THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1988 by The American Society for Biochemistry and Molecular Biology, Inc.

Vol. 263, No. 34, Issue of December 5, pp. 18220–18224, 1988
Printed in U.S.A.

Characterization of the Interaction *in Vivo* of Tissue-type Plasminogen Activator with Liver Cells*

(Received for publication, June 14, 1988)

Johan Kuiper‡, Marlies Otter§, Dingeman C. Rijken§, and Theo J. C. van Berkel‡¶

From the ‡Division of Biopharmaceutics, Center for Bio-Pharmaceutical Sciences, Sylvius Laboratories, University of Leiden, Leiden, The Netherlands and the §Gaubius Institute, TNO, Leiden, The Netherlands

The interaction *in vivo* of ^{125}I-labeled tissue-type plasminogen activator (t-PA) with the rat liver and the various liver cell types was characterized. Intravenously injected ^{125}I-t-PA was rapidly cleared from the plasma ($t_{1/2}$ = 1 min), and 80% of the injected dose associated with the liver. After uptake, t-PA was rapidly degraded in the lysosomes. The interaction of ^{125}I-t-PA with the liver could be inhibited by preinjection of the rats with ovalbumin or unlabeled t-PA. The intrahepatic recognition site(s) for t-PA were determined by subfractionation of the liver in parenchymal, endothelial, and Kupffer cells. It can be calculated that parenchymal cells are responsible for 54.5% of the interaction of t-PA with the liver, endothelial cells for 39.5%, and Kupffer cells for only 6%. The association of t-PA with parenchymal cells was not mediated by a carbohydrate-specific receptor and could only be inhibited by an excess of unlabeled t-PA, indicating involvement of a specific t-PA recognition site. The association of t-PA with endothelial cells could be inhibited 80% by the mannose-terminated glycoprotein ovalbumin, suggesting that the mannose receptor plays a major role in the recognition of t-PA by endothelial liver cells. An excess of unlabeled t-PA inhibited the association of ^{125}I-t-PA to endothelial liver cells 95%, indicating that an additional specific t-PA recognition site may be responsible for 15% of the high affinity interaction of t-PA with this liver cell type.

It is concluded that the uptake of t-PA by the liver is mainly mediated by two recognition systems: a specific t-PA site on parenchymal cells and the mannose receptor on endothelial liver cells. It is suggested that for the development of strategies to prolong the half-life of t-PA in the blood, the presence of both types of recognition systems has to be taken into account.

The tissue-type plasminogen activator (t-PA)[1] plays a central role in the extrinsic fibrinolytic system by catalyzing the conversion of plasminogen to plasmin (1–3). Subsequently, plasmin proteolytically degrades the fibrin network associated with blood clots. The activation of plasminogen by t-PA is markedly increased in the presence of fibrin (4, 5). t-PA is therefore considered as a promising thrombolytic agent. t-PA can be isolated from extracts of several tissues and the supernatant of a melanoma cell line (1, 6–8). Recently, the application of t-PA in acute myocardial infarction was stimulated because recombinant DNA technology enabled a wide availability of t-PA for clinical studies. A major drawback for the clinical use of t-PA is its very short plasma half-life of 1–4 min, which is due to an active uptake system residing in the liver (9–15). At present, site-specific mutagenesis of recombinant t-PA is actively explored as a possible mechanism to increase the plasma half-life (16–19). An alternative approach is the design of specific inhibitors of the t-PA uptake by the liver. Until now, the exact interaction of t-PA with the liver is relatively unexplored, and even the cell type which is responsible *in vivo* for the avid interaction of t-PA with the liver is unknown.

The complete amino acid sequence of t-PA, elucidated with the use of cDNA clones (20), indicates that t-PA is a single chain glycoprotein (530 amino acids, M_r = 67,000) which can be proteolytically spliced in a two-chain protein, consisting of a heavy (M_r = 38,000) and a light (M_r = 31,000) chain (6–8). Three *N*-glycosylation sites have been identified: on amino acid Asn-117, the oligomannose type of glycan, and on the amino acids Asn-184 and Asn-448, the *N*-acetyllactosamine type of glycan (21).

