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Modulation of Platelet Activating Factor-Induced Glycogenolysis in the Perfused Rat Liver after Administration of Endotoxin *In Vivo*[1]

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The effect of endotoxin treatment *in vivo* on platelet activating factor (PAF)-induced glycogenolysis was studied in the perfused rat liver. The addition of PAF (20 nM) to the perfusate increased glucose production concomitant with suppression of oxygen consumption in control rats without endotoxin treatment. At 6 h after endotoxin administration, PAF caused severe suppression of oxygen consumption, but glucose production was greatly inhibited. At 24 h after endotoxin treatment, PAF caused less suppression of oxygen consumption than the control, and glucose production was partially restored. The metabolic responses in the control rat were abolished by the simultaneous presence of cyclooxygenase- and lipoxygenase-inhibitors. Combined use of leukotriene (LT) D_4- and thromboxane (Tx) A_2-receptor antagonists inhibited the metabolic responses in the rat given endotoxin 6 h before. The efflux of Tx B_2 during PAF-infusion decreased 24 h after endotoxin treatment, and Tx A_2 receptor antagonist, but not LT D_4 receptor antagonist, prevented the suppression of oxygen consumption. These results suggest that different eicosanoids are involved in PAF-induced glycogenolysis in different stages of endotoxemia, and that LT D_4 may also play a role in PAF-induced glycogenolysis.

Key words: Kupffer cells, leukotriene, lipopolysaccharide, prostaglandin, thromboxane.

PAF (1-*o*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphocholine) is a unique phospholipid having diverse and potent effects on several biological systems as an allergy and inflammation mediator (*1-3*). PAF has also been shown to have powerful effects on liver metabolism *in vivo* (*4, 5*). In the perfused rat liver, PAF at concentrations of 0.02-20 nM induces marked changes in vasocontraction, oxygen consumption and glycogenolysis (*3, 6-12*). As PAF does not elicit any respiratory or glycogenolytic effects in isolated hepatocytes (*6*), liver non-parenchymal cells, which possess PAF receptors (*3, 13*), might be responsible for these PAF actions in liver metabolism.

It is known that PAF stimulates prostaglandin (PG) production in cultured Kupffer cells (*14*). Moreover, inhibitors of prostanoid synthesis suppress the glycogenolytic response in the perfused rat liver (*7-9*), and PG D_2 and $F_{2\alpha}$ directly stimulate glycogenolysis in the hepatocyte (*15, 16*). Based on these findings, PG is proposed to be a mediator of PAF-induced glycogenolysis. However, it has also been reported that PAF produces a classical ischemia-induced phosphorylase activation in a certain region of the liver due to vasoconstriction by PAF itself (*3, 10*). Hence, the participation of PG in PAF-induced glycogenolysis needs to be reinvestigated. It is also uncertain whether lipoxygenase products, such as leukotriene (LT), are involved in the glycogenolysis induced by PAF (*7*).

A variety of cells and tissues have been shown to synthesize PAF. In the liver, PAF synthesis is stimulated by infusion of heat-aggregated IgG (*17*) *in vitro*, and ischemia/reperfusion (*18*) and bile duct ligation (*19*) *in vivo*. The latter report indicates the involvement of an endotoxin in the PAF production. Moreover, in the septic state, PAF appears in extrahepatic tissues such as peritoneal macrophages and spleen cells (*20*), so that it might enter the liver through the portal vein. The metabolic response to PAF in the liver can be down-regulated by PAF itself and β-adrenergic agonists (*3, 11, 12*), whose concentrations increase in sepsis. Thus, it is expected that the metabolic response to PAF in the liver is down-regulated or modulated in sepsis.

In the present study we examined PAF-induced glycogenolysis in the perfused liver of control and endotoxin-administered rats in order to clarify the role of eicosanoids in the metabolic responses to PAF and possible alterations in sepsis. We found that PG, thromboxane (Tx), and LT D_4

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Abbreviations: LPS, lipopolysaccharide; LT, leukotriene; NDGA, nordihydroguaiaretic acid; PAF, platelet activating factor; PG, prostaglandin; Tx, thromboxane.

were mediators of PAF-induced glycogenolysis in the control, but that the relative importance of these individual mediators varied in a time-dependent manner after endotoxin injection.

MATERIALS AND METHODS

Materials—Lipopolysaccharide (LPS) from *Escherichia coli* (serotype 026:B6) and nordihydroguaiaretic acid (NDGA) were purchased from Sigma Chemical (St. Louis, MO, USA). I-BOP (a Tx A_2 receptor agonist) was bought from Cayman Chem. (Ann Arbor, MI, USA). Ibuprofen and LT D_4 were obtained from Wako Pure Chemical (Osaka). PAF, ONO-NT-126 (a Tx A_2 receptor antagonist), and ONO-1078 (a LT D_4 receptor antagonist) were generously provided by Ono Pharmaceutical (Osaka). Other reagents were of analytical grade.

