Angiotensinogen: An Acute-Phase Protein?

Meinhard Soden, Christoph Klett, Thomas Hasmann, Eberhard Hackenthal

Abstract
Angiotensinogen has been assumed to be an acute-phase protein, because some forms of acute inflammation, e.g., the injection of lipopolysaccharide or cellite or partial heptectomy, increased the hepatic synthesis of angiotensinogen. In addition, the well-characterized nephrectomy-induced stimulation of angiotensinogen was thought to represent an acute-phase reaction. To evaluate this hypothesis, we examined changes in angiotensinogen secretion by the isolated perfused rat liver after the systemic administration of turpentine or lipopolysaccharide as well as in response to nephrectomy or sham nephrectomy. Comparison was made with the secretion of two typical acute-phase proteins, α1-acid glycoprotein and α2-macroglobulin, and with the secretion of the negative acute-phase protein albumin. All forms of experimental surgery stimulated the secretion of both control acute-phase proteins several-fold. In contrast, the response of angiotensinogen was not uniform; lipopolysaccharide and bilateral nephrectomy stimulated secretion twofold to threefold, sham nephrectomy had no effect, and turpentine decreased the secretion to 30% of the control level. A similar inhomogeneity was found in an additional experiment performed to analyze the direct effects of interleukin-1 or interleukin-6 on the secretion of angiotensinogen by freshly isolated hepatocytes. Interleukin-6 increased but interleukin-1 decreased the mRNA and secretion of angiotensinogen, whereas both cytokines increased the secretion of both acute-phase proteins. Because of this nonuniform behavior of angiotensinogen, it is premature to classify angiotensinogen as an acute-phase protein until a specific function for angiotensinogen during acute inflammation is known. (Hypertension. 1994;23[suppl I]:I-126-I-130.)

Key Words • isolated perfused liver • hepatocytes • turpentine • lipopolysaccharide • acute-phase proteins • interleukins • angiotensinogen • albumin

The acute-phase reaction is the first overall response of the organism to acute or chronic tissue injury and is characterized by the secretion of several cytokines derived from activated macrophages, monocytes, Kupffer cells, and lymphocytes. These cytokines activate defense mechanisms, such as the upregulation of body temperature, stimulation of gluconeogenesis, and the activation of B and T lymphocytes, which promote the local inflammatory reaction while at the same time assisting the organism in limiting the consequences of local injury and in initiating defense and repair mechanisms. Another important cytokine-mediated response is the induction of constitutively secreted acute-phase proteins (APPs) by the liver. Because many of these APPs are protease inhibitors, the hepatic acute-phase response is thought to be essential for the limitation of an inflammatory process by inhibiting the enzymes that mediate or contribute to the inflammatory reaction. For some APPs, other specific functions, e.g., transport, radical-scavenger, or antioxidant properties, have been described. Sufficient and necessary characteristics for an APP are (1) the induction of its synthesis and (2) a specific function during inflammation.1-5 Bing2 was the first to implicate angiotensinogen, a major component of the renin-angiotensin system, in the acute-phase response because several forms of experimental inflammation, such as the injection of lipopolysaccharide (LPS) or of cellite, or partial heptectomy increased the plasma concentrations of angiotensinogen. This hypothesis was supported by several related observations, such as an increase in hepatic angiotensinogen mRNA after LPS injection or nephrectomy,7 increased plasma angiotensinogen concentrations during chronic infections in humans,8 and an amino acid homology between angiotensinogen and certain APPs with serine protease inhibitory function.9 Conversely, the response of angiotensinogen secretion has not yet been examined in experimental models of an acute-phase reaction other than that induced by LPS injection. Furthermore, there is as yet no indication that angiotensinogen has protease inhibitory properties or that it plays a role in the inflammatory process. To elucidate a possible participation of angiotensinogen in an acute-phase response, we investigated the changes in plasma concentrations and hepatic secretion rates of angiotensinogen in response to two generally accepted experimental inflammatory models: the injection of LPS or turpentine. For comparison, the response of α1-acid glycoprotein (AGP) and α2-macroglobulin (AMG), two well-characterized APPs used as standards in the rat,1-5 and the response of albumin, a so-called negative APP, were examined. In addition, we analyzed the hepatic secretion rates and plasma concentrations of angiotensinogen, albumin, AGP, and AMG in nephrectomized and sham-nephrectomized rats to determine whether it is the loss of the kidneys or the surgical trauma that induces the stimulation of angiotensinogen secretion in response to nephrectomy. Because interleukin-1 (IL-1) and interleukin-6 (IL-6) have been identified as important cytokines for the acute-phase response of the liver, the direct effects of IL-6 and IL-1 on the secretion of AGP, AMG, and angiotensinogen were also examined in freshly isolated rat hepatocytes in vitro.