Replacement of Asn-448 or Asn-117 by a Glu and Gln, respectively (not carrying a carbohydrate chain), resulted in a significantly prolonged plasma half-life of t-PA (16, 17), which indicates that carbohydrate chains may be involved in the interaction of t-PA with the liver. Recent evidence *in vitro*, however, indicates that carbohydrate moieties are not involved in the interaction of t-PA with isolated rat parenchymal liver cells, and a novel high affinity system for t-PA uptake in parenchymal liver cells was postulated (22). Besides carbohydrate receptors on parenchymal cells, the liver contains receptors recognizing mannose-terminated glycoproteins on liver endothelial and Kupffer cells (23, 24).

The aim of the present study was to establish *in vivo* the nature of the recognition sites in the liver which are responsible for the rapid uptake of t-PA. In addition, the cell types in the liver which are responsible for the specific recognition of t-PA were identified.

EXPERIMENTAL PROCEDURES

Materials

Nycodenz was obtained from Nycomed A/S Oslo, Norway. Collagenase type I, mannan, *N*-acetyl-D-galactosamine, bovine serum albumin (type V), fetuin (type IV), and agarose-bound neuraminidase (from *Clostridium perfringens*, type VI-A) were obtained from Sigma. Ovalbumin was obtained from Serva Feinbiochemica, Heidelberg, Federal Republic of Germany. *N*-Acetyl-α-D-glucosamine was from Janssen, Beerse, Belgium. ^{125}I (carrier-free) was from Amersham.

* This work was supported by Grant 86.057 from the Netherlands Heart Foundation and an Established Investigatorship (to D. C. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Div. of Biopharmaceutics, Sylvius Laboratories, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

[1] The abbreviations used are; t-PA, tissue-type plasminogen activator; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Asialofetuin was enzymatically prepared by incubating fetuin, dissolved in 0.1 M sodium acetate buffer, pH 5.5, with agarose-bound neuraminidase (20 milliunits/ml) for 4 h at 37 °C. A minimum of 80% of the sialic acid residues, assayed as described earlier (25), was removed by this procedure.

Methods

Isolation and Labeling of t-PA—Tissue-type plasminogen activator (more than 99% two-chain) was purified from Bowes melanoma cells as described by Kluft *et al.* (26). Radiolabeling of t-PA was done using the iodogen method (27), and the labeled protein was isolated as described earlier (10).

In Vivo Plasma Clearance and Liver Association of t-PA—Male Wistar rats (8–10 weeks old, weighing 250-300 g) were anesthetized by an intraperitoneal injection of 20 mg of Nembutal. The abdomen was opened, and radiolabeled t-PA (50 ng) was injected into the vena penis. Various competitors were injected into the vena cava inferior 2 min prior to injection of radiolabeled t-PA. The body temperature of the rats was monitored under the experimental conditions as the rectal temperature and maintained at 36.5-37 °C using an infrared lamp. At the indicated times after injection of radiolabeled t-PA, 0.3 ml of blood was taken from the vena cava inferior using heparinized syringes. Blood samples were centrifuged for 2 min at 10,000 × *g*. 10% trichloroacetic acid-precipitable and 10% trichloroacetic acid-soluble radioactivity was counted in aliquots of plasma. At the indicated times, liver lobules were tied off and excised. After weighing the lobule and counting the radioactivity, total liver uptake was calculated after measuring the liver weight at the end of the experiment. The amount of liver that was tied off at each time point was 2-3%, so that at the end of the experiment less than 10% of the total liver was removed.

Cell Isolation Procedures—Rats were anesthetized, and radiolabeled t-PA was injected as described above. Five min after injection of radiolabeled t-PA (50 ng), the vena porta was cannulated, and a liver perfusion was started using Hanks' buffer (plus 1.6 g of HEPES/liter) at 8 °C. After 8 min of perfusion (flow rate 14 ml/min), a lobule was tied off for determination of total liver uptake. In order to separate parenchymal from nonparenchymal cells, the liver was perfused with collagenase (0.05%, w/v) at 8 °C. To separate endothelial from Kupffer cells, the liver residue obtained from the collagenase digestion was further digested by stirring with Pronase (0.25%, w/v) at 8 °C exactly as described earlier (28). Calculation of the contribution of the various liver cell types to total liver uptake was performed as described (28). As found earlier for a number of substrates (28–30), no loss of cell-bound label or formation of acid-soluble radioactivity occurred during the low temperature cell isolation procedure, leading to a quantitative recovery of radioactivity associated with the isolated liver cells as compared to the total liver association. This was checked for each individual liver cell isolation by comparing the calculated (from the relative contribution of the various cell types) and the determined total liver association.

