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Original Paper

Action of α-Adrenoceptors in Liver Blood Flow Regulation during Treadmill Exercise in Dogs

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Abstract

The purpose of this study was to determine how the liver blood flow is maintained during running in dogs and to examine the sympathetic system that regulates hepatic circulation during dynamic exercise. Heart rate (HR), mean blood pressure (MBP), superior mesenteric arterial flow (SMAF), the external iliac arterial flow (EIAF), the hepatic arterial flow (HAF), and the portal venous flow (PVF) were measured during treadmill exercise at 8km/h with a 9% grade. HR, MBP, and EIAF increased significantly, while SMAF and PVF decreased significantly during exercise. HAF fell slightly, but not significantly. This response of HAF was reduced significantly by pretreatment with yohimbine and was eliminated by combining prazosin with the yohimbine. PVF responses were unmodified by α_1 and/or α_2 adrenoceptor blockers. These results suggest that blood flow to the splanchnic organs of the dog was decreased, while HAF was kept constant by a negative feedback mechanism mediated by the presynaptic α_2-adrenoceptor during dynamic exercise.

Introduction

The onset of dynamic exercise induces increases in mean arterial blood pressure and cardiac output, and elicits the redistribution of blood flow from inactive to active tissues by sympathoexcitation [1]. The redistribution of blood flow is characterized by an increase in the blood flow to working skeletal muscles and is accompanied by a reciprocal decrease in flow to the splanchnic organs, such as the stomach, pancreas and intestine [2, 3, 4]. The decrease in the arterial blood flow to the splanchnic organs was caused by an increase in sympathetic nerve activity during exercise [1, 5]. Several studies have reported that hepatic arterial flow, one of the splanchnic

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organs, is maintained relatively constant compared with that of other splanchnic organs during dynamic exercise [3, 4, 6, 7, 8]. However, the mechanism by which hepatic arterial blood flow is maintained has yet to be clarified.

Blood flow to the liver is supplied by the portal vein, which is a functional vessel, and the hepatic artery, which is a nutrient vessel. Functional interaction between the two vessels maintains total hepatic blood flow at a constant level [9, 10, 11]. It was previously reported that adrenoceptors participate in the reciprocal interaction between the hepatic artery and the portal vein in anesthetized dogs [12]. Pharmacological evidence has suggested that α_2-adrenoceptors, located at presynaptic sites, are involved in a negative feedback mechanism through which the neurotransmitter norepinephrine can regulate its own release [13, 14, 15, 16]. Therefore, this study was done to determine whether hepatic blood flow changes during dynamic exercise in dogs. In order to clarify the sympathetic mechanism that regulates hepatic circulation during exercise, α_1-and α_2-adrenergic blockers were systemically administered before running.

Method

Animal Preparation Eleven male and female adult beagle dogs (9-14 kg) were used in this study. The dogs were trained to run untethered on a motor driven treadmill (Okazai Co., Japan) for two to three weeks. The experimental procedures were carried out according to guidelines for the Care and Use of Animals in the Field of Physiological Sciences approved by the council of Physiological Society of Japan and the Guidelines on Animal Experiments at Yamanashi Medical University.

Surgical Procedure Sterile surgery was performed for chronic instrumentation of the animals. The dogs were anesthetized with pentobarbital sodium (25 mg/kg), intubated, and artificially ventilated with a Harvard respirator. Two types of preparations were used. In four dogs, a midline abdominal incision was made to expose the blood vessels supplying the intestine and the left hindlimb skeletal muscle. Two transient ultrasonic blood flow probes were placed around the superior mesenteric artery and the left external iliac artery, close to their origins from the aorta. In seven other dogs, the blood vessels supplying the liver were exposed with a right lateral incision. The blood flows of the portal vein (PVF) and the arterial branches to the hepatic lobus, which we designated hepatic arterial flow (HAF), were monitored with transient ultrasonic blood flow probes, simultaneously and chronically. To measure arterial blood flow to the liver alone, both the right gastric artery and gastroduodenal artery were ligated, because the common hepatic artery terminates as the small right gastric artery and the much larger gastroduodenal artery in dogs [17]. At the end of the experiment, it was confirmed that no necrosis had been induced in the gastrointestinal tract as a result of these ligatures.