Animals and Treatments—Male Sprague-Dawley rats weighing 180–210 g were used in all experiments. The experimental protocols followed the institution's and the National Research Council's criteria for the care and use of laboratory animals in research. In some experiments, rats were given intravenous injections of LPS (1 mg/kg of body weight dissolved in saline) in the tail vein between 09:00 and 10:00 h. Animals were fed *ad libitum*.

Liver Perfusion—Liver perfusion was started between 09:00 and 12:00 h, unless rats were not given injections of LPS. Liver perfusion was performed as described previously (*21, 22*). In brief, after anesthesia with pentobarbital (50 mg/kg), the portal vein and inferior vena cava were cannulated, and the liver was perfused at 32°C at a constant flow rate (3.5 ml/min/g liver) with oxygenated Krebs-bicarbonate buffer in a flow-through mode. The buffer was continuously gassed with a humidified mixture of 95% O_2–5% CO_2 at 32°C. The perfusion medium consisted of 115 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM Na_2SO_4, 25 mM $NaHCO_3$, and 2.5 mM $CaCl_2$.

Other Methods—The concentration of oxygen in the effluent was continuously monitored with Clark-type electrodes, and oxygen utilized by the liver was estimated. Glucose in the perfusate was assayed by the method of Bergmeyer and Bernt (*23*). PG D_2 and Tx B_2 in the perfusate were extracted with ethylacetate, then assayed with EIA kits (Cayman Chem.).

Statistics—Values are expressed as means±SEM, and significant differences between means were analyzed by ANOVA.

RESULTS

The effects of PAF on oxygen consumption and glucose production were examined in the perfused liver of control or LPS-injected rats. When PAF (20 nM) was added to the perfusion medium in control rats, glucose production increased in a biphasic manner (Fig. 1A), while oxygen consumption was suppressed (Fig. 1B). In rats administered LPS, the liver consumed more oxygen relative to the control (130.5±2.1 *vs.* 90.6±5.6 μmol/h/g of liver) and released less glucose (3.7±1.6 *vs.* 17.9±2.3 μmol/h/g of liver) in the basal state at 6 h after the injection. PAF infusion elicited suppression of about 50% of total oxygen consumption, which returned to basal levels upon ending the infusion (Fig. 1B). However, PAF-induced glucose production was severely inhibited when compared to the control but increased especially after cessation of infusion (Fig. 1A). At 24 h after administration of LPS, the basal oxygen consumption (90.4±2.9 μmol/h/g of liver) in the liver had returned to control level, but the basal glucose production (11.9±0.9 μmol/h/g of liver) was still depressed. PAF infusion again suppressed oxygen consumption, though the suppression was only transient and slight (Fig. 1B), and increased glucose production (Fig. 1A). These results indicated that, even in a sepsis state, PAF could induce glycogenolysis in the liver without apparent down-

