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Eicosanoid-mediated increase in glucose and lactate output as well as decrease and redistribution of flow by complement-activated rat serum in perfused rat liver

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Rat serum, in which the complement sytem had been activated by incubation with zymosan, increased the glucose and lactate output, and reduced and redistributed the flow in isolated perfused rat liver clearly more than the control serum. Heat inactivation of the rat serum prior to zymosan incubation abolished this difference. Metabolic and hemodynamic alterations caused by the activated serum were dose dependent. They were almost completely inhibited by the cyclooxygenase inhibitor indomethacin and by the thromboxane antagonist 4-[2-(4-chlorobenzenesulfonamide)-ethyl]-benzene-acetic acid (BM 13505), but clearly less efficiently by the 5'-lipoxygenase inhibitor nordihydroguaiaretic acid and the leukotriene antagonist *N*-{3-[3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-propoxy]-4-chlorine-6-methyl-phenyl}-1H-tetrazole-5-carboxamide sodium salt (CGP 35949 B). Control serum and to a much larger extent complement-activated serum, caused an overflow of thromboxane B_2 and prostaglandin $F_{2\alpha}$ into the hepatic vein. It is concluded that the activated complement system of rat serum can influence liver metabolism and hemodynamics via release from nonparenchymal liver cells of thromboxane and prostaglandins, the latter of which can in turn act on the parenchymal cells.

During inflammatory diseases severe metabolic impairment of intermediary metabolism and hemodynamics occurs locally at the site of inflammation as well as systemically during severe septicaemia or endotoxinaemia. In the course of these processes the complement system can be activated via a specific pathway that is initiated by antigen · antibody complexes, or by an unspecific, so called alternate pathway, that constitutes an autocatalytic process [1]. The alternate activation can be enhanced by cell-wall particles from yeast (zymosan) or Gram-negative bacteria [1]. The activation of the complement system *in vivo* causes the synthesis of a variety of very potent mediators of inflammation and leads to the formation of a lytical membrane-bound complex. Whereas most of the components of the activated complement system are very unstable in the serum or are inactivated almost immediately after their formation [1], so that they can be active only near their site of formation, others seem to escape the rapid inactivation and can exert effects in remote organs [1].

Several agents which can activate the complement system either via the classic or the alternate pathway, e. g. heat-aggregated immunoglobulins [2], zymosan [3, 4] or endotoxins [5], have been shown in rat liver perfused with a serum and cell-free medium, to increase glucose and lactate output and portal pressure directly without the intervention of the complement system. The purpose of this study was to examine in the perfused rat liver, whether rat serum in which the complement system had been activated with zymosan via the alternate pathway, could modulate liver metabolism and hemodynamics and which mediators were possibly involved. It was found that complement-activated rat serum increased glucose and lactate output and reduced, as well as redistributed, the flow. Metabolic and hemodynamic changes were accompanied by an overflow of thromboxane B_2 (TXB_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) into the hepatic vein and could be inhibited by indomethacin or the thromboxane antagonist, BM 13505.

MATERIALS AND METHODS

Chemicals

Dimethylsulfoxide (Me_2SO), indomethacin, nordihydroguaiaretic acid (NDGA), zymosan and trypan blue were purchased from Sigma (Deisenhofen, FRG). Enzymes and coenzymes for the metabolite assays were bought from Boehringer (Mannheim, FRG), who also provided the thromboxane antagonist, BM 13505 as a gift. The Merck Glucose System was obtained from Merck (Darmstadt, FRG). CGP 35949 B, was a gift from Ciba-Geigy (Basel, Switzerland). Radioimmunoassays for TXB_2 and $PGF_{2\alpha}$ were bought from Amersham (Braunschweig, FRG). The carboxypeptidase inhibitor D,L-mercapto-methyl-3-guanidinoethylthiopropanoic acid was from Calbiochem (Frankfurt, FRG). All other chemicals were from commercial sources and of analytical grade.

