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# DIFFERENTIAL EFFECTS OF INFLAMMATION MODELS ON RAT T-KININOGEN AND RAT ANGIOTENSINOGEN

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**Abstract**—The effect of different experimental models of inflammation on plasma concentrations of T-kininogen and angiotensinogen was examined in the rat. T-kininogen, a major phase protein which inhibits cysteine proteinase is increased in all cases of induced inflammation: administration of lipopolysaccharide and turpentine, bilateral nephrectomy or sham-operation and intraperitoneal injection of peanut oil. Angiotensinogen, the renin-substrate, is increased by lipopolysaccharide but is decreased by turpentine. Sham-operation or peanut oil injection have no effect on angiotensinogen whereas, bilateral nephrectomy and dexamethasone increase its concentration. Therefore, angiotensinogen is regulated differently than T-kininogen during inflammation.

The concentration of many plasma proteins changes considerably in animals during acute inflammation. Haptoglobulin, $\alpha_1$-antitrypsin, $\alpha_1$-acid glycoprotein and fibrinogen increase whereas albumin and transferrin decrease [1, 2]. In response to inflammation, induced by turpentine-treatment or lipopolysaccharide (LPS), the major acute phase protein ($\alpha_1$MAP) increases about 15-fold in rat plasma [3, 4]. The comparison of cDNA structures of $\alpha_1$MAP, $\alpha_1$-cysteine proteinase inhibitor and T-kininogen showed that these three proteins were similar if not identical [3, 5–7]. During inflammation, the increase of $\alpha_1$MAP in rat plasma is secondary to an increase in the level of translatable liver mRNA for the protein [4]. On the other hand, mRNA of angiotensinogen, the renin substrate, was found surprisingly to be markedly increased in rat liver after induction of acute inflammation by LPS injection [8]. Until now, the sole function of angiotensinogen has been considered to be as the precursor protein of the biologically active peptide angiotensin which plays an important role in the regulation of blood pressure and electrolyte balance. No role is known for the inactive form of the molecule, des-angiotensin I-angiotensinogen. The large carboxyl terminal portion of angiotensinogen was found to be related at the amino-acid level to that of $\alpha_1$-antitrypsin [9], a serine proteinase inhibitor. Moreover, it has been reported that the angiotensinogen gene and the $\alpha_1$-antitrypsin gene share an identical structural organization in the number and position of the introns [10]. On account of these similarities and the increase of its mRNA in the liver after LPS-treatment, it has been suggested that the angiotensinogen may have an additional function as a factor in the inflammation reaction [8].

In this study, we have compared the effect in the rat of two inflammatory agents, turpentine and LPS from *Escherichia coli*, on plasma concentration of T-kininogen and angiotensinogen. The level of these two proteins was also compared in the rat either after bilateral nephrectomy or after administration of dexamethasone, circumstances known to increase plasma angiotensinogen concentration.

## MATERIALS AND METHODS

Male Wistar rats weighing 200–300 g were used and different groups were studied: (1) control rats which received no treatment; (2) rats subcutaneously injected with 0.5 ml of 9‰ NaCl per 100 g body weight; (3) turpentine-treated rats: animals were killed 3, 6, 12, 24 and 48 hr after the administration of 0.5 ml of turpentine per 100 g body weight, subcutaneously; (4) LPS-treated rats: animals were killed, 3, 6, 12, 24 and 48 hr after the injection of LPS from *E. coli* (Sigma) 100 $\mu$g per 100 g body weight, intraperitoneally; (5) bilaterally nephrectomized or sham-operated rats were killed 24 hr after the operation; (6) dexamethasone-treated rats: rats were injected with 10 $\mu$g dexamethasone (Sigma) in 10% ethanol and 90% peanut oil once a day for two days. A control group received the vehicle alone (10% ethanol, 90% peanut oil). Animals were killed 24 hr after the second injection.

All the rats were killed by decapitation and blood was collected in polyethylene tubes containing 1:10 volume of 10% sodium citrate. Plasma was separated from cells by centrifugation at 700 *g* for 10 min.

