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LEUKOTRIENES INCREASE GLUCOSE AND LACTATE OUTPUT AND DECREASE FLOW IN PERFUSED RAT LIVER

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In isolated perfused rat liver leukotriene C_4 and D_4 but not B_4 and E_4 enhanced glucose and lactate output and lowered perfusion flow similar to the thromboxane A_2 analogue U46619, extracellular ATP and prostaglandin $F_{2\alpha}$. The kinetics of the metabolic changes caused by leukotriene C_4 and D_4 resembled those effected by U46619 and ATP but not those elicited by prostaglandin $F_{2\alpha}$; the kinetics of the hemodynamic changes were similar only to those caused by U46619. The results show that leukotrienes could be important modulators of hepatic metabolism and hemodynamics and point to a complex intra-organ cell-cell communication between non-parenchymal and parenchymal cells.

Leukotrienes are potent mediators of inflammation and tissue trauma as well as circulatory and respiratory dysfunction (1-4). The dihydroxylated leukotriene B_4 exerts its actions predominantly on polymorphonuclear leukocytes and mononuclear phagocytes; it causes chemotaxis, chemokinesis, aggregation and adherence. The cysteinyl leukotrienes C_4, D_4 and to a lesser extent E_4 exert their effects mainly on smooth muscles in heart, lung and kidney; they cause vasoconstriction, bronchoconstriction and plasma extravasation. Leukotrienes are produced and released predominantly by macrophages, monocytes, neutrophils, eosinophils and mast cells following various stimuli (1-4). They have a very short half life in blood and are therefore regarded mainly as local rather than circulating hormones. They are excreted after catabolism mainly in lung, liver and kidney via the hepatic pathway into bile and thence feces, and via the renal pathway into urine (4-6).

Leukotrienes appear to be involved as mediators also in liver diseases. In the endotoxin/D-galactosamine model of fulminant hepatitis in mice several inhibitors of leukotriene synthesis or actions were highly effective in reducing the histological signs of and the rise of cellular enzyme activities in plasma resulting

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from liver injury (4). In D-galactosamine-induced hepatitis in rats a leukotriene synthesis inhibitor was found to protect against inflammation (4). During the early phase of acute alcoholic hepatitis a chemotactic leukotriene may mediate leukocytic infiltration of the liver parenchyma (7). Leukotrienes formed during sepsis, severe trauma, burn injury, viral hepatitis and allergic reactions may contribute to intrahepatic cholestasis by promoting edema around bile ducts (4).

The effects of leukotrienes have so far not been studied in isolated liver preparations such as the perfused organ, hepatocyte suspensions or cultures (cf.1-4, 8). Therefore, it was the object of the present study to investigate possible actions of leukotrienes on hepatic metabolism and hemodynamics in the isolated perfused rat liver. It was found that the cysteinyl leukotrienes C_4 and D_4 clearly increased glucose and lactate output and lowered perfusion flow.

MATERIALS AND METHODS

All chemicals were of reagent grade and from commercial sources. Enzymes and bovine serum albumin (BSA) were from Boehringer (D-6800 Mannheim), leukotrienes and prostaglandin $F_{2\alpha}$ were from Sigma (D-8024 Deisenhofen) and the thromboxane A_2 analogue U46619 and the LTD_4-antagonist CGP 35949 B were generous gifts of Upjohn (D-6148 Heppenheim) and Ciba Geigy (CH-4002 Basel), respectively. The leukotriene methyl esters were hydrolyzed with a 5% solution of potassium carbonate for 3 h, then diluted into a Krebs-Henseleit buffer containing 0.5% BSA and 20 mM HEPES instead of sodium carbonate to an appropriate concentration for infusion. Prostaglandin $F_{2\alpha}$ was first dissolved in saline to a concentration of about 6 mM and the thromboxane analogue in 36 mM sodium bicarbonate under shaking for 2 h at 4°C to a concentration of 60 µM and then diluted in the perfusion buffer to an appropriate concentration for infusion.

The livers of male Wistar rats (150-200 g; Winkelmann, D-4791 Borchen) were perfused at constant pressure *in situ* without recirculation via the portal vein in a 37°C cabinet using a Krebs-Henseleit bicarbonate buffer containing 5 mM glucose, 2 mM lactate, 0.2 mM pyruvate and 0.1% BSA equilibrated with 95% (v/v) O_2 and 5% CO_2. Flow was measured by fractionating the effluent. One week before the experiments the animals were subjected to a 12 h day-night rhythm with free access to food (standard diet 1320 of Altromin, D-4917 Lage); they were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body weight) (9).