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Abbreviations. BM 13505, 4-[2-(4-chlorobenzenesulfonamide)-ethyl]-benzeneacetic acid; CGP 35949 B, *N*-{3-[3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-propoxy]-4-chlorine-6-methyl-phenyl}-1H-tetrazole-5-carboxamide sodium salt; Me_2SO, dimethylsulfoxide; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; NDGA, nordihydroguaiaretic acid; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2.

Enzymes. Glucose dehydrogenase (EC 1.1.1.47); lactate dehydrogenase (EC 1.1.1.27); glutamate pyruvate transaminase (EC 2.6.1.2).

Note. Dedicated to Professor Karl Decker, Freiburg, on the occasion of his 65th birthday.

Animals

Male Wistar rats were bought from Winkelmann (Borchen, FRG) and kept on a 12-h day-night rhythm (light from 7 a.m. to 7 p.m.) for at least one week before the experiment with free access to water and a rat standard diet from Ssniff (Soest, FRG).

Preparation of rat serum

Blood was collected from the inferior caval vein of anaesthetized rats that were to be sacrificed for organ sampling in other studies. After complete clotting, the blood was centrifuged at $5000 \times g$ and the serum decanted. Serum from several rats was pooled and frozen in aliquots at $-20\,^{\circ}C$ for later use. It was stored for no longer than two weeks. The complement system was activated immediately before the perfusion experiment via the alternate pathway by incubation of the serum with zymosan (2 mg/ml, 1 h, 37 °C). Zymosan, which previously had been shown to increase glucose output and perfusion pressure in perfused rat liver by itself [3, 4], was then completely removed by centrifugation at $27000 \times g$ for 30 min. The successful removal of zymosan by centrifugation was ascertained by infusion of the supernatant of zymosan-treated Krebs-Henseleit buffer, which was inactive. Control serum was treated in exactly the same manner, but without zymosan. Control serum or activated serum were diluted in perfusion medium, 1:3 if not otherwise stated and infused at a rate of 2 ml/min, i.e. about 1/15 of the total perfusion flow.

Molecular mass determination of the active component of zymosan-treated rat serum

The bulk of protein was removed from rat serum which had been activated by incubation with zymosan, by precipitation with 10% (mass/vol.) polyethyleneglycol 6000 at pH 5.0. The supernatant (5 ml) of the precipitation was chromatographed on a Sephadex G75 column (2.5 cm diameter, 90 cm length) equilibrated with 25 mM Tris/HCl, pH 7.4. The flow rate was 18 ml/h, the fraction size was 2.25 ml. The column was calibrated with bovine serum albumin (68 kDa), chymotrypsinogen (25 kDa), myoglobin (17 kDa) and cytochrome c (12.5 kDa). Pools of 20 fractions each corresponding to roughly the molecular mass range ≥ 68 kDa, < 68 kDa $- \geq 25$ kDa, < 25 kDa $- \geq 17$ kDa, < 17 kDa $- \geq 10$ kDa, < 10 kDa, were lyophilized, redissolved in 4 ml water, filtered through a micropore filter (0.2 μm) and infused as described for activated serum.

Perfusion

Rats (160 – 190 g) were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg). Livers were perfused *in situ* at a rate of about 30 ml/min via the portal vein, using a pressure constant, non-recirculating perfusion system described previously [6]. The perfusion medium was a Krebs-Henseleit buffer pH 7.4, 37 °C, equilibrated with O_2/CO_2 95:5 (by vol.), containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate, and in some experiments additionally 0.1% (mass/vol.) bovine serum albumin, 0.1% (by vol.) Me_2SO and indomethacin, NDGA, BM 13505 or CGP 35949 B at the concentrations indicated.

Metabolite determination

Glucose was determined employing a commercial test kit based on the glucose dehydrogenase method (the Merck Glucose System) [7], lactate was measured in a combined optical test with lactic dehydrogenase and glutamic pyruvic transaminase in a basic glutamine buffer [8]. TXB_2 and $PGF_{2\alpha}$ were determined with radioimmunoassays from Amersham according to the instructions of the company.

