Control of glycogenolysis and blood flow by arterial and portal adrenaline in perfused liver

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In isolated liver from fed rats, simultaneously single-pass-perfused via both the hepatic artery (80 mmHg, 30–35 % flow) and the portal vein (10 mmHg, 70–65 % flow), adrenaline was infused either singly or jointly via the hepatic artery or the portal vein in the absence or presence of the α_1 -blocker prazosin and the β_2 -blocker butoxamine. It was found that: (1) arterial adrenaline caused increases in glucose and lactate output which were slower in onset, smaller in peak height but longer in duration than did portal adrenaline; (2) arterial adrenaline elicited a much more pronounced decrease in flow and increase in pressure in the ipsilateral vessel than did portal adrenaline, and arterial, but not portal, adrenaline elicited qualitatively similar alterations also in the contralateral vessel; (3) arterial adrenaline caused metabolic changes mainly via α_1 -receptors, with β_2 -receptors playing a permissive role via haemodynamic alterations, whereas portal adrenaline acted only via α_1 -receptors; (4) arterial adrenaline decreased arterial flow via α_1 -receptors counteracted via β_2 -receptors and operated on portal flow as portal adrenaline only via α_1 -receptors; and (5) arterial adrenaline was extracted to a far greater extent than portal adrenaline. The results indicate that the hepatic artery and the portal vein can function as independent sites of hormonal signal input, which interact by complex, still undefined, mechanisms.

INTRODUCTION

The isolated liver perfused conventionally via the portal vein only, isolated hepatocyte suspensions and primary hepatocyte cultures are widely used for the study of the mechanism and regulation of liver metabolism. With these experimental models the possible importance of the hepatic artery and the portal vein as independent, yet interacting, sites of input of substrates and hormonal and nervous signals cannot be studied. Therefore a perfusion system was developed that allows the simultaneous supply of the isolated liver via both the hepatic artery and the portal vein with physiological flow rates and pressures [1–3].

Using this bivascular perfusion system it was shown that stimulation of the arterial nerve plexus caused about 2–3 times greater increases in glucose and lactate output and decreases in arterial and portal flow than did stimulation of the portal nerve plexus [2]. Stimulation of both plexuses caused metabolic responses which were equal to, but haemodynamic responses which were less than, the sum of the responses to individual plexus stimulation. Selective stimulation of the arterial or portal plexus caused a decrease not only in the ipsilateral but also in the contralateral flow. In addition, it was demonstrated that, in liver perfused via the portal vein only, all nerve effects were inhibited by the α_1 -blocker prazosin, whereas the β -blocker propranolol was without effect, and that in liver perfused via the artery and portal vein all actions were antagonized totally by the α_1 -blocker and also largely by the β -blocker [3]. This led to the conclusion that under physiological conditions of hepatic blood supply the sympathetic liver nerves act via α_1 -receptors and that their action can be modulated via β -receptors.

Since in liver not only stimulation of the arterial and portal nerve plexus but also the application of a hormone via the artery and the portal vein might cause different metabolic and haemodynamic alterations, the actions and fate of adrenaline applied via either vessel were studied. It was found that the kinetics and extents of the metabolic and haemodynamic alterations, the receptor involvement and the degree of hormone extraction were clearly different with arterially and portally

administered adrenaline. A preliminary report has been published [4].

EXPERIMENTAL

Materials

All chemicals were of reagent grade and from commercial sources. Enzymes and BSA were purchased from Boehringer (Mannheim, Germany), D-(–)adrenaline sulphate was from Serva (Heidelberg, Germany), prazosin from Pfizer (Karlsruhe, Germany), and butoxamine from Wellcome (Burgwedel, Germany).

Animals

Male Wistar rats (200–240 g) were obtained from Winkelmann (Borchen, Germany). At least 1 week before the experiment they were subjected to a 12 h-day/night rhythm with free access to food and water and rat standard diet from Ssniff (Soest, Germany). All experiments were started at 09:00 h. The animals were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body wt.).