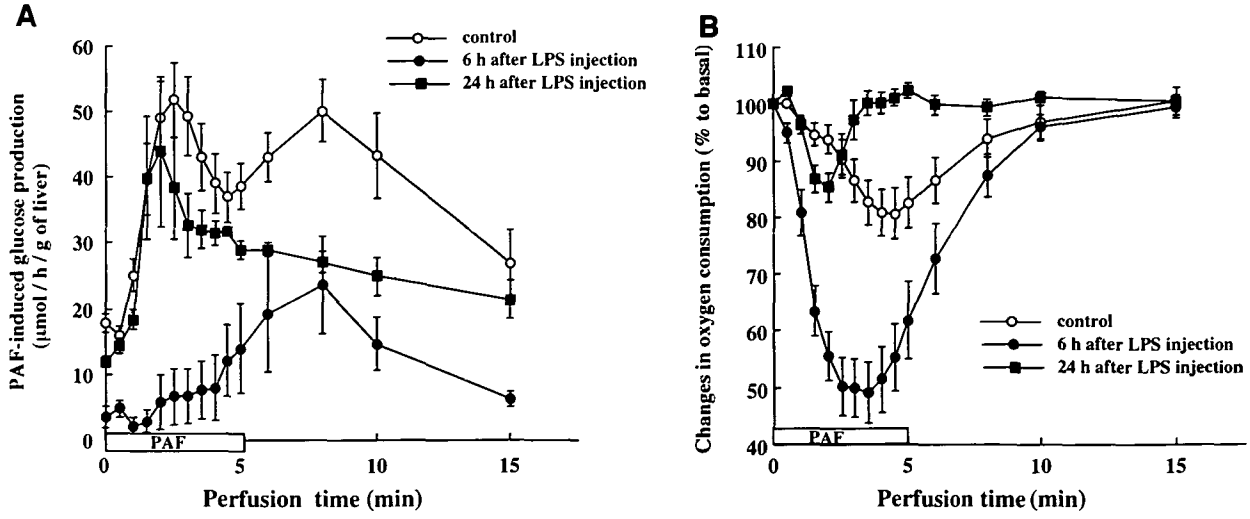


Fig. 1. **Modulation of PAF-induced increase in glucose production and suppression of oxygen consumption in the perfused liver after administration of LPS *in vivo*.** Rat livers were perfused as described in "MATERIALS AND METHODS." After 30 min of pre-perfusion (time zero), PAF (20 nM) was infused for 5 min (box) in control rats ($n=7$, ○), 6 h ($n=5$, ●), or 24 h ($n=5$, ■) after rats had been given intravenous LPS (1 mg/kg of body weight). Glucose released into the perfusate (A) and relative changes in oxygen consumption (B) are expressed as the means±SEM from separate experiments. Oxygen consumption in each perfused liver at time zero refers to 100%. There are significant differences between the control and LPS-injected groups when analyzed by ANOVA.

regulation, although there was some modulation of PAF-induced metabolic responses.

To clarify the mechanisms of the modulation, we examined the possibility that the amounts of PG and Tx produced by PAF altered during endotoxemia. PAF enhanced release of PG D_2 and Tx B_2, a metabolite of Tx A_2, from the liver (Fig. 2). At 6 h after the injection, similar amounts of both eicosanoids were released. However, PAF induced a lesser amount of Tx B_2 after 24 h, while production of PG D_2 was unchanged (Fig. 2). Thus, at least 6 h after LPS-injection, the amounts of eicosanoids produced

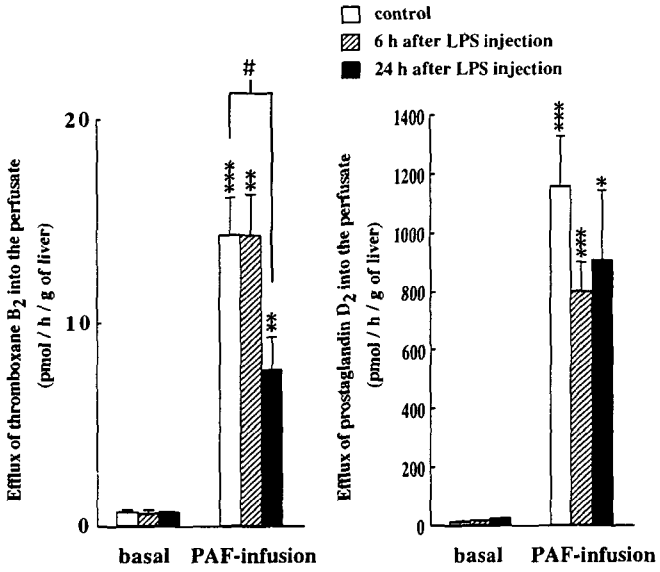


Fig. 2. **Efflux of thromboxane B_2 and prostaglandin D_2 into the perfusate before and during infusion of PAF.** Control rat livers (open column), and those 6 h (hatched column) and 24 h (closed column) after injection of LPS were perfused as described in the legend of Fig. 1. The perfusate was pooled before (basal) and during PAF-infusion (1.5–3.5 min after the start of infusion) in control and LPS-injected rats. Thromboxane B_2 (left panel) and prostaglandin D_2 (right panel) in the perfusate were measured. Significant differences between before and during the infusion are expressed by $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$, and those between the control and LPS treatment by $^{\#}p<0.05$.

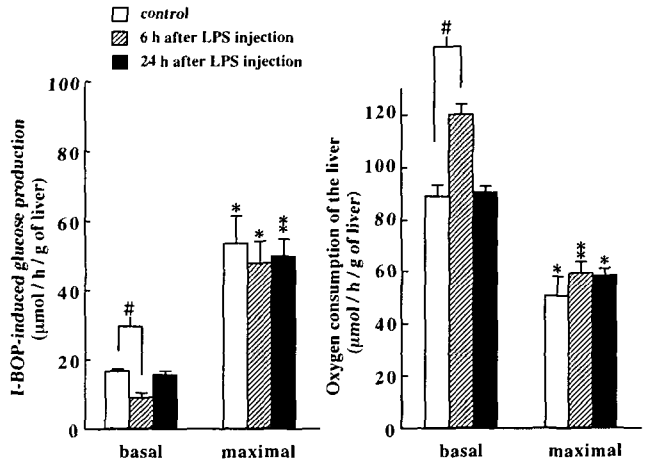
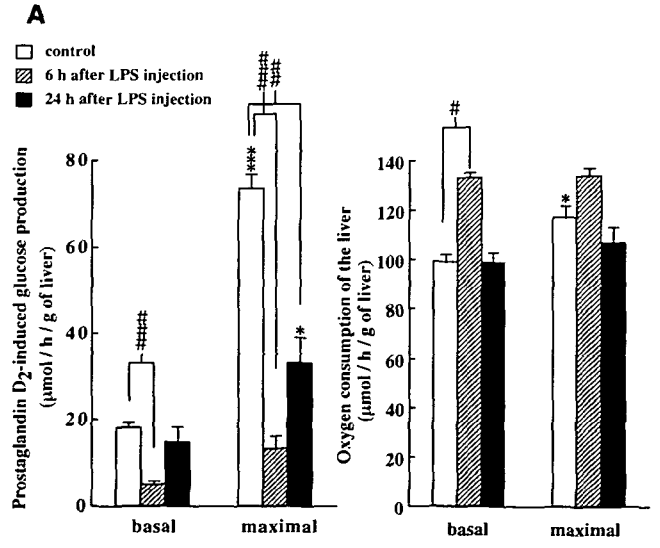


