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ROLE OF AFFERENT NERVES AND SENSORY PEPTIDES IN THE MEDIATION OF HEPATIC ARTERY BUFFER RESPONSE.

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Intrahepatic arteries are richly innervated by both adrenergic and sensory vanilloid-sensitive (capsaicin-sensitive) fibers. Stimulation of capsaicin sensitive fibers has been shown to dilate the intrahepatic vessels by both releasing sensory neuropeptides and by modulating the adrenergic tone. However the participation of capsaicin-sensitive fibers in the mediation of the hepatic artery buffer response (HABR) has not been investigated yet. To explore the involvement of sensory innervation and sensory neuropeptides in the HABR, the experiments were performed on capsaicin-denervated Wistar rats. In addition, we used selective CGRP and tachykinin receptor antagonists to test the participation of CGRP, substance P and NK-A in HABR in the rat. In anesthetized rats the hepatic artery blood flow (HABF), microcirculatory hepatic blood flow (HBF) and portal blood flow (PBF) were determined. The HABR was induced by partial occlusion of the portal vein and maintaining the PBF at 10% of its control preocclusive value. In the control HABR the hepatic artery blood flow increased by 89% ($p< 0.005$) whilst the HBF at the same time decreased by 32% ($p< 0.005$) in comparison to preocclusive HABF and HBF values. In sensory-denervated rats the resting HBF and PBF were increased by 23% ($p< 0.05$) and 34% ($p< 0.05$), respectively in comparison to the control HBF and PBF values. In this group the induction of the HABR increased the hepatic artery blood flow by only 55% ($p< 0.05$), whilst the HBF was reduced by 45% ($p< 0.05$). Pretreatment with CGRP 8-37 (CGRP receptor antagonist) and NK-1 but not NK-2 nor NK-3 receptor antagonists significantly reduced the HABF by 43% ($p< 0.05$) and 25 % ($p< 0.05$) as compared to the HABF value in the control HABR group. These findings support the hypothesis that the hepatic artery buffer response induced by reduction of the portal inflow to the liver by 90% is partially mediated by activation of capsaicin-sensitive sensory fibers in the liver, probably due to local tissue ischemia and hypoxia. The observed vasodilation in the vascular bed of the hepatic artery is due to stimulation of CGRP and NK-1 receptors.

Key words: *hepatic artery buffer response, hepatic blood flow, sensory neurons, capsaicin, vanilloid receptors.*

INTRODUCTION

The hepatic artery buffer response (HABR) is the compensatory mechanism of the hepatic circulation which buffers the effects of the portal blood flow changes on total hepatic blood flow. It was first described by Lautt in 1981 (1) and since then many mechanisms have been described regarding this issue. As the relationship between the portal vein and hepatic artery blood flow was clearly not a reciprocal flow relationship Laut *et al.* (1) coined the term HABR. The first mechanism postulated was of myogenic origin. This hypothesis assumed that a change in the portal flow would produce a change in the portal pressure that would be sensed by the hepatic artery, however the changes in portal pressure were very minor even in the face of very large changes in the portal flow (2). Other theories included metabolic regulation and washout hypothesis, which were introduced by Laut and Legare in 1986 (3). As the main metabolic agent responsible for the compensatory mechanism of the HABR, adenosine had been postulated. Adenosine was shown to dilate the hepatic artery, even when injected into the portal vein and adenosine receptor blockade had been shown to significantly reduce the HABR in the experimental animals (3). Richter *at al.* (4) in 2001 re-evaluated the role of adenosine in mediating the HABR in the rats by using 8-phenyltheophylline as a competitive antagonist. Pretreatment of animals with the adenosine antagonist 8-phenyltheophylline completely blocked the hepatic arterial buffer response with the consequence of decreased tissue oxygenation and increased heterogeneity of sinusoidal perfusion in the rats. They also found, that diameters of the terminal hepatic arterioles (THAs), terminal portal venules (TPVs) and sinusoids did not change during HABR, indicating that the reduction in resistance to the hepatic artery blood flow is located upstream and may function via hepatic arteriolo-portal venular shunts, resulting in equal distribution of microvascular blood flow and oxygen delivery under conditions of restricted portal vein.