Liver perfusion and adrenaline infusion

The liver was perfused *in situ* without recirculation in a 37 °C cabinet via both the portal vein and the hepatic artery. The portal vein, the aorta and the inferior caval vein were cannulated, and all branching vessels of the aorta and the common hepatic artery were ligated. Rats were killed after cannulation of the portal vein and before cannulation of the aorta and inferior caval vein by an intracaval injection of pentobarbital (60 mg/kg body wt.). The preparation technique has been described in more detail previously [1,2]. Thus a bivascular perfusion system was established with a low-pressure component via the portal vein (10–15 mmHg, 70–65 % of total flow) and a high-pressure component via the artery (70–90 mmHg, 30–35 % of total flow) [1–3]. In both systems a physiological total flow rate of about 2 ml/min per g was maintained [5,6]. The experiments were started after a pre-perfusion period of 30 min. Adrenaline was

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Table 1. Effects on glucose and lactate balance of single and joint arterial and portal application of adrenaline in perfused rat liver

Values are means $\pm$ S.E.M. of 5 experiments each. Adrenaline was infused to the sinusoidal concentration shown, and substrate balance was measured as described in Fig. 1. The adrenaline-dependent alterations (increase in output positive) were evaluated by two different procedures as outlined in the Experimental section: the peak values and the area under the curve (AUC). Statistics (Student's *t* test for unpaired values): portal versus arterial application ^b*P* < 0.01, ^c*P* < 0.005; joint versus arterial application ^x*P* < 0.05; joint versus portal application ^y*P* < 0.05.

Mode of adrenaline application (0.1 μ M single, 0.2 μ M joint) via	Alteration in glucose balance		Alteration in lactate balance	
	Peak value (μ mol/min per g)	AUC (μ mol/g)	Peak value (μ mol/min per g)	AUC (μ mol/g)
(1) Hepatic artery	0.69 $\pm$ 0.03	7.3 $\pm$ 0.4	0.32 $\pm$ 0.02	3.6 $\pm$ 0.2
(2) Portal vein	1.16 $\pm$ 0.04 ^c	7.5 $\pm$ 0.3	0.83 $\pm$ 0.12 ^b	5.6 $\pm$ 0.4 ^b
(3) Sum of (1) and (2)	1.85 $\pm$ 0.05	14.8 $\pm$ 0.5	1.15 $\pm$ 0.12	9.2 $\pm$ 0.45
(4) Hepatic artery and portal vein	1.56 $\pm$ 0.06 ^{x,y}	14.2 $\pm$ 0.4 ^{x,y}	0.62 $\pm$ 0.04 ^x	6.0 $\pm$ 0.6 ^x

infused between 40 and 45 min of perfusion via the artery, the portal vein or simultaneously via both vessels with the same constant infusion rate. Thus with only arterial or only portal infusion after mixing of arterial and portal blood the same sinusoidal concentration of 0.1 or 1 μ M was established. With simultaneous arterial and portal infusion, a sinusoidal concentration was reached which was twice that with only arterial or only portal infusion. Owing to the lower arterial than portal flow, the concentration in the vessels before mixing of arterial and portal blood was different; compared with the sinusoidal value, it was about 3 times higher in the artery and 1.5 times higher in the portal vein. In inhibitor studies 0.5 μ M-prazosin and/or 0.5 μ M-butoxamine were added to the perfusion medium throughout the whole experiment. Total perfusion flow was quantified by fractionating the effluent into calibrated tubes. The portal flow was measured with a SMS-302 flowmeter from Hellige (Freiburg, Germany) placed into the portal inflow. The arterial flow was obtained as the difference between total and portal flow. Arterial and portal pressures were measured with a PC-pressure interface V1 from Meyerholz (Aurich, Germany) placed into the arterial and portal inflow.

Perfusion medium

The basic perfusion medium was a Krebs–Henseleit buffer containing 25–30% (v/v) bovine erythrocytes, 5 mM-glucose, 2 mM-lactate, 0.2 mM-pyruvate and 0.5% BSA. The medium was equilibrated with O₂/CO₂/N₂ (13:5:82), thus mimicking arterial oxygen partial pressures. The effluent medium was collected after an initial period of 30 min and cooled on ice.

Determination of metabolites

After centrifugation of the erythrocyte-containing perfusion medium, metabolites were measured in the supernatant by standard enzymic techniques [7]: glucose with glucose dehydrogenase and lactate with lactate dehydrogenase and glutamate–pyruvate transaminase. The output of adrenaline was determined by using the h.p.l.c. system with electrochemical detection from Waters (Eschborn, Germany). This method allowed a sensitive and selective detection of catecholaminergic metabolites [8].