Fig. 4. ***Effects of a thromboxane A_2 receptor agonist on glucose production and oxygen consumption in the perfused liver of control and endotoxin-treated rats.*** Control rat livers (open column), and those 6 h (hatched column) and 24 h (closed column) after injection of LPS were perfused as described in "MATERIALS AND METHODS." After 30 min of pre-perfusion, I-BOP (10 nM) was infused for 5 min. Maximal changes in glucose production (left panel) and oxygen consumption (right panel) are expressed as means ± SEM of three independent experiments. Significant differences between the basal and maximal values are indicated by $^{*}p<0.05$; $^{**}p<0.01$, and those between control and LPS treatment by $^{\#}p<0.05$.



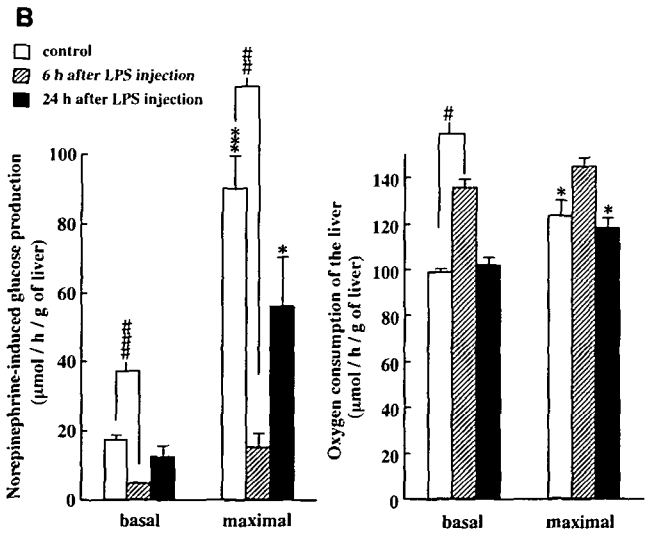


Fig. 3. **Effect of prostaglandin D_2 and norepinephrine on glucose production and oxygen consumption in the perfused liver of control and endotoxin-treated rats.** Control rat livers (open column), and those 6 h (hatched column) and 24 h (closed column) after injection of LPS were perfused as described in "MATERIALS AND METHODS." After 30 min of pre-perfusion, prostaglandin D_2 (5 μM) was infused for 5 min (A). After a 20-min interval, norepinephrine (100 nM) was infused for 5 min (B). Maximal changes in glucose production (left panel) and oxygen consumption (right panel) are expressed as means ± SEM of three independent experiments. Significant differences between the basal and maximal values are indicated by $^{*}p<0.05$; $^{***}p<0.001$, and those between control and LPS treatment by $^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$.

by PAF did not account for the modulation.

We next examined whether PG D_2 and Tx A_2 receptor agonist could cause glycogenolytic responses after LPS injection. PG D_2 stimulated glycogenolysis in the control liver (Fig. 3A) without suppression of oxygen consumption (Fig. 3A). However, PG D_2 hardly increased glucose production 6 h after the injection and enhanced glucose production to some extent after 24 h (Fig. 3A). Similar suppression and recovery were observed in norepinephrine-induced glucose production in LPS-injected rats (Fig. 3B). In contrast to PG D_2, the Tx A_2 receptor agonist I-BOP increased glucose production in the liver 6 h after LPS injection to the same extent as the control liver (Fig. 4). I-BOP also suppressed oxygen consumption of the liver (Fig. 4), and the glucose production followed the relief of the suppression (figure not shown). At 24 h after LPS injection, I-BOP induced similar changes in glucose production and oxygen consumption (Fig. 4).