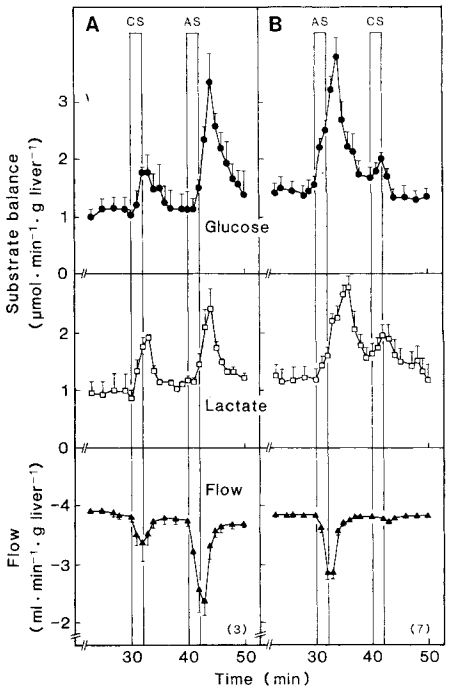

Fig. 1. *Increase in glucose and lactate output, and reduction of flow after infusion of control and complement-activated rat serum in perfused rat liver.* Rat livers were perfused *in situ* via the portal vein with a Krebs-Henseleit buffer, pH 7.4, containing 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate. At the times indicated, control serum (CS) or complement-activated serum (AS) of the rat was infused at a rate so that its final dilution was 1:45. Perfusate was collected from the inferior caval vein every minute, the flow rate being measured as the volume of each fraction. Glucose and lactate output ($\mu mol \cdot min^{-1} \cdot g\ liver^{-1}$) were calculated according to the formula: [posthepatic concentration ($\mu mol \cdot ml^{-1}$) – prehepatic concentration ($\mu mol \cdot ml^{-1}$)] × flow ($ml \cdot min^{-1} \cdot g\ liver^{-1}$). Values are means ± SEM of the number of experiments given in parentheses

RESULTS

Increase in glucose and lactate output as well as reduction and redistribution of perfusion flow by complement-activated rat serum

Diluted (1:3) control and activated rat serum were infused in the isolated perfused rat liver at 1/15 of the rate of perfusion flow, i.e. to a final dilution of 1:45 for 2 min, each with an interval of 8 min. The control serum increased glucose and lactate output and decreased the flow slightly (Fig. 1 A). Rat serum, in which the complement system had been activated via the alternate pathway with zymosan, increased glucose and lactate output and decreased the flow strongly, the alterations being clearly larger than those observed with the control serum (Fig. 1 A). To exclude a sensitization of the liver as reason for the larger effects in the second infusion period, complement-activated serum was infused in the first period and control serum in the second period (Fig. 1 B). The increase in glucose and lactate output and the decrease in flow were the same

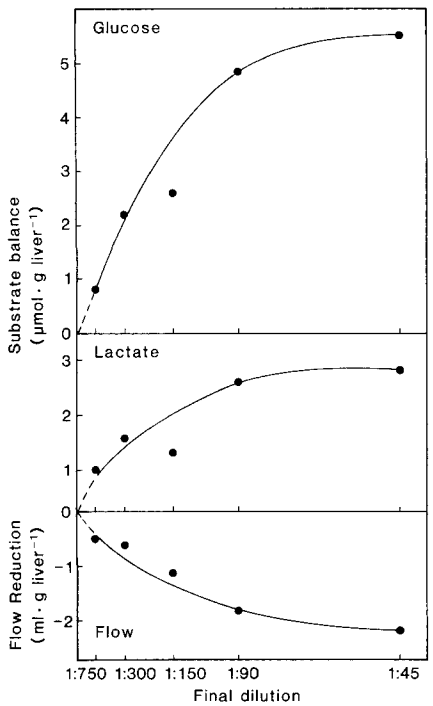

Fig. 2. *Dose dependence of the increase in glucose and lactate output, and the reduction of flow after infusion of complement-activated rat serum in perfused rat liver.* Livers were perfused as described in Fig. 1. Activated rat serum was infused as a first stimulus to the dilution indicated. The increases in metabolite output and the reduction in flow (areas under the curve) are shown. The values are from a single series of experiments

with infusion of activated serum during the second and first period; however, the effects of the control serum were largely diminished with application during the second period. Activated serum no longer decreased the flow in the presence of the calcium antagonist nifedipine, which has been shown previously to prevent hemodynamic changes by sympathetic-nerve stimulation, circulating noradrenaline and $PGF_{2\alpha}$ [9]; yet, it still increased glucose and lactate output (not shown). This precludes hypoxia as a major cause for the increases in metabolite output by activated serum.