Evaluation of data

The effects of adrenaline on metabolism and haemodynamics were evaluated by either measuring the difference between the peak values and the basal values or calculating the area under the curve (AUC) of the adrenaline-dependent alterations. When baselines of metabolic and/or haemodynamic parameters after

infusion differed from those of the pre-stimulation period, both baselines were connected for evaluation of the area under the curve, as indicated in the Figures. Significance was tested with Student's *t* test for paired or unpaired values.

RESULTS

In the present study the influence of arterial and portal adrenaline on hepatic glucose and lactate metabolism and on haemodynamics, its mechanism of action via α_1 - and β_2 -receptors and its extraction were investigated in rat liver perfused simultaneously via the hepatic artery and the portal vein. Adrenaline was infused via the hepatic artery or the portal vein to reach a sinusoidal concentration of 0.1 μ M and also simultaneously via both inflows to a sinusoidal concentration of 0.2 μ M. The concentration in the hepatic artery was 0.3 μ M and that in the portal vein 0.15 μ M, since the arterial flow amounted to about 35% and the portal flow to about 65% of total flow.

Effects of arterial adrenaline

Metabolism. Arterial adrenaline increased glucose output and shifted lactate uptake to a slight output (Fig. 1, Table 1).

Haemodynamics. Arterial adrenaline decreased arterial flow strongly but portal flow only slightly (Fig. 1, Table 2). Similarly, it increased arterial pressure clearly and portal pressure only minimally (Fig. 1, Table 3).

Effects of portal adrenaline

Metabolism. Portal adrenaline enhanced glucose output and shifted lactate uptake to output faster, reaching higher transient maxima than with arterial adrenaline (Fig. 1, Table 1). The extents of the changes (AUC) in glucose balance were the same with both sites of application, but those in lactate balance were greater after portal than after arterial infusion (Fig. 1, Table 1).

Haemodynamics. Portal adrenaline decreased portal flow and increased portal pressure slightly with similar kinetics to arterial adrenaline, yet it had no effect on arterial flow and pressure (Fig. 1, Tables 2 and 3). The extents of the alterations (AUC) in total flow were very much smaller after portal than after arterial adrenaline (Fig. 1, Table 2).

Effects of jointly applied arterial and portal adrenaline

Metabolism. Arterial plus portal adrenaline enhanced glucose output and shifted lactate uptake to output with a time course similar to the alterations by portal, but not to those by arterial,

Fig. 1. Effects of arterial and portal adrenaline on glucose and lactate balance, on arterial, portal and total flow and on arterial and portal pressure as well as extraction of adrenaline in rat liver perfused simultaneously via hepatic artery and portal vein

Livers were perfused with a Krebs-Henseleit bicarbonate buffer containing 30% (v/v) erythrocytes, 5 mM-glucose, 2 mM-lactate and 0.2 mM-pyruvate. Adrenaline (A) was infused either separately via the hepatic artery or portal vein to a calculated sinusoidal concentration of 0.1 μM or jointly via both vessels to a sinusoidal concentration of 0.2 μM . Owing to the different flow rates in the two afferent vessels, the adrenaline concentrations were 0.3 μM in the hepatic artery and 0.15 μM in the portal vein. Substrate balance is given by [concn. in hepatic vein (in $\mu\text{mol/ml}$) $\times$ flow (in ml/min per g)] - [concn. in portal vein (in $\mu\text{mol/ml}$) $\times$ portal flow (in ml/min per g)] + concn. in hepatic artery (in $\mu\text{mol/ml}$) $\times$ arterial flow (in

adrenaline (Fig. 1). The extent of the changes (AUC) in glucose output was equal to the sum of the changes after arterial and portal adrenaline alone; yet the extent of the changes (AUC) in lactate balance was the same with arterial, portal or joint hormone application (Fig. 1, Table 1).

Haemodynamics. Arterial plus portal adrenaline decreased arterial flow and increased arterial pressure with the same kinetics as arterial adrenaline alone, and altered portal flow and pressure with the same kinetics as portal adrenaline alone (Fig. 1). The extents of the haemodynamic changes (AUC) after arterial plus portal adrenaline were not equal to the sum of the changes after selective application of arterial and portal adrenaline (Fig. 1, Tables 2 and 3).

