Stimulation of oxygen uptake by prostaglandin E₂ is oxygen dependent in perfused rat liver

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Qu, Wei, Zhi Zhong, Gavin E. Arteel, and Ronald G. Thurman. Stimulation of oxygen uptake by prostaglandin E₂ (PGE₂) is oxygen dependent in perfused rat liver. Am. J. Physiol. 38: G542–G549, 1998.—The aim of this study was to determine if the effect of prostaglandin E₂ (PGE₂) on hepatic oxygen uptake was affected by oxygen tension. Livers from fed female Sprague-Dawley rats were perfused at normal or high flow rates (4 or 8 ml·g⁻¹·min⁻¹) to vary local oxygen tension within the liver lobule. During perfusion at normal flow rates, PGE₂ (5 µM) infusion increased oxygen uptake by about 50 µmol·g⁻¹·h⁻¹; however, when livers were perfused at high flow rates, the increase was nearly twice as large. Simultaneously, glucose output was increased rapidly by about 50%, whereas glycogenosis was decreased about 60%. When flow rate was held constant, increases in oxygen uptake due to PGE₂ were proportional to oxygen delivery. Infusion of PGE₂ into livers perfused at normal flow rates increased state 3 rates of oxygen uptake of subsequently isolated mitochondria by about 25%; however, rates were increased 50–75% in mitochondria isolated from livers perfused at high flow rates. Thus it is concluded that PGE₂ stimulates oxygen uptake via mechanisms regulated by oxygen tension in perfused rat liver. High flow rates also increased basal rates of oxygen uptake; this increase was prevented by inactivation of Kupffer cells with GdCl₃. In addition, conditioned medium from Kupffer cells incubated at high oxygen tension (75% oxygen) stimulated oxygen uptake of isolated parenchymal cells by >30% and elevated PGE₂ production about twofold compared with Kupffer cells exposed to normal air-saturated buffer (21% oxygen). These effects were blocked completely by both indomethacin and nisoldipine. These data support the hypothesis that oxygen stimulates Kupffer cells to release mediators such as PGE₂ which elevate oxygen consumption in parenchymal cells, possibly by mechanisms involving cyclooxygenase and calcium channels.

Kupffer cells; eicosanoids; hypermetabolic state

PROSTAGLANDINS, which are metabolites of arachidonate, are locally acting hormones that initiate a multitude of physiological actions in nearly all mammalian tissues (6, 33). They have important roles in cell-to-cell signal propagation between nonparenchymal and parenchymal cells in liver (30). It is well known that Kupffer cells are the major source of hepatic eicosanoids and that hepatocytes have receptors for a variety of different classes of eicosanoids (5, 16, 35). Eicosanoids produced by hepatic nonparenchymal cells have long been known to participate in metabolic regulation of processes such as carbohydrate release by parenchymal cells (3). Recently, Qu et al. (32) demonstrated that Kupffer cells were activated by ethanol and endotoxin to release prostaglandin E₂ (PGE₂), which stimulated oxygen uptake in parenchymal cells. PGE₂ added directly to hepatic parenchymal cells also caused a dose-dependent increase in oxygen consumption (32); however, the precise mechanisms by which PGE₂ stimulates oxygen uptake remain unclear.

Cells in various zones of the liver lobule exist at different oxygen tensions due to a natural oxygen gradient (17). Also, hepatocytes located near the portal vein take up oxygen at rates two-to-three times faster than cells located near the central vein (21). Interestingly, hormones that increase intracellular calcium stimulate oxygen uptake predominantly in regions of the liver lobule where oxygen tension is the lowest (23). Oxygen also plays an important role in the regulation of metabolism by hormones (17). For example, Kizaki and Thurman (17) demonstrated that glucagon increased oxygen uptake of mitochondria subsequently isolated from the perfused liver about twice as much at high than at normal flow rates because of increased oxygen delivery. Addition of glucagon to suspensions of mitochondria, however, had no effect on oxygen uptake. Thus the effect of glucagon on mitochondria must be “remembered” by the organelle during the isolation procedure (17), suggesting some permanent alteration such as phosphorylation. In contrast, oxygen tension had little effect on oxygen uptake in isolated hepatocytes and had virtually none in isolated mitochondria (26). Therefore, we hypothesize that oxygen stimulates Kupffer cells to release mediators such as PGE₂, which elevates oxygen consumption in parenchymal cells. The purpose of this study was to determine if PGE₂ stimulates oxygen uptake in an oxygen-dependent manner in the isolated perfused liver and if nonparenchymal cells participate in this phenomenon.

