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The Heterogeneous Response of the Bivascularly Perfused Rat Liver to Adenosine

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ABSTRACT. The heterogeneity of the liver parenchyma in relation to the metabolic response to adenosine was investigated using the bivascularly perfused rat liver in the anterograde and retrograde modes. Adenosine was infused into livers from fed rats according to four experimental protocols: (A) anterograde perfusion, adenosine via the portal vein; (B) anterograde perfusion, adenosine via the hepatic artery; (C) retrograde perfusion, adenosine via the hepatic vein; and (D) retrograde perfusion, adenosine via the hepatic artery. Due to the very pronounced concentration gradients generated by metabolic transformation, the infused adenosine attained maximal concentrations in different regions with each experimental protocol. The sinusoidal mean transit times ($\bar{t}_s$) were not changed by adenosine in anterograde perfusion, but were increased in retrograde perfusion. It was concluded that the vasoconstrictive elements are localized essentially in the presinusoidal region. Glucose release stimulation presented two kinetic components. The first one was rapid in both onset and decay with a peak around 30 sec; the second one developed more slowly (several minutes). The factors of the first kinetic component are possibly generated in the presinusoidal region or in the first periportal cells. The initial decrease in oxygen consumption seemed to be localized in the region just after the intrasinusoidal confluence of the ramifications of the portal vein and hepatic artery. Indomethacin decreased glucose release stimulation by adenosine in both anterograde and retrograde perfusion only when DMSO was the vehicle. The participation of eicosanoids in the generation of the effects of adenosine seems to be less important than hitherto believed. BIOCHEM PHARMACOL **58**;3:397–409, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. bivascular liver perfusion; adenosine; glycogenolysis; glycolysis; oxygen uptake; zonation of metabolic effects; zonation of metabolic uptake

Backward and forward monovascular perfusion of the rat liver has proven to be a useful technique for investigating metabolic heterogeneities along the hepatic acinus. Bivascular perfusion in the anterograde and retrograde modes, however, represents a refinement of this technique because it allows one to supply certain specific regions of the hepatic acini with substrates, inhibitors, or agonists. In the rat liver, the confluence of the ramifications of the hepatic artery and of the portal vein may occur in presinusoidal as well as intrasinusoidal regions [1, 2]. The intrasinusoidal confluence provides direct access of hepatic arterial flow to sinusoids downstream from the portal triads [2]. This intrasinusoidal confluence occurs at different positions in the sinusoidal beds. However, the cell spaces that can be reached with [^{3}H]water via the hepatic artery in retrograde perfusion of the rat liver present only minor fluctuations from specimen to specimen [1, 3]. The same occurs with the metabolic response to substrates and agonists when they are infused according to this experimental protocol [1, 3–6]. This indicates that the frequency distributions of the positions of the intrasinusoidal confluences are similar in livers from different rats. On the other hand, the rat liver hepatocytes that can be reached through the hepatic artery in anterograde and retrograde perfusion have different metabolic properties [1, 4–6]. In general terms, the behaviour of the cells that can be reached in retrograde perfusion is that expected for periportal hepatocytes, according to the model of metabolic zonation of the liver parenchyma [7]. Using this technique in a preceding work, we investigated the heterogeneous response of the liver cells along the hepatic acinus to ATP [8], which is mainly a P_2-purinergic agent, and to AMP [9], which is believed to act via both P_1- and P_2-purinergic receptors.

Morimoto *et al.* [10], using monovascular anterograde

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† *Abbreviations:* $\bar{t}_s$, sinusoidal mean transit time; t_o, transit time in the large vessels plus collecting system; $Q(t - t_o)$, normalized hemoglobin outflow curve; A_{250}, A_{260}, A_{275}, and A_{293}, absorbances at 250, 260, 275, and 293 nm, respectively; A, adenosine; X, xanthine; HI, hypoxanthine/inosine; U, uric acid; C_A, adenosine concentration; C_{HI}, hypoxanthine plus inosine concentration; C_X, xanthine concentration; C_U, uric acid concentration; ε^{HI}_{250}, ε^{HI}_{260}, ε^{HI}_{275}, and ε^{HI}_{293}, molar extinction coefficients of hypoxanthine/inosine at various wavelengths; ε^{A}_{250}, ε^{A}_{260}, ε^{A}_{275}, and ε^{A}_{293}, molar extinction coefficients of adenosine at various wavelengths; ε^{X}_{250}, ε^{X}_{260}, ε^{X}_{275}, and ε^{X}_{293}, molar extinction coefficients of xanthine at various wavelengths; ε^{U}_{250}, ε^{U}_{260}, ε^{U}_{275}, and ε^{U}_{293}, molar extinction coefficients of uric acid at various wavelengths.

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and retrograde perfusion, have presented evidence that liver cells respond heterogeneously to adenosine. This agonist produces, among other effects, glycogenolysis stimulation, vasoconstriction, and changes in ion fluxes [11, 12]. According to Morimoto *et al.* [10], the heterogeneous response to adenosine is mainly related to the fact that its metabolic effects are mediated by both cyclic AMP, generated intracellularly, and by eicosanoids, which were proposed to be generated in a periportal acinar compartment or in a presinusoidal portal compartment. Morimoto *et al.* [10] also proposed that only those eicosanoids produced under the stimulus of adenosine in anterograde perfusion can contribute significantly to the glycogenolytic response of the liver. Nukina *et al.* [13], on the other hand, have shown that Kupffer cells or other macrophages are essential for the effects of adenosine. When the function of these cells was impaired by previous treatment with gadolinium chloride, adenosine was no longer active in the liver. Kupffer cells and other macrophages are more concentrated in the periportal region of the liver [14, 15], a fact that reinforces the possibility of an unequal distribution of the effects of adenosine in the liver.

Due to the higher resolution of the bivascularly perfused rat liver in the anterograde and retrograde modes, we used this technique in the present study to obtain more detailed information about the zonation of the hepatic response to adenosine. In our experimental approach adenosine was infused either into the portal vein or into the hepatic artery in anterograde perfusion and into the hepatic vein or into the hepatic artery in retrograde perfusion at four different rates. Indomethacin was used as an inhibitor of eicosanoid formation. Changes in glucose release, oxygen uptake, and lactate production were measured, as well as the extraction of adenosine. The possible haemodynamic changes were analyzed by means of the multiple-indicator dilution technique. The results were analyzed in terms of the different regions of the liver that can be supplied with the agonist in each of the four possible combinations of perfusion modes (anterograde or retrograde) and infusion routes (portal or hepatic vein and hepatic artery).

MATERIALS AND METHODS

Materials

The liver perfusion apparatus was built in the workshops of the University of Maringá. Adenosine, xanthine, hypoxanthine, inosine, uric acid, hemoglobin, indomethacin, ibuprofen, and all enzymes and coenzymes used in the enzymatic assays were purchased from the Sigma Chemical Co. [^{3}H]Water was purchased from E. I. Du Pont de Nemours. All other chemicals were from the best grade available (98 to 99.8% purity).

Liver Perfusion

Male albino rats (Wistar), weighing 180–220 g, were fed *ad lib.* with a standard laboratory diet (Purina®). For the surgical procedure, the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg).

Hemoglobin-free, non-recirculating bivascular liver perfusion was performed either in the anterograde mode (entry via the portal vein plus hepatic artery and exit via the hepatic vein) or in the retrograde mode (entry via the hepatic vein plus hepatic artery and exit via the portal vein). The surgical technique was described elsewhere [4]. *In situ* perfusion was performed, the flow being maintained constant by two peristaltic pumps. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer (pH 7.4) containing 25 mg/mL of BSA, saturated with a mixture of oxygen and carbon dioxide (95:5) by means of a membrane oxygenator with simultaneous temperature adjustment (37°). The flow through the portal vein (anterograde) or the hepatic vein (retrograde) was between 28 and 32 mL/min. The flow through the hepatic artery was between 2 and 3 mL/min. All perfusion experiments were initiated in the anterograde mode. Retrograde perfusion was established by changing the direction of flow at 15–20 min before initiating sampling of the effluent perfusate.

The recovery of the fluid pumped into the hepatic artery was monitored by using [^{3}H]water as indicator. In general, [^{3}H]water was infused into the hepatic artery at a rate of approximately 1.5 μCi/min.