Extraction of adrenaline

When adrenaline was infused for 5 min at a total flow rate of 2 ml/min per g of liver via the artery or portal vein to a sinusoidal concentration of 0.1 μM , or via both vessels to a sinusoidal concentration of 0.2 μM , a total of 1000 or 2000 pmol/g of liver respectively was applied. With arterial application less than 100 pmol/g, with portal application about 600 pmol/g and with arterial and portal application about 700 pmol/g were found in the effluent (AUC) (Fig. 1). Thus arterial adrenaline was extracted during a single liver pass by more than 90%, whereas portal adrenaline was eliminated by only 40%. Arterial and portal adrenaline were extracted by 65%; the extraction was essentially the sum of extractions after separate infusion of arterial and portal adrenaline.

Effects of adrenergic antagonists on basal metabolism and haemodynamics

In order to characterize the mechanism of action of adrenaline, the α_1 -antagonist prazosin and the β_2 -blocker butoxamine were added separately or jointly to the perfusion medium throughout the whole experiment. For the investigations at 0.1 μM or 0.2 μM sinusoidal concentrations of arterial, portal or arterial plus portal adrenaline, 0.5 μM -prazosin and/or 0.5 μM -butoxamine were applied. Under basal conditions without hormone addition the sympathetic antagonists, added alone or together, influenced neither glucose and lactate balance nor arterial and portal flow. Yet prazosin significantly decreased arterial and portal pressure, whereas butoxamine significantly increased arterial pressure without modifying portal pressure; prazosin and butoxamine together increased arterial but decreased portal pressure (Table 4; cf. Figs. 2, 3 and 4). Apparently, under basal conditions without hormone addition, metabolism was not regulated via adrenergic receptors, but arterial resistance was controlled antagonistically via α_1 - and β_2 -receptors, and portal resistance exclusively via α_1 -receptors.

Mechanism of action of arterial adrenaline

Metabolism. The increase in glucose output and change in lactate balance by 0.1 μM arterial adrenaline were inhibited almost completely by 0.5 μM -prazosin, but also partially by 0.5 μM -butoxamine; no, or only clearly decreased, peak values were reached, respectively (Fig. 2). Related to the baselines in the control experiments, adrenaline in the presence of prazosin might still have caused a slight change of lactate balance (AUC); in the presence of butoxamine it elicited smaller alterations in both glucose and lactate balance (peak values and AUC) than in

ml/min per g]. A positive value indicates release, a negative value uptake. Values are means $\pm$ S.E.M. of 5 experiments each. Basal values without hormone addition are shown as broken lines.

Table 2. Effects on total, portal and arterial flow of single and joint arterial and portal application of adrenaline in perfused rat liver

Values are means $\pm$ S.E.M. of 5 experiments each. Adrenaline was infused to the sinusoidal concentration shown as described in Fig. 1. The adrenaline-dependent alterations (decrease in flow negative, increase in flow positive) were evaluated by two different procedures as outlined in the Experimental section: the peak values and the area under the curve (AUC). Statistics (Student's *t* test for unpaired values): arterial versus portal application ^c*P* < 0.001; joint versus arterial application ^x*P* < 0.05; joint versus portal application ^y*P* < 0.05.

Mode of adrenaline application (0.1 μ M single, 0.2 μ M joint) via	Alteration in arterial flow		Alteration in portal flow		Alteration in total flow	
	Peak value (ml/min per g)	AUC (ml/g)	Peak value (ml/min per g)	AUC (ml/g)	Peak value (ml/min per g)	AUC (ml/g)
(1) Hepatic artery	-0.55 $\pm$ 0.02	-4.3 $\pm$ 0.5	-0.10 $\pm$ 0.01	-0.8 $\pm$ 0.1	-0.62 $\pm$ 0.03	-5.0 $\pm$ 0.6
(2) Portal vein	-0.03 $\pm$ 0.04 ^c	-0.2 $\pm$ 0.1 ^c	-0.18 $\pm$ 0.04	-1.0 $\pm$ 0.2	-0.20 $\pm$ 0.06 ^c	-1.0 $\pm$ 0.4 ^c
(3) Sum of (1) and (2)	-0.58 $\pm$ 0.04	-4.5 $\pm$ 0.5	-0.28 $\pm$ 0.04	-1.8 $\pm$ 0.2	-0.82 $\pm$ 0.07	-6.0 $\pm$ 0.7
(4) Hepatic artery and portal vein	-0.65 $\pm$ 0.09 ^y	-3.3 $\pm$ 0.6 ^y	-0.34 $\pm$ 0.07 ^x	-1.7 $\pm$ 0.4 ^x	-0.96 $\pm$ 0.09 ^{x,y}	-4.9 $\pm$ 0.6 ^y