The flow reduction after infusion of activated serum was accompanied by a redistribution of the flow in the liver. Whereas under basal conditions the perfused liver was stained homogeneously with trypan blue infused to a final concentration of 0.2%, it was stained quite inhomogeneously during the action of activated serum (not shown). Some areas of the liver apparently were no longer perfused, whereas others remained perfused normally. After cessation of the infusion of activated serum, when the flow had returned to the basal level, the staining of the liver was homogeneous again.

Metabolic and hemodynamic alterations were dose dependent (Fig. 2). A significant increase in glucose output and reduction of flow could still be observed at a final dilution of 1:750 of the activated serum.

Heating the serum to 57 °C for 30 min, which is a generally accepted method to inactivate the complement proteases and thus to prevent complement activation [10, 11], prior to the treatment of the serum with zymosan, completely abolished the differences in action between zymosan-treated and control serum (Table 1). This shows that (a) the integrity of the complement system was necessary in order to generate the components that were responsible for the stronger metabolic and hemodynamic effects caused by zymosan-treated serum in comparison to control serum; (b) the zymosan particles, which by themselves interfere with liver metabolism and hemodynamics [3, 4] were completely removed from the serum after zymosan treatment. Heating of the serum to 57 °C for 30 min after activation with zymosan, i.e. after formation of the active components of the complement system, did not significantly diminish the capacity of activated serum to increase glucose and lactate output and to decrease the flow. This shows that the active components of the activated serum were heat-resistent and therefore could only be low molecular mass proteins (< 20 kDa), polypeptides or oligopeptides, or small molecular mass substances. This interpretation was further substantiated by gel-chromatographic analysis of the activated serum. Zymosan-treated serum was subjected to a precipitation with 10% polyethyleneglycol; the supernatant was chromatographed on Sephadex G75. The components responsible for the increase in glucose and lactate output, and reduction of the flow, were found in pools corresponding to the molecular mass range of < 25 kDa $- \geq 17$ kDa and < 17 kDa $- \geq 10$ kDa. The latter showed a somewhat higher potency than the former (not shown).

The inhibitor of serum carboxypeptidase D,L-mercaptomethyl-3-guanidinoethylthiopropanoic acid [12], that was reported to prevent the degradation of the anaphylatoxins C3a and C5a in human serum (M. Oppermann, F. Liebmann, M. Schulze and O. Götze, unpublished results) and to enhance the effect of human anaphylatoxin C3a in perfused rat liver [13], did not influence the extent of the metabolic and hemodynamic alterations (not shown).

Inhibition of the metabolic and hemodynamic effects of activated serum by eicosanoid-synthesis inhibitors and receptor antagonists

The increments of increases in glucose and lactate output and reduction of perfusion flow by activated serum over control serum were reduced by indomethacin (20 μM), an inhibitor of cyclooxygenase [14] and thus prostanoid synthesis, by more than 90% (Fig. 3), whereas they were reduced by NDGA (20 μM), an inhibitor of lipoxygenase [15] and thus leukotriene synthesis by only about 50%. Likewise the thromboxane A_2 (TXA_2) receptor antagonist BM 13505 (20 μM) [16], which blocked the metabolic and hemodynamic effects of the TXA_2 analog U46619 in perfused rat liver and did not unspecifically interfere with the actions of leukotriene D_4 (LTD_4) or $PGF_{2\alpha}$ (M. Iwai and K. Jungermann, unpublished results), almost completely abolished the differences in the action between activated serum and control serum. However, the leukotriene C_4 (LTC_4)/LTD_4 receptor antagonist CGP 35949 B (1 μM) [17], which completely blocked the metabolic and hemodynamic actions of LTC_4 and LTD_4 in perfused rat liver [18, 19] and also partially inhibited those of the TXA_2 agonist U46619 (G. P. Püschel, unpublished results), reduced the differences between activated and control serum in glucose output only by about 50% and left the lactate output and the flow reduction almost unaffected. Thus it appeared that the metabolic and hemodynamic effects of complement-activated rat serum were mediated primarily by TXA_2.