The eicosanoids and ATP were infused to the final concentration as indicated in the figures and the table. Glucose and lactate were measured in the influent and effluent with standard enzymatic techniques.

RESULTS

Rat livers were perfused at constant pressure via the portal vein. After a pre-perfusion period of 30 min the experiment was

started. Leukotrienes, prostaglandin $F_{2\alpha}$ and the thromboxane A_2 analogue U46619 were infused for a period of 5 min from 40-45 min of perfusion. The leukotrienes were infused also for a second, third and fourth stimulation period of 5 min with 10 min intervals.

Influence of leukotrienes on carbohydrate metabolism

The cysteinyl leukotriene D_4 (LTD_4) increased glucose and lactate output; it caused half-maximal effects (area under the curve = AUC) already at the low concentration of 1 nM (Fig. 1). The maximal changes (peak values) of glucose and lactate metabolism were reached about 7 min after LTD_4 addition. The cysteinyl leukotriene C_4 but not E_4 elicited similar metabolic alterations as LTD_4 when infused at a concentration of 20 nM, which was saturating for LTD_4 (Table 1; Fig. 1). The dihydroxylated LTB_4 infused at a concentration of 20 nM was without effect (Table 1).

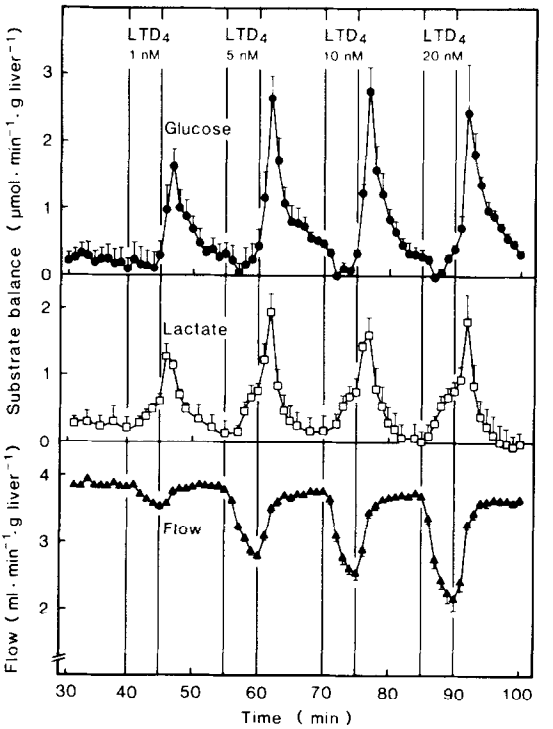

Fig. 1. Glucose and lactate balance and perfusion flow in perfused rat liver following repetitive infusions of leukotriene D_4. Livers were perfused in situ without recirculation with a Krebs-Henseleit bicarbonate buffer containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. Leucotriene D_4 (LTD_4) was infused for the time periods and to the final concentrations indicated. Substrate balance is given by [concentration in hepatic vein - concentration in portal vein (µmol x ml^{-1})] x flow (ml x min^{-1} x g $liver^{-1}$). Values are means ± SEM of three experiments.

Table 1 Alteration of glucose and lactate balance and of perfusion flow by leukotrienes in perfused rat liver

Parameter	LTB_4	LTC_4	LTD_4	LTE_4
Increase in		µmol x g liver^{-1} (area under the curve)		
glucose output	0.2 ± 0.1	7.6 ± 1.1	8.2 ± 1.5	0.4 ± 0.1
lactate output	0.1 ± 0.1	5.3 ± 0.7	6.4 ± 0.6	0.3 ± 0.2
Decrease in		ml x g liver^{-1} (area under the curve)		
flow	n.d.	-8.0 ± 0.7	-7.6 ± 0.6	-0.2±0.2

Livers were perfused and leukotrienes were infused to a final concentration of 20 nM each as described in fig. 1. Values are means ± S.E.M. of three experiments each. n.d. = not detectable.

