Liver injury after an aggressive encounter in male mice

Olga Sánchez,* Meritxell Viladrich,† Ignasi Ramirez, and Maria Soley

Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

Submitted 14 February 2007; accepted in final form 28 August 2007

Liver injury after an aggressive encounter in male mice. Am J Physiol Regul Integr Comp Physiol 293: R1908–R1916, 2007. First published August 29, 2007; doi:10.1152/ajpregu.00113.2007.—Acute and intense psychological stressors induce cell damage in several organs, including the heart and the liver. Much less is known about social stress. In male mice, aggressive behavior is the most common social stressor. It is remarkable that upon fighting, submandibular salivary glands release a number of peptides into the bloodstream including epidermal growth factor (EGF). We showed previously that released EGF protects the heart from cell damage in this particular stressful situation. Here, we studied the effect of an aggressive encounter on the liver and whether EGF has a similar effect on this organ. An aggressive encounter in male mice caused inflammatory response and a transient increase in plasma alanine and aspartate transaminase activities. At 3 h, focal infiltration of neutrophils was observed in liver parenchyma. These cells accumulate on eosinophilic hepatocytes, which may correspond to dying cells. A few hours later, evidence of necrotic lesion was observed. Surgical excision of submandibular glands, sialoadenectomy, did not prevent the rise in plasma EGF concentration and did not affect the increase in plasma transaminase activities. Neither did the administration of tyrphostin AG-1478 (inhibitor of EGF receptor kinase) alter the increase in plasma alanine transaminase activity. However, it did enhance the rise in both aspartate transaminase and creatine kinase activity, suggesting heart damage. We conclude that an aggressive encounter causes mild liver damage and that released EGF does not protect this organ, in contrast to its effect on the heart.

corticosterone; tumor necrosis factor-α; interleukin-6; Kupffer cells; liver glycogen

THE RELATIONSHIP BETWEEN STRESSFUL life and health is well documented (6). Among the many consequences of chronic stress, it was shown that it is associated with exacerbated stroke outcome in mice (20), with increased liver metastasis (57), increased susceptibility to endotoxic shock (44), and impaired antiviral immunity in wounded animals (18). Not only is chronic stress unhealthy, also short-term stress episodes may damage some organs.

Acute and intense psychological stressors induce gastric ulceration and heart injury in rodents (29, 47). Several acute stressors alter also liver structure. Thus, stressors as different as restraint and forced exercise induce formation of autophagic vacuoles among several ultrastructural modifications (46). This is associated with DNA oxidative damage (2), lipid peroxidation (30), protein oxidation (35), and, ultimately, the loss of hepatocyte integrity, as indicated by the rise in plasma transaminase activities (22). All these alterations may be caused by catecholamines. Sustained elevation of plasma noradrenaline by means of miniosmotic pump implantation in peritoneal cavity causes hepatocyte injury and depresses liver function (56). In recent years, it became clear that liver injury, caused by stress, is the consequence of an inflammatory response (51). Both physical and psychological stressors elevate plasma IL-6 (58) and increase hepatic IL-6 expression (28).

Acute social stress insults, aggression between males to establish dominance, is a very common stressor in laboratory mice. Few studies addressed the consequences of such a behavior on organ integrity. Matte (37) reported a rise in plasma creatine kinase in regrouped male mice fighting for several hours, we could not reproduce such an increase, although we did observe a moderate increase in plasma lactate dehydrogenase and transaminase activities (47).

Since Bing and Poulsen (9) described that aggressive behavior in mice rises renin concentration in plasma, several laboratories have studied the influence of such behavior on other releasable submandibular salivary gland peptides. Thus, in 1981, Nexo et al. (39) reported a similar increase in plasma epidermal growth factor (EGF), and in 1986, Aloe et al. (3) published their results on the increase in plasma nerve growth factor (NGF) in fighting related to male mice regrouping. The biological role of these submandibular factors in this particular stressful condition has not been studied in detail. In a nonfighting model of psychosocial stress, Lee et al. (33) concluded that the hypertensive response was due almost exclusively to control of the renin-angiotensin system. Concerning the role of NGF, it was suggested that it may be involved in the survival and differentiation of immature progenitor cells in the brain (23), as well as in the basal activity of nerve, immune, and endocrine cells (4).