*T-kininogen determination.* The plasma concentration of T-kininogen was measured by a direct radioimmunoassay (RIA) as described previously [11]. In brief, pure T-kininogen was prepared from plasma of turpentine-treated rats and antibodies directed against pure rat T-kininogen were raised in rabbits by multiple intradermic injections [5]. T-kininogen was labeled with $^{125}$I in the presence of iodogen [12]. $^{125}$I-T-kininogen (10,000 dpm in 200 $\mu$l), T-kininogen antibody (1:90,000, 200 $\mu$l) and samples at the appropriate dilution (100 $\mu$l) were incubated in 0.1 M Tris–HCl, pH 8.5 containing 1 mM EDTA and 2 mg/ml BSA. After 24 hr of incubation at 4°, bound T-kininogen was precipitated in

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the presence of 1 mg bovine γ-globulin by 1 ml of 20% polyethylene glycol (mol. wt 8000, Sigma), and dissolved in the same buffer. The mixture was centrifuged and the precipitate counted. Each assay was run in triplicate.

*Angiotensinogen determination by direct RIA.* Direct RIA of angiotensinogen was performed as previously described [13]. Pure angiotensinogen was prepared from plasma of bilaterally nephrectomized rats [14]. Antibodies against pure rat angiotensinogen were those previously described [14]. This assay measures angiotensinogen as well as des-angiotensin I-angiotensinogen.

Plasma renin activity (PRA) was measured by incubation of 100 μl of plasma for 1 hr at pH 6.5 followed by RIA of the angiotensin I generated as previously described [15].

*Statistics.* Results were expressed as mean ± SE. Treated-groups were compared to controls using analysis of variance followed by the Newman–Keuls multiple range test [16]. Student's *t*-test was done where applicable. Differences for $P < 0.05$ were considered significant.

## RESULTS

### *Comparative effect of LPS and turpentine on T-kininogen and plasma renin system parameters*

Figure 1A shows the time course of the effect of LPS and turpentine on the plasma concentration of T-kininogen. No change was observed 3 hr after LPS treatment but at 6 hr a small increase (40%) but already significant ($P < 0.01$) appeared (348 ± 52 μg/ml plasma compared to the control: 250 ± 19). The increase continued up to 48 hr and reached 1483 μg/ml plasma, about 6-fold the basal T-kininogen concentration. Administration of turpentine increased T-kininogen concentration significantly at 6 hr (+100%, $P < 0.001$). The increase continued up to 48 hr and reached 3010 μg/ml plasma, about 12-fold the basal T-kininogen concentration. Injection of 9% NaCl did not change T-kininogen concentration in the plasma.

Figure 1B presents the time course of the effect of LPS and turpentine on plasma concentration of angiotensinogen determined by direct RIA. After 6 hr of LPS treatment, angiotensinogen level increased significantly from 44.9 ± 2.0 to 83.7 ± 6.2 μg/ml plasma ($P < 0.001$). At 12 hr, angiotensinogen concentration reached 93.9 ± 19.2 and remained elevated during the following 36 hr. Turpentine treatment did not increase angiotensinogen level, but a slight decrease was observed which became significant ($P < 0.02$) at 24 and 48 hr.

As illustrated in Fig. 2, PRA significantly decreased ($P < 0.05$) 3 hr after LPS treatment whereas it increased at 3 and 6 hr after turpentine administration ($P < 0.02$).

### *Effect of nephrectomy and dexamethasone on T-kininogen and angiotensinogen*

Plasma concentration of T-kininogen increased in bilaterally nephrectomized rats from 174 ± 13 to 718 ± 29 μg/ml, $P < 0.001$. However, the same increase was showed in sham-operated rats, (694 ± 32), $P < 0.001$ (Table 1). Plasma angiotensinogen concentration increased about 4-fold after bilateral nephrectomy while it did not change after a sham-operation.