The kinetics of the metabolic alterations caused by 20 nM LTC_4 and LTD_4 were very similar to those elicited by 100 nM U46619, the thromboxane A_2 analogue, and by 100 µM ATP, in that the maximal alterations were reached about 7 min after onset of the stimulus (Fig. 2). The kinetics of the metabolic changes

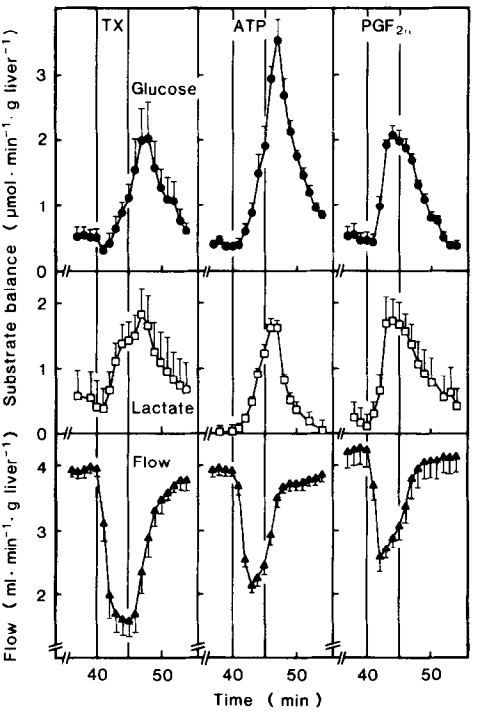

Fig. 2. Glucose and lactate balance and perfusion flow in perfused rat liver following infusion of the thromboxane A_2 analogue U46619, of ATP and of prostaglandin $F_{2\alpha}$. Livers were perfused as described in Fig. 1. For the indicated time periods U46619 (TX), ATP or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were infused to final concentrations of 100 nM, 100 µM and 10 µM, respectively. Substrate balance is defined in Fig. 1. Values are means ± SEM of four experiments.

differed from those effected by 10 µM prostaglandin $F_{2\alpha}$, because with this stimulus the maximal alterations were reached already 3 min after the start of stimulation (Fig. 2).

Influence of leukotrienes on perfusion flow

Leukotriene D_4 decreased perfusion flow; it caused half-maximal effects (AUC) at concentrations around 5 nM (Fig. 1). The maximal change (peak value) was reached after 5 min at the end of the stimulation period. When infused at concentrations of 20 nM, LTC_4 caused similar hemodynamic changes; LTE_4 and LTB_4 were essentially ineffective (Table 1).

The kinetics of the hemodynamic alterations caused by 20 nM LTD_4 and LTC_4 were similar to those caused by 100 nM U46619, in that the maximal changes (peak values) were reached at the end of the 5 min stimulation period (Fig. 2). The kinetics of the flow changes were clearly different from those caused by 100 µM ATP and 10 µM prostaglandin $F_{2\alpha}$, because with the latter two stimuli flow was maximally reduced (peak values) already after 2-3 min and began to return to the pre-stimulation level inspite of continued stimulation thus showing an "escape" phenomenon (Fig. 2).

Specificity of leukotriene action

The metabolic and hemodynamic effects of 5 nM LTC_4 and LTD_4 were completely inhibited by 1 µM CGP 35949 B, a LTD_4-antagonist.

Lack of correlation between metabolic and hemodynamic alterations

The metabolic effects of LTC_4 and LTD_4 could be caused directly by leukotriene action on (almost) each or only on some hepatocytes with signal propagation through gap junctions or indirectly by leukotriene action on non-parenchymal cells causing the formation of other mediators, e.g. thromboxanes and prostaglandins, or eliciting hemodynamic changes, which could cause partial hypoxia and thus an increase in glycogenolysis in at least some parenchymal areas. The extents of the LTD_4-dependent metabolic were not correlated to those of the hemodynamic alterations (Fig. 1): 1 nM LTD_4 caused half-maximal metabolic but only minor hemodynamic changes and 20 nM LTD_4 elicited maximal metabolic but only submaximal hemodynamic changes. It can therefore be concluded that the indirect hemodynamic mechanism cannot play a major role in the action leukotrienes on carbohydrate metabolism.

DISCUSSION

It was found in the present study that the cysteinyl leukotrienes C_4 and D_4 but not E_4 or the dihydroxylated leukotriene B_4 stimulated glucose and lactate output and reduced flow in the isolated perfused rat liver (Fig. 1). Leukotrienes circulate in blood normally in picomolar concentrations; the metabolically and hemodynamically effective nanomolar concentrations may be reached only in very severe pathological situations (1-4). It is therefore most likely that leukotrienes affect liver metabolism and hemodynamics as local hormones generated within the organ. Since in the liver cysteinyl leukotrienes, i.e. LTC_4, LTD_4 and LTE_4, are formed by non-parenchymal cells such as the Kupffer cells of the sinusoids (8) and since the major metabolic pathways are catalyzed by the parenchymal cells, metabolic regulation involving leukotrienes is an example of a complex intra-organ cell-cell communication.

Mechanism of action of leukotrienes in liver

The present study allowed the conclusion that the hemodynamic alterations caused by LTC_4 and LTD_4 cannot be a major cause for the metabolic effects. This conclusion is in line with the previous demonstration that the decrease in flow was not a major cause for the nerve stimulation-dependent metabolic alterations either (10).