Some roles of the EGF secreted during fighting episodes may be indirectly suggested. It may accelerate wound healing (5, 12), and it may protect the gastrointestinal tract from ulcerogenesis. In fact, such a role in the acute psychological stress model of combined water immersion and restraint was reported (11, 29). We directly addressed whether EGF secreted during aggressive encounters in mice protects the heart. We found that the inhibition of EGF receptors with tyrphostin AG-1478 made intruder mice susceptible to suffer heart lesions after an aggressive encounter with a resident mouse (42).

Mouse liver contains the largest number of EGF receptors described in nontransformed cells (50). A protective function against alcohol-induced injury (19) and LPS-induced hepatitis (16) was reported. Therefore, we decided to 1) characterize lesions produced in the liver by aggressive encounters and 2) to study whether secreted EGF protects the liver as it does in the heart.

* Olga Sánchez and Meritxell Viladrich contributed equally to this article and so should be considered joint main authors.

Address for reprint requests and other correspondence: M. Soley, Dept. de Bioquímica I Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, 08028-Barcelona, Spain (e-mail: msoley@ub.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MATERIAL AND METHODS

Animals

Adult Swiss-CD1 mice were obtained from Interfauna (Barcelona, Spain). All animals were male (35–40 g), fed ad libitum, and kept under a constant 12:12-h light-dark cycle (lights on at 0800) and in controlled conditions of humidity (45–55%) and temperature (23 ± 1°C). All experimental procedures were approved by the Committee on Animal Care of the University of Barcelona and by Conselleria de Ramadera i Pesca, Generalitat de Catalunya.

Aggressive Encounters

After a period of adaptation to the animalarium, mice were kept for 15 days in individual cages (14 dm²). On the day of the experiment (starting at 9 AM), each mouse (the intruder) was introduced into the cage of an older (and bigger) mouse (the resident), which had been isolated in smaller cages (7.5 dm²) for at least 21 days. Residents had been tested for aggressiveness before the experiment. The encounter lasted for up to 3 h, time enough to establish dominance relationship.

Usually, the intruder acquired submissive status showing a passive behavior in a corner of the cage. On the contrary, the resident became dominant moving constantly around the cage. Intruders were either immediately killed or returned to their home cages. In some experiments, mice were either sialoadenectomized or sham-operated (24) 1 wk before isolation. In another experiment, intruder mice received an intraperitoneal injection of the EGF receptor inhibitor tyrphostin AG-1478 (Calbiochem, Merck Eurolab, Granada, Spain) (0.25 mg/kg dissolved in 10% DMSO) 20 min before the experiment (42). In another experimental series, intruder mice received anti-EGF antibodies by either an sc injection of 0.15 ml of rabbit anti-mEGF serum 48 h before the experiment, or an intraperitoneal injection of 0.1 ml of rabbit anti-mEGF serum 2 h before the experiment. Rabbit serum to mEGF was obtained as in (24).

Endotoxic Shock After an Aggressive Encounter

To study whether an aggressive encounter affected the response to an endotoxic shock, mice were isolated as indicated above and exposed or not (control animals) to a 2-h aggressive encounter. Then, the animals were returned to their home cages, and 1 h afterward, they received an intraperitoneal injection of bacterial LPS (from Salmonella abortus equi, Sigma-España, Madrid, Spain) (0.625 µg/kg combined with d-(+)-galactosamine (Calbiochem, Merck, Barcelona, Spain) 750 mg/kg). Eight hours after the burst, animals were killed to obtain samples. Preliminary experiments showed that alanine transaminase (ALT) reached the highest activity 8 h after the burst and decreased thereafter (not shown).

Sampling and Analysis

To obtain samples, mice were anesthetized (sodium pentobarbital 60 mg/kg). Blood was collected into heparinized syringes from the inferior vena cava. Blood plasma was obtained by centrifugation. A sample was processed as indicated (24) for EGF quantification. Lactate dehydrogenase, aspartate aminotransferase, alanine amino-transferase, and creatine kinase activities in plasma were determined as previously described (47). Plasma corticosterone was determined as indicated (43). Plasma TNF-α and IL-6 concentration was determined by ELISA using commercial kits (Biosource International, Camarillo, CA). Immediately after bleeding, the liver was perfused in situ with PBS. Then, an ~500 mg piece was fixed in 4% paraformaldehyde and embedded in paraffin to obtain 7-µm-thick sections. The rest of the liver and submandibular salivary glands were frozen in liquid nitrogen, and stored at −80°C until further processed (in less than 1 wk).

