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Hemodynamic Characteristics of Extracellular UTP in the Perfused Rat Liver

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Uridine 5'-triphosphate (UTP) is stored in the granules of cells such as platelets and is released into the extracellular space upon cell stimulation. Extracellular UTP is known to influence many biological processes. We investigated the hemodynamic effects of UTP on the perfused rat liver and characterized its receptors. Liver perfusions were performed in a recirculation system under constant pressure (28 cmH_2O). The perfusion flow and oxygen consumption rate were measured at 30 second intervals. UTP decreased the perfusion flow and the oxygen consumption rate, dose-dependently. UTP-induced changes were transient and disappeared in about 10 minutes. Suramin (P_2-purinergic antagonist, 100 uM) and indomethacin (cyclooxygenase inhibitor, 20 uM) blocked UTP-induced hemodynamic changes significantly. The effects of UTP were also inhibited when Kupffer cells were damaged with treatment of gadolinium chloride (10 mg/kg iv). L-NAME (1 mM), a potent inhibitor of nitric oxide synthase, markedly enhanced and prolonged the contractile response of UTP in the hepatic vessel. These results suggest that UTP acts mainly on suramin-sensitive UTP receptors on the Kupffer cell through prostanoid synthesis. The nitric oxide systems in the endothelium seem to counteract the vasoconstrictile action of UTP in the hepatic circulation.

Key Words: Kupffer cells, liver perfusion, nitric oxide, UTP

It is known that extracellular nucleotides play an important role in the regulation of many cell functions. These nucleotide-induced responses are mediated by purinoceptors on the plasma membrane of target cells. Purinoceptors have been divided into two classes: one preferring adenine nucleotides and designated P_2 receptors, and the other designated P_1 receptors which are preferentially stimulated by adenosine (Burnstock and Kennedy, 1985). Further subtypes have been described on a pharmacological basis (Burnstock, 1990). Some purine and pyrimidine nucleotides, and their analogues can selectively activate different P_2 receptors. Recently, a new nomenclature scheme has been proposed for the P_2 purinoceptors, based on their predicted structure and signal transduction mechanism (Dubyak and El-Moatassim, 1993).

Various effects of extracellular uridine and uracil nucleotides have been reported in different kinds of cells. These responses range from the relaxation or contraction of various smooth muscle preparations, to the inhibition of O_2 uptake, stimulation of glucose output, and the enhancement of superoxide or nitric oxide formation (Seifert and Schultz, 1989; O' Connor, 1992). Extracellular UTP, a pyrimidine nucleotide is known as a potent vasoactive substance, and it has been found to be much more vasoactive than other nucleotides in certain vasculatures (Shirasawa *et al.* 1983;

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Hardevo *et al.* 1987). Regulation of vascular tone by UTP is believed to be mediated by the activation of either the P_{2U} purinoceptors or the pyrimidinoceptors, which are distinct from the other purinergic P_2 receptors.

In perfused rat liver, it is known that extracellular nucleotides show hemodynamic responses, glycogenolytic responses, and ion flux responses across the cell membrane. Of these responses, the UTP-induced hemodynamic effects are the most prominent (Haussinger *et al.* 1987; Haussinger *et al.* 1988). However, what remains unclear is which types of cells contribute mainly in the hepatic microcirculatory systems with the stimulation of UTP, and what types of receptors are activated. Therefore, this study was conducted to investigate the hemodynamic actions of UTP and to characterize the receptors activated by UTP in the perfused rat liver.

MATERIALS AND METHODS

Liver perfusion

All perfusion employed Sprague-Dawley rats (300~350 g) regardless of sex. The rats were anesthetized with pentobarbital sodium (30 mg /kg) administered intraperitoneally. The abdominal cavity was opened widely through midline and midtransverse incisions. The inferior vena cava and portal vein were isolated, and 500 USP units of heparin were injected intravenously. After ligating the abdominal vena cava above the left renal vein, a PE-260 polyethylene catheter was inserted into the portal vein and tied in place with a 3.0 silk suture. The thorax was then opened by a transverse incision just above and along the line of the diaphragm and by a longitudinal cephalad incision. A PE-280 polyethylene catheter was inserted and secured in the thoracic vena cava via penetration of the right atrium. The liver was rapidly excised, transferred onto a liver platform, and placed in the perfusion chamber maintained constantly at 37℃. The liver perfusion was performed through the portal vein at a constant pressure (28 cmH_2O). The basal perfusion flow rate was approximately 4 ml/min/g.