Subcellular Fractionation—Rats used for tissue fractionation were pretreated with leupeptin (5 mg injected intravenously) 60 min prior to injection of radiolabeled t-PA (50 ng). Fractionation of total liver was performed 30 min after injection of the radiolabeled t-PA exactly as described (31).

Assay of enzyme activity in each fraction was performed as described (32).

RESULTS

Plasma Half-life and Liver Association—Radiolabeled tissue-type plasminogen activator showed, when injected intravenously, a plasma half-life of less than 1 min (57 s) (Fig. 1). t-PA interacted primarily with the liver, and, after 6 min, 80.7 ± 0.2% (mean of three experiments ± S.D.) of the injected dose was recovered in the liver. Between 10 and 30 min after injection, the liver-associated radioactivity decreased to 14%. This effect was accompanied by an increase in trichloroacetic acid-soluble radioactivity in plasma (6% of the injected dose at 29 min after injection of t-PA). Besides the liver, no other organs (spleen, kidney, lung, bone marrow) contributed significantly to the plasma clearance of t-PA.

Specificity and Affinity of t-PA Uptake in the Liver—To investigate the possible involvement of carbohydrate-specific

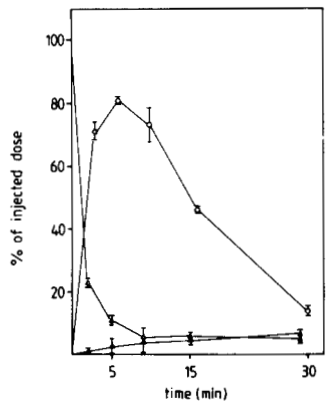

FIG. 1. **Liver association and plasma clearance of ^{125}I-t-PA.** Radiolabeled t-PA was injected into anesthetized rats. At the indicated times, liver association (○) and plasma clearance was determined. In plasma, both 10% trichloroacetic acid (▲) and trichloroacetic acid-precipitable (△) radioactivity was determined. Data represent the mean of three experiments ± S.D.

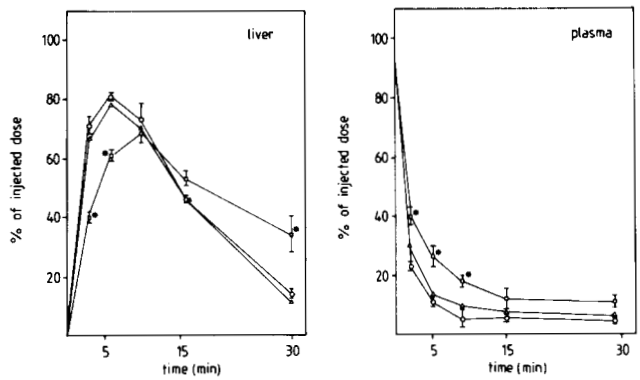

FIG. 2. **Effect of preinjection of asialofetuin and ovalbumin on the liver uptake and plasma clearance of ^{125}I-t-PA.** Ovalbumin (□, 20 mg), asialofetuin (△, 20 mg), or solvent (○) was injected 2 min prior to the injection of ^{125}I-t-PA. Liver uptake and plasma clearance of t-PA were determined under the various conditions. The *bars* represent mean ± S.D. (n = 3). *Asterisk* indicates significant difference from control ($p < 0.05$).

receptors in the liver uptake of t-PA, various glycoproteins were tested for their ability to inhibit the liver uptake of t-PA (Fig. 2). Asialofetuin, which interacts with the galactose receptor on hepatocytes, was not able to inhibit the plasma clearance nor the liver uptake of radiolabeled t-PA. Ovalbumin, however, a mannose-terminated glycoprotein, did change the plasma half-life of t-PA from less than 1 min to more than 2 min. Liver uptake of t-PA at 3 min after injection was lowered from 70% to 40% of the injected dose.

Asialofetuin only interacts with the galactose receptor on parenchymal liver cells, whereas *N*-acetylgalactosamine also blocks the uptake by the galactose-particle receptor from Kupffer cells (29). In Fig. 3, the effect of *N*-acetylgalactosamine, *N*-acetylglucosamine, and mannan is illustrated. Only mannan could inhibit the liver uptake and prolong the plasma half-life of t-PA.