Since PG D_2 failed to stimulate glycogenolysis, Tx A_2 might mediate PAF-induced responses 6 h after LPS injection. However, NT-126 (100 nM), a Tx A_2 receptor antagonist, partially blocked the suppression of oxygen consumption but not the glucose production by PAF (Fig. 5). The concentration of NT-126 used was sufficient to block I-BOP (10 nM)-dependent responses. These results indicated that other compounds such as LT might act as a mediator of PAF (7). To test this idea, ONO-1078, an LT D_4 receptor antagonist, was infused. ONO-1078 prevented the suppression of oxygen consumption by PAF, but not the glucose production (Fig. 5). The glucose production by PAF was inhibited only when both antagonists were present simultaneously (Fig. 5). Moreover, exogenous LT D_4 stimulated glycogenolysis 6 h after LPS injection (Fig. 6). Taken together, these results suggested that Tx and LT D_4, but not PG, produced by PAF caused the metabolic responses 6 h after LPS injection.

As it is uncertain whether LT D_4 played a role in PAF-induced metabolic responses in the control, we tested effects of various inhibitors of the eicosanoid metabolism on PAF-induced metabolic responses in the control rat. At the concentrations used, no inhibitors affected norepinephrine-induced glucose production and changes in oxygen consumption (data not shown). Ibuprofen, a cyclooxygenase inhibitor, inhibited the PAF-induced PG D_2 efflux completely, but NDGA, a lipoxygenase inhibitor, did not (Fig. 7A), confirming the specific effects of the inhibitors. Ibuprofen partially prevented glucose production by PAF but failed to prevent the suppression of oxygen consumption (Fig. 7B). In contrast to ibuprofen, NDGA inhibited these changes in oxygen consumption and reduced the glucose production (Fig. 7B). In the presence of both inhibitors, PAF failed to produce any metabolic responses (Fig. 7B). Since simultaneous blockade of both cyclooxygenase and lipoxygenase was necessary to completely prevent PAF-induced glucose production (Fig. 7B), it is possible that glucose production in the presence of an inhibitor (for example, NDGA alone) was induced through alternative eicosanoids (Tx A_2 and PG D_2) produced by PAF.

Next the effects of ONO-1078 and NT-126 were determined. Each antagonist somewhat reduced glucose produc-

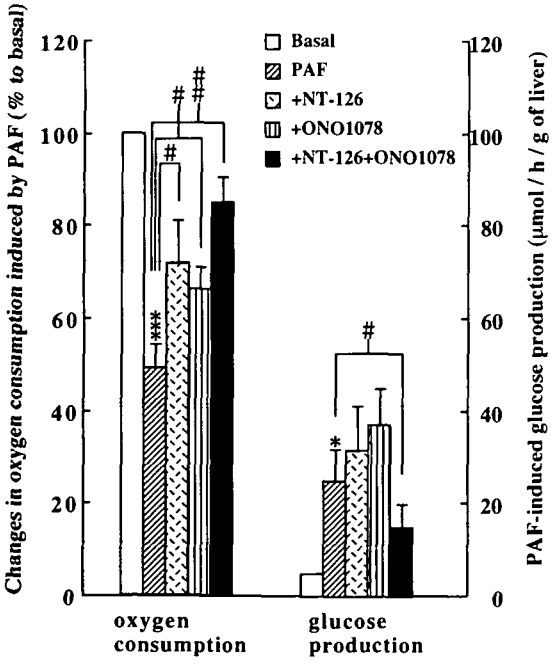


Fig. 5. **Effects of a leukotriene D_4 receptor antagonist and a thromboxane A_2 receptor antagonist on PAF-induced changes in oxygen consumption and glucose production in the perfused rat liver 6 h after administration of endotoxin.** The liver 6 h after LPS injection was perfused as described in Fig. 1. ONO-1078 (100 ng/ml, striped column), NT-126 (100 nM, dot column), or both antagonists (closed column) was infused from 5 min before the PAF-infusion. Maximal changes in oxygen consumption relative to the basal level (left panel) and glucose production (right panel) induced by infusion of PAF are summarized. Data are expressed as means ± SEM. Significant differences between the basal and maximal values are indicated by $^{*}p<0.05$; $^{***}p<0.001$; and those between PAF and inhibitor treatment by $^{\#}p<0.05$; $^{\#\#}p<0.01$.