Table 3. Effects on arterial and portal pressure of single and joint arterial and portal application of adrenaline in perfused rat liver

Values are means $\pm$ S.E.M. of 5 experiments each. Adrenaline was infused to the sinusoidal concentration shown as described in Fig. 1. The adrenaline-dependent alterations (increase in pressure positive, decrease negative) were evaluated by two different procedures as outlined in the Experimental section: the peak values and the area under the curve (AUC). Statistics (Student's *t* test for unpaired values): portal versus arterial application ^b*P* < 0.01, ^c*P* < 0.005; joint versus arterial application ^x*P* < 0.05; joint versus portal application ^y*P* < 0.05.

Mode of adrenaline application (0.1 μ M single, 0.2 μ M joint) via	Alteration in arterial pressure		Alteration in portal pressure	
	Peak value (mmHg)	AUC (mmHg · min)	Peak value (mmHg)	AUC (mmHg · min)
(1) Hepatic artery	17.4 $\pm$ 1.2	137.6 $\pm$ 30.4	1.8 $\pm$ 0.1	13.0 $\pm$ 1.9
(2) Portal vein	-0.9 $\pm$ 0.37 ^c	-4.1 $\pm$ 3.1 ^b	2.5 $\pm$ 0.5	14.4 $\pm$ 3.0
(3) Sum of (1) and (2)	16.5 $\pm$ 1.26	133.5 $\pm$ 30.5	4.3 $\pm$ 0.5	27.4 $\pm$ 3.6
(4) Hepatic artery and portal vein	8.6 $\pm$ 0.4 ^{x,y}	46.0 $\pm$ 3.2 ^{x,y}	2.2 $\pm$ 0.4	11.1 $\pm$ 2.3

Table 4. Arterial and portal pressure under basal conditions in the absence and presence of the α_1 -antagonist prazosin or the β_2 -antagonist butoxamine

Livers were perfused as described in Fig. 1. When indicated, the medium contained 0.5 μ M-prazosin or 0.5 μ M-butoxamine. Values are means $\pm$ S.E.M. of *n* experiments. Statistics (Student's *t* test for unpaired values): inhibitor versus control experiments ^a*P* < 0.05.

Adrenergic antagonist	<i>n</i>	Pressure (mmHg)	
		Hepatic artery	Portal vein
-	26	73.9 $\pm$ 0.9	14.4 $\pm$ 0.2
Prazosin	13	62.6 $\pm$ 0.7 ^a	10.7 $\pm$ 0.2 ^a
Butoxamine	12	88.6 $\pm$ 0.6 ^a	14.3 $\pm$ 0.3
Prazosin + butoxamine	3	93.2 $\pm$ 2.6 ^a	10.3 $\pm$ 0.5 ^a

the controls (Fig. 2). The inhibitory effects of butoxamine could not be tested with lower concentrations of adrenaline (e.g. 10 nM), since these caused too small changes in glucose and lactate balance for reliable inhibitor studies (results not shown). The decreased metabolic alterations by 0.1 μ M-adrenaline in the presence of 0.5 μ M-butoxamine were very similar to the changes by the 10-fold higher dose of 1 μ M-adrenaline in the absence of any receptor blocker (*n* = 4; results not shown). These decreased metabolic alterations were not the result of the adrenaline-dependent block of arterial flow (Fig. 2; cf. below), because they could not be elicited by a ligation-caused cessation of arterial flow (results not shown).

Haemodynamics. The decrease in arterial flow and the elevation of arterial pressure by arterial 0.1 μ M-adrenaline were completely blocked and tended to be reversed to a slight increase and decrease, respectively, by 0.5 μ M-prazosin (Fig. 2). In the presence of butoxamine, adrenaline caused an almost complete cessation of arterial flow; the decrease (AUC) in arterial flow was about 4-fold greater than, and the increase (AUC) in arterial pressure was similar to, that in the control experiments (Fig. 2). The slight decrease in portal flow and the increase in portal pressure by arterial adrenaline were inhibited by prazosin; they were also no longer detectable in the presence of butoxamine, probably owing to the essentially total inhibition of arterial flow, so that only insufficient amounts of arterial adrenaline could reach the contralateral vessel (Fig. 2).