Measurements of perfusion flow

The perfusion flow rate was measured by a calibrated spherical float meter (Gilmont Instrument Inc., Great Neck, NY, U.S.A.). A Gilmont Flowmeter F-1200 (accuracy, ±2%) was used for the measurement of portal vein perfusion flow. The real perfusion flow rate (ml/min) was calculated from the scale reading on the flow meter via a calibration chart. The changes of perfusion flow by UTP were measured at 30 second intervals.

Measurements of oxygen consumption

Oxygen tension was measured on a biological oxygen monitor (YSI model 53). Two Clark-type oxygen probes were employed; one at the site entering and the other at the site leaving the liver. Oxygen tension differences between the two probes indicated oxygen uptake by the liver. Oxygen consumption rates were calculated from measurements of oxygen uptake and perfusion flow. The changes of oxygen consumption were measured at the same time as those of perfusion flow.

$GdCl_3$ treatment

Gadolinium chloride ($GdCl_3$) was dissolved in physiological saline at pH 3.0 and injected into the tail vein (10 mg/kg) 24 h before perfusion. Upon intravenous injection, $GdCl_3$ became particulate and was taken up exclusively by macrophages, thus selectively destroying the Kupffer cells (Bouma and Smit, 1989).

Solutions and drugs

Krebs-Ringer bicarbonate buffer (KRB) was used as the perfusion medium. The solution contained (in mM) NaCl 117, KCl 4.7, $CaCl_2$ 1.91, KH_2PO_4 1.19, $MgSO_4$ 1.44, and $NaHCO_3$ 24.8. KRB was saturated with an O_2/CO_2 (95:5 v/v) gas mixture, and pH was adjusted to 7.4 at 37℃. Ca^{2+}- free KRB was made by omitting $CaCl_2$ in the solution. All nucleotides used were prepared as stock solutions in cold saline at a concentration of 50 mM. Gadolinium chloride was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI, U.S.A.), and

Suramin was obtained from Biomol Research Company (Plymouth Meeting, PA, U.S.A.). 2-methylthio-ATP was purchased from RBI (Natick, MA, U.S.A.) and all other drugs were purchased from Sigma (St. Louis, MO, U.S.A.).

Statistical analysis of data

Results are presented as the mean±the standard error of the mean (S.E.M.). The statistical significance of the data was determined by t-tests of paired observations. P value<0.05 were considered statistically significant.

RESULTS

Effects of UTP on portal perfusion flow and hepatic oxygen consumption

In perfused rat liver, the portal perfusion flow was decreased concentration-dependently when UTP was added to the perfusate (Fig. 1A). The maximal response following the administration of UTP was attained within 1 ~2 min, after which there was a slow recovery. After the perfusion flow was restored to a near-basal level, the re-addition of UTP reproduced a similar magnitude of response. UTP also caused a marked, dose-dependent inhibition of hepatic oxygen consumption (Fig. 1B). There was a linear relationship between the UTP-induced decrement of portal perfusion flow and the extent of inhibition of oxygen uptake.

Effects of P_2 purinoceptor antagonists on hepatic hemodynamic changes by UTP

We used P_2 purinoceptor antagonists such as suramin and reactive blue 2 (RB-2) in order to test whether portal hemodynanic changes induced by UTP was due to the activation of the P_2 purinoceptor. Basal portal perfusion flow and hepatic oxygen consumption was not affected by prior treatment with suramin and RB-2. Suramin (100 uM) reduced the UTP-induced changes in perfusion flow and oxygen consumption rate (Fig. 2, 3). Suramin also reduced the ATP-induced inhibition in perfusion flow from 14.3±0.6 ml/min to 4.7±0.4 ml/min,