All the dogs were implanted with two polyvinyl catheters (Argyle, 16G). One catheter was placed in the subclavian artery through a branch of the axillary artery. The other was placed in the subclavian vein for injecting drugs. The catheters and flow probe cables were tunneled

subcutaneously to exit through the skin on the dorsum between the scapulae. The exposed ends of the catheters were plugged, wrapped in gauze, and protected with a denim jacket fastened over the trunk of the dog. Antibiotics (penicillin G) were given 5-7 days postoperatively. The catheters were flushed daily and filled with heparinized saline. All experiments were performed after the dogs recovered from surgery and were well familiarized with standing and running on the treadmill .

Hemodynamic Measurements Mean systemic blood pressure (MBP) was measured with a pressure transducer (Nihon Kohden, AP-620G), which was connected to the catheter implanted in the subclavian artery on the day of an experiment. Superior mesenteric arterial flow (SMAF) and left external iliac arterial flow (EIAF) or HAF and PVF were measured by connecting the flow probes to a mobile ultrasonic blood flowmeter (T201, Transonic System Inc.). Heart rate (HR) was measured using a cardiotachometer (Nihon Kohden, AT-601G) triggered by the electrical pulse wave signal picked up by an arterial transient ultrasonic flowmeter. All parameters were displayed simultaneously on a physiograph (Nihon Kohden, WT-645 G) and recorded on a heat-pen writing recorder (Nihon Kohden, 06124).

Experimental Protocol Experiments were started 10-14 days after surgery. Treadmill exercise was conducted in the morning after an 18-h fast, and were not started until the baseline levels of all recorded variables were stable. Hemodynamic variables were measured before, during, and after exercise at an exercise intensity of 8km/h with a 9% grade. Each treadmill exercise period lasted 5 min, and recovery was observed for 15 min. One run was performed on each experimental day, with a one-day interval between runs. After all experiments were completed, the animal was anesthetized and the flow probe was calibrated in situ. To investigate changes in regional blood flow induced by treadmill excercise, SMAF and EIAF were measured in 4 dogs and PVF and HAF in 7 dogs.

To clarify the sympathetic regulation of hepatic circulation during treadmill exercise, Bolus injections of two subtypes of α-adrenoceptor blockers were done in 7 dogs. All drugs were injected intravenously 15min before running was begun. Experimental groups were divided as follows: 1) control group with saline injection (1ml); 2) α_2-adrenergic blocking group, pretreatment with yohimbine hydrochloride (Sigma, St. Louis: 0.2mg/kg); 3) combined α_1- and α_2-adrenoceptor blocking group, pretreatment with yohimbine (0.2mg/kg) plus prazosin (as an α_1-adrenoceptor blocker: 0.2mg/kg, Tito Pfizer, Japan), as shown in Fig. 1. Pharmacological effectiveness of blocking was confirmed by a lack of response to agonists before and after the iv injection. The agonists used in this study were clonidine (0.5μg-1.0μg/kg) for yohimbine and norepinephrine (0.05μg-0.1μg/kg) for prazosin. The antagonistic effects of all blocking agents lasted for at least 45min.

Statistical Analysis All data recordings were analyzed at 1-min intervals. The effects of each α-adrenoceptor blocker on the response of HAF and PVF during exercise were expressed as differences from the base line values at 0 min just before running. All the data are presented as means± SE. Statistical analysis of measured variables was carried out by a one-way analysis of