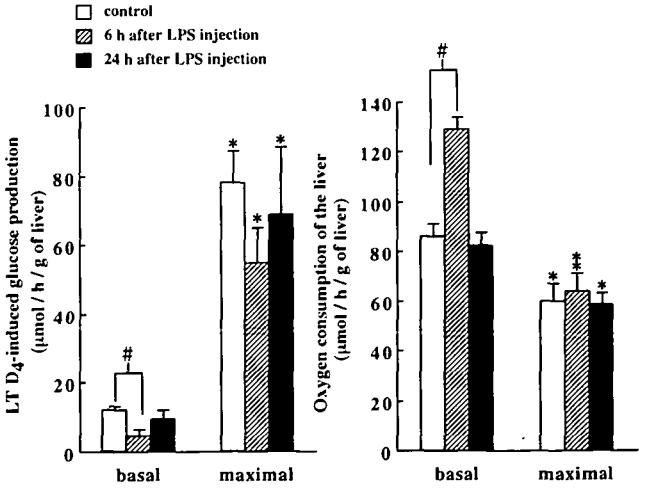


Fig. 6. **Effects of leukotriene D_4 on glucose production and oxygen consumption in the perfused liver of control and endotoxin-treated rats.** Control rat livers (open column) and those 6 h (hatched column) and 24 h (closed column) after injection of LPS were perfused as described in "MATERIALS AND METHODS." After 30 min of pre-perfusion, LT D_4 (20 nM) was infused for 5 min. Maximal changes in glucose production (left panel) and oxygen consumption (right panel) are expressed as means ± SEM of three independent experiments. Significant differences between the basal and maximal values are indicated by $^{*}p<0.05$; $^{**}p<0.01$, and those between control and LPS treatment by $^{\#}p<0.05$.

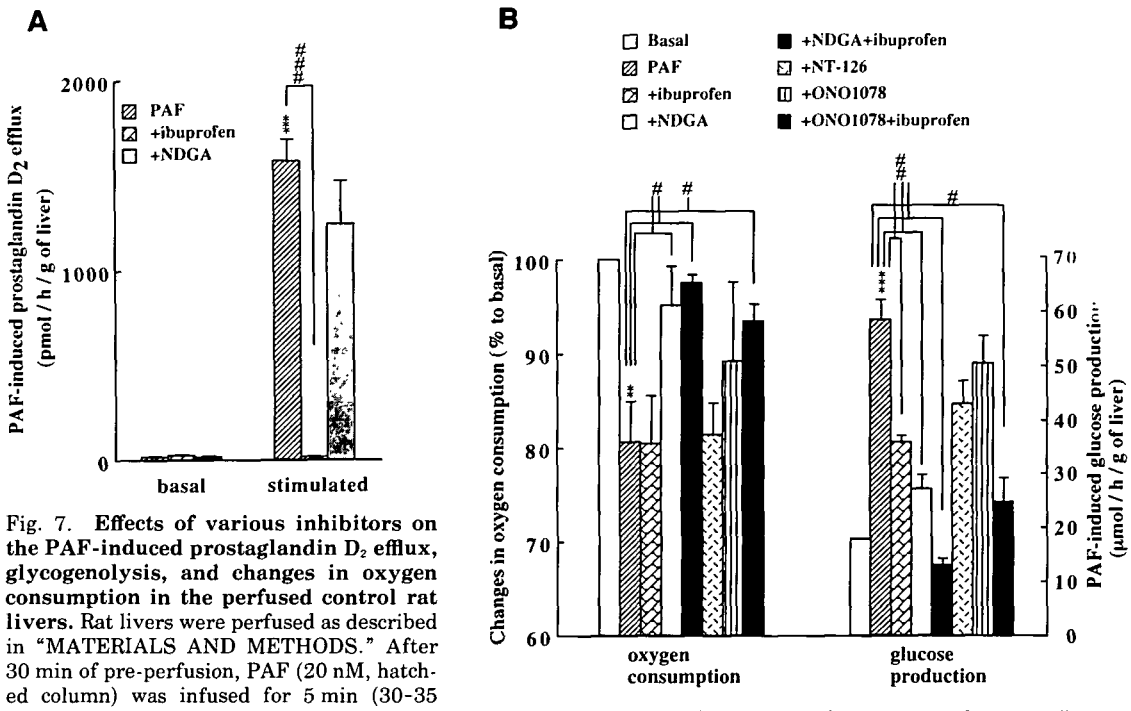


Fig. 7. **Effects of various inhibitors on the PAF-induced prostaglandin D_2 efflux, glycogenolysis, and changes in oxygen consumption in the perfused control rat livers.** Rat livers were perfused as described in "MATERIALS AND METHODS." After 30 min of pre-perfusion, PAF (20 nM, hatched column) was infused for 5 min (30-35 min). Ibuprofen (20 μM) was infused 15 min before the infusion of PAF ($n=4$, checked column). NDGA (10 μM) was infused with ($n=4$, dark gray column) or without ($n=4$, gray column) ibuprofen from 15 min before the PAF infusion. ONO-1078 (100 ng/ml, $n=4$, striped column), NT-126 (100 nM, $n=3$, dotted column), or ONO-1078 and ibuprofen ($n=4$, closed column) was infused from 5 min before the PAF-infusion. The perfusate was pooled before and during PAF-infusion (1.5-3.5 min after the start of infusion), and prostaglandin D_2 in the perfusate was measured (A). Maximal changes in glucose production (B, right panel) and oxygen consumption (B, left panel) are expressed as means ± SEM. Statistical significances of the differences between the basal and maximal values are indicated by $^{**}p<0.01$; $^{***}p<0.001$; and those between the presence and absence of inhibitors by $^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$.

tion but did not significantly prevent the suppression of oxygen consumption (Fig. 7B). Combined use of ONO-1078 and ibuprofen, which might inhibit Tx A_2 production, also blocked PAF-induced metabolic responses (Fig. 7B). Although we failed to detect LT D_4 in the perfusate, the results obtained using inhibitors and Tx B_2 measurement indicated that LT D_4 and Tx A_2 were responsible for the suppression of oxygen consumption in the control rats. These results indicated that PAF induced metabolic responses in the liver through production of PG, Tx, and LT D_4 in the control rats.