Experimental Protocols

Livers from fed rats were used in all experiments. Adenosine was infused during 20 min according to four experimental protocols: (A) anterograde perfusion (portal vein → hepatic vein) and adenosine infusion via the portal vein; (B) anterograde perfusion (portal vein → hepatic vein) and adenosine infusion via the hepatic artery; (C) retrograde perfusion (hepatic vein → portal vein) and adenosine via the hepatic vein; and (D) retrograde perfusion (hepatic vein → portal vein) and adenosine via the hepatic artery. The infusion rates were in the range between 0.059 and 0.7 $\mu mol\ min^{-1}$ (g liver wet weight)$^{-1}$. These infusion rates would correspond to initial portal concentrations between 20 and 200 μM. [^{3}H]Water was infused simultaneously with adenosine when this compound was introduced into the hepatic artery. The recovery of [^{3}H]water was used for monitoring the recovery of the fluid pumped into the hepatic artery, a parameter that allows the determination of the true rate of adenosine infusion. The effluent perfusate generally was sampled in 2 min intervals. The exception was the first 2 min immediately following the onset of adenosine infusion, where sampling was performed in 30-sec intervals.

Indicator-Dilution Experiments

The sinusoidal mean transit times were evaluated by means of the indicator-dilution technique [16, 17]. Hemoglobin was used as an indicator for the extracellular space. The outflowing perfusate was deviated into a flow-through cell,

conveniently placed in a spectrophotometer connected with a recorder. At different times, 100 μL of Krebs/Henseleit-bicarbonate buffer containing 3 mg hemoglobin was injected rapidly into the portal vein (anterograde perfusion) or into the hepatic vein (retrograde perfusion) by means of a precision syringe. The changes in absorbance at 555 nm (the isosbestic point of hemoglobin; $\varepsilon = 13.5$ mM^{-1} cm^{-1}) were recorded as a function of time. The $\bar{t}_s$† were evaluated from the traced curves by numerical procedures, which include numerical integration [8], using the following formula:

$$\bar{t}_s = \int_{to}^{\infty} (t - t_o) \cdot Q(t - t_o)dt \quad (1)$$

In Equation 1, t represents the time after injection, t_o is the transit time in the large vessels plus the collecting system, and $Q(t - t_o)$ is the normalized hemoglobin outflow curve. Normalization was accomplished by dividing the amount of hemoglobin appearing at each time by the total injected amount. $Q(t - t_o)$ has, thus, the dimensions of fraction per unit time. As shown by Goresky *et al.* [18], the distribution of the transit times in the large vessels plus the collecting system, t_o, is very sharp and can be approximated by a single time. The time of appearance of hemoglobin is a fairly good approximation for t_o.

Analytical Procedures

Samples of the effluent perfusion fluid were collected according to the experimental protocol and analyzed for glucose, lactate, and pyruvate by standard enzymatic procedures [19–21].

The oxygen concentration in the outflowing perfusate was monitored continuously employing a Teflon-shielded platinum electrode adequately positioned in a plexi-glass chamber at the exit of the perfusate.

[^{3}H]Water in the influent and effluent perfusate was measured by liquid scintillation spectroscopy. The scintillation solution was a mixture of toluene and ethanol (2:1) containing 2,5-diphenyloxazol (5 g/L) and 2,2′-*p*-phenylene-bis-[5-phenyloxazol] (0.15 g/L).

Adenosine in the effluent perfusate was measured spectrophotometrically. The absorbance (A) of the deproteinized effluent perfusate was measured at four wavelengths: 250, 260, 275, and 293 nm. These wavelengths correspond to the absorption maxima of hypoxanthine/inosine (HI), adenosine (A), xanthine (X), and uric acid (U). For adenosine up to 200 μM in the inflowing perfusate, inosine and uric acid are by far the most important metabolites absorbing in the range between 250 and 293 nm. Xanthine and hypoxanthine appear only in tracer amounts as revealed by high-pressure liquid chromatography using a Shim-Pack (CLC-ODs) 15 cm-length column (Shimadzu). Elution was started with aqueous 0.044 mM KH_2PO_4 (pH 6.0), which gradually was mixed with aqueous 0.044 mM KH_2PO_4 (pH 7.0) + methanol (70:30) until a final proportion of 50% was reached. The concentration of adenosine (C_A) was obtained by solving the following equation system for C_A:

$$\begin{aligned}
\varepsilon^{HI}_{250}C_{HI} + \varepsilon^{A}_{250}C_A + \varepsilon^{X}_{250}Cx + \varepsilon^{U}_{250}C_U &= A_{250} \\
\varepsilon^{HI}_{260}C_{HI} + \varepsilon^{A}_{260}C_A + \varepsilon^{X}_{260}Cx + \varepsilon^{U}_{260}C_U &= A_{260} \\
\varepsilon^{HI}_{275}C_{HI} + \varepsilon^{A}_{275}C_A + \varepsilon^{X}_{275}Cx + \varepsilon^{U}_{275}C_U &= A_{275} \\
\varepsilon^{HI}_{293}C_{HI} + \varepsilon^{A}_{293}C_A + \varepsilon^{X}_{293}Cx + \varepsilon^{U}_{293}C_U &= A_{293}
\end{aligned} \quad (2)$$

The various ε values (e.g. ε^{HI}_{250} and ε^{A}_{250}) represent the molar extinction coefficients of hypoxanthine/inosine (HI), adenosine (A), xanthine (X), and uric acid (U) at 250, 260, 275, and 293 nm, respectively. They were determined with standard solutions for the specific conditions of the perfusion fluid.

The dissolution of indomethacin was checked spectrophotometrically. Indomethacin presents characteristic absorption peaks at 227 and 267 nm when dissolved in perfusion fluid containing 5 μM BSA. The absorption coefficient at 227 nm (ε_{227}) in Krebs/Henseleit-bicarbonate buffer, as determined from the slope of the linear relation between absorbance and concentration in the range up to 10 μM, is 21.9 mM^{-1} cm^{-1}. This value is similar to that reported for the ethanolic solution of indomethacin.

Metabolic rates were calculated from input–output differences and the total flow rates and were referred to the wet weight of the liver.

RESULTS

Transformation of Adenosine

In Fig. 1 the fractions of adenosine that reappeared in the effluent perfusate were represented against the time after the onset of the infusion for each of the four experimental protocols. The perfusion modes and the infusion routes are indicated on the top of each graph as well as the mean infusion rates, expressed as μmol min^{-1} (g liver wet weight)$^{-1}$. It is apparent that the kinetics of adenosine extraction depends on the infusion route and perfusion mode. In general terms, the following sequence of decreasing fractional extraction is valid: protocol C (hepatic vein) > protocol A (portal vein) > protocol B (hepatic artery; anterograde) > protocol D (hepatic artery; retrograde). With protocol C almost no adenosine was recovered in the effluent perfusate for infusion rates up to 0.3 μmol min^{-1} g^{-1}. When adenosine was infused into the portal vein (Fig. 1A; protocol A), all curves showed a maximum at 1.5 min after the onset of the infusion. At the highest infusion rate (corresponding to approximately 200 μM in the portal perfusate), this maximum was nearly 60%. After this maximum, the recovery declined progressively and tended to stabilize at much lower values at the end of the infusion. For infusion rates up to 0.37 μmol min^{-1} g^{-1}, the final recovery was minimal. It should be noted that the

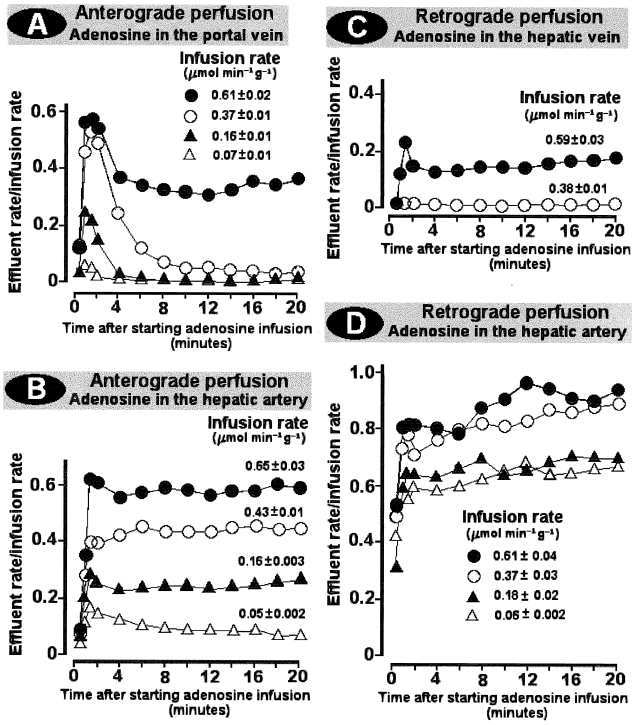

FIG. 1. Adenosine recoveries as a function of time in the bivascularly perfused rat liver. Bivascular liver perfusion was performed as described in Materials and Methods. Adenosine was infused during 20 min at different rates and according to four different protocols, as indicated on each graph. The rates of adenosine infusion into the hepatic artery were calculated from the recovery of the simultaneously infused [^{3}H]water. Adenosine was measured spectrophotometrically as described in Materials and Methods. The recoveries are expressed as fractions of the infusion rates. Each curve represents the mean of 3–4 liver perfusion experiments.

phenomenon of initial maximum recovery was also observable with protocol C, but it is less evident with protocol B and practically nonexistent with protocol D. It must be added that steep concentration gradients of adenosine were formed along the hepatic acini under several conditions, i.e. the adenosine concentrations in the outflowing perfusate were frequently much lower than those in the inflowing perfusate. These concentration gradients were more pronounced at low adenosine concentrations and when protocol C was employed. With infusion rates up to 0.18 μmol min^{-1} g^{-1}, excepting protocol D, the outflowing adenosine concentration never surpassed 30% of the inflowing concentration.