The kinetics and extents of the haemodynamic alterations by arterial 0.1 μ M-adrenaline in the presence of 0.5 μ M-butoxamine were similar to the haemodynamic changes caused by the 10-fold larger dose of arterial 1 μ M-adrenaline in the absence of any antagonist (*n* = 4; results not shown).

Apparently arterial adrenaline increased glucose and lactate release via α_1 -receptors with an indirect permissive involvement of β_2 -receptors, which antagonized the α_1 -mediated decrease in flow and therefore increased the access of arterial adrenaline to the sinusoids (Table 5).

Mechanism of action of portal adrenaline

Metabolism. The increase in glucose output and the shift from lactate output to release caused by portal adrenaline were inhibited completely by prazosin, but not by butoxamine (Fig. 3).

Fig. 2. Effects of the α_1 -antagonist prazosin and the β_2 -antagonist butoxamine on the metabolic and haemodynamic alterations caused by arterial adrenaline in rat livers perfused simultaneously via hepatic artery and portal vein

Livers were perfused and adrenaline (A) was infused via the hepatic artery to a sinusoidal concentration of $0.1 \mu\text{M}$ as described in Fig. 1. The arterial adrenaline concentration was $0.3 \mu\text{M}$. When indicated, the medium contained $0.5 \mu\text{M}$ -prazosin (PRAZ) or $0.5 \mu\text{M}$ -butoxamine (BUTO). Substrate balance is defined in Fig. 1: a positive value indicates release, a negative value uptake. Values are means $\pm$ S.E.M. of 5 control experiments or 4 experiments in the presence of prazosin or butoxamine. Basal values without hormone addition are shown as broken lines.

Fig. 3. Effects of the α_1 -antagonist prazosin and the β_2 -antagonist butoxamine on the metabolic and haemodynamic alterations caused by portal adrenaline in rat livers perfused simultaneously via hepatic artery and portal vein

Livers were perfused and adrenaline (A) was infused via the portal vein to a sinusoidal concentration of $0.1 \mu\text{M}$ as described in Fig. 1. The portal adrenaline concentration was $0.15 \mu\text{M}$. When indicated the medium contained $0.5 \mu\text{M}$ -prazosin (PRAZ) or $0.5 \mu\text{M}$ -butoxamine (BUTO). Substrate balance is defined in Fig. 1: a positive value indicates release, a negative value uptake. Values are means $\pm$ S.E.M. of 5 control experiments or 4 experiments in the presence of prazosin or butoxamine. Basal values without hormone addition are shown as broken lines.

Table 5. Involvement of adrenergic receptors in the actions of arterial and portal adrenaline on hepatic metabolism and haemodynamics

Abbreviations: A, hepatic artery; P, portal vein.

Parameter	Adrenaline application ...	Alteration and receptor involvement		
		Via A	Via P	Via A and P
Metabolism				
Glucose output		Increase via α_1	Increase via α_1	Increase via α_1
Lactate output		Permissive effects via β_2		
Haemodynamics				
Arterial flow		Decrease via α_1 , antagonized via β_2	No change	Decrease via α_1
Arterial pressure		Increase via α_1 , antagonized via β_2	No change	Increase via α_1
Portal flow		Decrease via α_1	Decrease via α_1	Decrease via α_1
Portal pressure		Increase via α_1	Increase via α_1	Increase via α_1

Fig. 4. Effects of the α_1 -antagonist prazosin and the β_2 -antagonist butoxamine on the metabolic and haemodynamic alterations caused by arterial plus portal adrenaline in rat livers perfused simultaneously via hepatic artery and portal vein

Livers were perfused and adrenaline (A) was infused via the hepatic artery and the portal vein to a sinusoidal concentration of $0.2 \mu\text{M}$ as described in Fig. 1. The arterial concentration was $0.3 \mu\text{M}$, and the portal concentration $0.15 \mu\text{M}$. When indicated, the medium contained $0.5 \mu\text{M}$ -prazosin (PRAZ) or $0.5 \mu\text{M}$ -butoxamine (BUTO). Substrate balance is defined in Fig. 1: a positive value indicates release, a negative value uptake. Values are means $\pm$ S.E.M. of 5 control experiments, 4 experiments in the presence of prazosin or butoxamine, or 3 experiments in the presence of prazosin plus butoxamine. Basal values without hormone addition are shown as broken lines.