The plasma level of T-kininogen increased significantly after intraperitoneal injection of peanut oil from 174 ± 13 to 453 ± 71 μg/ml, $P < 0.001$. The administration of dexamethasone in peanut oil as vehicle decreased significantly ($P < 0.02$) this effect of peanut oil on T-kininogen (263 ± 33 μg/ml—Table 1). As expected, angiotensinogen concentration (33.9 ± 1.6 μg/ml) doubled during dexa-

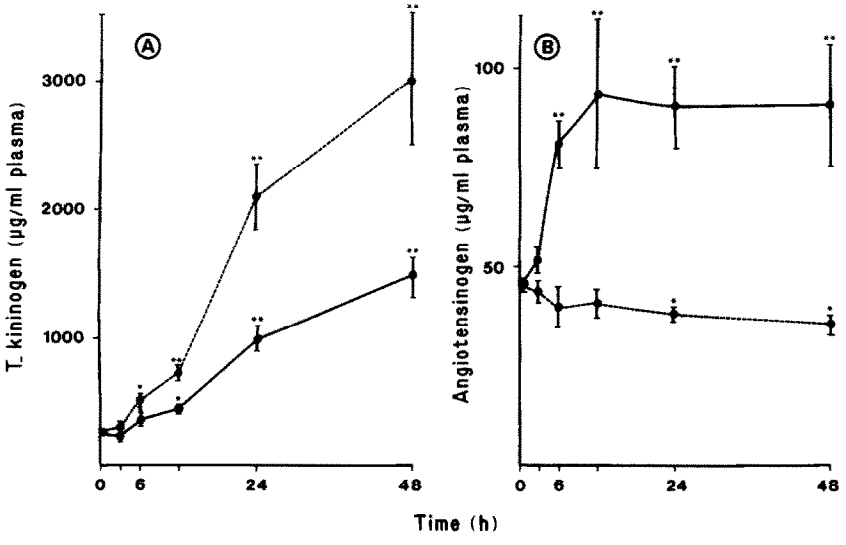


Fig. 1. Time-course of the effect of LPS (●——●) and turpentine (●----●) on plasma T-kininogen (A) and angiotensinogen (B) concentration. Points are mean values for 5 rats for treated-groups and 20 for the controls. Rats were injected intraperitoneally with LPS (100 μg/100 g body weight) or turpentine (0.5 ml/100 g body weight). Controls received 9‰ NaCl. *$P < 0.02$; **$P < 0.001$.

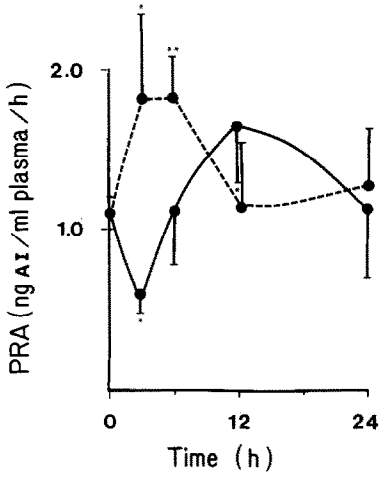


Fig. 2. Time-course of the effect of LPS (●——●) and turpentine (●----●) on plasma renin activity (PRA). The conditions are the same as in Fig. 1. AI: angiotensin I. *$P < 0.02$; **$P < 0.001$.

methasone treatment while it did not change after peanut oil, 66.6 ± 7.0 μg/ml and 40.6 ± 4.8 μg/ml, respectively.

## DISCUSSION

The results of the present study demonstrated that in several circumstances of inflammation induced in the rat, the plasma concentration of T-kininogen is increased. A single intraperitoneal injection of LPS increased the basal level of T-kininogen concentration 6-fold. The role of T-kininogen in the inflammatory response in rats is not known at this time. It is possible that T-kininogen is a substrate for either lysosomal enzymes such as Cathepsin D or an unknown protease which release T-kinin, a key mediator of inflammation. Experiments *in vivo* have shown that $B_1$ receptors are induced in rabbits by intravenous injection of LPS [17]. Moreover, in rabbits treated with LPS, des-Arg$^9$BK, which is inactive in normal animals, becomes a potent vasodilator and hypotensive agent [18]. In fact, the two T-prekininogen mRNAs but not the LMW and HMW prekininogen mRNAs are markedly increased after induction of acute inflammation by LPS [4]. However, no indication has been provided concerning the mechanism involved in the activation of liver mRNA.