At 24 h after administration of LPS, the effect of PAF on hepatic oxygen consumption was smaller than in the control (Figs. 1B and 8) and the release of Tx B_2 by PAF was reduced to about half of that in the control (Fig. 2). In these rats, ONO-1078 had no effect on the metabolic changes induced by PAF (Fig. 8), indicating that LT D_4 might play a minor role in PAF-induced responses after 24 h. In contrast, NT-126 alone decreased PAF-induced glucose production and prevented the suppression of oxygen consumption (Fig. 8). Since the responsiveness of Tx A_2 receptor agonist was unaltered after LPS injection (Fig. 4), the smaller changes in oxygen consumption induced by PAF might have been due to lowered production of Tx A_2. Moreover, a significant amount of PG D_2 was released by PAF (Fig. 2), and exogenous PG D_2 partly recovered its effect with an increase of glucose production (Fig. 3). All these results suggested that the metabolic responses to PAF 24 h after LPS administration were mainly mediated by PG and Tx.

DISCUSSION

The present study indicated that (a) glycogenolytic responses of the perfused liver to PAF were caused by PG, Tx, and LT D_4 in control rats, (b) Tx and LT D_4, but not PG, were involved in the response 6 h after rats were injected with LPS, and (c) PG and Tx, but not LT, elicited the response 24 h after LPS-injection.

Our results confirmed that PAF stimulates glycogenolysis in normal rat liver (*3, 6-12*). At the concentration used in this study (20 nM), PAF has been thought to induce vasoconstriction directly in certain regions of the liver, thereby causing the classical ischemic response of phosphorylase interconversion (*10*). However, the findings that inhibition of the lipoxygenase pathway by NDGA abolishes the vascular effects of PAF (*7*) and prevents the suppression of oxygen consumption (this study) suggest that LT is involved in vascular response to PAF. Since the selective LT D_4 receptor antagonist mimicked the effect of NDGA, peptide-LT may have already been produced. PAF is known to stimulate production of LT D_4 in some cell types (*3*), and Kupffer cells possess PAF receptors like peritoneal macrophages (*3, 13*). Although PAF alone does not stimulate LT D_4 production in cultured Kupffer cells, it enhances A23187-induced LT D_4 production (*24*). In the perfused rat liver, infusion of both phorbol ester and A23187 produces LT D_4 (*25*). PAF induces hydrolysis of phosphoinositides in Kupffer cells (*3*), which implies activation of protein kinase C. Furthermore, PAF induces Ca^{2+}-flux in the cells (*26*).

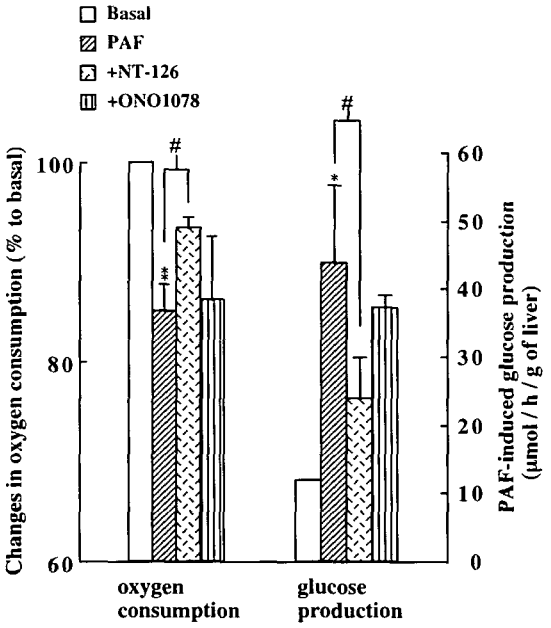


Fig. 8. **Effects of a leukotriene D_4 receptor antagonist and a thromboxane A_2 receptor antagonist on PAF-induced changes in oxygen consumption and glucose production in the perfused rat liver 24 h after administration of endotoxin.** The liver 24 h after LPS injection was perfused as described in Fig. 1. ONO-1078 (100 ng/ml, striped column) or NT-126 (100 nM, dot column) was infused from 5 min before the PAF-infusion. Maximal changes in oxygen consumption relative to the basal level (left panel) and glucose production (right panel) induced by infusion of PAF are summarized. Data are expressed as means ± SEM. Significant differences between the basal and maximal values are indicated by $^*p<0.05$; $^{**}p<0.01$; and those between PAF and receptor antagonists by $^{\#}p<0.05$.