Haemodynamics. The slight decrease in portal flow and the increase in portal pressure elicited by portal adrenaline were completely prevented by prazosin and not significantly influenced by butoxamine. Arterial flow and pressure were not affected by portal adrenaline (Fig. 3).

Apparently, portal adrenaline caused metabolic and haemodynamic alterations only via α_1 -receptors without affecting arterial flow and pressure (Table 5).

Mechanism of action of jointly applied arterial and portal adrenaline

Metabolism. The increase in glucose release and the shift from lactate uptake to output by arterial plus portal adrenaline were completely inhibited by prazosin, not affected by butoxamine and again totally blocked by the simultaneous addition of both antagonists (Fig. 4).

Haemodynamics. The decrease in arterial and portal flow and

the increase in arterial and portal pressure by arterial plus portal adrenaline were completely inhibited by prazosin, not affected by butoxamine and totally prevented by prazosin plus butoxamine (Fig. 4).

Apparently, the metabolic alterations by arterial plus portal adrenaline were mediated only via α_1 -receptors; surprisingly, the changes in arterial haemodynamics were transduced only via α_1 -receptors without apparent involvement of 'dilatatory' β_2 -receptors (Table 5).

DISCUSSION

Metabolic actions of arterial and portal adrenaline

The present results can be compared only with the metabolic effects of separate application of arterial and portal noradrenaline [9] and of selective stimulation of the arterial and portal nerve plexus as studied in the perfused rat liver [2,3], since the effects of arterial and portal adrenaline on hepatic metabolism have not been investigated previously, either *in vivo* or *in vitro*.

The kinetics of the alterations in glucose and lactate balance by arterial and portal adrenaline were different; the changes were faster in onset and greater in peak height, but shorter in duration, after portal than after arterial hormone application, so that the extents (AUC) were almost the same (Fig. 1, Table 1). Similar observations were made after infusion of arterial and portal noradrenaline [9]. Yet after arterial nerve stimulation the alterations were clearly greater than after portal stimulation [2,3]. This greater efficacy of arterial nerve stimulation can be explained by a stronger development and/or higher density of efferent sympathetic fibres in the arterial nerve plexus [10–14]. Similar changes with respect to kinetics and extents by arterial and portal catecholamines might be expected in view of the generally accepted concept [15–17] that arterial and portal blood mix before or upon entering the sinusoids, as found in rats and other animals [10,16–21] and as indicated in cats by the same extraction of arterial and portal Indocyanine Green [22] or the same stimulation of bile flow by arterial and portal taurocholate [23]. The differences in the kinetics of the metabolic actions of arterial and portal adrenaline (Fig. 1) and noradrenaline [9] were probably due to the differences in the haemodynamic changes and in the elimination of the catecholamines (see 'Model for the differences...' below).

The adrenaline concentrations of $0.1 \mu\text{M}$ used in this study were clearly higher than the physiological values of 1–10 nM [24]; this was necessary because, for unknown reasons, the perfused liver does not respond to physiological catecholamine concentrations as first reported many years ago [25].

Receptor specificity of the metabolic alterations by arterial and portal adrenaline

Arterial and portal adrenaline increased glucose and lactate release directly via α_1 -receptors. β_2 -receptors appeared to have a permissive function only with arterial, but not with portal, adrenaline (Figs. 2 and 3, Table 5). Similar observations were made with arterial and portal noradrenaline [9]. The sympathetic nerves of both the arterial and portal plexuses stimulated glucose and lactate release via α_1 -receptors under the permissive action of β -receptors which were not accessible in the conventional portal perfusion [3].

The concept of a direct involvement of α_1 -receptors and probably an indirect role of β_2 -receptors in the activation of glucose output by arterial catecholamines and of only α_1 -receptors in the changes by portal catecholamines contrasts with the widely accepted view that only receptors on the hepatocytes proper, which have the same accessibility from the sinusoids, are involved

in the regulation of metabolism by circulating hormones. Therefore the possibility has to be considered that in this case β_2 -receptors located only in the artery and regulating arterial inflow, and thus tissue accessibility, are indirectly involved in the regulation of metabolism (see 'Model for the differences...' below).