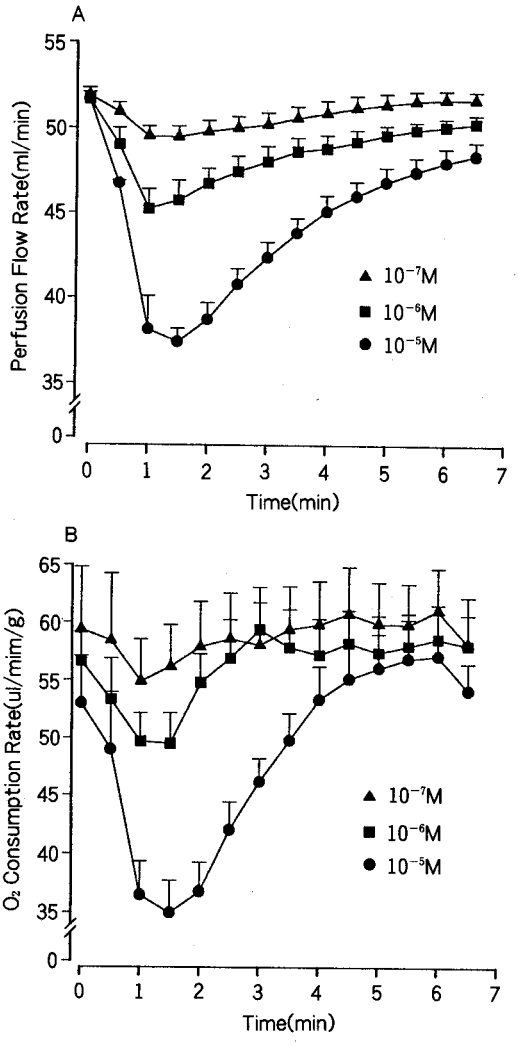

Fig. 1. ***A:*** *Effects of UTP on perfusion flow in the perfused rat liver. Livers were perfused with KRB (pH 7.4, 37℃) in a recirculation system. After 30 min of perfusion, UTP was added into the perfusate.*
B: *Effects of UTP on oxygen uptake in perfused rat liver. The data represents mean± S.E.M. from six livers.*

but no such effect was shown with phenylephrine, an α-adrenergic agonist (Fig. 2). In contrast to UTP, an equivalent concentration of ATP increased the hepatic oxygen consumption rate. Suramin also reduced the increase of the oxygen consumption rate by ATP (Fig. 3). RB-2, known as P_{2y}-selective an-

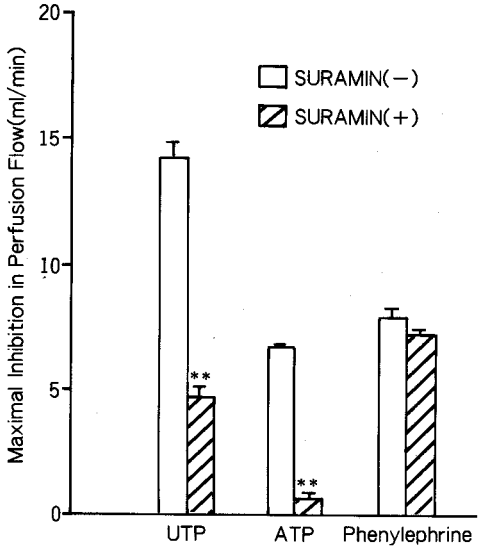

*Fig. 2. Effects of purinoceptor antagonist, 100 uM suramin on the 10 uM UTP, 10 uM ATP and 5 uM phenylephrine-induced inhibition of perfusion flow. ** denotes P<0.01 for comparison with control. The data represents mean±S.E.M. from six livers.*

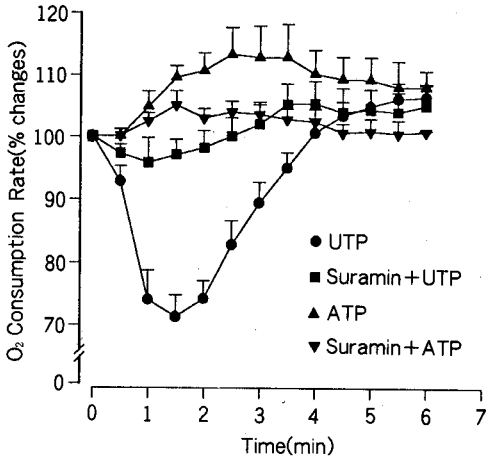

Fig. 3. Effects of 100 uM suramin on % changes in oxygen consumption rate by 10 uM UTP and 10 uM ATP. The data represents mean±S.E.M. from six livers.

tagonist, inhibited the hepatic hemodynamic changes by UTP, ATP, and 2-methythio-ATP (2-MeSATP, a P_{2y}-selective agonist), but it was not a specific purinergic blocker since RB-2 also inhibited the effect of phenylephrine (data not shown).