Collectively, it is plausible that activation of both Ca^{2+} and protein kinase C-dependent pathways by infusion of PAF may elicit the LT D_4 production in perfused rat liver. Moreover, the Tx A_2 receptor antagonist prevented the suppression of oxygen consumption, and PAF enhanced the production of Tx. Since exogenous Tx and LT D_4 induce marked hemodynamic action in the liver (*27*), which is reflected by the changes in oxygen consumption, our results suggested that PAF caused vasoconstriction indirectly through the production of LT D_4 and Tx in the liver.

Two mechanisms may be considered for PAF-induced glycogenolysis. Since hypoxia is a trigger of glycogenolysis (*28*), one mechanism may involve restriction of oxygen in certain regions of the liver by vasoconstriction, through PAF-mediated production of LT D_4 and Tx, as described above. Alternatively, at lower concentrations, PG is suggested to be a mediator of PAF-induced glycogenolysis, since inhibitors of prostanoid synthesis suppress the glycogenolytic response in the perfused rat liver (*7-9*). As PG D_2 and PG $F_{2\alpha}$ directly stimulate glycogenolysis in the hepatocyte (*15, 16*), and these PGs detected in the perfusate are highly correlated with glucose production (*21*), PG produced by PAF may be responsible, at least in part, for the glycogenolysis in the perfused rat liver.

Hypermetabolism is generally associated with sepsis. Administration of an endotoxin causes transient hyperglycemia with rapid mobilization of glycogen stores. As sepsis proceeds, hypoglycemia develops, probably because of inhibition of gluconeogenesis and depletion of glycogen stores (*29, 30*). The elevation of basal oxygen consumption and glucose production seen 6 h after endotoxin treatment imply hypermetabolism and the decrease of glycogen stores. These must be the underlying mechanisms responsible for the relative ineffectiveness of PG D_2 and norepinephrine in activating phosphorylase. Interestingly, only the classical ischemic response of phosphorylase interconversion by LT D_4 and Tx affected glucose production, though the mechanism is unknown.

The development of liver failure after sepsis or septic shock remains a serious clinical problem, and the cause of this hepatic dysfunction remains incompletely understood. It has been postulated that disturbance of hepatic microcirculatory blood flow and the resulting transient local hypoxia, which occur *in vivo* during sepsis (*31*), contribute to liver failure in the same way as in ischemia/reperfusion-induced liver injury (*18*). One of the likely mediators of the effects of endotoxins (*32*) and ischemia (*18*) is PAF, which is not only produced in liver but also introduced into the liver from extrahepatic tissues through the portal vein (*19, 20*). The present results indicated that both Tx and LT D_4 were involved in the glycogenolysis by PAF 6 h after endotoxin treatment, implying that these eicosanoids themselves, rather than PAF, induced vasoconstriction. It is worth noting that antagonists of LT D_4, as well as PAF antagonists, improve survival of endotoxin-intoxicated rats (*33*). Endotoxins also induce production of LT D_4 *in vivo* (*34*), which may be a potent inducer of liver injury (*35*). Moreover, cyclooxygenase inhibitors and Tx A_2 receptor antagonists attenuate the degree of endotoxin-induced liver injury (*36*). Therefore, local hypoxia in the liver under septic conditions might occur *via* a mechanism by which PAF, induced by an endotoxin from intrahepatic and extrahepatic tissues, causes the production of LT D_4 and Tx and, thereby, vasoconstriction.

The reason why production of TX A_2 is reduced 24 h after administration of an endotoxin is unclear. Although endotoxins are known to inhibit contraction of vascular smooth muscle *in vitro* (*37*), their contribution *in vivo* may be of less importance, because exogenous LT D_4 and Tx A_2 agonist showed the same effect in endotoxin-administered rats as in control rats. Another plausible explanation is that glucocorticoid may reduce the production of these compounds, since administration of an endotoxin *in vivo* elevates the plasma corticosterone concentration (*38*), while dexamethasone inhibits phospholipase A_2 (*39*) and cyclooxygenase (*40*). A similar decrease in Tx production was reported in macrophages prepared from endotoxin-tolerant rats (*41*).

In acute endotoxin-tolerant rats, hyposensitivity to a subsequent endotoxin challenge is suggested to be mainly due to the down-regulation of LPS-induced cytokine release *in vivo* (*42*). The acute endotoxin tolerance also down-regulates superoxide anions released by the perfused liver and isolated hepatic nonparenchymal cells (*43*). The reduced production of eicosanoids inducing vasocontraction in response to PAF would also contribute to such phenomena.

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