METHODS

Experimental animals and liver perfusion. Female Sprague-Dawley rats (200–220 g) were allowed free access to laboratory chow and tap water. For some experiments, GdCl₃ (10 mg/kg) dissolved in acidified saline (pH 3.0) was injected into the tail vein 24 h before perfusion. Details of the perfusion technique have been described elsewhere (34). Briefly, livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°C) saturated with an oxygen-carbon dioxide mixture (95:5) in a nonrecirculating system. Perfusate was pumped into the liver via a cannula inserted in the portal vein, and effluent perfusate was collected via a cannula placed in the inferior vena cava. Effluent perfusate was channeled past a Teflon-shielded, Clark-type platinum electrode to determine oxygen tension. Rates of oxygen uptake or metabolite production were calculated from the difference between the influent and effluent oxygen concentration, liver wet weight, and flow rate. Samples of effluent perfusate were collected and analyzed for glucose, lactate, and pyruvate by standard enzymatic techniques (1). Livers were perfused at normal flow
rates of ~4, at medium flow rates of 6, or at high flow rates of 8 ml·g liver−1·min−1. For some experiments, the perfusate oxygen concentration was varied at constant flow rate (Table 1). At all flow rates studied, lactate dehydrogenase, an index of cell injury, could not be detected in the effluent perfusate, indicating that changes in flow did not affect tissue viability.

Isolation of mitochondria. Mitochondria were isolated from livers perfused at normal, medium, or high flow rates in the presence or absence of PGE₂ (5 µM) by standard techniques of differential centrifugation. Livers were homogenized at 0–1°C in a buffer consisting of 0.225 M mannitol, 0.075 M sucrose, and 0.1 mM EDTA, pH 7.0, using a Teflon-glass homogenizer. Nuclear and cellular debris were removed by centrifugation at 2,000 g for 10 min, and the supernatant was centrifuged subsequently at 10,000 g for 10 min. The mitochondrial pellet was washed twice in 20 ml of buffer and was resuspended at protein concentrations of 25–35 mg/ml (17, 31).

Measurement of mitochondrial oxygen uptake. Mitochondrial oxygen uptake was measured at 25°C with a Teflon-shielded, Clark-type oxygen electrode in 2 ml of a buffer (pH 7.2) containing (in mM) 100 KCl, 50 sucrose, 20 Tris·HCl, and 5 Tris phosphate, and 10 µM rotenone (9). State 4 rates of respiration were initiated by the addition of succinate (1 µmol) and correspond to electron flux in the absence of ADP. State 3 rates of respiration occur when ADP (0.5 µmol) is added and reflect near maximal rates of ATP synthesis (4). Mitochondrial protein was determined colorimetrically using BSA as the standard (12).

Isolation and culture of Kupffer cells. Kupffer cells were isolated and cultured as described by Pertoft and Smedsrod (29). Briefly, rats were anesthetized with pentobarbital (60 mg/kg ip), and the liver was isolated and perfused in a nonrecirculating system with calcium-free Krebs-Ringer-HEPES buffer containing 0.5 mM EGTA (pH 7.4, 37°C) for 10 min. The liver was then perfused with Krebs-Ringer-HEPES buffer containing 0.02% type IV collagenase (Sigma Chemical, St. Louis, MO) for 6 min. Liver cells were dispersed by gentle shaking and separated from other adherent endothelial and stellate cells were collected 15 min later, and 3 ml of fresh culture medium were used to wash each dish (29). These fractions were pooled, and the number of cells in the fraction was counted. The number of attached Kupffer cells was calculated by subtracting the number of cells removed from the number of cells seeded to each dish. The volume of medium was adjusted to yield 2 × 10⁶ cells/ml. All flat cells on the culture dish phagocytosed 1-µm latex beads, verifying that they were Kupffer cells (9). The viability of Kupffer cells was assessed by light microscopy and uptake of trypan blue which routinely exceeded 90%.