Overflow of TXB_2 and $PGF_{2\alpha}$ into the hepatic vein

Control serum contained 1.3 ± 0.2 μM TXB_2 and 68 ± 12 nM $PGF_{2\alpha}$ (SEM, $n = 6$). The levels of these pro-

Table 1. *Increase in glucose and lactate output, and reduction in flow after infusion of control and complement-activated rat serum heat treated before or after activation*
Rat livers were perfused as described in Fig. 1. Control serum (CS) and complement-activated serum (AS) of the rat were heated to 57 °C for 30 min either before or after incubation of the serum with zymosan. Values are means ± SEM of the number of experiments in parentheses. The control experiments were compared to the experiments with heat treated sera in the Student's t-test for unpaired samples

Experiment	Serum	Increase in metabolite output		Decrease in flow
		glucose	lactate	
		μmol · g liver^{-1}		ml · g liver^{-1}
Control (8)	CS	2.94 ± 0.78	2.17 ± 0.20	−0.54 ± 0.12
	AS	8.25 ± 0.84	3.52 ± 0.35	−3.39 ± 0.29
Heat treatment prior to activation (6)	CS	2.3 ± 0.6	2.3 ± 0.4	−0.2 ± 0.15
	AS	1.45 ± 0.27[a]	1.75 ± 0.27[a]	−0.01 ± 0.09[a]
Heat treatment after activation (4)	CS	3.86 ± 0.25	2.1 ± 0.56	−0.78 ± 0.18
	AS	7.72 ± 0.2	2.75 ± 0.42	−3.44 ± 0.39

[a] $p < 0.001$

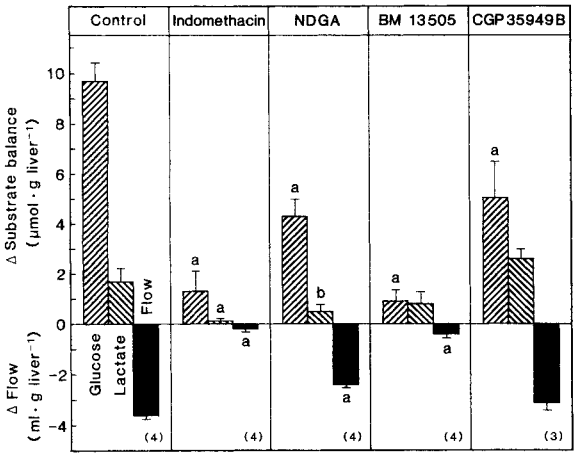

Fig. 3. *Increase in glucose and lactate output, and decrease in flow by complement-activated rat serum in the presence of the cyclooxygenase inhibitor indomethacin, the 5'-lipoxygenase inhibitor NDGA, the TXA_2 antagonist BM 13505 and the LTC_4/LTD_4 antagonist CGP 35949 B, in perfused rat liver.* Rat livers were perfused as described in Fig. 1, except that 0.1% bovine serum albumin, 0.1% Me_2SO and where indicated, one of the inhibitors, were included in the perfusion medium. The final concentrations were 20 μM each with indomethacin, NDGA and BM 13505, 1 μM with CGP 35949 B. The differences between glucose and lactate output and the flow after control serum and complement-activated serum (areas under the curve) are shown. Values are means ± SEM of the number of experiments given in parentheses. The significance was tested in the Student's t-test for unpaired samples, a, $p \leq 0.01$; b, $p \leq 0.05$