The role of high affinity binding sites specific for t-PA in the interaction of t-PA with the liver was explored by injection of an excess of unlabeled t-PA 2 min prior to radiolabeled t-PA (Fig. 4). Ten mg of t-PA per kg of body weight clearly diminished the liver uptake of labeled t-PA. After preinjection of 20 mg of t-PA/kg of body weight, the liver uptake of radiolabeled t-PA did not exceed 22% of the injected dose. Up to 30 min after injection, 50% of the radiolabeled t-PA was still present in the circulation.

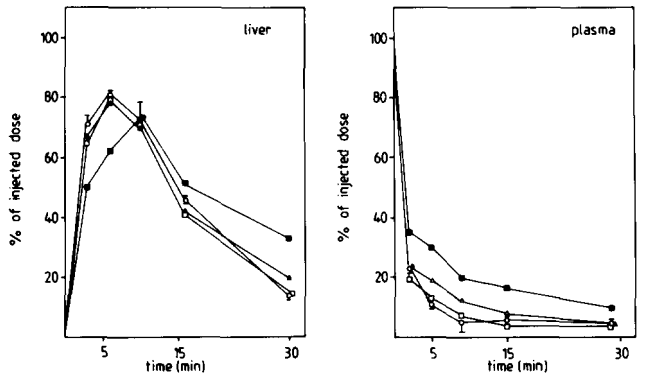

FIG. 3. **Effect of various carbohydrates on the liver uptake and plasma clearance of ^{125}I-t-PA.** *N*-acetylgalactosamine (□, 0.5 mmol), *N*-acetylglucosamine (△, 0.5 mmol), mannan (■, 5 mg), or solvent (○, control) was injected 2 min prior to injection of radiolabeled t-PA. Under the various conditions, liver uptake and plasma clearance of ^{125}I-t-PA was determined at the indicated times. *Bars* represent mean ± S.E. (n = 3).

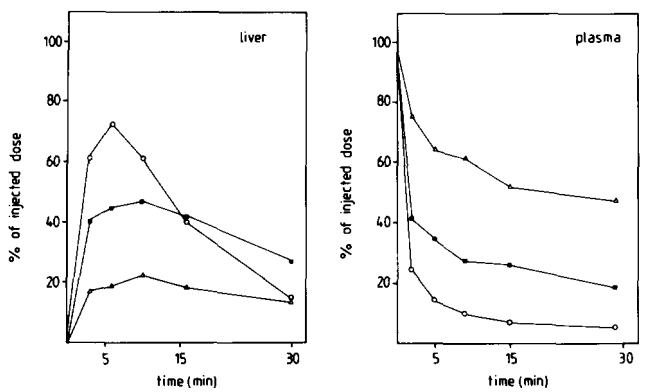

FIG. 4. **Effect of preinjection of unlabeled t-PA on the liver uptake and plasma clearance of radiolabeled t-PA.** One ml of solvent (0.3 M L-arginine) containing 0 (○, control), 10 (■), or 20 (△) mg of t-PA/kg of body weight was injected 2 min prior to injection of ^{125}I-t-PA. Liver uptake and plasma clearance of ^{125}I-t-PA were determined at the indicated times.

TABLE I

Relative contribution of the different liver cell types to the total liver uptake of t-PA

Liver cell isolation was started 5 min after an intravenous injection of ^{125}I-t-PA. Multiplication of the per cent of the injected dose (i.d.)/mg of cell, with the amount of protein that each liver cell type contributes to total liver protein, results in the t-PA uptake (% of total liver) by each cell type. Data represent the mean of three experiments ± S.D.

Cell type	Uptake of t-PA	
	% i.d. × 10^3/mg cell protein	*% total liver*
Parenchymal	31.9 ± 2.4	54.5 ± 4.8
Endothelial	651.2 ± 71.5	39.5 ± 3.8
Kupffer	132.0 ± 29.0	6.1 ± 1.2