Measurement of parenchymal cell oxygen uptake. Hepatocytes were isolated from rat livers according to the method of Pertoft and Smedsrod (29). Briefly, livers were perfused with 0.02% collagenase (Sigma) for 6–8 min until the tissue surrounding each lobe became detached from the parenchyma. The liver was placed in cold buffer, and hepatocytes were dispersed by gentle shaking and separated from other cells and liver debris by centrifugation at 50 g for 2 min. Pellets were subsequently washed with Krebs-Henseleit bicarbonate buffer and collected by centrifugation at 50 g for 2 min (32). Viability of hepatocytes was assessed by light microscopy and uptake of trypan blue which routinely exceeded 90%. Kupffer cells isolated from normal rats were cultured in 60-mm culture dishes with RPMI 1640 at 37°C in a 5% carbon dioxide atmosphere for 4 h. Subsequently, oxygen was increased to 75% oxygen-5% carbon dioxide for 4 h. In some experiments, indomethacin [5 µM, an inhibitor of cyclooxygenase (COX)] or nisoldipine (4 µM, a calcium channel blocker) was added before exposure to oxygen. Conditioned medium was collected and incubated with parenchymal cells isolated from untreated rats in a closed chamber (2 ml) fitted with a Clark-type oxygen electrode, and changes in oxygen concentration were measured.

Measurement of PGE₂ in conditioned medium from cultured Kupffer cells. Isolated Kupffer cells were cultured as previously described. Samples of conditioned medium were analyzed for PGE₂ (20, 32) by competitive RIA using 125I-PGE₂ from Advanced Magnetics (Cambridge, MA). Although this antibody reacts with PGE₁, there is less than 2% cross-reactivity with other prostaglandins, arachidonic acid, and thromboxane.

Statistical analysis. Student’s t-test or ANOVA was used as appropriate. Differences were considered significant at P < 0.05.

Results

Effect of PGE₂ on hepatic oxygen uptake and carbohydrate metabolism in perfused livers from fed rats. Livers from fed rats were perfused at normal, medium, and high flow rates to deliver oxygen to the organ at various rates. The effect of PGE₂ on oxygen uptake, glucose output, and glycolysis (lactate plus pyruvate

Table 1. Effect of PGE₂ on oxygen uptake, glycolysis, and glucose production in livers perfused at various flow rates and different oxygen tensions

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>%O₂</th>
<th>Basal O₂ Uptake</th>
<th>Maximal Rates of O₂ Uptake</th>
<th>% Increase</th>
<th>Lactate + Pyruvate Production</th>
<th>Glucose Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>After PGE₂</td>
<td>Basal</td>
<td>+PGE₂</td>
<td>Basal</td>
</tr>
<tr>
<td>Normal</td>
<td>95</td>
<td>111±7</td>
<td>151±11*</td>
<td>36</td>
<td>29±12</td>
<td>23±8</td>
</tr>
<tr>
<td>Medium</td>
<td>95</td>
<td>117±15</td>
<td>173±17*</td>
<td>48</td>
<td>76±15</td>
<td>45±8</td>
</tr>
<tr>
<td>High</td>
<td>95</td>
<td>128±14</td>
<td>207±4*</td>
<td>62</td>
<td>103±10</td>
<td>37±2*</td>
</tr>
<tr>
<td>High</td>
<td>50</td>
<td>102±5</td>
<td>146±4*</td>
<td>43</td>
<td>98±6</td>
<td>39±9</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol·g⁻¹·h⁻¹; n = 4 livers in each group. Livers were perfused at normal (3–4 ml·g⁻¹·min⁻¹), medium (5–6 ml·g⁻¹·min⁻¹), and high (7–8 ml·g⁻¹·min⁻¹) flow rates for 20 min. Prostaglandin E₂ (PGE₂; final concentration 5 µM) was infused with precision infusion pump in experiments typified by Figs. 1 and 2. For some experiments perfusate oxygen concentration was adjusted at 50%.