Table 2. *Balance of TXB_2 and $PGF_{2\alpha}$ in perfused rat liver during infusion of control serum, complement-activated serum and authentic compounds*
Rat livers were perfused as described in Fig. 1. 20 μM indomethacin together with 0.1% bovine serum albumin and 0.1% Me_2SO were included where indicated. TXB_2 and $PGF_{2\alpha}$ concentrations were determined by radioimmunoassays in the infused serum and effluent. Values are the amounts (determined by the area under the curve, AUC), of TXB_2 and $PGF_{2\alpha}$ infused between 30 and 32 min and leaving the liver between 30 and 40 min (Fig. 4). The elimination was determined by infusion of a known amount of authentic TXB_2 or $PGF_{2\alpha}$ and measurement of the amount of TXB_2 or $PGF_{2\alpha}$ leaving the liver in the following 10 min. Values are means ± SEM of 3 experiments each. CS, control serum; AS, complement-activated serum; Indo, indomethacin; n.d., not detectable

Compound	Infusate	Input ± Indo	Output		Elimination
			−Indo	+Indo	
		pmol · g liver^{-1} (AUC)			%
TXB_2	TXB_2	−	−	−	82 ± 7
	CS	282 ± 48	38 ± 4	27 ± 4	−
	AS	277 ± 40	125 ± 12	32 ± 11	−
$PGF_{2\alpha}$	$PGF_{2\alpha}$	−	−	−	94 ± 1
	CS	15 ± 3	36 ± 5	n.d.	−
	AS	15 ± 3	127 ± 8	n.d.	−

stanoids, which were probably formed during the process of blood clotting, were identical in activated serum, i.e. 1.3 ± 0.2 μM TXB_2 and 72 ± 19 nM $PGF_{2\alpha}$ (SEM, $n = 6$). Thus, during infusion of control or activated serum due to the 1:45 dilution, the final concentrations of 28 nM TXB_2 and 1.5 nM $PGF_{2\alpha}$ were reached. TXB_2 is considered an inactive degradation product of the active TXA_2. When infused to a final concentration of about 30 nM for 2 min, TXB_2 had no metabolic or hemodynamic effects (not shown). During a single path through the liver about 82% of 30 nM TXB_2 was degraded to products not detectible in the assay employed (Table 2). $PGF_{2\alpha}$ had previously been shown not to cause any metabolic or hemodynamic effects at nanomolar concentrations [20–22]. When infused to a final concentration of 10 μM for 5 min, about 94% of $PGF_{2\alpha}$ was degraded during a single passage through the liver to products that were not detectible with the assay used (Table 2). These findings suggest that TXB_2 and $PGF_{2\alpha}$, present in the control and activated serum, did not cause any significant alterations and that they were eliminated largely (TXB_2) or almost completely ($PGF_{2\alpha}$) during their passage through the liver.

280 pmol TXB_2 was infused with the control serum/g liver during a 2-min period from 30–32 min and about 38 pmol TXB_2 was found in the effluent during a 10-min period from 30–40 min (Table 2). The peak of the outflow was observed after 2 min at the end of the infusion period (Fig. 4A). Less than 20% of the TXB_2 infused with the control serum left the liver; a similar percentage of elimination was observed in