Effects of Adenosine on Sinusoidal Mean Transit Times

The mean transit time of an indicator is directly proportional to its distribution space [16]. Thus, it should be possible to detect sinusoidal vasoconstriction or vasodilation by measuring the sinusoidal mean transit times ($\bar{t}_s$). For this reason, the $\bar{t}_s$ values were measured before and at different times after the onset of adenosine infusion by means of the indicator-dilution technique, as described in Materials and Methods and in the legend to Fig. 2. In Fig. 2, the sinusoidal mean transit times, measured with four different combinations of perfusion modes and adenosine infusion routes, were represented against the time after the onset of adenosine infusion. Two infusion rates were examined, namely 0.15 and 0.65 μmol min^{-1} g^{-1} (Fig. 2, A and 2B, respectively). Figure 2 reveals that the $\bar{t}_s$ values in anterograde and retrograde perfusion were substantially different, confirming previous studies [8]. When adenosine was infused in anterograde perfusion, no significant changes were found irrespective of the infusion route (portal vein or hepatic artery). Maximally it could be said that at an infusion rate of 0.65 μmol min^{-1} g^{-1} small tendencies toward shorter mean transit times were apparent at 1.5 min. In retrograde perfusion, on the other hand, significant increases in $\bar{t}_s$ occurred only when adenosine was introduced into the hepatic artery at an infusion rate of 0.65 μmol min^{-1} g^{-1}. When adenosine was infused at 0.15 μmol min^{-1} g^{-1}, there was no more than a small tendency toward longer sinusoidal mean transit times. The changes caused by adenosine were not stable over the entire infusion period. Actually, there was a peak at 1.5 min with a steady decline afterwards. It should be mentioned that the effects of adenosine on the sinusoidal mean transit times, although qualitatively similar, were less pronounced than those found with ATP [8].

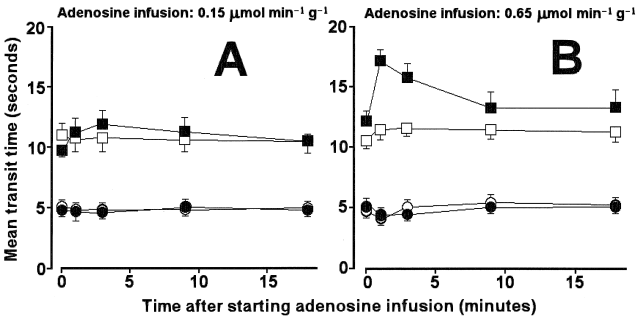

FIG. 2. Effects of adenosine on the sinusoidal mean transit times. Adenosine was infused at two different rates as indicated on the top of each panel. The outflowing perfusate was deviated into a flow-through cell, conveniently placed in a spectrophotometer connected with a recorder. At different times, 100 μL of Krebs/Henseleit-bicarbonate buffer containing 3 mg hemoglobin was injected rapidly into the portal vein (anterograde perfusion) or into the hepatic vein (retrograde perfusion) by means of a precision syringe. The changes in absorbance at 555 nm (the isosbestic point of hemoglobin; ε = 13.5 mM^{-1} cm^{-1}) were recorded as a function of time. The mean transit times were evaluated from the traced curves by standard numerical procedures and corrected for the transit times in the large vessels and in the tubing systems before reaching the spectrophotometer. The latter were evaluated as the time of appearance of hemoglobin in the flow-through cell. Key: anterograde perfusion and adenosine via the portal vein (protocol A), ○——○; anterograde perfusion and adenosine via the hepatic artery (protocol B), ●——●; retrograde perfusion and adenosine via the hepatic vein (protocol C), □——□; and retrograde perfusion and adenosine via the hepatic artery (protocol D), ■——■. Each series represents the mean ± SEM of 4–6 liver perfusion experiments.

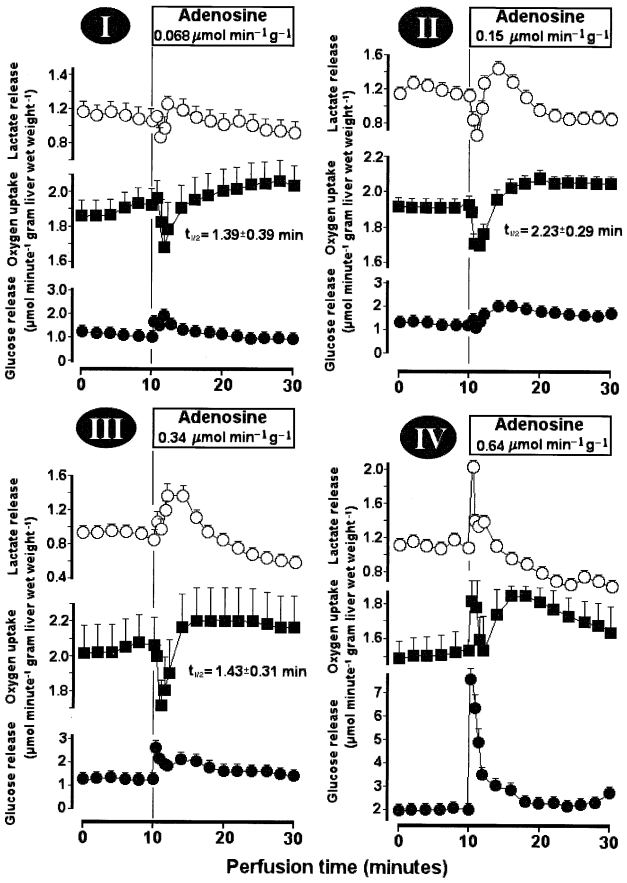

FIG. 3. Time–course of the metabolic changes caused by adenosine infused into the portal vein (anterograde perfusion) at four different rates (protocol A). Bivascular liver perfusion was performed as described in Materials and Methods. Adenosine was infused during 20 min at different rates, as indicated. Lactate and glucose in the effluent perfusate were measured by standard enzymatic procedures. Oxygen was monitored polarographically. All rates were expressed as μmol min^{-1} (g liver wet weight)$^{-1}$. Each graph shows the mean of 3–5 liver perfusion experiments. Horizontal bars are SEM.