Cellular Distribution of t-PA Association in Liver—The association of t-PA with the various liver cell types was determined at 5 min after injection. The highest association of t-PA is found with liver endothelial cells (Table I). A 20-fold higher amount of t-PA per mg of cell protein is associated with endothelial liver cells as compared to parenchymal cells. Endothelial cells, however, contribute only 3.3% to total liver protein (for parenchymal cells this value is 92.5%). Taking into account the contribution of the various liver cell types to total liver protein (3.3% for endothelial, 92.5% for parenchymal, and 2.5% for Kupffer cells (28–30)), the contribution of the various liver cell types to the total liver uptake of t-PA can be calculated. It appeared that parenchymal and endothelial liver cells contributed 54.5% and 39.5% to the total liver uptake of t-PA, respectively (Table I). The contribution of Kupffer cells to the liver uptake of t-PA was very small (only 6.1%). To evaluate the high affinity recognition systems by the various cell types, cell isolations were performed after preinjection of an excess unlabeled t-PA. In addition, cell isolations were performed after preinjection of ovalbumin in order to assess a possible role of mannose-mediated recognition of t-PA by one of the liver cell types (Fig. 5). Ovalbumin inhibited predominantly the uptake of radiolabeled t-PA in the nonparenchymal cells. Uptake of t-PA in the endothelial cells was inhibited for 80% and in the Kupffer cells for 60%. Uptake of t-PA by parenchymal cells, on the other hand, was only inhibited for 20%. Preinjection of 20 mg of t-PA/kg of body weight inhibited the amount of labeled t-PA, which becomes associated to each liver cell type for more than 80%.

Processing of t-PA—The intracellular processing of t-PA *in vivo*, possibly by a lysosomal pathway, was studied by pretreating the rats with leupeptin (Fig. 6). Leupeptin had no effect on the initial liver association and plasma clearance of t-PA, but the processing of t-PA by the liver was clearly inhibited. This resulted in a residual liver level of 51% of the injected dose at 30 min after t-PA injection for leupeptin

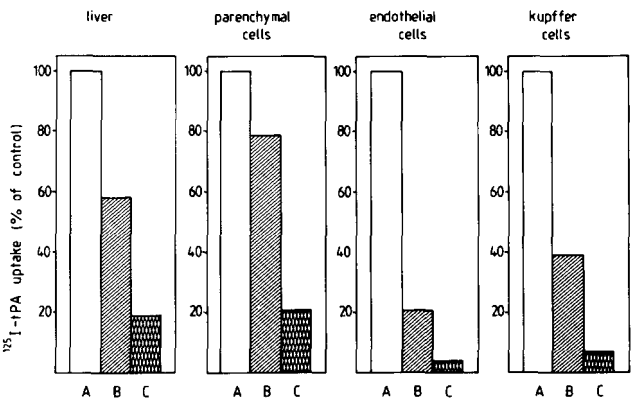

FIG. 5. **Effect of ovalbumin and excess of unlabeled t-PA on the association of ^{125}I-t-PA to the liver, parenchymal, endothelial, and Kupffer cells.** Rats were injected with ^{125}I-t-PA after preinjection with either ovalbumin (20 mg, *B*), t-PA (20 mg/kg of body weight, *C*), or solvent (control, *A*) at $t = -2$ min. At 5 min, the liver cell isolation procedure was started (see "Experimental Procedures").

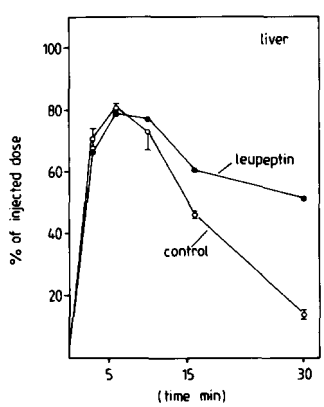

FIG. 6. **Effect of leupeptin on the liver association of t-PA.** Liver association of ^{125}I-t-PA in control (○) and leupeptin-treated (●) rats was determined at the indicated times. Leupeptin was injected 60 min prior to injection of ^{125}I-t-PA.

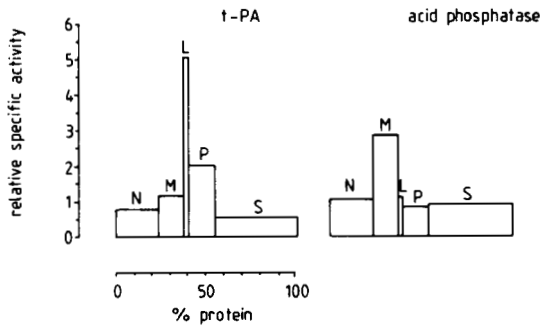

FIG. 7. **Distribution of t-PA in subcellular fractions of the liver.** ^{125}I-t-PA was injected into rats pretreated with leupeptin (5 mg, 60 min prior to injection of ^{125}I-t-PA). Thirty min after injection of ^{125}-t-PA, a subcellular distribution was started as described (27). *N* = nuclear fraction, *M* = heavy mitochondrial fraction, *L* = light mitochondrial fraction, *P* = microsomal fraction, and *S* = final supernatant.