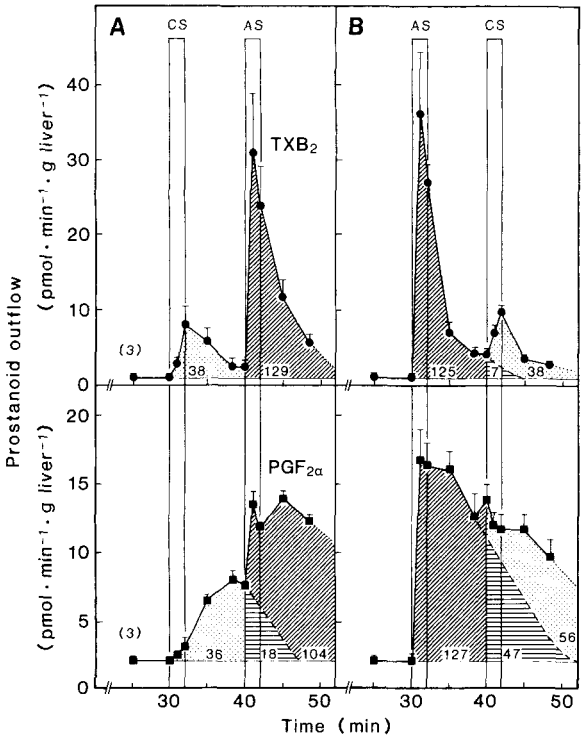

Fig. 4. *Release of TXB_2 and $PGF_{2\alpha}$ into the hepatic vein following infusion of control and complement-activated rat serum in perfused rat liver.* Livers were perfused as described in Fig. 1. At the times indicated control serum (CS) or complement-activated serum (AS) of the rat were infused to a final dilution of 1:45. TXB_2 and $PGF_{2\alpha}$ outflow (pmol · min^{-1} · g $liver^{-1}$) were calculated according to the formula [concentration in the hepatic vein (pmol · ml^{-1})] × [flow ml · min^{-1} · g $liver^{-1}$]. The total outflow (pmol · g $liver^{-1}$) is indicated in the area under the curve. Values are means ± SEM of the number of experiments given in parentheses

perfusions with authentic TXB_2. The same 280 pmol TXB_2 was infused with activated serum/g liver from 30 – 32 min, but about 125 pmol TXB_2 was released between 30 and 40 min (Table 2). The peak of outflow was reached already after 1 min (Fig. 4A). Similar results were obtained when activated serum was infused prior to control serum (Fig. 4B). In the presence of the cyclooxygenase inhibitor indomethacin, after infusion of control or activated serum 27 ± 6 pmol $TXB_2 \cdot g^{-1}$ and 32 ± 11 pmol $TXB_2 \cdot g^{-1}$ was found in the effluent (Table 2). Again less than 20% of the TXB_2 infused with control or activated serum left the liver, which corresponded to the elimination of authentic TXB_2. Thus, the higher amount of TXB_2 found in the effluent after infusion of activated serum probably was due to a *de novo* formation of TXA_2 in response to activated serum. However, it cannot be excluded that a decrease of elimination of TXB_2 infused with activated serum was caused by the reduction and redistribution of flow and thus also contributed to the increased amount of TXB_2 leaving the liver.

15 pmol $PGF_{2\alpha}$ was infused with control and activated serum/g liver during a 2-min period from 30 – 32 min. Since $PGF_{2\alpha}$ infused with the serum was degraded almost completely during its passage through the liver, the amount leaving the liver, corresponded to that part of newly formed $PGF_{2\alpha}$ which was not degraded intrahepatically. After infusion of control serum, 36 ± 5 pmol $PGF_{2\alpha} \cdot g^{-1}$ (Table 2) left the liver in a 10-min period between 30 and 40 min. The peak value was reached only 8 min after the onset of the infusion (Fig. 4A). Activated serum caused a total outflow of 127 ± 8 pmol $PGF_{2\alpha} \cdot g^{-1}$ between 30 and 40 min (Table 2), the peak values already being reached within the first minute (Fig. 4B). Since after a first stimulus the $PGF_{2\alpha}$ overflow returned towards basal levels only very slowly and had not reached prestimulation values at the start of the second stimulation, the overflow in the second period can be quantitated only by approximate extrapolation. Activated serum given as a second stimulus caused a further rapid increase in $PGF_{2\alpha}$ output (Fig. 4A), whereas control serum given as a second stimulus caused only a delay of the decay of $PGF_{2\alpha}$ overflow after activated serum as a first stimulus (Fig. 4B). In the presence of the cyclooxygenase inhibitor indomethacin, no $PGF_{2\alpha}$ was released from the liver either after control or after activated serum (Table 2). Thus the stimulation of $PGF_{2\alpha}$ formation by activated serum was fast, and that by the control serum was slow and smaller, independent of the order of stimulation.