Kinetics of the Metabolic Effects of Adenosine

Figures 3–6 illustrate the results found in experiments conducted according to each of the four experimental protocols described in Materials and Methods. Figure 3 illustrates the metabolic effects of adenosine when this compound was infused into the portal vein in anterograde perfusion (protocol A). It shows the changes caused by four different adenosine infusion rates in glucose release, lactate production, and oxygen uptake. The rates of adenosine infusion are indicated in the horizontal bars. The mean basal rates, i.e. metabolic rates in the absence of adenosine, measured in 34 anterograde perfusion experiments were the following: glucose release, 1.39 ± 0.05 μmol min^{-1} g^{-1}; oxygen uptake, 1.81 ± 0.04 μmol min^{-1} g^{-1}; and lactate production, 1.10 ± 0.02 μmol min^{-1} g^{-1}. During the first 2 min of adenosine infusion, the outflowing perfusate was sampled in 30-sec intervals. This procedure allowed us to verify that the whole process of glucose release stimulation by adenosine infused into the portal vein is likely to be a biphasic phenomenon. At an infusion rate of 0.34 μmol min^{-1} g^{-1} (panel III), two peaks can be clearly distinguished, the first one appearing as early as 30 sec after the onset of the infusion and the second one a few minutes later. When the infusion was increased further to 0.64 μmol min^{-1} g^{-1} (panel IV), the first peak was very high and sharp. The second peak appeared solely as a shoulder in the fall-off kinetics of the first peak. Oxygen uptake and lactate production also revealed a complex pattern. For infusion rates up to 0.34 μmol min^{-1} g^{-1}, oxygen uptake suffered a small but significant and reproducible decrease during the first 90 sec. This decrease was followed by a recovery to values slightly above the basal ones. The times for half-maximal recovery ($t_{1/2}$), ranging between 1.39 and 2.23 min, are given on each graph. At an infusion rate of 0.64 μmol min^{-1} g^{-1}, the transient decrease was preceded by a rapid stimulation. The peak time of this rapid stimulation, 30 sec, was simultaneous with the peak time of lactate production and glucose release. It should be noted that the kinetics of lactate production was similar to that of glucose release when adenosine was infused at 0.34 or 0.64 μmol min^{-1} g^{-1}. On a mole per mole basis, the increases in lactate production were less pronounced than those of glucose release. The increases in lactate production were concomitant with decreases in pyruvate production (not shown).

The infusion of adenosine into the hepatic artery in anterograde perfusion (protocol B) produced the responses shown in Fig. 4. A characteristic of the results obtained with this protocol was the biphasic response of glucose release for all infusion rates. The first maximum appeared at 30 or 60 sec after the onset of the infusion; the second one at 4 or 6 min. For all infusion rates the second peak with protocol B was more pronounced than the corresponding peak with protocol A (Fig. 3). Oxygen uptake also presented a transient decrease shortly after the onset of the infusion. With protocol B this decrease was more pronounced, however, and more persistent in time, as can be concluded from a comparison of the times for half-maximal recovery, which are given in Figs. 3 and 4. The values were between 2.53 and 3.35 min, and they were statistically different from those found with protocol A ($P < 0.05$). At the end of the infusion, oxygen uptake invariably stabilized at levels slightly above the basal values (before adenosine infusion). Lactate production was also transiently increased with protocol B, but to a relatively smaller extent than glucose release. At least for the infusion range between 0.16 and 0.7 μmol min^{-1} g^{-1}, lactate production suffered a transient decrease shortly after starting the infusion, a decrease that was parallel with the similar decrease in oxygen uptake.

The effects observed in retrograde perfusion are shown in Figs. 5 and 6. The metabolic rates in the absence of adenosine, measured in 37 retrograde perfusion experiments, were the following: glucose release, 0.90 ± 0.03 μmol min^{-1} g^{-1}; oxygen uptake, 1.97 ± 0.03 μmol min^{-1}

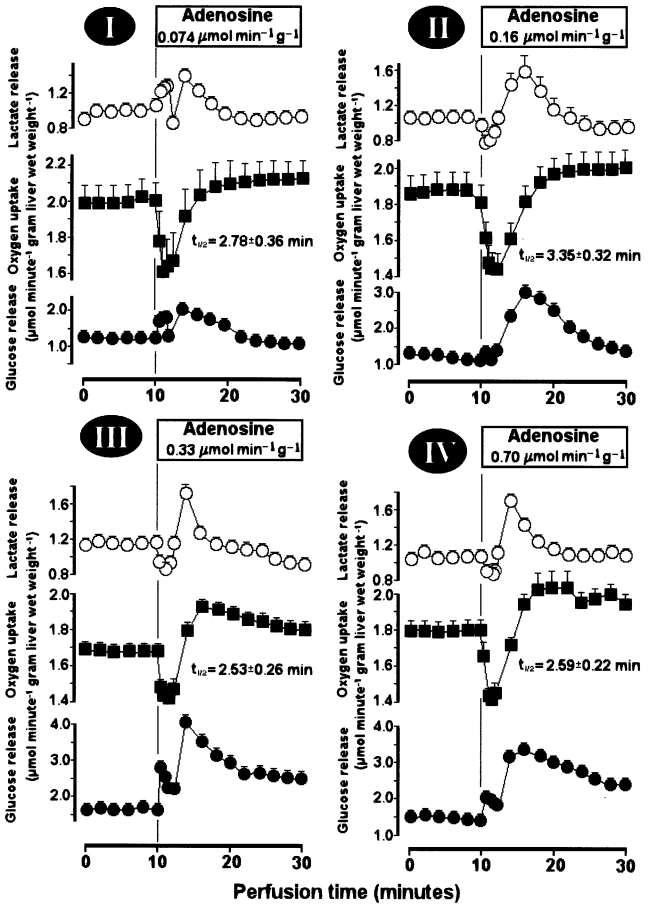

FIG. 4. Time–course of the metabolic changes caused by adenosine infused into the hepatic artery in anterograde perfusion (protocol B). Bivascular liver perfusion was performed as described in Materials and Methods. Adenosine was infused during 20 min at four different rates, as indicated. Lactate and glucose in the effluent perfusate were measured by standard enzymatic procedures. The oxygen concentration in the outflowing perfusate was monitored polarographically. All rates were expressed as μmol min^{-1} (g liver wet weight)$^{-1}$. Each graph shows the mean of 3–5 liver perfusion experiments. Horizontal bars are SEM.

g^{-1}; and lactate production, 0.93 ± 0.02 μmol min^{-1} g^{-1}. Basal glucose release in retrograde perfusion was substantially smaller than in anterograde perfusion, an observation that confirms the results obtained by Morimoto *et al.* [10]. When infused into the hepatic vein (protocol C; Fig. 5) adenosine acted basically as a stimulator: glucose release, lactate production and oxygen uptake all were increased without transient decreases or lag phases. The changes were clearly concentration-dependent and very small, practically nonexistent, at low rates of infusion. The effects on oxygen uptake were stable, but those on glucose release and lactate production tended to present a single maximum. A comparison of the changes in oxygen uptake revealed that protocol C produced the most pronounced stimulations and a well-defined concentration–effect relationship. Infusion of adenosine into the hepatic artery (protocol D; Fig. 6) produced quite pronounced changes if one takes into account that only a minor fraction of the liver cells can be reached when this protocol is employed (0.18 mL/g; [3]). The increases in glucose release were transient with single maxima. Oxygen uptake suffered small transient decreases immediately after the onset of the infusion. These transient decreases are similar to those found with protocol B, but considerably smaller. Lactate production was also transiently increased.

Extra Glucose Release and the Rates of Adenosine Infusion

Glucose release stimulation presented biphasic kinetics when adenosine was infused into the portal vein and into the hepatic artery in anterograde perfusion. Furthermore, the peak values varied considerably in time, more specifically, between 30 sec and 8 min. For these reasons it seems inadvisable to use the maximum rates of glucose release stimulation in the analysis of concentration versus effect relationships. In place of the maximum rates of glucose

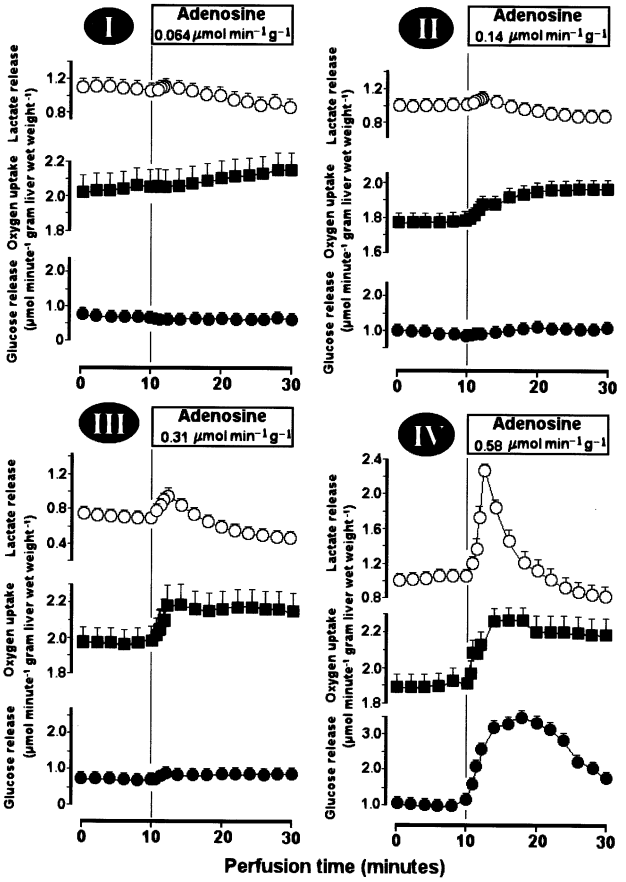

FIG. 5. Time–course of the metabolic changes caused by adenosine infused into the hepatic vein (retrograde perfusion; protocol C). Bivascular liver perfusion was performed as described in Materials and Methods. Adenosine was infused during 20 min at four different rates, as indicated. Lactate and glucose in the effluent perfusate were measured by standard enzymatic procedures. The oxygen concentration in the outflowing perfusate was monitored polarographically. All rates were expressed as μmol min^{-1} (g liver wet weight)$^{-1}$. Each graph shows the mean of 4–6 liver perfusion experiments. Horizontal bars are SEM.