Despite the participation of adenosine in the hepatic artery buffer response, this ATP derivative had been additionally postulated as the mediator of the autoregulatory mechanisms of the hepatic artery. If arterial pressure is decreased, this leads to the reduction of the arterial blood inflow and the accumulation of adenosine, which is responsible for vasodilation. Another aspect of HABR is that this regulatory phenomenon is seen in a fully denervated liver (5) and in transplanted human livers (6, 7).

These observations, however do not exclude the fact that neural factors could influence the hepatic artery buffer response as the neurogenic relaxation due to stimulation of the perivascular nerves, which have been described in the isolated arteries (8). The intrahepatic arteries are richly innervated by both adrenergic (8) and sensory vanilloid-sensitive (capsaicin-sensitive) fibers containing CGRP, Substance P and other tachykinins (10, 11).

Phillips et al. (8) describing the mechanisms underlying vasodilatory responses in small arteries of the rat found that after blockade of the effects of the sympathetic nerves and cholinergic neurotransmission, the hepatic nerve stimulation produced a large dilatation which could be abolished with capsaicin and significantly reduced by the CGRP peptide antagonist CGRP 8-37. Stimulation of capsaicin sensitive fibers has been shown to dilate the intrahepatic vessels by both releasing sensory neuropeptides and by modulating the adrenergic tone (8, 11, 12). CGRP is postulated as the main vasodilatatory mediator.

The vasodilatatory action of CGRP upon the hepatic artery is related to the increase in cyclic AMP in the smooth muscle cells and seems to be independent of nitric oxide release from the endothelium (13) as it was next demonstrated by Bratveit and Helle (14). However in our previous experimental work on anesthetized rats we showed (15) that nitric oxide seems to be one of several mediators of the hepatic artery buffer response.

The main goal of our study was to examine the role of the afferent vanilloid-sensitive (capsaicin-sensitive) nerves and sensory peptides in the mediation of the hepatic artery buffer response (HABR). To explore the participation of sensory innervation and sensory neuropeptides in the HABR we performed experiments on capsaicin-denervated and vanilloid 1 receptor blocked Wistar rats. In addition, we used selective CGRP and tachykinin receptor antagonists to test the role of CGRP and tachykinins in the mediation of HABR in the rat.

MATERIAL AND METHODS

Experiments were performed on 80 fasted Wistar rats weighing 200-230 g Animals were fasted, but were allowed access to water for 24 h before the onset of experiments. Rats were anesthetized with intraperitoneal injection of pentobarbital (Vetbutal, 50 mg/kg). The jugular vein was exposed and a cannula of 0.8 mm diameter filled with saline was inserted to administer 0.9% NaCl (2 ml/h) and supplemental doses of anesthetics. The trachea was exposed and incised for placing a tube, which was connected to rodent ventilator (UGO Basile, Italy). Respiration rate was estimated at 50/min with tidal volume of 10ml/kg. Body temperature was maintained at 37.5° by warming rats with a heating pad, controlled by a rectal thermistor and regulator (Fine Science Tools TR - 100). During the experiments, the systemic mean arterial pressure (AP) was monitored via a saline filled catheter inserted into the right carotid artery and connected to a strain gauge transducer (Saunders and Wickers). AP values were expressed in mmHg or percent change of control. A midline laparotomy was performed to expose the hepatic porta and the surface of the liver. Microcirculatory hepatic blood flow (HBF) was measured using the laser-Doppler probe 407 (Periflux 4001 Master, Sweden), equipped with a silicon cuff, which enabled stabilization against respiratory movements. Laser-Doppler signal was recorded and averaged within 5-sec periods. HBF values were recorded and analyzed with the use of Perisoft Computer Program and expressed in PU or percent change of control. The main trunk of the portal vein was exposed for placement of an ultrasonic flow probe on the vessel to estimate the mean portal blood flow (PBF). The hepatic artery blood flow (HABF) and portal blood flow (PBF) were estimated using

ultrasonic blood flowmeter (Altron T206, USA). PBF and HABF values were expressed in ml/min or percent change of control.