Effects of extracellular calcium ion on UTP-induced hemodynamic changes

UTP-induced hemodynamic change was not influenced when some Ca^{2+}-channel blockers such as verapamil and nimodipine (20 uM) were included into the perfusate prior to treatment with UTP. However, the depletion of Ca^{2+} from the KRB solution markedly inhibited the UTP-induced hemodynamic changes (Fig. 4).

Effects of indomethacin and gadolinium chloride treatment

Indomethacin (20 uM), an inhibitor of cyclooxygenase, reduced the maximal inhibition in perfusion flow by UTP from 14.9±0.6 ml/min

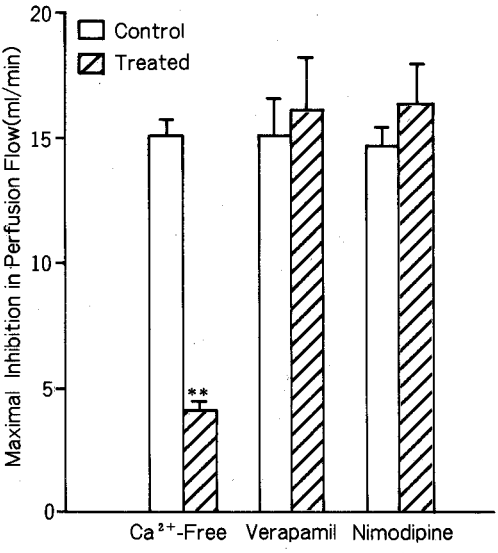

*Fig. 4. Effects of Ca^{2+} free and Ca^{2+} channel antagonists(10 uM verapamil, 10 uM nimodipine) on the UTP-induced inhibition in perfusion flow. ** denotes P<0.01 for comparison with control. The data represents mean±S.E.M. from six livers.*

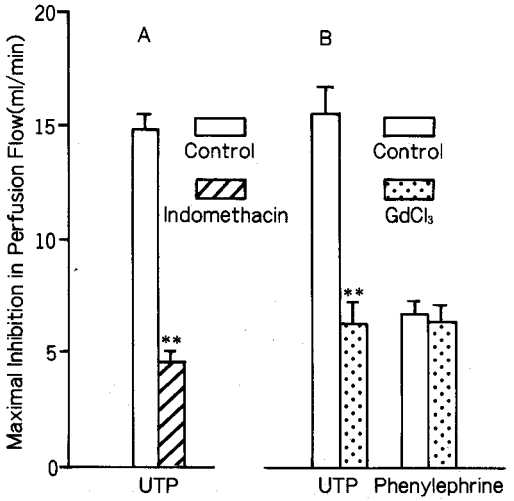

Fig. 5. *Effects of indomethacin (A) and $GdCl_3$ (B) on 10 uM UTP-induced inhibition in perfusion flow. Rats were injected intravenously with $GdCl_3$ (10 mg/Kg) 24 h before perfusion to destroy Kupffer cells. ** denotes $P<0.01$ compared with control. The data represents mean± S.E.M. from four livers.*

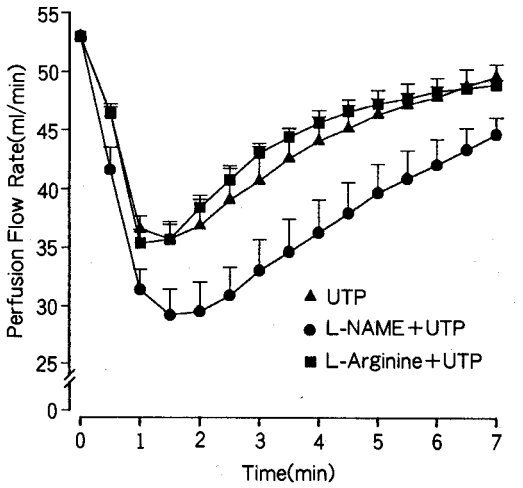

Fig. 6. *Effects of the L-NAME (1 mM) and L-arginine (1 mM) on the 10 uM UTP-induced inhibition of perfusion flow. The data represents mean±S.E.M. from six livers.*