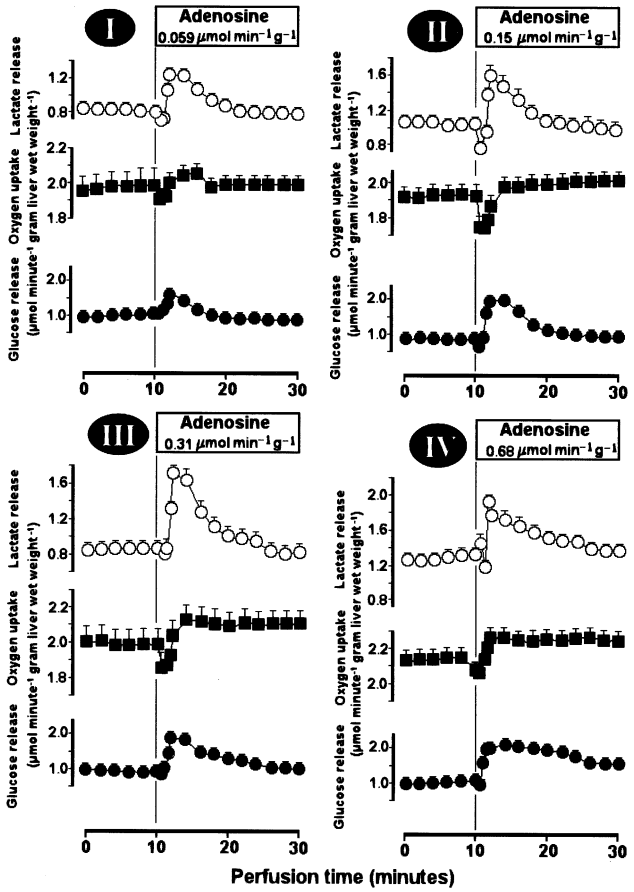

FIG. 6. Time–course of the metabolic changes caused by adenosine infused into the hepatic artery in retrograde perfusion (protocol D). Bivascular liver perfusion was performed as described in Materials and Methods. Adenosine was infused during 20 min at four different rates, as indicated. Lactate and glucose in the effluent perfusate were measured by standard enzymatic procedures. Oxygen in the outflowing perfusate was monitored polarographically. All rates were expressed as μmol min^{-1} (g liver wet weight)$^{-1}$. Each graph shows the mean of 4–6 liver perfusion experiments. Horizontal bars are SEM.

release, we have used the extra amounts of glucose that were released during the 20 min of adenosine infusion. The latter can be evaluated by numerical procedures as the area under the response versus time curves. This has been done for all data obtained in this work, and the results obtained with each of the four experimental protocols are shown in Fig. 7. In these graphs, the amounts of extra glucose (μmol g^{-1}) that were released during the infusion of adenosine were represented against the rates of adenosine infusion. The general pattern revealed by Fig. 7 is quite complex, each experimental protocol presenting its own characteristics. In anterograde perfusion, the effects of adenosine were more pronounced when adenosine was infused into the hepatic artery (protocol B). This is valid for all infusion rates. For rates of adenosine infusion between 0.07 and 0.33 μmol min^{-1} g^{-1}, glucose release with protocol B (hepatic artery) was approximately twice that found with protocol A (portal vein). At the highest infusion rate, the difference was reduced to approximately 30%. In retrograde perfusion, the main distinguishing feature was the parabolic relationship observed with protocol C (hepatic vein). Glucose release stimulation was minimal for infusion rates up to 0.3 μmol min^{-1} g^{-1}, but increased enormously when the infusion rate was further increased to 0.58 μmol min^{-1} g^{-1}. Actually, this was the strongest and the most persistent glucose release stimulation observed in the present study. With protocol D (adenosine in the hepatic artery), stimulation of glucose release was surprisingly high if one takes into account the small cellular spaces that can be reached (less than 30% of the total cell space [3]). For the whole infusion range the extra glucose release with protocol D was very similar to that of protocol A (portal vein). And, for infusions in the range up to 0.3 μmol min^{-1} g^{-1}, protocol D was also clearly superior to protocol C.

Influence of Indomethacin on the Effects of Adenosine

According to Morimoto *et al.* [10], ibuprofen, an anti-inflammatory agent and inhibitor of eicosanoid synthesis, decreases glucose release stimulation by adenosine in anterograde but not in retrograde perfusion when present at a concentration of 50 μM. To find out if this observation could be reproduced in our experimental system, we performed a series of experiments in which inhibitors of eicosanoid synthesis were infused prior to the infusion of adenosine. Several investigators have infused indomethacin or ibuprofen as a concentrated solution in DMSO because these substances are practically insoluble in pure water [10, 22, 23]. However, like most carboxylic nonsteroidal anti-inflammatories, indomethacin and ibuprofen can be dissolved easily in alkaline salt solutions such as the Krebs/Henseleit-bicarbonate buffer, especially when it contains BSA [24–26]. In our first experiments, we infused 50 μM ibuprofen (dissolved in the perfusion fluid) into the portal vein (protocol A). We found no effect on glucose release stimulation by adenosine (not shown). However, oxygen uptake was stimulated by ibuprofen before adenosine infusion (0.21 ± 0.053 μmol min^{-1} g^{-1}), an effect that is most probably the consequence of the uncoupling action of this compound when present at the relatively high concentration of 50 μM. Uncoupling of oxidative phosphorylation is a common property of most carboxylic anti-inflammatories [24–26]. For this reason, we decided to use indomethacin, which is said to be active on eicosanoid synthesis at much lower concentrations [22]. Figure 8 shows the results obtained with protocol A, i.e. when 100 μM adenosine was infused into the portal vein (anterograde perfusion) in the presence of indomethacin. The infusion of adenosine was preceded by a 20-min period of indomethacin infusion. Indomethacin (final concentration 10 μM) was introduced into the perfusion fluid in three different ways: (a) directly dissolved in the perfusion fluid; (b) infusion of a concentrated solution (4 mM) in pure DMSO; (75 μL/min) by means of a precision pump; and (c) infusion of a concentrated solution (500 μM) in Krebs/Henseleit-bicarbonate buffer containing 12% DMSO; (600 μL/min)

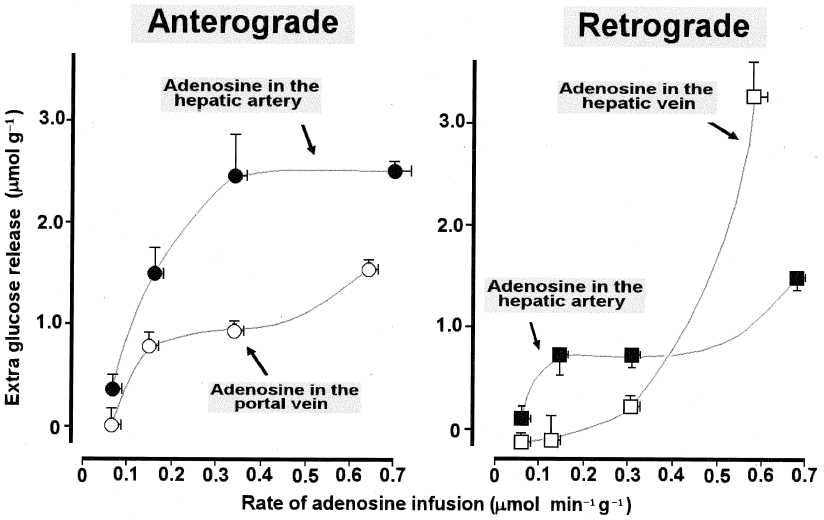

FIG. 7. Extra amounts of glucose released under the stimulus of adenosine in four different experimental protocols in the bivascularly perfused rat liver. The extra amounts of glucose released under the influence of adenosine were calculated as the areas under the time–response curves shown in Figs. 3–6 and plotted against the infusion rates. Key: anterograde perfusion and adenosine via the portal vein (protocol A), ○——○; anterograde perfusion and adenosine via the hepatic artery (protocol B), ●——●; retrograde perfusion and adenosine via the hepatic vein (protocol C), □——□; and retrograde perfusion and adenosine via the hepatic artery (protocol D), ■——■. Each experimental point represents the mean (± SEM) of 3–6 liver perfusion experiments.