Temporal 90% occlusion of the portal vein was made with the use of vascular occluder (Aesculap, Germany) under the control of the portal blood flow, recorded using an ultrasonic blood flowmeter (Altron T206, USA). The data were statistically analyzed with non-parametric Cruskall-Wallis Anova test with significant difference at $p< 0.05$. Experimental procedures conducted during the study conform to guidelines of Animals Research Committee of Jagiellonian University.

Depending on the experimental model the animals were assigned to one of the experimental groups.

In Group I (n=10), after completing the surgical procedures and stabilizing hemodynamic parameters, HBF, PBF, HABF and AP values were recorded. These values served as the control for the next experimental groups.

In Group II of rats (n=10) , temporal occlusion of the portal vein was performed with the use of vascular occluder (Aesculap, Germany) under the control of the portal blood flow to test the hepatic artery buffer response (control HABR group). In this group, the hepatic artery blood flow and microcirculatory hepatic blood flow were measured in response to 90% reduction of portal blood inflow.

In Group III of rats (n=10), temporal occlusion of the portal vein and thus the HABR was analyzed in Capsaicin sensory denervated animals. Capsaicin (Fluka) was applied as 1% solution in 5 consecutive doses up to the cumulative dose of 175 mg/kg 10 days before experiments under the ether anesthesia. The vehicle for capsaicin consisted of 10% ethanol, 10% of Tween 80 and 80% of physiological saline. The results from this group were compared to those obtained in the control HABR group.

In Group IV of rats (n=10), temporal occlusion of the portal vein and thus the HABR was analyzed in ruthenium red pretreated animals (vanilloid receptors VR-1 antagonist) given at the dose of 1.5 mg/kg i.v.

In Group V of rats (n=10), temporal occlusion of the portal vein and thus the HABR was analyzed in CGRP 8-37 (CGRP-1 receptor subtype blocker) (50 µg/kg i.v.) pretreated rats.

In Group VI of rats (n=10), temporal occlusion of the portal vein and thus the HABR was analyzed in CP-99,994 (NK-1 receptor antagonist) (1mg/kg i.v.) pretreated rats.

In Group VII of rats (n=10), temporal occlusion of the portal vein and thus the HABR was analyzed in MEN 10376 (NK-2 receptor antagonist) (Sigma) (100 µg/kg i.v.) pretreated rats.

In Group VIII of rats (n=10), temporal occlusion of the portal vein and thus the HABR was analyzed in SB 218795 (NK-3 receptor antagonist) (1mg/kg i.v.) pretreated rats.

RESULTS

In Group I (n=10), after completing the surgical procedures and stabilizing hemodynamic parameters, the control HBF, PBF, HABF and AP values were recorded. These values served as the control for the next experimental groups. Control HBF was 311+ 35 PU. Using ultrasonic blood flowmeter the control PBF and HABF values were 14 ± 2.3 ml/min and 2.4 ± 0.33 ml/min, respectively. The control control mean arterial pressure (AP) value was 104 ± 11 mmHg.

In the second group (n=10), a typical response of the hepatic artery was observed after reducing the portal blood inflow to the liver by 90%. In this group an increase in the HABF by 89% ($p< 0.005$) was recorded, whilst the HBF in this group