Haemodynamic actions of arterial and portal adrenaline

The effects on hepatic haemodynamics of catecholamines applied separately via either the artery or the portal vein and of stimulation of the arterial nerve plexus [26,27] have been investigated previously in cats [26], dogs [26–29] and rats [30] *in vivo*. In the perfused rat liver the effects of arterial and portal noradrenaline [9] and of separate stimulations of the arterial and portal nerve plexus [2,3] were studied.

The haemodynamic alterations by arterial and portal adrenaline were clearly different. Arterial adrenaline was much more effective than portal adrenaline in decreasing flow and increasing pressure in the ipsilateral vessel (Fig. 1). Similar observations were made with noradrenaline [9] and hepatic nerve stimulation [2].

Arterial adrenaline (Fig. 1, Table 2) and noradrenaline [9], as well as stimulation of the arterial and portal nerve plexuses [2], decreased flow also in the contralateral vessel. Whereas decreases in flow in the contralateral vessel by stimulation of the arterial or portal plexuses can be explained by assuming that nerve fibres of the arterial and portal plexus each reach the contralateral vessel, it is unknown by which mechanism circulating catecholamines can affect the contralateral vessel. Myogenic or metabolic autoregulatory mechanisms in the contralateral vessel without involvement of α - and β -receptors appear possible. However, such mechanisms are not in accord with the present results (Fig. 2) and other observations, since the haemodynamic actions of circulating catecholamines on the contralateral vessel could be specifically inhibited by α - or β -antagonists [3,4,29]. Alternatively, the flow effects on the contralateral vessel might be due to an activation of an intrahepatic nervous system [31] which might sense the catecholamine in the portal or arterial bed, respectively, and alter flow in the contralateral vessel. The catecholamines might reach the resistance sites of the portal venules from the arterial blood by filtration processes depending on the hydrostatic pressure or by a unidirectional arteriportal flow via the hepatic arterioles which also provide the vasa vasorum of the terminal portal veins [29,32–34]. These models would explain why arterial, but not portal, noradrenaline decreased flow in the contralateral vessel; it would not explain why *in vivo* portally infused dopamine [28] and isoprenaline [28,29,35] were able to decrease and enhance, respectively, arterial flow. Thus it appears necessary to assume that diffusion can take place in either direction independently of the hydrostatic pressure, perhaps in the region of the portal triads where the arterioles and the portal venules are intimately intertwined.

Receptor specificity of haemodynamic actions of arterial and portal adrenaline

Arterial adrenaline decreased arterial and portal flow via α_1 -receptors, with β_2 -receptors antagonizing only the action on arterial flow (Fig. 2, Table 5). Portal adrenaline decreased portal flow exclusively via α_1 -receptors (Fig. 3, Table 5). A similar receptor involvement was observed after application of arterial and portal noradrenaline [9], whereas the actions of the sympathetic nerves of both nerve plexuses on arterial and portal flow were mediated exclusively via α_1 -receptors [2,3]. The participation of β -receptors only in the regulation of arterial flow was indicated also in previous studies *in vivo* mainly in dogs [27,36,37].

Model for the differences in the metabolic alterations by arterial and portal adrenaline

The extent of a metabolic change (AUC) will depend on the amount of parenchyma perfused and on the average hormone concentration in the tissue. Arterial adrenaline decreased flow into the sinusoids more strongly than did portal adrenaline (cf. [9,38]); moreover, it was eliminated more efficiently (Fig. 1). Thus arterial adrenaline reached less tissue at a lower concentration but for a longer time, whereas portal adrenaline reached more tissue at a higher concentration but for a shorter time. Therefore the total changes (AUC) were similar, although the peak values were different.

Since the decrease in arterial but not in portal flow into the sinusoids via α_1 -receptors had to be antagonized via β_2 -receptors in order to maintain some inflow (Fig. 2), β_2 -receptors had an indirect permissive role in the metabolic actions of arterial adrenaline (Table 5).

In conclusion, the present study has shown that the hepatic artery and the portal vein function as independent sites not only of nervous but also of hormonal signal input, with complex mutual interactions, which still have to be defined both anatomically and biochemically.

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