In some experiments, parotid and thyroid glands, kidneys, heart, and a sample of skeletal muscle from the leg were excised and stored at −80°C. Tissues were homogenized in PBS. After centrifugation (100,000 g for 60 min at 4°C), the supernatant was stored at −40°C for EGF quantification (24). To study whether heparin-binding EGF-like growth factor (HB-EGF) interferes with the EGF assay, HB-EGF (Sigma-Aldrich España, Tres Cantos, Madrid) was dissolved in PBS containing 0.1% BSA. Serial dilutions were prepared and analyzed for competition with adsorbed EGF for binding to EGF-antiserum (24).

Macrophage (Kupffer Cell) Staining in Liver Sections

Seven-micrometer liver sections were collected on glass slides coated with gelatin. After deparaffination and hydration, the slides were rinsed in PBS (3 × 5 min). To inactivate endogenous peroxidase, the slides were incubated (10 min) at room temperature with 0.5% H2O2 in PBS containing 5% Triton X-100 and 10% methanol.

After rinsing (4 × 5 min in PBS and 5 min in PBS containing 0.2% Triton X-100), we blocked nonspecific binding sites for 2 h at room temperature in a humidified chamber, with 10% goat serum (Gibco-BRL, Invitrogen, El Prat de Llobregat, Spain) in PBS containing 0.5% Triton X-100, 0.2 M glycine, and 0.2% gelatin. Slides were rinsed briefly in 0.1 M Tris-HCl pH 7.6 and incubated with the primary antibody [rat anti-rat IgG conjugated with horseradish peroxidase, mouse adsorbed (AbD-Serotec, Bionova), diluted 1/25 in PBS containing 10% goat serum, 0.5% Triton X-100, 0.2% gelatin, and 0.02% sodium azide] overnight at 4°C in a humidified chamber. After rinsing (2 × 5 min in 0.2 Triton X-100 in PBS), sections were incubated with the secondary antibody [goat anti-rat IgG] (AbD-Serotec, Bionova), diluted 1/50 in PBS containing 10% goat serum, 0.5% Triton X-100, 0.2% gelatin, and 0.02% sodium azide] and immediately counterstained with methyl green. After a brief dehydration process, the slides were mounted in DPX. Sections were analyzed in a Zeiss Axiosstar-Plus microscope.

Neutrophil Staining in Liver Sections

Seven-micrometer liver sections were collected on glass slides coated with poly-l-lysine. After deparaffination and hydration, the slides were incubated (2 × 30 min) in 0.34% NaBH4 to reduce basal fluorescence and rinsed in PBS (3 × 5 min). Liver sections were permeabilized (10 min at room temperature in trypsin-EDTA solution; Gibco-BRL, Invitrogen) and rinsed (2 × 5 min in PBS and 5 min in 0.2% Triton X-100 in PBS). Sections were blocked (2 h at room temperature in a humidified chamber) with 10% goat serum in PBS containing 0.52 M NaH2PO4, 0.025% 3’3’-diaminobenzidine and 0.17% nickel ammonium sulfate. The reaction was stopped with 0.1 M Tris-HCl pH 7.6 and immediately counterstained with methyl green. After a brief dehydration process, the slides were mounted in DPX. Sections were analyzed in a Zeiss Axiosstar-Plus microscope.

Statistical Analysis

All results are the means ± SE of the number of animals indicated in each figure. The statistical significance of differences was determined by one-way or two-way ANOVA and post hoc Tukey’s test, depending on the experimental design.
RESULTS

An aggressive encounter induced an activation of the hypothalamus-pituitary-adrenal axis in mice as shown by the increase in plasma corticosterone concentration (Fig. 1). There was also a transient increase in plasma TNF-α during the encounter, although the large variation found at 30 min made differences nonsignificant. However, there was a progressive increase in plasma IL-6 and in transaminase activities (AST and ALT). If mice were returned to home cages, plasma AST and ALT activities remained high for several hours. But 24 h after the encounter, these activities were as low as controls (Fig. 2).

Histological examination of liver sections stained with hematoxylin-and-eosin revealed that, as early as 3 h but also 8 h after the encounter, there were scattered small focal points of infiltrated cells on single hepatocytes (Fig. 3D) or on a small group of cells (Fig. 3E). These focal points were more frequently observed near the edge of the lobe. After 8 h, large necrotic areas near the lobe edge (Fig. 3F) were also observed in three out of five animals.

It is known that polymorphonuclear leukocytes (neutrophils) are rapidly recruited on dying hepatocytes and cooperate in both apoptotic and necrotic death (27). We observed that apoptotic and necrotic death processes were as low as controls (Fig. 4).