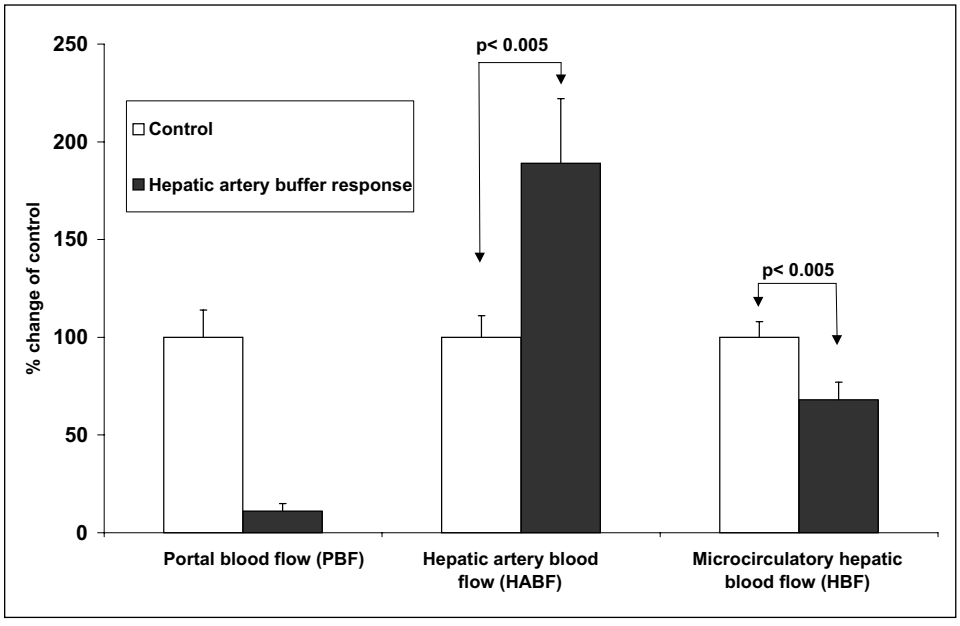

Fig. 1. Portal (PBF), hepatic artery (HABF) and hepatic microcirculatory (HBF) blood flow in the control group and during hepatic artery buffer response (HABR). Data are presented as means ± 1 S.D. 90 % reduction of portal blood flow significantly ($p < 0.005$) increased hepatic artery blood flow.

decreased by 32% ($p < 0.005$) in comparison to the control HBF values (*Fig.1*). The HABF and HBF values in this group served as the control values for the next groups with the hepatic artery buffer response (control HABR group) (*Fig.1*)

In the third group of rats (n=10), Capsaicin (Fluka) was applied as 1% solution in 5 consecutive doses up to the cumulative dose of 175 mg/kg 10 days before experiments under the ether anesthesia. This pharmacological maneuver is known to block the vanilloid-sensitive neurons and is referred to as sensory denervation. In sensory-denervated rats (capsaicin pre-treated) the reduction in the portal blood inflow to the liver by 90% (HABR) increased the hepatic artery blood flow (HABF) by only 55% ($p < 0.05$), whilst the microcirculatory hepatic blood flow (HBF) was reduced by 45% ($p < 0.05$) in comparison to the corresponding HABF and HBF values before the onset of the partial occlusion of the portal vein (*Fig.2* and *3*).

In the fourth group of rats (n=10), ruthenium red (1.5 mg/kg i.v.) was administered intravenously to block the vanilloid VR-1 receptors before the onset of the partial occlusion of the portal vein. Administration of ruthenium red did not significantly changed the resting PBF, HABF and HBF values in this group suggesting that stimulation of vanilloid-sensitive neurons does not play a role in the control of resting hepatic blood perfusion. However when the portal blood inflow to the liver was reduced by 90%, the hepatic artery blood flow (HABF) values

increased by only 52% ($p< 0.05$) (significantly lower in comparison to the control HABR group). In this group the HBF was reduced by 13% ($p< 0.05$) in comparison to the HBF value in the control HABR group (*Fig. 2* and *3*). The HABF and HBF values observed in this group upon stimulation of the HABR were not different (N.S.) in comparison to the corresponding HABF and HBF values in sensory-denervated rats. (*Fig.2* and *3*)

In the fifth group (n=10), CGRP 8-37 was used to test the hepatic artery buffer response in CGRP receptor blocked animals. CGRP 8-37 was administered intravenously at the dose of 50 μg/kg 15 min before the onset of the partial occlusion of the portal vein.

Administration of CGRP 8-37 did not significantly change the resting PBF, HABF and HBF values in this group suggesting that stimulation of CGRP receptors does not play a role in the control of resting hepatic blood perfusion.

However when the portal blood inflow to the liver was reduced by 90%, the hepatic artery blood flow (HABF) values increased by only 44% ($p< 0.05$) (significantly lower in comparison to control HABR group). In this group the HBF was reduced by 12% ($p< 0.05$) in comparison to the HBF value in control HABR group (*Fig. 2* and *3*). The HABF and HBF values observed in this group upon stimulation of the HABR were not different (N.S) in comparison to the corresponding HABF and HBF values in sensory-denervated rats. (*Fig.2* and *3*)