*P < 0.05 for comparison with basal. †P < 0.05 for comparison with values from livers perfused at normal flow rates in same column by ANOVA.
production) in livers perfused at normal and high flow rates from PGE2-treated rats is depicted in Figs. 1 and 2 and is summarized in Table 1. At normal flow rates, basal rates of oxygen uptake were 100–110 µmol·g⁻¹·h⁻¹. The subsequent infusion of PGE2 (5 µM) increased respiration gradually to peak values around 150 µmol·g⁻¹·h⁻¹ (Fig. 1A); basal rates of glucose output ranged from 40 to 50 µmol·g⁻¹·h⁻¹, and PGE2 increased glucose output by about 35 µmol·g⁻¹·h⁻¹ (Fig. 1B, Table 1). Concomitantly, basal rates of production of lactate plus pyruvate (glycolysis) were 29 µmol·g⁻¹·h⁻¹. PGE2 did not influence glycolysis significantly at normal flow rates (Fig. 1B, Table 1); however, a tendency for a decrease was observed. In livers perfused at high flow rates (Fig. 2A, Table 1), basal rates of oxygen uptake of 128 µmol·g⁻¹·h⁻¹ were nearly doubled by PGE2. Thus PGE2 stimulated oxygen uptake about twofold more in livers perfused at high than at normal flow rates. However, PGE2 infused at concentrations of 10 µM in livers perfused at normal flow rates only increased oxygen uptake from 113 to 159 µmol·g⁻¹·h⁻¹; values are similar to those observed with 5 µM PGE2. Thus the results observed at high flow rates cannot be explained by changes in PGE2 delivery. Moreover, glucose output was increased rapidly from 58 to 95 µmol·g⁻¹·h⁻¹ by infusion of PGE2, whereas glycolysis was decreased from 103 to 37 µmol·g⁻¹·h⁻¹ (Fig. 2B, Table 1). When flow rate was held constant and oxygen was varied at 50% influent oxygen concentration, the basal rate of oxygen uptake was 102 ± 5 µmol·g⁻¹·h⁻¹. The subsequent infusion of PGE2 (5 µM) increased respiration gradually to peak values around 146 ± 4 µmol·g⁻¹·h⁻¹. However, when the perfusate was saturated with 95% oxygen, the subsequent infusion of PGE2 (5 µM) increased respiration gradually from basal levels of 128 to peak values of 207 µmol·g⁻¹·h⁻¹ (Table 1). Thus, at constant flow, the response to PGE2 was nearly twofold greater at 95% than at 50% oxygen.

Relationship between stimulation of oxygen uptake by PGE2 and average hepatic oxygen concentration. As the oxygen concentration in the liver was increased by elevating flow, the response of respiration to PGE2 was increased in a flow rate-dependent manner, reaching values around 100 µmol·g⁻¹·h⁻¹. This stimulation of...
Effect of oxygen delivery on PGE₂-stimulated mitochondrial respiration. In mitochondria isolated from livers perfused at normal flow rates, state 3 rates of respiration were increased about 25% by prior PGE₂ infusion, whereas state 4 rates were not affected (Table 2). Rates of respiration were also increased by prior PGE₂ infusion in mitochondria isolated from livers perfused at high flow rates; however, the effect was much larger. For example, state 3 rates of respiration were increased about 60% by PGE₂ at high flow rates (Table 2), whereas state 4 rates were also increased about 40%. Thus, PGE₂ stimulated oxygen uptake two- to threefold more in mitochondria isolated from livers perfused at high than at normal flow rates. However, addition of PGE₂ to mitochondria directly had no significant effect on either state 3 or 4 rate of respiration. For example state 3 rate of respiration was 16.9 ± 1.8 with PGE₂ addition vs. 16.2 ± 1.2 nmol·min⁻¹·mg protein⁻¹ in controls. State 4 values were 54.9 ± 8.5 with PGE₂ vs. 48.5 ± 3.0 nmol·min⁻¹·mg protein⁻¹ in controls.