Quantitative analysis of dying hepatocytes (number of leukocyte infiltration focal points) in hematoxylin-and-eosin-stained sections showed that one out of 345,000 hepatocytes (we estimated a mean 9,000 hepatocytes per low-powered field) undergoes a death process in the liver of control mice. This number increased six-fold early after an aggressive encounter and remained high several hours afterward (Fig. 4D), which means that the number of dying hepatocytes did not exceed 1 out of 32,000.

Aggressive behavior markedly increases EGF concentration in plasma of male mice (32, 39, 47). We previously demonstrated that such a high EGF concentration protects the heart from suffering injury (42). Therefore, we studied next whether EGF has a similar role in the liver. To determine whether EGF from submandibular glands has such a role in the liver, we studied the effect of an aggressive encounter in control (sham-operated) and sialoadenectomized mice. Sialoadenectomy affected neither plasma corticosterone rise nor liver glycogen depletion (Fig. 5). We studied also the effect of sialoadenectomy on the increase of both IL-6 concentration and transaminase activities in plasma (Fig. 6). Surgical removal of submandibular glands 3 wk before the aggressive encounter had no effect on these parameters.

As expected, the aggressive encounter decreased submandibular glands EGF content in sham-operated control mice (Fig. 7). Unexpectedly, we did not find significant differences in either plasma or liver EGF concentration between sham-operated and sialoadenectomized mice. We had demonstrated that our ELISA did not cross-react with nerve growth factor or transforming growth factor-α (24). We extended the analysis to the closely related HB-EGF and found that it did not interfere with the EGF assay either (data not shown).

To study whether sialoadenectomy altered the distribution of EGF in tissues, we obtained samples of plasma, urine, parotid and thyroid glands, liver, kidneys, heart, and skeletal muscle from either sham-operated or sialoadenectomized mice 3 wk after surgery. In these fluids and tissues, we measured EGF concentration. Results are shown in Fig. 8. Three weeks after surgery, plasma and urine levels of EGF were similar in sham-operated and sialoadenectomized mice. EGF content in parotid glands was lower in sialoadenectomized than in control animals. On the contrary, EGF content in thyroid glands and kidneys was higher in sialoadenectomized than in control mice.
Sialoadenectomy did not modify EGF content in liver, heart, and skeletal muscle.

Next, we studied the effect of the EGF receptor inhibitor tyrphostin AG-1478 (34). We had shown that AG-1478 blocked the EGF protective effect on heart injury induced by combined restraint and cold exposure in mice (42). Administration of AG-1478 at the time of the aggressive encounter did not modify the rise in plasma corticosterone or the decrease in liver glycogen (data not shown). The rise in plasma ALT was not affected by tyrphostin administration (Fig. 9). However, tyrphostin AG-1478 administration did result in an enhanced rise in both plasma AST and CK.

We studied also the effect of the administration of EGF antibodies before the aggressive encounter. ALT activity in plasma of nonfighting mice was 0.36 ± 0.04 nkat/ml. The injection of EGF antibodies (sc or ip) did not modify the effect of the aggressive encounter: 0.74 ± 0.04 nkat/ml in preimmune serum-injected mice; 0.75 ± 0.11 nkat/ml in subcutaneously injected mice; and 0.62 ± 0.04 nkat/ml in intraperitoneally injected mice.

Finally, we studied whether an aggressive encounter affected the response to an endotoxin shock. Eight hours after the endotoxin shock, plasma ALT activity was increased in both nonexposed and exposed mice (Fig. 10). However, the increase was higher in exposed (20-fold: from 0.5 to 10.8 nkat/ml) than in nonexposed (8-fold: from 0.3 to 2.5 nkat/ml) mice.

**DISCUSSION**

Aggressive encounter induces liver injury in male mice. We had found that fighting raised plasma transaminase activities in male mice (47). Here, we directly show that, indeed, an aggressive encounter induces necrotic lesions in liver parenchyma.

Circulating neutrophils infiltrate liver parenchyma in response to the production of inflammatory mediators (27). We observed a moderate, nonsignificant, and transient increase in plasma TNF-α, but a more sustained rise in plasma IL-6. As early as 3 h after the initiation of the aggressive encounter, we observed neutrophil infiltration surrounding damaged hepatocytes. This is an early stage in the death process of hepatocytes. Neutrophils contribute to hepatocyte death, inducing oxidative stress (27). Damaged hepatocytes may end in apoptotic or necrotic death (36). Histopathological observations showing...
extension of the death process to neighboring cells and the rise in plasma transaminase activities indicate that necrosis is the end stage of the death process induced by an aggressive encounter. However, this damage is moderate and transient because the number of dying hepatocytes did not exceed 0.003% of total hepatocyte population. In accordance, both AST and ALT activities in plasma dropped to control values 21 h after intruder mice returned to their home cages.