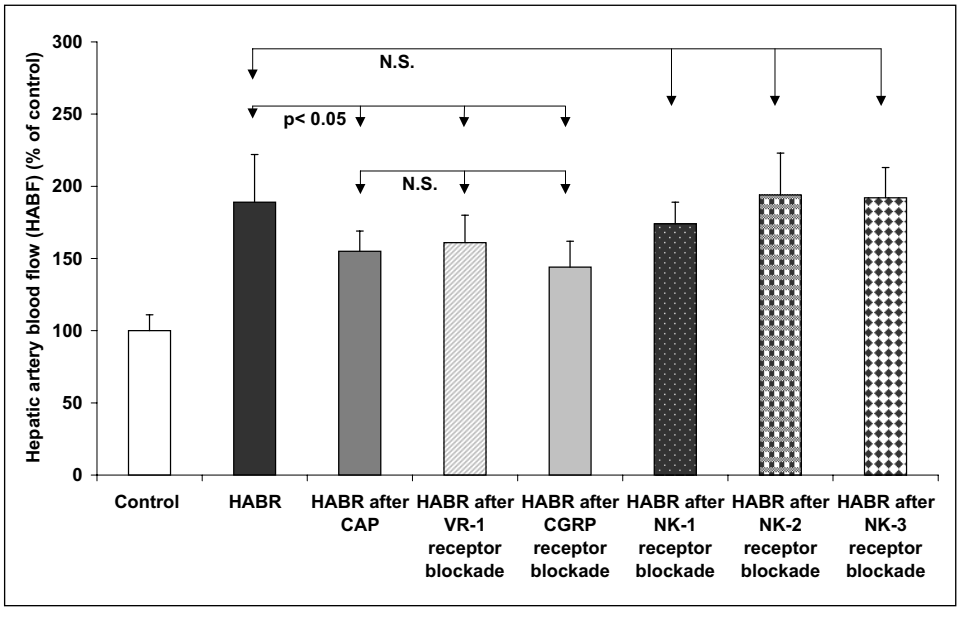

Fig. 2. Hepatic artery blood flow (HABF) in the control and hepatic artery buffer response (HABR) groups after sensory denervation by capsaicin (CAP) , vanilloid VR-1 receptor blockade, CGRP receptor blockade by CGRP 8-37, NK-1, NK-2 and NK-3 receptor blockade. Data are presented as means ± 1 S.D.

In the sixth group (n=10), CP-99,994 (NK-1 receptor antagonist) (1mg/kg i.v) was used to test the hepatic artery buffer response in NK-1receptor blocked animals. CP-99,994 was administered intravenously at the dose of 1 mg/kg before the onset of the partial occlusion of the portal vein.

Administration of NK-1 receptor antagonist itself did not significantly change the resting PBF, HABF and HBF values in this group suggesting that stimulation of NK-1 receptors does not play a role in the control of the resting hepatic blood perfusion.

However when the portal blood inflow to the liver was reduced by 90%, the hepatic artery blood flow (HABF) values increased by 74% ($p< 0.05$) (significantly lower in comparison to control HABR group). In this group the HBF was reduced by 8% ($p< 0.05$) in comparison to the HBF value in control HABR group (*Fig. 2* and *3*). The HABF and HBF values observed in this group upon stimulation of the HABR were not different (N.S.) in comparison to the corresponding HABF and HBF values in sensory-denervated rats. (*Fig. 2* and *3*).

In the seventh and eighth group (n=10 in each group) of animals the administration of either NK-2 or NK-3 receptor antagonists did not significantly change the resting hepatic blood perfusion parameters. In addition the administration of both antagonists did not significantly modify the HABF and HBF values in response to partial (90%) reduction of the portal blood inflow to the liver. (*Fig.2* and *3*)