Inactivation of Kupffer cells prevents increases in oxygen uptake due to PGE₂ at high oxygen concentrations. To determine if Kupffer cells are involved in stimulation of oxygen uptake due to oxygen, livers were perfused at normal and high flow rates to vary the oxygen gradient. In some rats, GdCl₃ (10 mg/kg) was injected into the tail vein 24 h before perfusion. Results are from typical experiments that were repeated 4 times in each group. Values are means ± SE, n = 4. *P < 0.05 for comparison with normal flow rates by ANOVA. †P < 0.05 for comparison with high flow rate by ANOVA.

Table 2. Effect of PGE₂ on mitochondrial respiration

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>Addition</th>
<th>State 3</th>
<th>% Increase</th>
<th>State 4</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>None</td>
<td>48.5 ± 3.0</td>
<td>16.2 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGE₂</td>
<td>60.6 ± 4.0*</td>
<td>18.8 ± 1.3</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>None</td>
<td>45.6 ± 2.2</td>
<td>13.4 ± 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGE₂</td>
<td>77.0 ± 3.6†</td>
<td>22.7 ± 1.5‡</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE from 4 livers in each group. Mitochondria were isolated from livers perfused at normal (4 ml·g⁻¹·min⁻¹) or high (8 ml·g⁻¹·min⁻¹) flow rates in presence or absence of PGE₂ (5 µM) for 20 min. *P < 0.05 for comparison of values from livers perfused in absence of PGE₂ in same column. †P < 0.05 for comparison of values from livers perfused at normal flow rates in same column by ANOVA.
stimulated oxygen uptake over 30% more than medium from cells exposed to 21% oxygen. Moreover, this effect was blocked completely by indomethacin or nisoldipine (Fig. 5A). Elevated oxygen tension also increased PGE₂ production about 60% by cultured Kupffer cells, an effect that was also blocked by both indomethacin (5 µM) and nisoldipine (4 µM; Fig. 5B). However, when indomethacin and nisoldipine were added directly to parenchymal cells, oxygen uptake was 31.5 ± 1.5 and 31.9 ± 1.8 µl h⁻¹ 10⁶ cells⁻¹, respectively, compared with control values of 30.3 ± 0.5 µl·h⁻¹·10⁶·cells⁻¹. Thus indomethacin and nisoldipine do not directly affect parenchymal cells.

**DISCUSSION**

Stimulation of oxygen uptake by PGE₂ is dependent on oxygen tension in the liver. Recently, Qu et al. (32) reported that Kupffer cells were activated by ethanol and endotoxin to release mediators such as PGE₂, which stimulated oxygen consumption in parenchymal cells via mechanisms involving cell-cell communication. From this earlier work (32) as well as this study, it is clear that PGE₂ stimulates oxygen uptake in the liver (Fig. 1 and Table 1). Interestingly, this increase was two to three times greater in livers perfused at high compared with normal flow rates (Figs. 1 and 2, and Table 1) and was directly proportional to the average oxygen concentration when the flow rate was varied (Fig. 3). Previous work from this laboratory demonstrated that elevation of flow rate decreased the hepatic oxygen gradient and increased oxygen delivery to the organ (36). However, flow could modify more than just oxygen delivery. Therefore, flow rate was held constant and oxygen was varied. At 50% influent oxygen concentration, the basal rate of oxygen uptake was 102 ± 5 µmol·g⁻¹·h⁻¹. The subsequent infusion of PGE₂ (5 µM) increased respiration gradually to peak values around 146 ± 4 µmol·g⁻¹·h⁻¹. However, when the perfusate was saturated with 95% oxygen, the subsequent infusion of PGE₂ (5 µM) increased respiration gradually from basal levels of 128 µmol·g⁻¹·h⁻¹ to peak values of 207 µmol·g⁻¹·h⁻¹ (Table 1). At constant flow, the response to PGE₂ was nearly twofold greater than at 50% oxygen. Thus it is clear that increases in oxygen delivery to the liver than changes in flow rate are responsible for changes in respiration observed in this study. Moreover, PGE₂ increased oxygen uptake about twice as much in subsequently isolated mitochondria at high than at normal flow rates (Table 2). Thus the oxygen-dependent action of PGE₂ involves mitochondria and is a “remembered” event (i.e., it is not lost during the isolation procedure).