Methods

Animals
Experiments were performed on male Sprague-Dawley rats (Ivanovas, Kifelleg, Germany) weighing 180 to 240 g. They were kept on a standard diet and had free access to water.
Pretreatment of Animals

Animals either were pretreated with LPS (Escherichia coli 055:B5, Sigma Chemical Co, St Louis, Mo, 500 μg/kg IP) or turpentine (DAB 9, 5 mL/kg SC) or were subjected to bilateral nephrectomy or sham nephrectomy. At 0, 12, 24, 36, or 48 hours, six animals each were anesthetized by injection of 60 mg/kg IP pentobarbital sodium, and blood was collected from the tail vein into EDTA (0.2% final concentration) for the estimation of angiotensinogen, albumin, AGP, and AMG. Subsequently, the livers were prepared and connected to a perfusion system to determine the secretion rates of angiotensinogen, albumin, AGP, and AMG.

Isolated Liver Perfusion

Liver preparation and perfusion were performed as described previously.10 Livers were isolated and perfused for 5 minutes with a single-pass system at a flow rate of approximately 35 mL/min with a modified minimal essential medium (MEM with Earle’s salt mixture, Serva, Heidelberg, Germany). Subsequently, the liver was perfused for 4 hours with a recirculating system with 100 mL oxygenated MEM. At intervals of 1 hour, perfusate samples were taken to estimate concentrations of angiotensinogen, albumin, AGP, and AMG.

Preparation of Hepatocytes and Incubation Conditions

Hepatocytes were isolated by the collagenase technique of Seglen11 with several modifications described previously.10 Liver parenchymal cells were separated from endothelial and Kupffer cells by a Percoll density-gradient centrifugation. The hepatocyte fraction was resuspended in MEM to a density of 1.5x10^6 cells/mL and incubated in 30-mL aliquots in 400-mL round-bottomed flasks under standard conditions. At hourly time intervals, aliquots were taken for the estimation of angiotensinogen and of both APPs in the cell-free supernatant and of angiotensinogen mRNA in the cell pellet.

Analytic Procedures

Angiotensinogen mRNA

Total RNA was isolated either from hepatic tissue samples (3 g) taken from separate animals that had not been included in the perfusion experiments or from isolated cells (2.3x10^6 cells). RNA was extracted by LiCl/urea, and the content of angiotensinogen mRNA was determined routinely by liquid hybridization using a 716-nucleotide [32P]uridine triphosphate–labeled cRNA of angiotensinogen as described in detail by Hellmann et al.12 The integrity of the RNA preparations was checked on ethidium bromide agarose gels. Quantification was performed by liquid scintillation and comparison to a standard calibration curve. Additionally, with a representative number of RNA samples, an RNase protection assay was performed using a [32P]uridine triphosphate–labeled cRNA of 290 nucleotides (from vector pRAG03) for liquid hybridization. Subsequently, aliquots were subjected to proteinase K digestion (125 μg per tube, 15 minutes, 37°C) and were run on a 5% polyacrylamide gel containing 7 mol/L urea.

Angiotensinogen

Angiotensinogen concentrations in liver perfusates and in hepatocyte supernatants were measured indirectly by radioimmunoassay of angiotensin I after quantitative conversion of angiotensinogen to angiotensin I by an excess of hog renin, as described previously.10 Subsequently, the livers were prepared and connected to a recirculating system with 100 mL oxygenated MEM. At intervals of 1 hour, aliquots were subjected to proteinase K digestion (125 fig per tube, 15 minutes, 37°C) and were run on a 5% polyacrylamide gel containing 7 mol/L urea.

Acute-Phase Proteins and Albumin

APPs were analyzed by an enzyme-linked immunosorbent assay as described recently.14 Both APPs were isolated from plasma of rats injected with turpentine 20 hours previously. Polyclonal antibodies were obtained by immunization of New Zealand White rabbits. Concentrations were calculated from linear standard calibration curves. Albumin concentrations were calculated by a specific radioimmunoassay according to Stuzmann et al.13