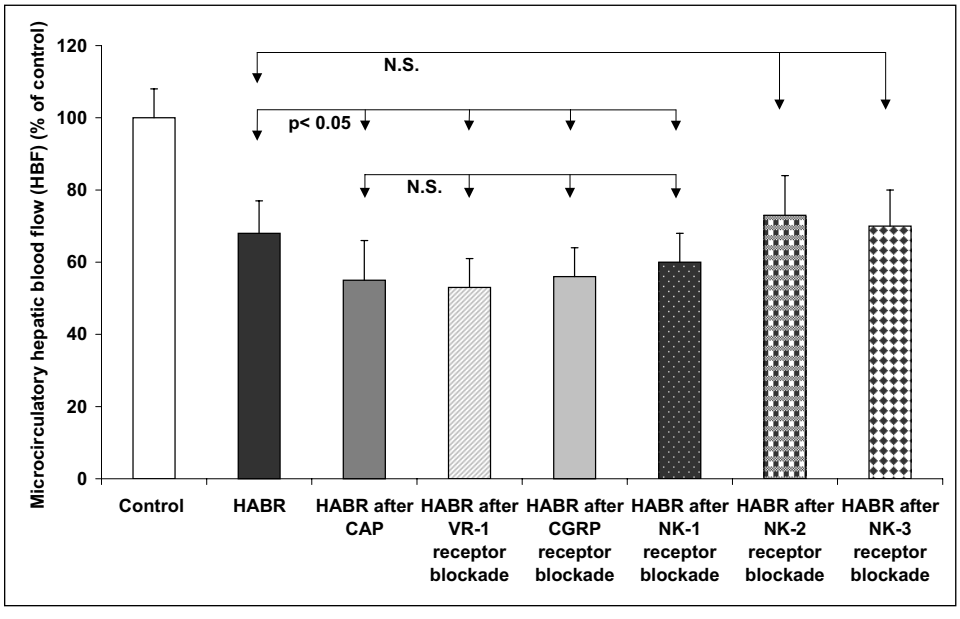

Fig. 3. Hepatic microcirculatory blood flow (HBF) in the control and hepatic artery buffer response (HABR) groups after sensory denervation by capsaicin (CAP) , vanilloid VR-1 receptor blockade, CGRP receptor blockade by CGRP 8-37, NK-1, NK-2 and NK-3 receptor blockade. Data are presented as means ± 1 S.D.

DISCUSSION

Using a model of afferent vanilloid-sensitive (capsaicin-sensitive) denervated rats we found that these nerves participate in the mediation of the hepatic artery buffer response (HABR) in the rats. In addition, using CGRP and tachykinin receptor antagonists to test the participation of CGRP and tachykinins in the HABR in the rat, we showed that CGRP is the mediator of vasodilatatory action of vanilloid-sensitive neurons with minor role of endogenous tachykinins in this vascular response.

To induce the HABR in the rats we reduced the portal blood flow by about 90% using a vascular occluder under the simultaneous control of the blood flow to stabilize the PBF at 10% of control values. This maneuver induced the HABR which was characterized by 89% increase in the HABF in comparison to its control values. These findings are consistent with those reported by Richter et al. (4). In addition we found that 90% reduction of the PBF was accompanied by only 32% ($p< 0.05$) reduction in the hepatic microcirculatory blood flow. The observed reduction in the HBF of only 32% ($P< 0.05$) in our experimental animals is a result of both the occlusion of the portal vein and hepatic artery buffer response.

Lautt (2) describing the intrinsic regulation of the hepatic blood flow demonstrated that the hepatic arterial inflow is only one of the mechanisms maintaining hepatic oxygen supply. Another mechanism is related to the oxygen extraction from the hepatic arterial and portal venous blood, which is able to maintain the oxygen delivery to the liver during reduction of the PBF. Therefore the HABR serves to protect oxygen delivery to the hepatocytes by increasing hepatic artery blood flow.

The hepatic microvasculature consists of four components: the terminal hepatic arterioles (THAs), terminal portal venules (TPVs), sinusoids and post sinusoidal efferent venules. Richter et al. (4) found that diameters of the terminal hepatic arterioles (THAs), terminal portal venules (TPVs) and sinusoids do not change during the HABR, indicating that reduction in resistance to the hepatic artery blood flow is located upstream and may function via the hepatic arteriolo-portal venular shunts, resulting in equal distribution of microvascular blood flow and oxygen delivery under conditions of the restricted portal vein blood flow.