The question then arises, how does PGE₂ increase oxygen uptake in the liver? About 25% of the increase can be accounted for by enhanced demand of mitochondrial oxidative phosphorylation for oxygen to compensate for reduced extramitochondrial ATP production due to inhibition of glycolysis (17). For example, at high flow rates, oxygen uptake was increased from 128 to 207 µmol·g⁻¹·h⁻¹, whereas glycolysis was reduced from 103 to 37 µmol·g⁻¹·h⁻¹. It is known that glycolysis from glycogen yields a net 1.5 mol ATP/mol lactate plus pyruvate produced (17). Thus the decrease of glycolysis is equivalent to 1.5 × (103 – 37) = 99 µmol ATP·g⁻¹·h⁻¹. In contrast, consumption of 79 µmol oxygen·g⁻¹·h⁻¹ by the mitochondrial respiratory chain produces 426 µmol ATP·g⁻¹·h⁻¹. Thus decreased glycolytic ATP production can only account for about 25% (99 of 426) of the increase in oxygen uptake due to PGE₂. However, factors responsible for the predominant fraction (i.e., 75%) of the increase in oxygen uptake due to PGE₂ remain unclear. DeRubertis et al. (7) demonstrated that high oxygen tension (95% oxygen-5% carbon dioxide) stimulated cAMP production about 6- to 10-fold in the inner medulla of the kidney and that the cAMP regulatory system was sensitive to tissue oxygen tension (24). The PGE₂-induced increase in oxygen uptake involves EP₂ receptors, G proteins, the cAMP signal transduction pathway, protein kinase A, and mitochondria (W. Qu, L. M. Graves, and R. G. Thurman, unpublished data). Thus enhanced cAMP production may explain the rest of the increase in oxygen uptake caused by PGE₂.

![Fig. 5. Stimulation of parenchymal cell oxygen uptake and PGE₂ production by conditioned medium from Kupffer cells exposed to high oxygen tension. Isolated Kupffer cells were cultured in RPMI 1640 medium with 21% O₂-5% CO₂ for 4 h, then in fresh medium equilibrated with either 21 or 75% O₂ for an additional 4 h. A: parenchymal cell oxygen consumption measured as described in METHODS. B: PGE₂ content in conditioned medium measured as described in METHODS. Values are means ± SE, n = 5. *P < 0.05 for comparison with 21% O₂ by ANOVA. #P < 0.05 for comparison with 75% O₂-treated group alone by ANOVA. **95% O₂ was toxic to cultured Kupffer cells under these conditions. Indo, indomethacin (5 µM); Niso, nisoldipine (4 µM).](image-url)
Involvement of Kupffer cells in mechanisms of increased oxygen uptake. It has been reported that cultured nonparenchymal cells produce a variety of eicosanoids from arachidonate (27, 28). Eicosanoids produced by hepatic nonparenchymal cells such as PGE2 participate in metabolic regulation of processes such as carbohydrate release by parenchymal cells (2). Recently, Qu et al. (32) demonstrated that oxygen uptake of parenchymal cells from normal rats was stimulated 30–40% by conditioned medium collected from Kupffer cells isolated from ethanol-treated rats. Therefore, intercellular communication in the liver is a potentially important mechanism for the regulation of hepatic metabolism (10, 14, 18, 19). In adult rats, the effect of oxygen on basal rates of oxygen uptake was small (Table 1). Thus oxygen may increase the sensitivity of parenchymal cells to PGE2. However, basal rates of oxygen uptake were nearly doubled after perfusion at high flow rates in livers from small rats (Fig. 4). Elevated oxygen uptake due to oxygen was blocked by treatment with GdCl3, supporting the hypothesis that Kupffer cells are involved. In addition, PGE2 added directly to isolated parenchymal cells increased oxygen uptake (32), supporting the hypothesis that Kupffer cells release eicosanoids in response to oxygen, which regulates oxygen metabolism in parenchymal cells.