treated rats, whereas only 14% of the injected dose was recovered at 30 min in control rats. The role of the lysosomes in the processing of t-PA was also investigated by performing a subcellular fractionation after pretreatment of the rats with leupeptin. The radioactive marker was found to be highly enriched in the lysosomal fraction (Fig. 7). Due to the pretreatment of rats with leupeptin, the lysosomal marker acid phosphatase which is in untreated rats recovered in the lysosomal fraction is now recovered in both the lysosomal and the mitochondrial compartment. A similar shift was found earlier for other lysosomal markers like β-glucuronidase and cathepsin D (30).

DISCUSSION

The clinical application of t-PA as a thrombolytic agent is highly stimulated by the abundant availability of this protein as a consequence of DNA technology. Pharmacokinetically, t-PA is characterized by a rapid removal from the plasma, which results in an initial half-life of 1–4 min in various species (9–15). In accordance with these data (9, 10), we determined in rats a $t_{1/2}$ value of about 1 min. The rapid removal of t-PA from the plasma is caused by a highly active uptake system residing primarily in the liver (9–15), and we recovered more than 80% of the injected dose in this organ.

The specificity of the t-PA uptake *in vivo* was clarified by competition studies. In agreement with earlier studies (9, 11), we found that the liver uptake of t-PA was not galactose-dependent. Preinjection of asialofetuin, which blocks the galactose receptor on parenchymal cells (33), and *N*-acetylgalactosamine, which blocks the galactose-specific uptake both in Kupffer and parenchymal cells (29), did not influence the plasma clearance nor the liver uptake of t-PA. Competition studies also indicated that *N*-acetylglucosamine groups are not involved in the uptake of t-PA by the liver. However, blockade of the mannose receptor by mannan or by the mannose-terminated glycoprotein ovalbumin prolonged the plasma half-life of t-PA and retarded the liver uptake, suggesting that mannose groups are in some way involved in the association of t-PA with the liver.

Preinjection of high doses of unlabeled t-PA (10–20 mg/kg of body weight) resulted in a pronounced decrease in liver-associated ^{125}I-t-PA, indicating that t-PA is recognized *in vivo* by a specific high affinity system. In order to specify the cell types in the liver responsible for this high affinity recognition of t-PA, we isolated the various liver cell types by a low temperature procedure, which was evaluated earlier (28–30), and determined the cell association of labeled t-PA or minus preinjection of the relevant competitor. Within the liver, parenchymal and endothelial cells were found to be responsible for the avid interaction of t-PA with the liver. The specificity and high affinity of t-PA association *in vivo* with the various liver cell types was indicated by the high inhibition caused by the preinjection of unlabeled t-PA. None of the sugar competitors significantly inhibited the association of t-PA to parenchymal cells. These results obtained *in vivo* support recently published data (22) on the uptake of recombinant t-PA in rat parenchymal cells *in vitro*. Bakhit *et al.* (22) established that glycopeptides, isolated from t-PA, or glycoproteins did not inhibit the uptake of ^{125}I-t-PA by isolated parenchymal cells, and their results suggested that uptake of t-PA by hepatocytes proceeded via a receptor specific for t-PA. Our *in vivo* data are in complete agreement with the postulated novel high affinity uptake system on rat parenchymal cells (22). In contrast, we identified that the uptake of t-PA by the endothelial liver cells is mainly exerted by a carbohydrate-specific system. Ovalbumin, possessing an oligomannose type of glycan identical with the carbohydrate group on amino acid 117 (Asn) of the t-PA molecule (21), was able to inhibit the endothelial cell uptake of t-PA *in vivo* for 80%. Mannose-specific uptake of t-PA by liver endothelial cells is in agreement with preliminary data of Einarsson *et al.* (34). In autoradiographic studies, other types of mannose-terminated glycoproteins were also shown to be taken up preferentially by liver endothelial cells (24). Carbohydrate-specific uptake of t-PA in liver endothelial cells may also explain the finding that t-PA variants, lacking one carbohydrate group, show a prolonged plasma half-life (17). Endothelial cell uptake could be inhibited for 95% by an excess of unlabeled t-PA. The fact that t-PA inhibited the endothelial cell uptake of radiolabeled t-PA to a slightly larger extent than ovalbumin may be explained by a higher affinity of the mannose receptor for t-PA than for ovalbumin. Differences in affinity were also described for other mannose-terminated glycoproteins (24). However, the additional 15% inhibition observed with t-PA as compared to ovalbumin may also be explained by the presence of an additional low amount of t-PA-specific sites on endothelial liver cells. Specific t-PA binding sites were recently described to be present on human umbilical vein endothelial cells (35, 36). The possible association of ^{125}I-t-PA with a specific t-PA binding site on endothelial liver cells may contribute maximally only 5% to total liver uptake of t-PA. The uptake of labeled t-PA by Kupffer cells was found also to be largely mannose-specific and was blocked by t-PA, but, as pointed out before, Kupffer cells contribute only to a minor extent to total liver uptake.