to 4.6±0.5 ml/min (Fig. 5A). In the liver, eicosanoids are produced exclusively in nonparenchymal cells and predominantly by Kupffer cells. To assess the involvement of Kupffer cells in the action of UTP, rats were treated with $GdCl_3$ (10 mg/kg) 24 h prior to the perfusion in order to destroy the Kupffer cells selectively. In the perfused liver of the $GdCl_3$-treated rat, inhibition in the perfusion flow by UTP was reduced from 15.6±1.1 ml/min to 6.3 ±0.9 ml/min, but the phenylephrine-induced response was not reduced (Fig. 5B).

Effects of nitric oxide on liver hemodynamic changes by UTP

To test the role of nitric oxide (NO) in the UTP-induced response, L-arginine (a substrate for nitric oxide synthase, 1 mM) and N^G-monomethyl-L-arginine (L-NAME, nitric oxide synthase inhibitor, 1 mM) were given 10 min before UTP was added to the perfusate. Perfusions with L-arginine and L-NAME prior to UTP administration had no direct effect on portal perfusion flow and hepatic oxygen consumption. However, L-NAME markedly enhanced and prolonged the contractile effects of UTP (Fig. 6).

DISCUSSION

In this study, we have shown that extracellular UTP acts mainly on suramin-sensitive UTP receptors on Kupffer cells via prostanoid synthesis, and that the nitric oxide systems in hepatic sinusoidal endothelial cells (SEC) may counteract the vasoconstrictile action of UTP in the hepatic microcirculation.

It is known that resting levels of extracellular UTP in solid tissues are in the range of 1×10^{-6}M, and that UTP acts as an extracellular signaling molecule (Keppler *et al.* 1970; Seifert and Schultz, 1989). The multiplicity and the widespread existence of receptors for uridine nucleotides and the presence of the nucleotides at concentrations capable of activating these receptors suggests that extracellular UTP involves a physiologically important process. Extracellullar UTP does not only activate the non-selective nucleotide receptors

(P_{2U} receptor) equipotently with ATP, but also activates the uridine nucleotide-selective (or pyrimidinergic) receptors (O'Connor *et al.* 1991; Lazarowski and Harden, 1994). In our experiments, UTP was much more vasoactive than other nucleotides such as ATPP (adenosine tetraphosphate), ATP, ADP, AMPCPP (α,β-methylene ATP), 2-methythio-ATP (2-MeSATP) and UDP in the perfused rat liver(data not shown). The threshold concentration of UTP-induced hepatic hemodynamic changes was below the micromolar concentration (Fig. 1). Because the nucleotide pyrophosphatase in the rat liver plasma membrane has a similar affinity for ATP and UTP (Bischoff *et al.* 1975), we assumed that UTP would be completely hydrolyzed during a single liver passage as was ATP (Haussinger *et al.* 1987).

Although specific antagonists for receptors for extracellular nucleotides have not been identified, suramin and reactive blue 2 (RB-2), which have been shown to block competitively the P_2 purinergic receptors in a number of tissues, have been widely used to distinguish each receptor subtype on a pharmacological basis (Dunn and Blakeley, 1988; Fedan and Lamport, 1990). Suramin also inhibits the uridine nucleotides-selective receptors (Lazarowski and Harden, 1994). As shown in Fig. 2 and 3, suramin inhibited the UTP-induced changes in portal perfusion flow and hepatic oxygen consumption. These effects were not observed by phenylephrine, an α-adrenergic agonist. The findings that the effects by UTP were much more potent than any other nucleotide, and that UTP and ATP had different effects of action on the hepatic microcirculation suggest that the effects by UTP are mediated by the activation of the UTP-selective receptors. Haussinger *et al.* (1987) demonstrated that sodium nitroprusside was largely ineffective in preventing the hemodynamic changes induced by UTP, except in the case of α-adrenergic stimulation and ATP. This could also reflect a different mode of action of these agents on the hepatic microcirculation. But the P_2 purinoceptor antagonists were not specific for the effects by UTP or ATP because suramin blocked not only the hemodynamic changes by UTP, but also those by ATP. RB-2 was not helpful as a purinergic antagonist in the perfused rat liver.