The present results do not allow to decide whether the leukotrienes acted directly on the parenchymal cells or indirectly by stimulating the production of a second signal such as a thromboxane or a prostaglandin. The latter indirect mode of action has been shown to play an important role e.g. in bronchoconstriction caused by leukotrienes in the guinea pig (3,4). The thromboxane analogue U46619 may be a better candidate for an intermediate signal than prostaglandin $F_{2\alpha}$ because of the similar kinetics of both the metabolic and hemodynamic changes caused by LTD_4 and U46619 (Fig. 1 and 2). U46619 (11) and prostaglandin E_2 (12) and $F_{2\alpha}$ (13) were shown very recently to increase glucose output and portal pressure in rat livers perfused at constant flow, which corresponds to the enhancement of glucose release and the decrease of flow observed here in livers perfused at constant pressure (Fig. 2). However, it is unclear whether U46619 and prostaglandin E_2 or $F_{2\alpha}$ can increase glucose release directly in isolated hepatocytes (11).

Since LTC_4 is rapidly converted to LTD_4 in liver by the action of the ectoenzyme γ-glutamyltranspeptidase (4-6), the

present data do not permit either to differentiate, whether LTC_4 or LTD_4 or both acted on receptors of the target cells involved in mediating the metabolic and hemodynamic responses.

Functions of leukotrienes in liver

Kupffer and mast cells under normal conditions and inflammatory cells infiltrating the liver under pathological conditions may contribute to intrahepatic leukotriene production (4,8). Under physiological conditions leukotrienes may be involved as mediators or modulators in the increase of hepatic glucose release caused by sympathetic nerve stimulation (9), extracellular ATP (9) or phagocytosis of zymosan (cell wall particles from yeast) by Kupffer cells (14) as well as in the hemodynamic alterations (decrease of flow in constant pressure perfusions or increase of portal pressure in constant flow perfusions) elicited by sympathetic nerve stimulation (9), by extracellular ATP (9), circulating noradrenaline (9) and zymosan phagocytosis (15), because all these effects were found to be inhibited by nordihydroguaiaretic acid, an inhibitor of lipoxygenase and thus of leukotriene formation.

In inflammatory liver diseases (cf. introduction) (4) leukotrienes formed intrahepaticly can be expected to contribute to the regulation of carbohydrate metabolism and hemodynamics, especially to the control of the local microcirculation in liver.

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REFERENCES

1) Samuelson, B. (1983) Science 220, 568-575
2) Hammarström, S. (1983) Ann. Rev. Biochem. 52, 355-377
3) Piper, P.J. (1984) Physiol. Rev. 64, 744-761
4) Keppler, D., Hagmann, W., Rapp, S., Denzlinger, C. and Koch, H.K. (1985) Hepatology 5, 883-891
5) Keppler, D., Huber, M., Weckbecker, G., Hagmann, W., Denzlinger, C. and Guhlmann, A. (1987) Adv. Enz. Regul. 26, 211-224
6) Huber, M. and Keppler, D. (1987) Eur. J. Biochem. 167, 73-79
7) Perez, H.D., Roll, F.J., Bissell, D.M., Shak, S. and Goldstein, I.M. (1984) J. Clin. Invest. 74, 1350-1357
8) Decker, K. (1986) in Cells of the Hepatic Sinusoid, (Kirn, A., Knook, D.L. and Wisse, E.), vol. 1, pp. 53-58, Kupffer Cell Foundation Rijswijk, The Netherlands
9) Iwai, M. and Jungermann, K. (1987) FEBS Lett. 221, 155-160

10) Beckh, K., Beuers, U., Engelhardt, R. and Jungermann, K. (1987) Biol. Chem. Hoppe Seyler 368, 379-386
11) Fisher, R.A., Robertson, S.M. and Olson, M.S. (1987) J. Biol. Chem. 262, 4631-4638
12) Buxton, D.B., Fisher, R.A., Briseno, D.L., Hanahan, D.J. and Olson, M.S. (1987) Biochem. J. 243, 493-498
13) Häussinger, D., Stehle, T., Tran-Thi, T.-A., Decker, K. and Gerok, W. (1987) Biol. Chem. Hoppe Seyler 368, 1509-1513
14) Dieter, P., Altin, J.G., Decker, K. and Bygrave, F.L. (1987) Eur. J. Biochem. 165, 455-460
15) Dieter, P., Altin, J.G. and Bygrave (1987) FEBS Lett. 213, 174-178