Recently Kurbel and al. (16) proposed a two circuits' model of the liver blood circulation, joining at the acinar level. The main circuit is a low-pressure portal circulation, delivering blood from the intestinal circulation to liver acini whilst the remaining one is the arterial hepatic circulation coming from the hepatic artery. In their theory each acinus is defined as a part of the liver tissue surrounding the end of a vascular stalk that contains terminal branches of the portal vein, arteries and bile ducts. Each acinus drains to 2 or 3 central venules. A group of acini draining through the same central venule forms a hepatic lobule. According to the level of hypoxia, central acinar portions (zone 1) are well oxygenated, intermediate

portions (zone 2) are moderately well oxygenated, while the peripheral acinar portion, near the central venule, (zone 3) is poorly oxygenated.

As most of the liver cells are in the well-oxygenated zone 1, survival of this zone is probably more important for the liver in case of interrupting portal venous inflow.

In the presented model, secretion of adenosine in each acinar zone parallels the level of hypoxia. The portal blood enters acinar space in the zone 1, while the arterial branches empty at the beginning of the zone 2. At physiological conditions the liver cells in zone 1 are well oxygenated by the portal blood and they have low adenosine secretion. Since most arterial branches empty more peripherally, Kurbel *et al.* (16) suggested that arterial branches are governed by the adenosine secretion from the upstream zone 1. Low portal flow would increase adenosine secretion from the zone 1 and thus dilate numerous downstream arterial resistance vessels. Since the main point for arterial inflow is concentrated downstream from the zone 1, in case of low portal pressure, or elevated upstream resistance, some of the arterial blood might leave the acinus in retrograde direction via the portal branch. This blood would mix with the blood in the portal branch and enter some other acinus as a part of the portal blood. These arteriolo-portal communications might be important in cases of low or none portal flow when the zone 1 is in hypoxia.

These observations of Kurbel and al. (16) incorporates and confirms the results of Richter *et al.* (4) who found that the hepatic arteriolo-portal venular shunts are responsible for maintaining the proper oxygen supply to the liver during the hepatic artery buffer response

The arteriolo-portal venular shunts theory is pivotal in understanding the constant delivery of the oxygen rich blood to the hepatocytes during the HABR, however the mechanisms which are responsible for opening the arteriolo-portal venular shunts and dilating small branches of the hepatic artery have not been completely understood.

Lautt *et al.* (17) showed that the HABR is primarily mediated by local adenosine concentrations, however this observation does not exclude other vasoactive factors which could participate in the mediation of the HABR. Among those factors, neural regulation of the hepatic vasculature was the main interest of the present study.

Our previous studies on Wistar rats (15) showed that nitric oxide is only one of the mediators of the hepatic artery buffer response, however we used a non-selective nitric oxide synthase inhibitor so that we could not specify the type of NO -synthase, which could be responsible for the mediation of the HABR. We also were not able to exclude that nitric oxide is endothelium-derived mediator of other vasoactive substances released locally upon reduction of the portal blood inflow.

Recently Kurosawa *et al.* (18) in their *in vivo* study showed that sympathetic denervation had no influence on the hepatic blood flow measured using laser-Doppler flowmetry, however upon stimulation there was a significant reduction of the HBF, which was mediated *via* α-1 receptors. Neither denervation nor stimulation of the hepatic vagal nerves elicited significant changes in the HBF,

which was consistent with the previous studies (19, 20). These results suggest that vagal stimulation does not participate in the control of the hepatic blood flow.

As it was stated in the introduction, intrahepatic arteries are richly innervated by both sympathetic (9) and sensory vanilloid-sensitive (capsaicin-sensitive) neurons containing CGRP, Substance P and other tachykinins (10).

Phillips *et al.* (9) showed that small branches of the rat hepatic artery possess a dense sympathetic innervation, which upon stimulation produces vasoconstriction due to the release of noradrenalin and ATP, and abolition of sympathetic and cholinergic stimulation uncovers a vasodilation. This vasodilatation was unaffected by NO inhibition and was abolished with capsaicin and significantly reduced by CGRP 8-37 strongly suggesting that the vanilloid-sensitive neurons could be responsible for the observed effects.

Stimulation of the vanilloid-sensitive fibers has been shown to dilate the intrahepatic vessels by both releasing sensory neuropeptides and by modulating the adrenergic tone (8). However the vasodilatory response due to stimulation of sensory neurons did not result from the release of Substance P and other tachykinins since it was not affected by tachykinin receptor antagonists.