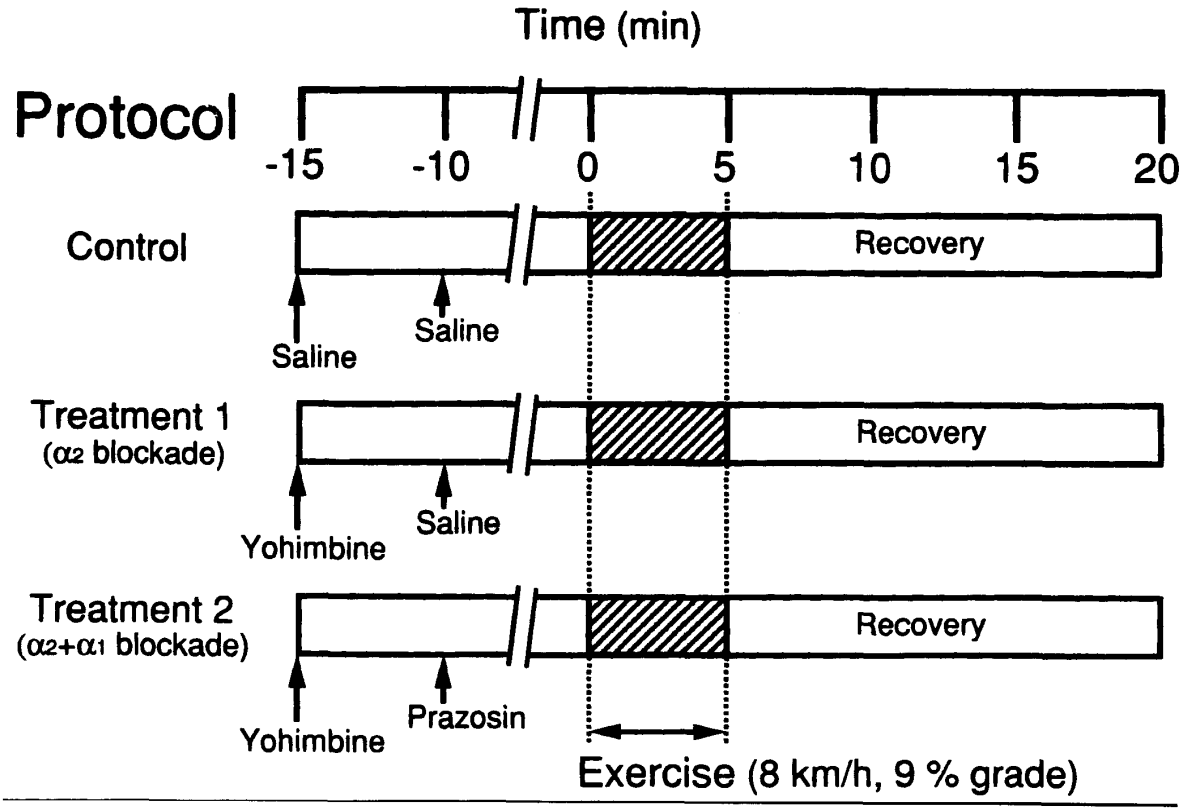

Fig. 1 Schematic representation of experimental protocol. Control, control group with saline injection; Treatment 1, α_2-adrenergic blocking group, pretreatment with yohimbine hydrochloride; Treatment 2, combined blocking group of α_1- and α_2-adrenocepters, pretreatment with yohimbine plus prazosin.

variance (ANOVA). The paired t-test was used to further delineate differences indicated by the ANOVA. $P<0.05$ was considered significant.

Results

Regional distribution of blood flow of dogs during dynamic exercise at 8km/h and 9% grade

The changes in the blood flow to the intestine, the skeletal muscle of the left hindlimb, and the liver before, during, and after treadmill exercise at 8 km/h and 9% grade, are shown in Fig. 2. The left panel shows the mean data (n=4) for HR, MBP, EIAF, and SMAF during the experimental period (Fig. 2A). The HR, MBP, and EIAF increased immediately after the onset of running. In contrast, SMAF decreased rapidly in response to the treadmill exercise. All measured variables reached steady-state values at 3 minutes after the onset of exercise and the changes in HR, MBP, EIAF, and SMAF persisted throughout the 5 minute period of the treadmill exercise. Three minutes after the start of running, HR increased from 113±4 beats/min at rest to 186±11 beats/min, MBP increased from 121±2 mmHg to 141±3 mmHg, EIAF increased from 54± 5 ml/min to 133±8 ml/min, and SMAF decreased from 271±11 ml/min to 215±11 ml/min. These changes were significant when compared with resting levels before running, and returned to resting levels 15 minutes postexercise.

The right panel shows the mean data (n=7) for HR, MBP, HAF, and PVF for the experimental

Table 1 Base line values of heart rate (HR), mean blood pressure (MBP), hepatic arterial flow (HAF), and portal venous flow (PVF) after pharmacological blockade.

	HR (beats/min)	MBP (mmHg)	HAF (ml/min)	PVF (ml/min)
Control	111±4	113±4	99±14	334±70
Yohimbine	132±8*	122±3*	95±13	352±80
Yohimbine + Prazosin	143±10*	84±4	72±12*	289±75*

Values are means ± SE; n=7 dogs. * Significant differences ($p<0.05$) from control.

period (Fig. 2B). The HR and MBP also increased immediately after the onset of running. The increases in HR and MBP induced by the exercise were not significantly different from the responses shown in Fig. 2A. PVF decreased rapidly in response to the dynamic exercise, falling from 308±47 ml/min at rest to 210±35 ml/min at 3 minutes of exercise ($p<0.05$). This change persisted throughout the treadmill exercise and returned to resting levels postexercise. In contrast, the HAF response was not statistically significant after 3 minutes of the exercise and remained relatively constant compared with SMA and PVF.