The processing of t-PA by the liver *in vivo* was investigated by preinjection of the rats with leupeptin, an inhibitor of thiol proteases (37). Pretreatment of the rats with leupeptin significantly inhibited the decrease in total hepatic radioactivity at longer times after injection. A subcellular distribution study indicated that radioactivity accumulated in the lysosomal fraction. These data provide evidence that the high affinity recognition of t-PA in the liver *in vivo* is coupled to uptake and lysosomal processing of t-PA. Upon leupeptin treatment, the density profile of acid phosphatase is shifted due to the appearance of prominent autolysosomes as indicated earlier (38). In analogy with other ligands that are taken up by receptor-dependent uptake in the liver (28–30), it is clear that the newly formed endocytotic vesicles involved in the uptake of t-PA do not fuse with the pre-existing autolysosomes.

In conclusion, it can be stated that t-PA uptake in the liver *in vivo* is a process mediated by two types of recognition sites. Firstly, the parenchymal cells utilize a novel high affinity

system specific for t-PA. Secondly, the liver endothelial cells do perform t-PA uptake mainly by the mannose receptor, which recognizes carbohydrate groups on the t-PA molecule. In the development of further strategies to prolong the plasma half-life of t-PA, the existence of both recognition sites has to be taken into account. This implies that for site-specific mutagenesis, modification of amino acids involved in both uptake systems will be necessary. Similarly, for the development of inhibitors of the liver uptake of t-PA either inhibitors interacting with both systems or a combination of two inhibitors, each interacting with one system, will have to be developed.