Bratveit and Helle (14) demonstrated that vasodilator responses to CGRP and sensory mediated vasodilation was independent of the endothelium, however this does not exclude the participation of NO and other endothelium derived mediators in the modulation of sensory peptides release from the vanilloid-sensitive fibers. A vanilloid receptor is a thermo sensor, activated by a moderate noxious temperature between 42 and 53 centigrades, however studies on the cloned channel confirmed the previous indication that the capsaicin receptor is stimulated by low extra cellular pH (pH 6-5) (21, 22). This lowering of the pH is one of the results of portal inflow reduction and seems to be responsible for the activation of the vanilloid-sensitive neurons during the HABR in the rat.

In our experiments, in the groups pretreated with capsaicin and vanilloid 1 receptor antagonist, the HABR was significantly reduced, suggesting that the vanilloid sensitive neurons participate in the dilation of small branches of the hepatic artery and in the opening of the arteriolo-portal venular shunts. The observed vasodilatatory effect of the vanilloid-sensitive neurons seems to be mediated by the release of CGRP - the main vasodilator of the sensory neurons, however our experiments can not exclude other than CGRP factors, which could interfere with the HABR in the rat.

CGRP seems to be the primary vasodilatatory peptide released upon stimulation of the vanilloid-sensitive neurons in the digestive tract (23, 24, 25) and thus our results in the liver confirm the protective role of these neurons.

Our additional objective was to explore the role of endogenous tachykinins in the mediation of the HABR in the rat. Recently much attention has been directed toward endogenous tachykinins as the vasodilatatory mediators of the vanilloid-sensitive neurons (26). The tachykinins: substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are a family of peptides characterized by the presence of a

common C-terminal amidated sequence in their structure. These peptides are encoded by two genes termed TAC1 (SP, NKA) and TAC3 (NKB). The terminal amidated sequence is fundamental for the interaction of tachykinins with specific receptors (termed NK1, NK2 and NK3) and for producing most of their biological effects (26).

The tachykinin family has recently been extended by the discovery of a third tachykinin gene, encoding previously unknown mammalian tachykinins (hemokinin 1, endokinin A and endokinin B) which have tachykinin NK1 receptor selectivity. This and the identification of other tachykinin-like peptides such as C14TKL-1 and virokinin (26) will need a quantative data defining the role of new-discovered tachykinins in the mediation of hepatic vascular responses.

Abundant evidence indicates that NK-1 receptors play a mediatory role in the dilation of the hepatic arteries (8) however the exact role of NK-1 mediated vasodilation will need a further explanation. Therefore we decided to use selective tachykinin receptor antagonists to explore the role of NK-1, NK-2 and NK-3 receptors in the maintenance of the hepatic microcirculatory blood flow upon reduction of portal venous inflow. Using a NK-1 tachykinin receptor antagonist we showed that endogenous tachykinins may also play a role in the mediation of the HABR in the rat, however the reduction of the hepatic artery blood flow in the rats pretreated with NK-1 receptor antagonist was not as evident as that shown in CGRP 8-37 pretreated animals.

Using either NK-2 or NK-3 receptor antagonist we found that stimulation of both receptors does not play a significant role in the modulation of the hepatic arterial vascular resistance and thus in the control of the HABR in the rat. These data are consistent with the previous observations of Phillips *et al.* (8) who found no evident role of endogenous tachykinins in the nerve-mediated vasodilation of the hepatic arteries.

Reduction of the portal blood inflow to the liver stimulates not only the vanilloid sensory neurons but has also an influence on nitric oxide release from the vascular endothelium (15,27), however the experimental data regarding other than neural factors suggest that vascular regulation of the gastrointestinal circulation is a complex mechanism and needs further intensive investigations.

We conclude that reduction of the portal blood inflow to the liver by inducing local hepatic ischemia and hypoxia stimulates the intrahepatic sensory fibers, which are partly responsible for the HABR in the rats. Among the possible neuropeptide mediators that are released from sensory endings, CGRP seems to be the most potent vasodilator during the HABR in the rats. In addition, the observed vasodilatatory response during the hepatic artery buffer response is partly mediated by tachykinins acting on NK-1 (but not NK-2 and NK-3) receptors, however the vasodilatatory response of endogenous tachykinins is minor in comparison to the effect of CGRP release.

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