Effects of α-adrenoceptor blockers on the hepatic circulation during treadmill exercise Table 1 shows the base-line HR, MBP, HAF and PVF levels in each treated group before exercise. HR increased significantly in the yohimbine-treated and yohimbine- plus prazosin-treated groups, compared with the base-line level (111±4 beats/min) in the control group. MBP was elevated significantly in the yohimbine-treated group but fell significantly in the yohimbine- plus prazosin-treated group, compared with the base-line level (113±4 mmHg) in the control group. The base-line HAF level in the control group was 99±14 ml/min, which did not differ significantly from the yohimbine-treated group. However, HAF decreased significantly in the yohimbine plus prazosin-treated group. PVF fell significantly in the yohimbine- plus prazosin-treated group but there were no significant differences between the yohimbine-treated group and the control group.

The differences in the response of HAF and PVF due to the injection of yohimbine or combined yohimbine plus prazosin became apparent soon after the onset of running (Fig. 3). Pretreatment with yohimbine caused a decrease in HAF to 34±3 ml/min after 3 minutes of running period, and the decrease, observed throughout the 5 minute running, was significantly different from the control group (Fig. 3A). The decrease in HAF due to yohimbine was canceled by combining prazosin with the yohimbine. After the exercise, HAF in the combined injection group did not recover to prerunning levels for 5 minutes. The decreases in PVF induced by the dynamic exercise were not significantly different under the three experimental conditions (Fig. 3B).

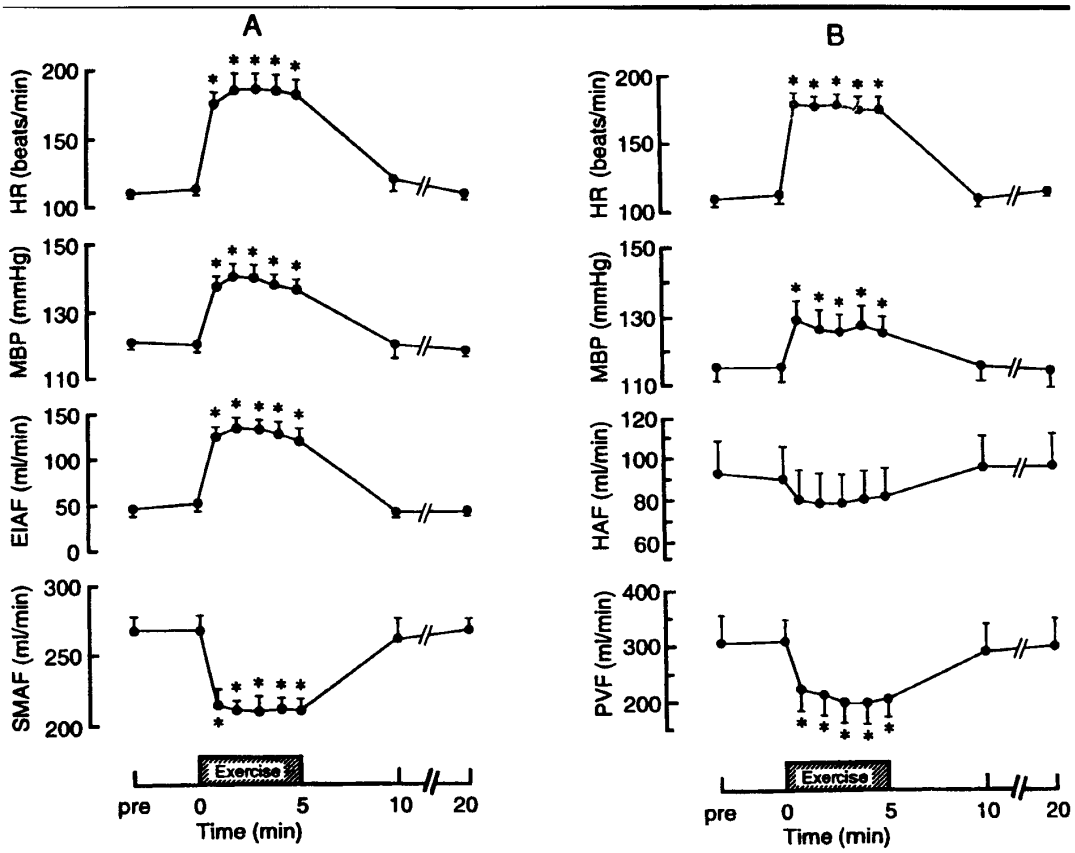

Fig. 2 Effects of treadmill exercise on hemodynamic valuables in beagle dog. A: Responses to HR, MBP, EIAF, and SMAF before, during, after treadmill exercise at 8km/h and 9% grade.(n=4) B: Responses to HR, MBP, HAF, and PVF during the experimental period. (n=7) * Significant differences ($p<0.05$) from the value of 0 min just before running. Means ± SE are given.