Table 1. Plasma concentration of T-kininogen and angiotensinogen in bilaterally nephrectomized and dexamethasone-treated rats

| | T-kininogen (μg/ml) | Angiotensinogen (μg/ml) |
|---|---|---|
| Control (13) | 174 ± 13 | 33.9 ± 1.6 |
| Peanut oil (10) | 453 ± 71[b] | 40.6 ± 4.8 |
| Dexamethasone (11) | 263 ± 33[a] | 66.6 ± 7.0[a] |
| Sham operated (11) | 694 ± 32[b] | 33.5 ± 2.7 |
| Binephrectomized (12) | 718 ± 29[b] | 166 ± 13.1[b] |

Each assay was made in triplicate and the data are presented as mean ± SE.

The number of animals is in parentheses.

Significance compared to controls: a = $P < 0.02$; b = $P < 0.001$.

Plasma angiotensinogen concentration increased about two-fold after LPS-treatment. This increase in plasma angiotensinogen is probably secondary to that of mRNA observed in the rat liver following the administration of *Escherichia coli* LPS [8], but also here the mechanism responsible for this rise is not known. It is possible that LPS acts directly on angiotensinogen synthesis or perhaps the increase of plasma angiotensinogen concentration may account for the decreased PRA at 3 hr after LPS-treatment allowing PRA to return to control levels. In this regard, angiotensinogen is a limiting factor of the renin substrate reaction in the rat and at low concentrations of substrate, the rate of the reaction depends on substrate concentration [19].

After a single injection of turpentine, the plasma level of T-kininogen increased to a level of 12-fold the basal concentration. We have demonstrated in an earlier study that turpentine increased only T-kininogen among the different rat kininogens [12]. Barlas *et al.* also reported that only T-kininogen was increased in plasma of Freund's adjuvant-treated rats [18]. The rise in rat plasma T-kininogen probably resulted from the increase in the liver of mRNA levels of $\alpha_1$MAP, which is closely related or identical to T-kininogen during acute inflammation induced by subcutaneous injection of turpentine [21]. On the contrary, it was found here that plasma angiotensinogen concentration decreased slightly but significantly at 24 and 48 hr after the administration of turpentine. This decrease was unexpected and it corresponds to a diminution of the angiotensinogen synthesis. It parallels the decrease of albumin and transferrin observed in response to inflammation [1, 2]. PRA, 24 hr after turpentine treatment, is only slightly increased compared to control (0 time) but it has diminished compared to 3 hr after injection in parallel with angiotensinogen concentration.

Plasma T-kininogen increased in all other situations where an inflammatory response was induced such as sham-operation and peanut oil administration. The glucocorticoids by their well known anti-inflammatory action [22, 23] decreased the effect of T-kininogen induction by peanut-oil. Binephrectomy *per se* did not increase further T-kininogen. In another set of experiments, it was also shown that binephrectomy as compared to sham-operation did not affect T-kininogen nor total kininogens as measured by kinin released after trypsin treatment (Suzuki *et al.*, submitted).

Angiotensinogen did not increase after sham-operation and peanut-oil administration. As expected, angiotensinogen increased after bilateral nephrectomy [24–26] and dexamethasone treatment [24]. The fact that neither turpentine, peanut oil nor sham-operation increased angiotensinogen makes it difficult to consider angiotensinogen as an inflammatory protein.

So, the reason for the increase in plasma angiotensinogen after LPS is due to an increase in angiotensinogen mRNA (8). The mechanism responsible for this increase, as for that of T-kininogen, is not

known. It could be a direct effect or via a mediator of inflammation. No data are available concerning the relationship between angiotensin, the active peptide after cleavage of angiotensinogen and inflammation. An alternative explanation would that angiotensinogen increases as a consequence of the decrease in PRA. In this regard, it has been reported that angiotensin II exerts a positive feed back effect on angiotensinogen synthesis [27].

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