by means of a precision pump. Panel A in Fig. 8 shows the changes in glucose release and panel B the changes in oxygen uptake (rates during adenosine infusion minus rate before adenosine infusion). Neither DMSO nor indomethacin alone affected glucose release or respiration, as shown by the inserts in Fig. 8 (panels A_1 and B_1). When indomethacin dissolved in the perfusion fluid was present, glucose release stimulation by adenosine was not inhibited, although the kinetics of the effects was different when compared with the control; the first peak was somewhat higher and the second one appeared merely as a shoulder in the fall-off kinetics, which was faster when compared with that of the control curve. When pure DMSO was the vehicle (75 μL/min), indomethacin strongly inhibited glucose release stimulation during the first 10 min. After this time, however, the control and indomethacin curves tended to become equal. When the vehicle was a mixture of Krebs/Henseleit-bicarbonate buffer and DMSO (600 μL/min), inhibition was also clear in the first 10 min, but not as pronounced as when the vehicle was pure DMSO. It should be noted that the rate of DMSO infusion was approximately the same in both conditions. As revealed by panel B of Fig. 8, oxygen uptake was also influenced by indomethacin, but in this case the effect of the vehicle was less pronounced. Basically, the initial transient decrease in oxygen uptake, which occurred shortly after the onset of adenosine infusion (see Fig. 3, panel III), was almost abolished. When pure DMSO was the vehicle, stimulation of oxygen uptake by adenosine was more pronounced, especially during the last 10 min of the infusion.

The results obtained with protocol C (retrograde perfusion) are illustrated by Fig. 9. Adenosine was infused into the hepatic vein at a concentration of 200 μM because this concentration produces a great increment in both glucose release and oxygen uptake (see Fig. 5, panel IV). Panel A in Fig. 9 reveals that indomethacin, when infused as a concentrated solution in DMSO, clearly inhibited glucose release stimulation by adenosine. In addition to inhibition, the kinetics of stimulation was also changed. When indomethacin was dissolved directly in the perfusion fluid, some inhibition was also apparent during the first minutes of adenosine infusion. This inhibition, however, was by far less than that found when DMSO was the vehicle. At the end of the infusion, glucose release in the presence of indomethacin tended to be higher when compared with the control, i.e. the kinetics of glucose release stimulation was actually changed by indomethacin. Oxygen uptake stimulation, finally, was not affected by indomethacin in retrograde perfusion (panel B in Fig. 9).

DISCUSSION

General Aspects

The results obtained in this work, besides confirming that the liver parenchyma responds heterogeneously to adenosine, reveal a very complex behaviour that cannot always be reconciled with the mechanisms that have been proposed for the action of this agonist [10, 12, 23]. In spite of their complexity, however, close examination and comparison of the results allow the approximate localization of regions in the hepatic parenchyma where certain primary or secondary effects of adenosine predominate. Furthermore, the results obtained with inhibitors of eicosanoid synthesis (indomethacin and ibuprofen) must be discussed in terms of their implications for the hypothesis that eicosanoids are involved in the mechanism by which adenosine stimulates glycogenolysis.

Transformation and Intrasinusoidal Localization of Adenosine

Extraction of adenosine was an inverse function of the infusion rate irrespective of the experimental protocol. This is to be expected from a saturable process. In general, concentration gradients were generated with all experimental protocols. These gradients were more pronounced with protocol C (retrograde, hepatic vein) than with protocol A (anterograde, portal vein). The different sinusoidal transit

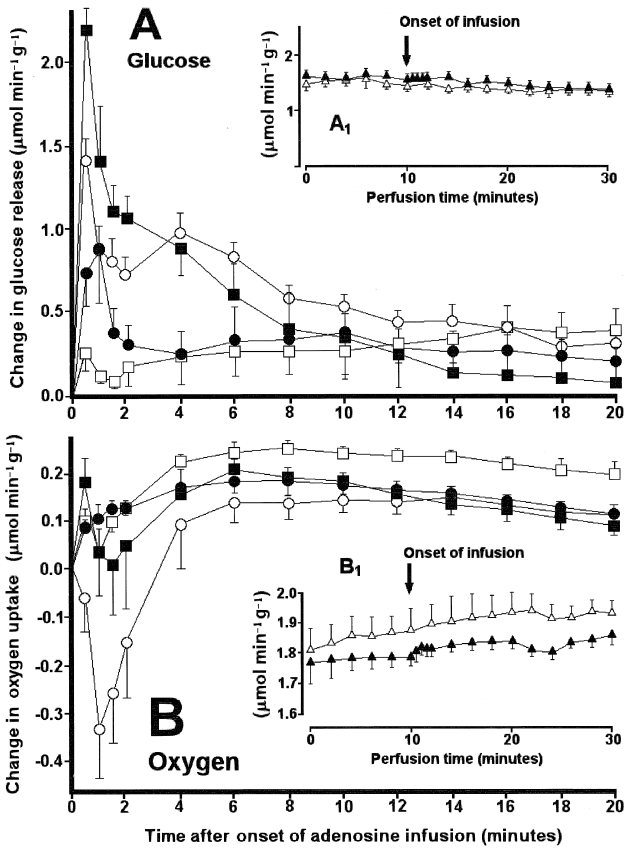

FIG. 8. Time–course of the effects of adenosine infused into the portal vein on glucose release (A) and oxygen uptake (B) in the presence and absence of indomethacin. Anterograde bivascular liver perfusion was performed as described in Materials and Methods. Adenosine (100 μM) was infused during 20 min in the absence (control) or in the presence of 10 μM indomethacin (final portal concentration). Indomethacin was introduced into the portal vein 20 min before the onset of adenosine infusion. The changes in glucose release and oxygen uptake are the differences between the rates during adenosine infusion and the rate before adenosine infusion. Each graph shows the mean of 4–6 liver perfusion experiments. Horizontal bars are SEM. Key: no indomethacin, ○——○; 10 μM indomethacin directly dissolved in the perfusion fluid, ■——■; indomethacin infused as a concentrated solution (500 μM) in DMSO/perfusion fluid (12/88% 600 μL/min), ●——●; and indomethacin infused as a concentrated solution (4 mM) in DMSO (75 μL/min), □——□. Inserts A_1 and B_1 show the mean results ± SEM (N = 3) of control experiments where DMSO alone (75 μL/min; □——□) or indomethacin (final concentration: 10 μM) dissolved in DMSO (△——△) was infused.

times in anterograde and retrograde perfusion are probably an important factor in determining these differences. In principle, longer transit times should produce higher single pass extractions.

The most striking characteristic of adenosine extraction is that it was time-dependent, being lower during the first minutes of the infusion especially when protocol A was employed. This is a phenomenon that cannot be explained by differences in sinusoidal mean transit times. It could be due to some regulatory or feedback mechanism. This is a question that needs to be clarified by further experimental work. However, it is worth mentioning that essentially the same phenomenon also was observed with ATP [8].