DISCUSSION

Mechanism of action of complement-activated rat serum

Complement-activated serum led to a much larger increase in glucose and lactate output, and reduction and redistribution of the flow, than the control serum (Fig. 1). The activation of serum was dependent on intact complement proteases, since it could be prevented by heat inactivation of the complement proteases prior to activation of the complement system (Table 1), whereas the active agent was resistant to heat inactivation. It eluted on gel-filtration chromatography in the molecular mass range below 25 kDa and above 10 kDa (not shown). Thus most likely one or several of the small heat-stable proteins, that are formed during complement activation, account for the metabolic and hemodynamic effects of activated serum, rather than the large heat-sensitive protein complexes that are also formed during complement activation. Among the small proteins the anaphylatoxins C3a (molecular mass about 9000) and C5a (molecular mass about 12000) seem to be likely candidates. It has been previously shown that the human anaphylatoxin C3a was capable of increasing glucose and lactate output, and perfusion resistance in perfused rat liver, whereas human anaphylatoxin C5a was inactive [13]. Meanwhile it could be demonstrated that the lack of activity of the human anaphylatoxin C5a in rat liver was most likely due to species incompatibilities, since it was active in perfused guinea-pig liver (G. Püschel, unpublished results).

The present results do not indicate whether C3a or C5a, or both, were active, yet they provide some indirect evidence. It appears more likely that C5a was the active serum component for the following reasons: (a) the fractions from the gel-chromatography separation of activated serum with a molecular mass below 10 kDa were inactive; (b) the carboxypeptidase inhibitor, D,L-mercaptomethyl-3-guanidinoethylthiopropanoic acid, did not enhance the effects of activated serum. This would have been expected if C3a had been the active agent, since C3a but not C5a loses its biological activity entirely after removal of the C-terminal arginine by serum carboxypeptidases [23 – 25]. Especially rat C5a-desArg is still very potent in contracting smooth muscle [24].

The active components of the complement system do not seem to act directly on the hepatocytes, vascular or sinusoidal cells, but indirectly via mediation by non-parenchymal cells. This view is supported by the observation that eicosanoids which in the liver can only be formed in non-parenchymal

cells [26] were involved in the action of complement-activated serum. The increase in glucose and lactate output, and perfusion resistance, could be abolished by the inhibitor of prostanoid synthesis, indomethacin, and by the TXA_2 antagonist, BM 13505 (Fig. 3), which would indicate a major role for TXA_2. Leukotrienes may also have a minor role, since a weak inhibition was also brought about by the lipoxygenase inhibitor NDGA and the LTC_4/LTD_4 antagonist CGP 35949 B (Fig. 2). This inhibition, however, may have been unspecific. NDGA has been shown to partially inhibit cyclooxygenase in the concentration used [27, 28], CGP 35949 B inhibited the increase in glucose and lactate output, and perfusion resistance, brought about by the thromboxane analog U 46619 (0.1 μM) completely at 10 μM and partially at 1 μM concentrations (G. Püschel, unpublished results). The thromboxane receptor antagonist BM 13505, in contrast, did not interfere with the action of LTD_4 (M. Iwai and K. Jungermann, unpublished results). Thus, thromboxanes and other prostanoids, but probably not leukotrienes had a major role in the signal chain elicited by complement-activated serum.

Further evidence is provided by the observed overflow of TXB_2 and $PGF_{2\alpha}$ into the hepatic vein (Fig. 4, Table 2). Thromboxane release alone could only account directly for the hemodynamic changes, but not for the metabolic changes, since TXA_2 does not alter glucose release in isolated hepatocytes in contrast to $PGF_{2\alpha}$ [29–31]. The increase in glucose and lactate output, that was inhibited with the TXA_2 antagonist, could then be caused by a release of for example $PGF_{2\alpha}$ from nonparenchymal cells in response to TXA_2.

Function of the action of complement-activated serum

Apparently a systemic activation of the complement system can take notable influence on liver metabolism and hemodynamics. The liver was not able to protect itself against the circulating peptide components of the activated complement system. This could be indicated by the high sensitivity of the liver towards activated serum, which caused an increase in glucose and lactate output already in a dilution as high as 750-fold (Fig. 3). Thus, it seems likely that concentrations of the active mediators that are sufficiently high can be formed pathophysiologically during septicaemia or endotoxaemia in patients or animals. A systemic activation of the complement system might therefore be a possible explanation for the increase of prostaglandin levels in livers of animals that had been injected with endotoxins intravenously [32] and possibly one of the mechanisms by which an increase in blood glucose is brought about in patients with severe sepsis.

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