The vasoconstrictor effect by UTP was greatly inhibited in the Ca^{2+}-free perfusion medium, but the presence of verapamil and nimodipine did not produce any inhibitory effect (Fig. 4). This may indicate that Ca^{2+} influx with the exception through the L-type calcium channel, or calcium mobilization from the intracellular calcium pool, is important in UTP-induced vasoconstriction in the perfused rat liver. Some nucleotides increase Ca^{2+} influx or trigger calcium mobilization in various types of cells via activation of purinoceptors on cell surface membranes. A Ca^{2+} influx or calcium mobilization by ATP in the perfused rat liver has already been reported (Haussinger *et al.* 1987; Lee and Filkins, 1988). Although there is little information on the role of Ca^{2+} in the UTP-induced vasoconstriction, it seems closely correlated with the release of eicosanoids causing vasoconstriction (Dieter *et al.* 1988). Because hepatocytes have been demonstrated to possess little or no capacity to form prostanoids (Kuiper *et al.* 1988), the nonparenchymal cells have been postulated to be the source of hepatic prostanoid secretion (Birmalin and Decker, 1984; Haussinger *et al.* 1988). In this study we demonstrated that the hemodynamic effects by UTP in the perfused rat liver were largely suppressed by indomethacin and pretreatment with $GdCl_3$ (Fig. 4). These findings indicate that prostanoids from Kupffer cells are the major mediators in the UTP-induced hemodynamic responses. Extracellular ATP and UTP stimulated prostanoid release from the perfused rat liver, the isolated Kupffer cells, and the SEC (Haussinger *et al.* 1988; Nukina *et al.* 1994). Hashimoto *et al.* (1995) identified the secretory profiles of prostanoids such as prostaglandin (PG) E_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, PGD_2, and thromboxane (TX) B_2 by Kupffer cells and SEC in primary cultures, both under basal condition and after stimulation with nucleotides. They revealed that PGE_2 was the major prostanoid secreted by the hepatic nonparenchymal cells under both conditions, and that ATP stimulated more major prostanoid secretion than any other adenine nucleotides,

adenosine, or UTP. The specificity for extracellular nucleotides for major prostanoid secretion did not correlate with the increased hemodynamic action by UTP compared to the other nucleotides including ATP. Therefore the UTP-induced stimulation of major prostanoids cannot fully explain the response of perfused liver to extracellular UTP. Although the reason for this discrepancy is unknown, it may be attributable to the possible roles of minor prostanoids, (i.e. not PGE_2), in UTP-induced hepatic hemodynamic changes or to signalling pathways other than via prostanoid synthesis.

It is known that nitric oxide (NO) causes hyporeactivity to vasopressors in some vasculatures (Sieber and Groszmann, 1992; Pastor and Billiar, 1995). In this study, we also showed that nitric oxide (NO) was involved in the UTP-induced hemodynamic changes. Takemura *et al.* (1995) reported that nucleotides and nucleosides exogenously applied into hepatic perfusate increased the rate of nitric oxide generation with no appreciable effect on the perfusion pressure. In our study, while L-arginine and N^G-nitro-L-arginine (L-NAME) had no direct effect on portal perfusion flow and hepatic oxygen consumption, L-NAME markedly enhanced and prolonged the contractile effect of UTP. L-arginine attenuated the decrease in perfusion flow by UTP to a lesser extent. This was likely due to the fact that the hepatic vasculature was already fully dilated in the basal perfusion condition (basal flow: about 4 ml/min/g). Under these conditions no further dilation was probably possible by the UTP- induced NO release.

In summary, we have shown that UTP mainly acts on suramin-sensitive UTP receptors, which is distinct from other classical P_2 purinoceptors, on nonparencymal cells such as Kupffer cell and SEC via prostanoid synthesis and NO production. The overall hemodynamic action by UTP was vasoconstriction predominantly in the perfused rat liver, and the nitric oxide systems in SEC may counteract the vasoconstrictile action of UTP in hepatic circulation.

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