Co., St. Louis, MO) was added to each supernatant. The supernatants were then lyophilized, resuspended in 2 ml water, and the GAGs were precipitated, following addition of 3 volumes of absolute ethanol, at 4 °C for 48 h. This method has been shown to recover 90% of the isolated glycosaminoglycans[8]. Aliquots of each sample were analyzed for total [35]S-sulfate incorporation. The labeled GAGs were then digested with chondroitinase ABC or AC-II (Miles Laboratories, Elkhart, IN) according to the method of Saito and coworkers[18]. The digests were chromatographed on Whatman 3MM filter paper in butanol, glacial acetic acid, and 1 N ammonia (2:3:1) for 18 h. The chromatograms were cut into 1-cm sections and analyzed for radioactivity in a liquid scintillation spectrometer. By these methods, [35]S-sulfate incorporation into total hepatic GAGs was determined as well as proportionate labeling of various subtypes (heparin/heparan sulfate, chondroitin 6-sulfate, chondroitin 4-sulfate, and dermatan sulfate).

Results. Total incorporation of [35]S-sulfate into liver glycosaminoglycans was measured before and at several intervals following intravenous administration of endotoxin. As shown in the table, there was an increase in the total incorporation of [35]S into glycosaminoglycans beginning as early as 12 h post-endotoxin administration and continuing throughout the 72-h period studied. There was a significant correlation between progression of the liver injury, as measured by time, and total GAG labeling ($r = 0.81$; $p < 0.001$).

Analysis of hepatic tissue from control animals not injected with endotoxin revealed a relative incorporation of [35]S of 81.1% into heparin/heparan sulfate, 5.1% into chondroitin 6-sulfate, 6.3% into chondroitin 4-sulfate, and 7.4% into dermatan sulfate. Over the 72-h period studied, the relative incorporation of [35]S-sulfate into heparin/heparan sulfate steadily decreased (table). There was no significant change in the relative distribution of [35]S in chondroitin sulfate (6-S or 4-S); however, the relative incorporation of [35]S into dermatan sulfate increased to 14.2 ± 1.86% from a control value of 7.4 ± 4.60% (table). This latter increase was statistically significant ($p < 0.05$); moreover, there was a significant correlation between labeling of dermatan sulfate and the time interval following liver injury ($r = 0.88$; $p < 0.001$).

Discussion. Freudenberg and co-workers have documented hepatic necrosis and hemorrhage with leukocyte infiltration following i.v. endotoxin administration[6], while others have postulated that repeated exposure to endotoxin may result in hepatic fibrosis[7]. In other models of hepatic fibrosis, investigators have noted an increase in all GAGs, with a proportionately greater increase in the amounts of dermatan sulfate[9,12,14]. In the present studies, a significant increase in [35]S-labeling of all glycosaminoglycans was noted, along with a significant increase in the relative proportion of [35]S incorporated into dermatan sulfate. It should be noted that differences in the pool-size of the radioactive sulfate between control and experimental animals could possibly account for the changes in total label incorporation observed in this study. However, the differences noted in the proportionate labeling of the various subtypes of glycosaminoglycans should be independent of any such pool-size alterations.

A similar pattern of [35]S-labeling of glycosaminoglycans was found in experimental models of pulmonary fibrosis[8,13], including rapid increases in total [35]S-GAGs and proportionate increases in [35]S-dermatan sulfate. Experimental endotoxin lung injury[19] also resulted in increases in total incorporation of [35]S into GAGs as well as proportionate increases in labeling of both dermatan sulfate and chondroitin 4-sulfate. The consistent finding of increased dermatan sulfate suggests that this particular glycosaminoglycan may play an important role in tissue repair.

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