Results

Effects of Experimental Inflammatory Surgery

Ex Vivo

Hepatic secretion rates of angiotensinogen, albumin, and APP were determined by the isolated perfused liver system. Livers were taken at 12, 24, 36, and 48 hours after treatment and connected to a recirculating perfusion system. During an observation period of 4 hours, the cumulative perfusate concentrations of both APPs as well as of angiotensinogen and albumin were determined. The highest deviations from control levels were found for AGP at 36 hours, for AMG at 24 hours, for albumin at 36 hours, and for angiotensinogen at 12 hours. Fig 1 demonstrates the maximal stimulatory or inhibitory responses of the secretion rates after the various types of experimental inflammation for AGP (Fig 1A), AMG (Fig 1B), albumin (Fig 1C), and angiotensinogen (Fig 1D). Under all conditions examined—LPS, turpentine, nephrectomy, or sham nephrectomy—the plasma concentrations of AGP and AMG increased significantly, although they differed in the magnitude of the response and in the time course. Control livers showed an AGP secretion of 51±5 fmol/ml liver. Turpentine, LPS, and nephrectomy induced similar peak increments in the secretion rates of AGP (12-, 10-, and 7-fold, respectively, P<.005 versus control rates), whereas in 17-hour sham-nephrectomized rats, only a 4.1-fold increase was observed (P<.005). The corresponding stimulation rates for AMG (control, 3±2 fmol/mg liver per hour) were 41-fold in turpentine-treated rats (P<.005), 21-fold in LPS-treated rats (P<.005), and 30-fold in nephrectomized rats (P<.005). Sham nephrectomy resulted in an 8-fold stimulation (P<.005) at 17 hours. Sham nephrectomy was obviously the least intense stimulus for eliciting an acute-phase response.

In accordance with the definition of albumin as a negative APP, its secretion (control, 1.7±0.1) was significantly decreased (P<.005) under all experimental conditions examined, with the exception of a sham nephrectomy. The most marked inhibition was found after turpentine injection (to 37% of control). Nephrectomy induced a decrease in angiotensinogen secretion to 60% of control, whereas in 17-hour sham-nephrectomized rats, only a 4.1-fold increase was observed (P<.005). The corresponding stimulation rates for AGP (control, 3±2 fmol/mg liver per hour) were 41-fold in turpentine-treated rats (P<.005), 21-fold in LPS-treated rats (P<.005), and 30-fold in nephrectomized rats (P<.005). Sham nephrectomy resulted in an 8-fold stimulation (P<.005) at 17 hours. Sham nephrectomy was obviously the least intense stimulus for eliciting an acute-phase response.

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Bilateral nephrectomy increased hepatic angiotensinogen secretion (control, 10±2 fmol/mg liver per hour) 2.6-fold (P<.005) and was similar to the LPS-induced stimulation (2.5-fold increase, P<.005). However, livers from turpentine-treated rats secreted significantly less angiotensinogen than controls (40%, P<.005). Although in turpentine-treated rats the liver weight increased by 20%, angiotensinogen secretion rates in this group were still significantly lower when calculated for the whole liver. This decrease in angiotensinogen secretion might not represent a direct effect of turpentine, because this agent failed to alter angiotensinogen mRNA and secretion when
added to a hepatocyte incubation system. Sham nephrectomy did not significantly alter angiotensinogen secretion, thus indicating that inflammatory processes might not be responsible for the nephrectomy-induced stimulation of angiotensinogen synthesis.

Under all experimental conditions, a good correlation existed between changes in plasma concentrations and alterations in hepatic secretion, suggesting that the observed changes in plasma concentrations of the four proteins are due to alterations in hepatic secretion rates and not to alterations in elimination kinetics, eg, in nephrectomized rats by the loss of renal elimination.

**Angiotensinogen mRNA**

To clarify whether the determined changes in the secretion rate of angiotensinogen represent alterations in the rate of synthesis, we measured the cytosolic concentrations of angiotensinogen mRNA in hepatic tissue samples taken from animals not included in the perfusion experiments but subjected to the same treatments. In control livers, a specific content of angiotensinogen mRNA between 5.1 and 5.8 pg/μg total RNA (Fig 1E) was measured. Peak changes were observed at 10 hours in all treatment groups. LPS injection induced a 3.5-fold and nephrectomy a 3.1-fold increase ($P<.005$). In contrast, in turpentine-treated rats, hepatic angiotensinogen mRNA was reduced to 48% of the control level ($P<.005$). Sham nephrectomy failed to influence the concentrations of angiotensinogen mRNA. The nonuniform behavior of angiotensinogen in response to the various inflammatory stimuli applied suggests that angiotensinogen may not be a typical APP; however, the decrease of angiotensinogen secretion may also be related to unspecific effects of turpentine not related to inflammation.

**Effects of IL-1 and IL-6**

To further elucidate a possible relation of angiotensinogen to APPs, we analyzed the effects of two main mediators of an acute-phase response, IL-1 and IL-6, on the secretion of both APPs and angiotensinogen in freshly isolated hepatocytes.