Discussion

The present study showed that hepatic arterial blood flow was kept relatively constant by the negative feedback mechanism mediated by presynaptic α_2-adrenoceptors during dynamic exercise. During dynamic running at a rate of 8 km/hour with a 9% grade, heart rate, mean blood pressure and external iliac arterial blood flow increased significantly, and superior mesenteric arterial and portal venous blood flow decreased significantly. Hepatic arterial blood flow was maintained relatively constant compared to the decreased blood flow to the other splanchnic organs. In contrast, hepatic arterial blood flow decreased significantly in response to an α_2-adrenoceptor antagonist at a similar level of exercise.

Dynamic exercise induces a redistribution of blood flow from inactive to active tissues. In previous reports, the data concerning regional blood flow responses to dynamic exercise have been inconsistent. Several studies have shown that blood flow to the splanchnic organs of the dog decreases during exercise [2, 6, 7, 18, 19]. In contrast, a number of studies have shown that blood flow to these areas remains unchanged [20, 21, 22]. Musch et al. [4] suggested the possibility that the apparent inconsistencies between studies may have been due in part to

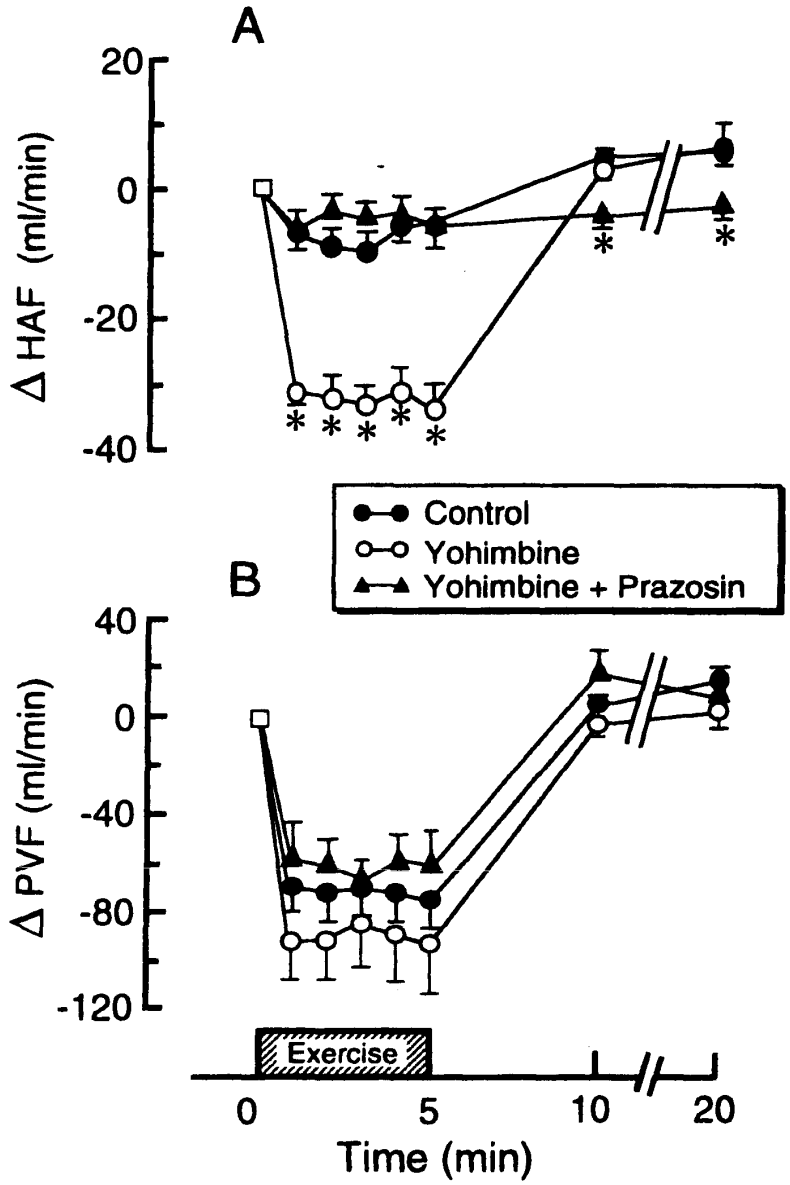

Fig. 3 Changes in HAF (△ HAF: A) and PVF (△ PVF: B) from the base line values of 0 min just before (□) treadmill exercise at 8km/h and 9% grade. Each was studied after saline administration (1ml) as control (●), after yohimbine (0.2 mg/k) as α_2-adrenocepter blocker (○), after yohimbine (0.2 mg/k) plus prazosin (0.2 mg/k) as α_1-adrenocepter blocker (▲). * Significant differences ($p<0.05$) from control. Means ± SE (n=7) are given.

different exercise protocols and/or the different methods used to measure blood flow. They reported that blood flow to the intestine, stomach, and pancreas decreased remarkably but that hepatic arterial blood flow was maintained during dynamic exercise at 30% to 50% of maximal O_2 consumption [4]. Others also reported that hepatic arterial blood flow was maintained relatively constant, but blood flow to splanchnic organs like the intestine, stomach, and pancreas was decreased significantly during moderate exercise [3, 4]. Sanders et al. reported that the blood flow response to each splanchnic organ was not necessarily the same, and the differences in function of the organs during exercise may account for the different responses [19]. In the

present study, superior mesenteric arterial blood flow and portal venous blood flow decreased significantly, whereas hepatic arterial blood flow did not change significantly during dynamic exercise at 8 km/hour with a 9% grade. Portal venous blood flow derives from the spleen, stomach, pancreas, and intestine. The exercise in this study produced an increase in heart rate from 113±4 beats/min to 186±11 beats/min. This increase in heart rate during exercise represents a mild to