Other stressful situations, including restraint (46) or tourniquet shock (55), induce liver damage. It appears that it always involves oxidative damage of cellular components, which is in agreement with the role of neutrophil recruitment in response to inflammatory mediators. A pivotal role of TNF-α production from Kupffer cells in the initial inflammatory response and thus in liver injury is well known (48). In keeping with this role of Kupffer cells, inhibition of these cells by gadolinium chloride prevented most of the damage induced in the liver by tourniquet shock (55). However, the precise mechanisms that induce activation of Kupffer cells in stressful situations are not completely understood. It was suggested that release catecholamines from autonomic nerve endings and from the adrenal medulla may be involved in the inflammatory response to stress in the liver (51). In fact, norepinephrine induces Kupffer cells to release TNF-α (59), and hepatic inflammation induced by carbon tetrachloride is attenuated by spinal transection or by administration of adrenergic blocking drugs (10). The other major arm of the stress response, the hypothalamic-pituitary-adrenal axis, appears to have an opposing effect since glucocorticoids were shown to be potent anti-inflammatory agents (53).

EGF does not protect the liver against aggressive encounter-induced injury. Aggressive encounters between male mice induce the largest increase in plasma EGF concentration ever reported (32, 39, 47). Other submandibular gland peptides like renin (9) and NGF (3, 32) are also released. There is a good correlation between renin and EGF release (39) and also between EGF and NGF secretion not only upon aggressive encounter (32) but also in circadian variation and after secretagogue-induced synthesis (49). Therefore, it is conceivable that all of these peptides follow a common route of secretion.

Fig. 4. Evidence of neutrophil recruitment on dying hepatocytes. We obtained serial slices of livers from animals exposed for 3 h to aggressive mice. Some animals were returned to their home cages and killed 5 h later. Several focal points of infiltrated leukocytes were identified in hematoxylin-and-eosin-stained sections (A1, B1, and C1) and analyzed for the presence of neutrophils (A2, B2, and C2) and macrophages (C3). D: number of dying hepatocytes (eosinophilic hepatocytes surrounded by leukocytes) were counted in 20 low-powered fields (×100 magnification) per slice. Four slices per animal were counted. Results are the means ± SE of five animals per group. The significance of the differences vs. control animals (zero time) was tested by one-way ANOVA and post hoc Tukey’s test. **P < 0.01.

Fig. 5. Effect of sialoadenectomy on the response of plasma corticosterone and liver glycogen during an aggressive encounter. Mice were sialoadenectomized (solid dots) 1 wk before isolation (15 days). Control animals were sham-operated (open dots). The animals were exposed to an aggressive encounter and killed at the indicated times. Plasma corticosterone (CS) and liver glycogen were determined. Results are the means ± SE of 12 animals per group. Two-way ANOVA indicated that there were no significant differences between sham-operated and sialoadenectomized mice.
We did not study the relationship between episodes of aggression and EGF concentration, but Aloe et al. (3) have found a positive correlation between the number of fighting episodes and plasma NGF concentration. It suggests that repeated stimulus results in a progressive accumulation not only of NGF, but also EGF and renin. This may explain why plasma EGF concentration after a prolonged aggressive encounter is much higher than after other stressful situations like immobilization (22). The main objective of our study was to determine whether this high amount of EGF in plasma protects the liver, as was found for the heart (42).

Sialoadenectomy is a tool for studying EGF function in whole animals (22, 25, 40, 52). As it was reported that sialoadenectomy prevented the rise of plasma EGF (32, 39), NGF (3, 32), and renin (8, 39) after aggressive encounters, we decided to study the effect of sialoadenectomy on the response to an aggressive encounter. Surprisingly, plasma EGF in sialoadenectomized mice reached even higher concentration, although differences with sham-operated mice were nonsignificant. We had reported that in our assay system neither NGF nor TGF-α cross-react with EGF. Here, we show that HB-EGF does not do so either. Therefore, the EGF measured in sialoadenectomized plasma may actually correspond to EGF secreted from some extra-submandibulary gland sources. We have found that EGF content in thyroid gland and kidneys (but not in liver, heart, and skeletal muscle) is higher in sialoadenectomized than in control mice. This is in keeping with previous reports (17, 43). On the contrary, the EGF content in parotid glands is lower in sialoadenectomized than in sham-operated mice, perhaps because of an enhanced secretion to saliva in those mice lacking submandibular glands. Our results suggest that not a single, but several organs, may take over the function of submandibular salivary glands as a source of plasma EGF.