REFERENCES

1. Rijken, D. C., and Collen, D. (1981) *J. Biol. Chem.* **256,** 7035–7041
2. Bachmann, F., and Kruithof, E. K. O. (1984) *Semin. Thromb. Haemostasis* **10,** 6–17
3. Danø, K., Andreasen, P. A., Grøndahl-Hansen, J., Kristensen, P., Nielsen, L. S., and Skriver, L. (1985) *Adv. Cancer Res.* **44,** 139–266
4. Matsuo, O., Rijken, D. C., and Collen, D. (1981) *Nature* **291,** 590–591
5. Collen, D., and Lijnen, H. R. (1984) *Arteriosclerosis* **4,** 579–585
6. Wallen, P., Bergsdorf, N., and Rånby, M. (1982) *Biochim. Biophys. Acta* **709,** 318–328
7. Rijken, D. C., Wijngaards, G., Zaal-de Jong, M., and Welbergen, J. (1979) *Biochim. Biophys. Acta* **580,** 140–153
8. Wallen, P., Pohl, G., Bergsdorf, N., Rånby, M., Ny, T., and Jörnwall, H. (1983) *Eur. J. Biochem.* **132,** 681–686
9. Emeis, J. J., Van den Hoogen, C. M., and Jense, D. (1985) *Thromb. Haemostasis* **54,** 661–664
10. Rijken, D. C., and Emeis, J. J. (1986) *Biochem. J.* **238,** 643–646
11. Fuchs, H. E., Berger, H., and Pizzo, S. V. (1985) *Blood* **65,** 539–544
12. Beebe, D. P., and Aronson, D. L. (1986) *Thromb. Res.* **43,** 663–674
13. Mohler, M. A., Tate, K., Bringman, T. S., Fuller, G., Keyt, B., Vehar, G., and Hotchkiss, A. J. (1988) *Fibrinolysis* **2,** 17–23
14. Nilsson, S., Einarsson, M., Ekvärn, S., Häggroth, L., and Matsson, Ch. (1985) *Thromb. Res.* **39,** 511–521
15. Korninger, C., Stassen, J. M., and Collen, D. (1981) *Thromb. Haemostasis* **46,** 658–661
16. Lau, D., Kuzma, G., Wei, C., Livingston, D. J., and Hsiung, N. (1987) *Biotechnology* **5,** 953–958
17. Collen, D., Stassen, J., and Larsen, G. (1988) *Blood* **71,** 216–219
18. Browne, M. J., Carey, J. E., Chapman, G. G., Tyrrell, A. W. R., Entwisle, C., Lawrence, G. M. P., Reavy, B., Dodd, I., Esmail, A., and Robinson, J. H. (1988) *J. Biol. Chem.* **263,** 1599–1602
19. Kalyan, N. K., Lee, S. G., Wilhelm, J., Fu, K. P., Hum, W.-T., Rappaport, R., Hartzell, R. W., Urbano, C., and Hung, P. P. (1988) *J. Biol. Chem.* **263,** 3971–3978
20. Pennica, D., Holmes, W. E., Kohr, W. J., Harkins, R. N., Vehar, G. A., Ward, C. A., Bennett, W. F., Yelverton, E., Seeburg, P. H., Heyneker, H. L., Goeddel, D. V., and Collen, D. (1983) *Nature* **301,** 214–221
21. Pohl, G., Kerne, L., Nilsson, B., and Einarsson, M. (1987) *Eur. J. Biochem. (Tokyo)* **170,** 69–75
22. Bakhit, C., Lewis, D., Billings, R., and Malfroy, B. (1987) *J. Biol. Chem.* **262,** 8716–8270
23. Steer, G. J., and Clarenburg, R. (1979) *J. Biol. Chem.* **254,** 4457–4461
24. Hubbard, A. L., Wilson, G., Ashwell, G., and Stukenbrok, H. (1979) *J. Cell Biol.* **83,** 47–64
25. Spiro, R. G. (1966) *Methods Enzymol.* **8,** 3–25
26. Kluft, C., Van Wezel, A. L., Van der Velden, C. A. M., Emeis, J. J., Verheijen, J. H., and Wijngaards, G. (1983) in *Advances in Biotechnological Processes* (Mizrahi, A., and Van Wezel, A. L., eds) Vol. 2, pp. 97–110, Alan R. Liss Inc., New York
27. Fraker, P. J., and Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80,** 849–857
28. Nagelkerke, J. F., Barto, K. P., and van Berkel, T. J. C. (1983) *J. Biol. Chem.* **258,** 12221–12227
29. van Berkel, T. J. C., Kruijt, J. K., Spanjer, H. H., Nagelkerke, J. F., Harkes, L., and Kempen, H.-J. M. (1985) *J. Biol. Chem.* **260,** 2694–2699
30. van Berkel, T. J. C., Kruijt, J. K., and Kempen, H.-J. M. (1985) *J. Biol. Chem.* **260,** 12203–12207
31. De Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955) *Biochem. J.* **60,** 604–617
32. Bergmeijer, H. U. (1970) *Methoden der Enzymatischen Analyse,* pp. 457–458, Verlag Chemie, Weinheim, Federal Republic of Germany
33. van Berkel, T. J. C., Dekker, C. J., Kruijt, J. K., and Van Eijk, H. G. (1987) *Biochem. J.* **243,** 715–722
34. Einarsson, M., Smedsrød, B., and Pertoft, H. (1985) *Thromb. Haemostasis* **54,** 270 (Abstr. P1601)
35. Beebe, D. P. (1987) *Thromb. Res.* **46,** 241–247
36. Barnathan, E. S., Kuo, A., Van der Keyl, H., McCrae, K. R., Larsen, G. R., and Cines, D. B. (1988) *J. Biol. Chem.* **263,** 7792–7799
37. Kirscke, H., Langner, J., Wiederanders, B., Ansorg, S., and Bohley, P. (1977) *Eur. J. Biochem.* **74,** 293–301
38. Furano, K., Ishikawa, T., and Kato, K. (1982) *J. Biochem. (Tokyo)* **91,** 1485–1494