moderate level of exercise for dogs. Thus, our experimental results agree with the previous observation that hepatic arterial blood flow is maintained constant compared to other splanchnic organs during moderate exercise [3, 4]. These results support the hypothesis that the distribution of blood flow to the splanchnic organs of dogs during dynamic exercise is heterogeneous and dependent on the dynamic exercise. However, the mechanism of the different responses in each organ has yet to be clearly defined.

Increased sympathetic vasoconstriction is responsible for the reduction in blood flow to the splanchnic organs during dynamic exercise [1, 5]. O'hagan et al. showed direct evidence that sympathetic nerve activity increases abruptly at the onset of treadmill exercise and that the increase in renal sympathetic nerve activity continues during the dynamic exercise [22]. Judging from the increase in heart rate of 73±7 beats/min from the baseline value, the exercise intensity of 8 km/hour with a 9% grade also caused an increase in sympathetic nerve activity during the treadmill exercise. Hepatic blood vessels receive a rich nerve supply from the anterior hepatic plexus along the common hepatic artery [25]. Hepatic arterial constriction induced by nerve stimulation can be reversed to a mild dilation by α-adrenoceptor blockers in the anesthetized cat or dog [23, 26]. This shows that α-adrenoceptors play a part in the vasoconstriction of the hepatic artery. There is pharmacological evidence that α-adrenoceptors are involved in a negative feedback mechanism through which the neurotransmitter norepinephrine can presynaptically regulate its own release [14, 15, 16]. A significant finding in this study was the decrease of hepatic arterial blood flow during exercise caused by an α_2-adrenoceptor antagonist. In addition, this decrease of hepatic arterial blood flow caused by the α_2-adrenoceptor antagonist disappeared when the α_2-adrenoceptor antagonist was administrated in combination with an α_1-adrenoceptor antagonist. These results suggest that presynaptic α_2-adrenoceptors play a role in inhibiting vasoconstriction in the hepatic vascular bed when sympathoexcitation is elicited by dynamic exercise. On the other hand, it was also shown that the portal venous blood flow, which derives from the spleen, stomach, pancreas, and intestine, decreased significantly during dynamic exercise. This decrease in portal venous blood flow was unmodified by an α_2-adrenoceptor antagonist or an α_2- plus α_1-adrenoceptor antagonist. This result suggests that α-adrenoceptors do not play a large role in the reduction of the portal venous blood flow during dynamic exercise.

In summary, this study showed that blood flow to the splanchnic organs of the dog decreased during dynamic exercise, while hepatic arterial blood flow was kept constant by the negative feedback mechanism mediated by presynaptic α_2-adrenoceptors.

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