Some experimental details may explain why we found a rise in plasma EGF in sialoadenectomized mice. Although in normal conditions, the rise in plasma EGF upon adrenergic stimulation depends on the release from submandibulary salivary glands in male mice (24). Tuomela (54) showed that in mice sialoadenectomized 30 days before the experiment, adrenergic stimulation raised plasma EGF but at a slower rate: it required 90 min to reach maximum concentration, while it took only 10 min in sham-operated mice. In previous studies (3, 32, 39), experiments were performed just 10 days after sialoadenectomy, and aggressive encounters lasted for 20 min. In none of these studies was a rise in plasma EGF or NGF concentration observed in sialoadenectomized mice. However, our experiments were performed 21 days after surgery, and aggressive encounters were prolonged to 3 h.

The liver contains the largest number of EGF receptors and is responsible for the clearance of EGF from plasma (26, 50). A decrease in clearance rate might explain the rise of plasma EGF concentration in sialoadenectomized mice during the aggressive encounter. Although our results concerning EGF content in the liver during the aggressive encounter argue against this hypothesis, more specific studies involving analysis of EGF receptor number and clearance rates are required to directly address this question.

The lack of effect of sialoadenectomy on the rise in plasma transaminase activities after aggressive encounter might be attributable to the rise in plasma EGF released from unknown extra-submandibulary gland sources. Therefore, to overcome this difficulty, we designed two additional experiments: administration of either the EGF receptor inhibitor tyrphostin AG-1478 or anti-EGF antiserum. AG-1478 was shown to be effective in blocking the effect of EGF on heart protection (42). We observed that AG-1478 induced a rise of creatine kinase...
and AST in plasma after an aggressive encounter. This suggests that the heart is affected by blockade of EGF receptor and points to the relevant role of EGF receptor in this organ.

Antiserum to EGF was shown to be effective in studies on the role of EGF in adipose tissue (1, 31). Because both experiments showed that neither treatment altered the rise in plasma ALT activity, we may conclude that EGF does not protect the liver from acute damage induced by prolonged aggressive encounters in male mice. This is in contrast to the heart or gastric mucosa in which EGF protects against acute stress-induced injury (11, 42). However, a lack of liver protection by exogenous EGF administration was also described by Caballero et al. (14) in a model of thioacetamide-induced multiorgan failure. These authors observed however, that exogenous EGF did protect the small intestine and kidneys.

Although our results show that EGF does not prevent necrotic cell death induced in the liver by an aggressive encounter, several reports showed that it does protect against TGF-β-induced (45) or Fas-induced (21, 38) apoptotic death. In agreement, we have observed a transient increase in hepatocyte apoptosis coincident with a transient disappearance of EGF shortly after salivary glandectomy (13). This suggests that EGF may protect the liver against some insults but not against others. The mechanisms beneath the selectivity of the protective role of EGF remain to be clarified.

**Perspectives and Significance**

Although the effect of an aggressive encounter inducing liver damage is mild, it predisposes to the toxic effect of bacterial LPS. Recent reports show that a variety of acute stressors (electric foot shock, restraint) and chronic stressors (social disruption) enhance xenobiotic-induced hepatotoxicity (15, 41, 44). These previous publications and our results suggest that stress of any type may exacerbate liver diseases. Our and previous studies leave many questions unresolved that deserve further investigation: How long does the effect of stress persist? Does stress predispose the liver also to tumor development? Does stress sensitize the liver to the toxic effect of low-dose long-lasting potential hepatotoxins (e.g., many pollutants)? Answers to these and other questions may help us understand an individual’s predisposition to liver diseases.
ACKNOWLEDGMENTS

We thank Robin Rycroft for editorial help.

GRANTS

This study was supported by Grants PB97-0936 and BF12002-03037 from the Dirección General de Enseñanza Superior e Investigación Científica, Ministerio de Educación y Ciencia, Spain.

REFERENCES


AGGRESSION-INDUCED LIVER INJURY

R1915

AJP-Regul Integr Comp Physiol • VOL 293 • NOVEMBER 2007 • www.ajpregu.org