In previous work we have analyzed the metabolic responses of the liver to the infusion of substrates or glucagon into the hepatic artery in anterograde and retrograde perfusion in terms of the different cell spaces that are accessible in each perfusion mode [4–6]. A characteristic of these studies was that concentration gradients were kept at a minimum by using high rates of infusion, so that all cells reached via the microcirculation were adequately supplied with substrates or glucagon. In the experiments performed in the present work, the adenosine concentration gradients were very pronounced so that a difference certainly existed between the cell space that could be reached via the microcirculation and the cell space effectively reached by a significant concentration of adenosine. It is obviously very

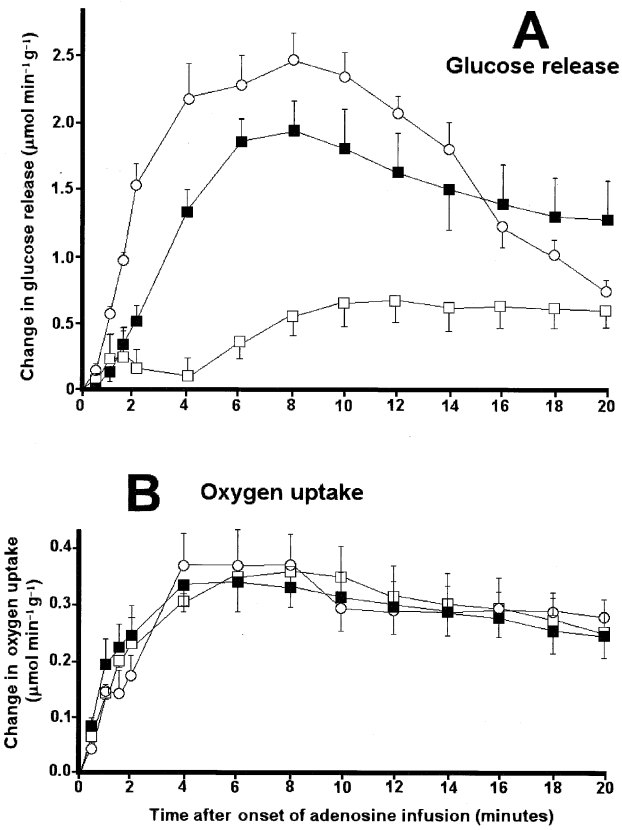

FIG. 9. Time–course of the effects of adenosine infused into the hepatic vein on glucose release (A) and oxygen uptake (B) in the presence and absence of indomethacin. Retrograde bivascular liver perfusion was performed as described in Materials and Methods. Adenosine (200 μM) was infused during 20 min in the absence (control) or in the presence of 10 μM indomethacin. Indomethacin was introduced into the hepatic vein 20 min before the onset of adenosine infusion. The changes in glucose release and oxygen uptake are the differences between the rates during adenosine infusion and the rate before adenosine infusion. Each graph shows the mean of 4–6 liver perfusion experiments. Horizontal bars are SEM. Key: no indomethacin, ○——○; 10 μM indomethacin directly dissolved in the perfusion fluid, ■——■; and indomethacin infused as a concentrated solution (4 mM) in DMSO (75 μL/min), □——□.

difficult to obtain an exact picture of the distribution of adenosine along the hepatic acini. The sites that received the highest concentrations of adenosine, however, can be inferred with some confidence from the extraction profiles shown in Fig. 1 and from the known characteristics of the microcirculation of the liver (see the Introduction). With protocol A (anterograde perfusion; adenosine in the portal vein), the regions within the hepatic acinus that were more intensely supplied with adenosine were the presinusoidal regions and the periportal hepatocytes localized in the most upstream regions. Depending on the position of the intrasinusoidal confluences of hepatic artery and portal vein, the hepatocytes in these regions were also supplied with adenosine, especially in the initial stages of the infusion. At the lowest infusion rates, regions situated downstream from the intrasinusoidal confluences of hepatic artery and portal vein were poorly supplied with adenosine. At the highest infusion rates, however, even perivenous hepatocytes were supplied with some adenosine. With protocol B (anterograde perfusion; adenosine in the hepatic artery), the liver cells situated just after the intrasinusoidal confluence of hepatic artery and portal vein were intensively supplied with adenosine. A certain amount must also have reached periportal hepatocytes situated upstream from the intrasinusoidal confluence; it is difficult to say if presinusoidal regions also were reached. With protocol C (retrograde perfusion; adenosine in the hepatic vein), mainly perivenous hepatocytes were supplied at low infusion rates. At the highest infusion rate used in this work (200 μM), however, periportal cells also were supplied with adenosine. With protocol D (retrograde perfusion, adenosine in the hepatic artery), finally, certainly only those periportal cells situated between the intrasinusoidal confluence of hepatic artery and portal vein were supplied with adenosine. Presinusoidal regions also were supplied, but due to the direction of flow, any product eventually formed in this region did not reach the hepatic parenchyma.

Haemodynamic Effects of Adenosine and Extraction Rates

The action of adenosine on haemodynamics generally has been monitored by means of pressure measurements [10, 11]. In the present work, we have monitored the haemodynamic changes caused by adenosine by measuring the sinusoidal mean transit times, because this parameter allows us to verify if the sinusoidal space is or is not changed when the agonist is introduced. The pattern that was obtained was very similar to that previously reported for ATP except that the effects of adenosine were much less pronounced [8]. Identical to the case of ATP, the present data allow the conclusion that adenosine increases vascular resistance only in the presinusoidal region. If vasoconstriction were present in the postsinusoidal region, the sinusoidal mean transit times should have been increased by adenosine in anterograde perfusion because constriction at the exit should cause the sinusoids to swell. Conversely, if the vasoconstrictive elements were localized along the sinusoidal beds, the sinusoidal mean transit times should have been decreased in anterograde perfusion. Actually, in anterograde perfusion, no significant effect on the sinusoidal mean transit times was found. The sinusoidal mean transit times were increased, however, in retrograde perfusion, an expected phenomenon if the vasoconstrictive elements were localized in the presinusoidal region. These effects of adenosine, however, were significant only when it was infused into the hepatic artery at a final concentration of 200 μM. This is consistent with the proposed localization of the vasoconstrictive elements, i.e. in the presinusoidal region. From the outflow profiles shown in Fig. 1, it can be easily deduced that the adenosine concentration in the presinusoidal region was much more elevated when it was infused in the hepatic artery (Fig. 1D) in retrograde perfusion as opposed to the infusion into the hepatic vein (Fig. 1C). When adenosine was infused into the hepatic vein at a concentration of 200 μM, the maximal effluent concentration (in this case equivalent to the presinusoidal concentration) was only 40 μM, but it was at least 160 μM when adenosine was infused into the hepatic artery.

Kinetics of Glucose Release Stimulation and the Heterogeneous Response along the Hepatic Acini

The biphasic kinetics of glucose release strongly suggests that two different signals are involved in glycogenolysis stimulation by adenosine. The first component was very rapid in onset and decay; the second one was relatively slow in both onset and decay. The first component appeared only in anterograde perfusion, and it became very pronounced when adenosine was infused into the portal vein at high concentrations. Actually, the first component increased progressively as the adenosine concentration was raised in the portal vein. All these observations suggest that the first and rapid stimulation of glycogenolysis depends on events occurring either in the presinusoidal region or in cells localized in the most upstream part of the periportal regions. It should be recalled that in retrograde perfusion events occurring in the presinusoidal region or in its vicinity are unlikely to have any marked influence on most parenchymal cells because of their position in relation to the flow direction. Furthermore, the fact that the first rapid increase in glycogenolysis was more prominent when adenosine was infused into the portal vein as opposed to the hepatic artery is probably related to the much higher concentration of the agonist in the presinusoidal or in the first periportal cells when the former infusion route was utilized.

The second component of glycogenolysis stimulation also predominates in the periportal zone. This conclusion can be drawn from a comparison of the results obtained with protocols C (retrograde/hepatic vein) and D (retrograde/hepatic artery). In retrograde perfusion and at low infusion rates (up to 0.3 $\mu mol\ min^{-1}\ g^{-1}$), extra glucose release was more pronounced when adenosine was infused

into the hepatic artery as opposed to infusion into the hepatic vein. Due to the almost complete single pass extraction with protocol C at infusion rates up to 0.3 μmol min^{-1} g^{-1}, only perivenous cells were supplied effectively with adenosine. The response to adenosine up to 0.3 μmol min^{-1} g^{-1} obtained with protocol C, consequently, can be attributed mainly to perivenous cells. The response with protocol D, on the other hand, was certainly generated solely in periportal cells because these cells were supplied with adenosine when this protocol was employed.

Vanstapel *et al.* [23] have emphasized the fact that, besides increasing glycogenolysis, adenosine and ATP also are able to inhibit glucose release from endogenous glycogen. These authors have attributed inhibition to a decrease in intracellular phosphate. This phenomenon, in addition to receptor desensitization, as proposed by Morimoto *et al.* [10], could be responsible, in part, at least, for the transient nature of the effects of ATP and adenosine. The data obtained in this work indicate that the glycogenolytic signals generated when adenosine entered the portal vein also were accompanied by the strongest inhibitory component. This conclusion receives support from a combination of events. With 0.64 μmol min^{-1} g^{-1} adenosine in the portal vein (protocol A), only the first and rapid burst in glucose release was observed (Fig. 3, panel IV). The peak value of this rapid burst was the most pronounced one observed in the present work. However, when the total extra amount of glucose release was computed and compared with that obtained with a similar adenosine infusion rate into the hepatic artery in retrograde perfusion (protocol D), they turned out to be practically equal (Fig. 7). In morphological terms, the cellular space that can be reached with protocol D is certainly much smaller than that with protocol A. The same is valid from the functional point of view, because the concentration gradients are relatively small at the highest infusion rates employed in this work (Fig. 1A). The simplest interpretation for this discrepancy is the assumption that the glycogenolytic signal generated when adenosine entered the portal vein also was accompanied by a strong inhibitory component so that at high adenosine concentrations the second signal for glucose release stimulation was practically absent.