**IL-6**

It is known from the literature that the full response of APP depends on the presence of threshold doses of dexamethasone, so we also included one experimental group in which a combination of IL-6 (500 U/mL) and dexamethasone (3 nmol/L) was applied, which per se had no effect on either APP or angiotensinogen secretion (see Fig 2). Control hepatocytes had a constant secretion rate of AGP (65 ± 9 fmol/mg per hour, Fig 2A), AMG (1.3 ± 0.3 fmol/mg per hour, Fig 2B), and angiotensinogen (100 ± 11 fmol/mg per hour, Fig 2C). Under experimental conditions, secretion rates were measured during the fifth hour of exposure. IL-6 alone (500 U/mL) significantly induced ($P<.005$) the secretion of both AGP (fivefold) and AMG (sixfold). Slightly higher secretion rates were obtained by the simultaneous presence of threshold concentrations of dexamethasone. Angiotensinogen secretion was stimulated 1.8-fold or 2.4-fold by IL-6 alone or in combination with dexamethasone, respectively ($P<.005$).
The group of APPs is a class of hepatic proteins whose plasma concentration and hepatic secretion rate become elevated in response to acute or chronic inflammation. It has been claimed that angiotensinogen belongs to this group, because several models of an acute inflammation, e.g., LPS or cellite injection or partial hepatectomy, increased the plasma concentrations of angiotensinogen. This view was supported by several observations; e.g., LPS was shown to increase angiotensinogen mRNA and secretion in experimental animals, and plasma concentrations of angiotensinogen were increased in patients suffering from chronic infections. Furthermore, for angiotensinogen, an amino acid homology of up to 30% with some APPs has been described; however, no specific function is known for angiotensinogen as yet under conditions of acute or chronic inflammation.

The results obtained demonstrate that turpentine and LPS injection elicited an acute-phase reaction, as evident from the increase in hepatic secretion rates of AGP and AMG, as well as from the concomitant decrease in the secretion rate of albumin. Overall, the response to turpentine appears to be stronger than that to LPS. A response of AGP and AMG, similar in magnitude to that induced by LPS, was also observed after bilateral nephrectomy, whereas sham nephrectomy resulted in smaller changes, indicating that the surgical trauma and subsequent inflammatory reaction inflicted by the sham nephrectomy were less severe than those induced by true nephrectomy. Secretion rates and the mRNA of angiotensinogen were increased to a similar extent after LPS and nephrectomy, indicating that the nephrectomy-induced stimulation of angiotensinogen synthesis may be the result of an acute-phase reaction.

These results tend to support the initial concept of Bing of the acute-phase association of angiotensinogen. These effects are consistent with reports on the presence of acute-phase response elements in the promoter region not only of some APP genes but also of angiotensinogen.
also of the angiotensinogen gene. In contrast, the lack of response to a sham nephrectomy, as well as the significant decrease in hepatic angiotensinogen mRNA and secretion observed in turpentine-treated rats in the face of a well-developed acute-phase response of the liver, is obviously not consistent with the putative role of angiotensinogen as an APP, because both LPS injection and turpentine injection in rats and mice are considered prototype models of the acute-phase reaction. Furthermore, a heterogeneous response, in the sense that LPS stimulates and turpentine suppresses the secretion of any of the APPs in the rat, has never been described. A similar heterogeneity, as for the various inflammatory models, was found when the effects of IL-1 and IL-6, two main mediators for the induction of the synthesis of APPs in the liver, on angiotensinogen synthesis were determined. Although both cytokines uniformly stimulate the synthesis of both APPs, IL-1 significantly suppresses the synthesis of angiotensinogen, whereas IL-6 stimulates it twofold to threefold. Because IL-6 stimulated and IL-1 inhibited angiotensinogen synthesis and because Kupffer cells had been separated from the parenchymal cell preparation by a Percoll density centrifugation, it is unlikely that the effects of IL-1 are the result of an IL-1-mediated Kupffer cell activation and an endogenous production of IL-6. Therefore, it seems that the acute-phase responsive element in the 5' flanking region of the angiotensinogen gene, which represents the target of IL-1-activated NFκB-like factors, is coupled to an inhibition rather than to a stimulation of angiotensinogen gene expression. The physiological consequences of this adverse regulation by the mediators of inflammatory processes are difficult to assess, because no specific function for angiotensinogen is known during acute or chronic forms of inflammatory diseases. One possible implication might be represented by the angiotensin peptides, which might interfere with several types of inflammation through stimulation of growth hormones, prostaglandins, or arachidonic acids; however, this view is still speculative.

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References

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