Oxygen stimulates PGE2 production by Kupffer cells, which increases oxygen uptake in hepatic parenchymal cells. Previous work from this laboratory showed that basal rates of oxygen uptake were about two times higher in periportal than in pericentral regions of the liver lobule when liver perfusion was in the anterograde direction. When the direction of perfusion was reversed, oxygen uptake was nearly three times higher in pericentral than in periportal regions. Thus rates of oxygen uptake were higher in "upstream" than in "downstream" regions of the liver lobule regardless of the direction of the flow (21, 22). These results support the hypothesis that oxygen tension regulates oxygen uptake in the liver. Later, Nakagawa et al. (26) observed that an increase in oxygen uptake due to arachidonate, which elevates intracellular calcium and is metabolized predominantly in Kupffer cells to eicosanoids, was two- to threefold greater at high than at low initial oxygen tensions (26). Furthermore, arachidonate increased oxygen uptake to a much greater extent in downstream than in upstream regions of the liver lobule. Collectively, these results suggest that the endogenous regulator(s) of oxygen metabolism in hepatic parenchymal cells is produced from arachidonate by Kupffer cells. In this study, it was demonstrated that conditioned medium collected from Kupffer cells exposed to high oxygen produced PGE2 and stimulated respiration in isolated parenchymal cells (Fig. 5). Therefore, the rapid stimulation in oxygen uptake by ethanol treatment and oxygen may have common pathways involving Kupffer cells. These data clearly support the hypothesis that Kupffer cells participate indirectly in the mechanism of elevated oxygen metabolism in hepatic parenchymal cells by producing mediators that stimulate parenchymal cell oxygen metabolism.

Because oxygen tension has little direct effect on oxygen uptake in isolated hepatocytes and no effect in isolated mitochondria other than to saturate cytochrome oxidase (25), whereas arachidonic acid stimulated oxygen uptake in perfused liver but not in isolated hepatocytes (26), it is proposed that an oxygen sensor exists in Kupffer cells that produces mediators such as PGE2 in response to oxygen, which stimulates oxygen uptake in parenchymal cells (Fig. 6). An interesting question arising from this work is what is the nature of this proposed oxygen sensor in Kupffer cells? Because the Michaelis constant of COX for oxygen is only 20 µM (21), it is an unlikely oxygen sensor. In contrast, COX is the rate-limiting enzyme in prostanoid synthesis, and oxidant stress is an inducer of COX-2 gene expression (11). This study showed that indomethacin, a nonspecific COX inhibitor, prevented stimulation of oxygen uptake due to conditioned medium from Kupffer cells exposed to high oxygen tension (Fig. 5). Thus COX-2 could be involved in an oxygen sensor mechanism. Furthermore, calcium is necessary for phospholipase A2 activation and eicosanoid synthesis (8). Nisoldipine, a calcium channel blocker, prevented stimulation of oxygen uptake in isolated hepatocytes (Fig. 6).

Fig. 6. Scheme depicting working hypothesis for how oxygen and PGE2 increase hepatic oxygen consumption. Oxygen increases intracellular calcium in Kupffer cells, which in turn activates phospholipase A2 and increases the rate of PGE2 synthesis most likely via mechanisms involving COX-2. PGE2 then acts on receptors in parenchymal cells to stimulate mitochondrial respiration via pathways involving cAMP. COX-2, cyclooxygenase-2; AA, arachidonic acid.
PGE₂-INDUCED STIMULATION OF O₂ UPTAKE IS O₂ DEPENDENT

oxygen uptake due to conditioned medium from Kupffer cells exposed to high oxygen tension (Fig. 5), suggesting that intracellular calcium could also be involved in an oxygen sensor mechanism. The determination of the precise pathways involved in a proposed oxygen sensor mechanism in Kupffer cells remains an important gap in our knowledge.

In conclusion, high oxygen tension stimulates Kupffer cells to release mediators such as PGE₂, which increases oxygen consumption in parenchymal cells. Furthermore, PGE₂ also stimulates oxygen uptake in parenchymal cells via oxygen-dependent pathways involving cAMP (Fig. 6).

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