There are other observations consistent with the above interpretation. The greatest extra glucose release observed in this work occurred when adenosine was infused at a rate of 0.58 μmol min^{-1} g^{-1} (200 μM) into the hepatic vein (retrograde, protocol C; Fig. 7). At this infusion rate, single pass extraction was 80%. Periportal cells, including those situated around the intrasinusoidal confluence of the ramifications of the hepatic artery and portal vein, were certainly supplied with adenosine. Even so, they must have received much less adenosine as compared with the condition where the agonist was introduced at a similar rate in the hepatic artery in anterograde perfusion (protocol B). Generation of an inhibitor signal in the presinusoidal or in the most upstream periportal regions could be an explanation for the greater response with protocol C at 200 μM adenosine as compared with protocol B at a similar concentration. As already stated above, when protocol B is employed, some adenosine can also reach the presinusoidal and the most upstream periportal regions via the presinusoidal confluence of the ramifications of hepatic artery and portal vein. On the other hand, the inhibitory signal should be weaker with protocol B as compared with protocol A because in the latter the concentration of adenosine is maximal in the presinusoidal and nearby periportal regions. Consequently, the glycogenolytic response in terms of extra glucose release should be superior with protocol B as compared with protocol A over the whole infusion range employed in the present work. The fact that this actually happened (Fig. 7) corroborates the view of an inhibitor signal being generated in presinusoidal or in the nearby periportal cells.

Eicosanoids and the Metabolic Action of Adenosine

The hypothesis that eicosanoids are involved in the mechanism of action of adenosine is based mainly on two observations: (a) adenosine stimulates eicosanoids release in the perfused liver (thromboxane and prostaglandin D_2), and (b) inhibitors of eicosanoid synthesis (indomethacin, ibuprofen) decrease the metabolic effects of adenosine [10, 11, 22]. There are also observations that do not agree so straightforwardly with the hypothesis of eicosanoid-mediated effects. Buxton *et al.* [11], for example, found that indomethacin was not able to prevent glycogen phosphorylase *a* stimulation by adenosine in spite of inhibiting glucose release stimulation.

Morimoto *et al.* [10] have claimed that ibuprofen decreases glucose release stimulation by adenosine only in anterograde perfusion. For this reason they have raised the hypothesis that eicosanoids participate in glucose release stimulation by adenosine only in anterograde perfusion. The sites of eicosanoid production are believed to be the Kupffer cells. However, Nukina *et al.* [13] have reported that adenosine did not stimulate eicosanoid release in cultured Kupffer cells, an effect that was exerted by ATP. Moreover, adenosine was able to induce ATP release in cultured parenchymal cells. Based on these observations, Nukina *et al.* [13] have proposed that the action of adenosine is actually the consequence of ATP release stimulation in parenchymal cells, which, in turn, stimulates eicosanoid release by Kupffer cells.

We were not able to reproduce the results of Morimoto *et al.* [10]. As revealed by Figs. 8 and 9, indomethacin inhibited the action of adenosine irrespective of the perfusion mode. The reason for this discrepancy seems to be the fact that Morimoto *et al.* [10] have used ibuprofen, which is less potent than indomethacin. As most anti-inflammatory agents, ibuprofen is likely to act as an uncoupler at the high concentration of 50 μM that was used by Morimoto *et al.* [10]. Consequently, if eicosanoids really participate in the action of adenosine, they do so irrespective of the perfusion

mode, anterograde or retrograde. On the other hand, it is highly significant that indomethacin (and also ibuprofen [10]) inhibited the action of adenosine infused into the portal vein only when DMSO was used as the vehicle. In principle, eicosanoid inhibition should not depend on the simultaneous presence of both indomethacin and DMSO. The combined action of indomethacin and DMSO could be nonspecific, i.e. not related to eicosanoid production. DMSO cannot be regarded as an inert substance. There are several reports in the literature about synergistic effects of DMSO with other drugs [27] and hormones [28]. By virtue of our results, thus, it seems more reasonable to conclude that only that portion of the glycogenolytic effect of adenosine which is sensitive to indomethacin infused in the absence of DMSO is mediated by eicosanoids. This excludes the first and rapid glycogenolytic burst observed when adenosine was infused into the portal vein. Eicosanoids could be partly responsible for the second and slow glycogenolytic effect of adenosine.

As mentioned above, Nukina *et al.* [13] proposed that the action of adenosine on eicosanoid release from Kupffer cells is an indirect one, mediated by ATP which is released from hepatocytes. Nukina *et al.* [13] have emphasized several similarities between the metabolic effects of ATP and adenosine. It must be mentioned, however, that there are other substances whose effects are similar to those of adenosine and ATP. For example, the action of endothelin, a potent peptide agonist in the liver, presents many similarities with the action of adenosine or ATP [29]. Furthermore, as detected in the bivascularly perfused rat liver, there are some differences between the actions of ATP and adenosine that are difficult to reconcile with the proposition of Nukina *et al.* [13]. In our previous work with ATP [8], we also found a biphasic kinetics of glucose release stimulation when this agonist was infused into the portal vein (protocol A). However, the first and rapid signal was present at low concentrations (20 μM); it decreased when the concentration was increased and was almost absent at high concentrations (200 μM), a condition where the second and slow signal was maximal. It is worth recalling that exactly the opposite occurred with adenosine, a fact that is difficult to reconcile with adenosine effects mediated exclusively by ATP released from parenchymal cells. Furthermore, we have presented evidence that the vasoconstrictive elements are localized in presinusoidal regions. No vasoconstriction has been detected in the sinusoids. In anterograde perfusion, substances released from parenchymal cells are unable to reach presinusoidal regions. Consequently, ATP produced in parenchymal cells cannot be responsible for vasoconstriction. It seems, thus, that if adenosine is really unable to increase glycogenolysis by itself, as suggested by the experiments of Nukina *et al.* [13], its action cannot be fully explained in terms of ATP release from parenchymal cells nor can it be fully explained in terms of eicosanoid production.

Heterogeneous Response of Oxygen Uptake to Adenosine

The initial transient decrease in oxygen uptake was sensitive to indomethacin in the absence of DMSO. It is possible, thus, that this effect is mainly dependent on eicosanoids. Oxygen uptake stimulation, on the other hand, which was more evident when protocol C was employed, was insensitive to indomethacin. The transient decrease in oxygen uptake inhibition was more pronounced and more persistent when protocol B was employed. This may be indicating that this effect predominates in regions situated shortly after the intrasinusoidal confluence of the ramifications of the hepatic artery and portal vein. The effect was also present, though much less pronounced, when protocol D was employed, an indication that its generation does not depend solely on effectors generated in presinusoidal regions.

Concluding Remarks

In conclusion, it can be said that the response of the liver parenchyma to adenosine is essentially heterogeneous. Vasoconstriction occurs in the presinusoidal region and oxygen uptake inhibition predominates in the periportal region situated around the intrasinusoidal confluence of the ramifications of the hepatic artery and portal vein. In kinetic terms, two different signals leading to glucose release stimulation could be distinguished. Both signals predominate in the periportal region. The experiments of Nukina *et al.* [13] with gadolinium chloride seem to be a strong indication that the Kupffer cells or other macrophages are important for glycogenolysis stimulation by adenosine. However, according to our results, the participation of eicosanoids in the generation of the effects of adenosine seems to be less important than hitherto believed, and ATP mediation is unable to explain differences between the effects of both agonists. Moreover, the conclusions of Nukina *et al.* [13] essentially are based on a specific action of gadolinium chloride on Kupffer cells. However, recent work reveals that gadolinium chloride also produces changes in hepatocytes [30] and that Kupffer cells are also likely to be involved in the hepatic endothelial cell functions and ultrastructural properties [31]. Consequently, the possibility that other, yet unknown, factors are involved in the mechanism of action of adenosine is a striking possibility to be explored by future work.

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