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Detection of acetazolamide-induced increase in organ blood flow in rabbits by laser flowmetry

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Abstract

Purpose. We investigated the mechanism by which inhibition of carbonic anhydrase (CA) increases organ blood flow.

Methods. Regional blood flow (rBF) in white rabbits anesthetized with ketamine/urethane was measured in the kidney, liver, stomach wall, and abdominal muscle by means of laser blood flow probes. Data obtained from rabbits receiving acetazolamide (AZ) to inhibit CA were compared with those obtained from rabbits ventilated with air containing increased concentrations of CO_2.

Results. Systolic blood pressure, body temperature, hemoglobin, and base excess were unaffected by either treatment. Inhalation of CO_2 increased blood flow in all organs tested as well as the cardiac output and PCO_2 but decreased pH. Inhibition of CA by AZ administration increased the rBF only in the liver and kidney and did not increase cardiac output or decrease pH.

Conclusion. Administration of AZ increased rBF in the tissues and organs that contained large amounts of CA without increasing the cardiac output or decreasing the pH, which suggests a direct local effect. A differential sensitivity to the retention of CO_2 is suggested as a possible mechanism of the selectivity of the increase in rBF.

Key words: Organ blood flow, Carbonic anhydrase, Carbonic anhydrase inhibitor, Acetazolamide, Laser flowmeter

Introduction

The enzyme carbonic anhydrase (CA) is present in red blood cells, lung, brain, liver, kidney, and other tissues and is essential to the balance between CO_2 and HCO_3^-. When CA activity is decreased by such inhibitors as acetazolamide (AZ), the conversion velocity to CO_2 from HCO_3^- is slowed, resulting in an imbalance between CO_2 and HCO_3^- in the pulmonary venous system. This imbalance is reduced as blood flows through the pulmonary veins to the systemic arteries. The result is a higher PCO_2 in the arterial blood ($PaCO_2$) than in the pulmonary capillaries, which are in equilibrium with the alveoli ($PaCO_2 \neq PETCO_2$). This difference between $PaCO_2$ and $PETCO_2$, [(a-ET)PCO_2], changes the ventilation/perfusion (V/Q) relation [1–3], stimulating respiration. The hypercapnia that accompanies the inhibition of CA produces a similar difference in PCO_2 between tissue and blood [4].

Retention of CO_2 creates a mild acidosis that alters cell membrane function [5–8]. Its direct local circulatory effects include decreases in cardiac contractility and systemic vasodilation. The central autonomic effects of an increase in PCO_2 such as vasoconstriction and an increase in heart rate are attributed to activation of the sympathetic nervous system. Blood flow in the brain, kidney, and liver is increased during hypercapnia [9–11]. Blood flow also appears to be increased in the organs and tissues during CA inhibition. Our objective was to evaluate the changes in regional blood flow (rBF) in organs of the rabbit following inhibition of CA induced by administration of AZ compared with the changes induced by inhalation of CO_2.

Materials and methods

Preparation of animals

A total of 25 white rabbits (2.3–2.5 kg) were used. All rabbits were anesthetized with ketamine hydrochloride (30 mg·kg^{-1} IM) and urethane (100 mg·kg^{-1} IM). Thirteen rabbits underwent tracheotomy and were paralyzed by injection of pancuronium bromide (0.4 mg·kg^{-1}). Ventilation was maintained at a constant rate (25 times·min^{-1}, 16 ml·kg^{-1}) with a respirator (Respira-

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tor SN-480-5; Shinai, Tokyo, Japan). The composition of the inspired air was 5% or 10% CO_2 in air during the measurement of changes in rBF. The remaining 12 rabbits were allowed to breathe spontaneously during the entire experiment.

The femoral artery was cannulated and connected to a pressure transducer (AP-601G, NEC, Tokyo, Japan; and Recti-Horiz-8K, Sanei, Tokyo, Japan) to record the systolic blood pressure (SBP) and to collect arterial blood for blood gas analysis (ABL-3; Radiometer, Copenhagen, Denmark). An ear vein was cannulated for infusion of fluids and injection of drug. After laparotomy and sternotomy, laser Doppler blood-flow probes (ALF21; Advance, Tokyo, Japan) [12–14] were placed on the surface of the liver, kidney, stomach, and abdominal muscle; and the probe of an electromagnetic blood flowmeter (MFV-2100; Nihon Koden, Tokyo, Japan) was placed on the ascending aorta to measure cardiac output. The abdomen was closed with clips. The rectal temperature (RT) was maintained at 38.0°C by use of a heating pad. The entire experiment lasted 45 min. The test animals were anesthetized and killed with an intravenous overdose of KCl.

Measurement of rBF

The laser Doppler flowmeter permits measurement of relative changes in rBF but does not provide absolute blood flow values. Arbitrary units of power output (millivolts) were chosen to express relative rBF values. We determined rBF, SBP, cardiac output (CO), RT, and base excess (BE) at baseline after the SBP and rBF had stabilized. AZ was injected (4 mg·kg^{-1}) at 10-min intervals to achieve a total dose of 12 mg·kg^{-1}. Values were recorded after each injection. In rabbits ventilated with CO_2, measurements were recorded 10 min after the CO_2 was introduced into the inspired air.

Statistical analysis

Data were expressed as the mean ± SE of values obtained from n rabbits. The significance of differences from baseline was analyzed by ANOVA and post hoc Dunnett's test, as appropriate. A level of $P < 0.05$ was considered statistically significant.

Results

No significant changes in SBP, RT, hemoglobin (Hb), PaO_2, or BE were observed during inhalation of CO_2 (Table 1). The pH decreased and the $PaCO_2$ and CO increased significantly after inhalation of 5% or 10% CO_2. The rBF in all of the organs monitored increased during inhalation of CO_2 (Figs. 1, 2). The pancuronium bromide caused no change in the variables measured.

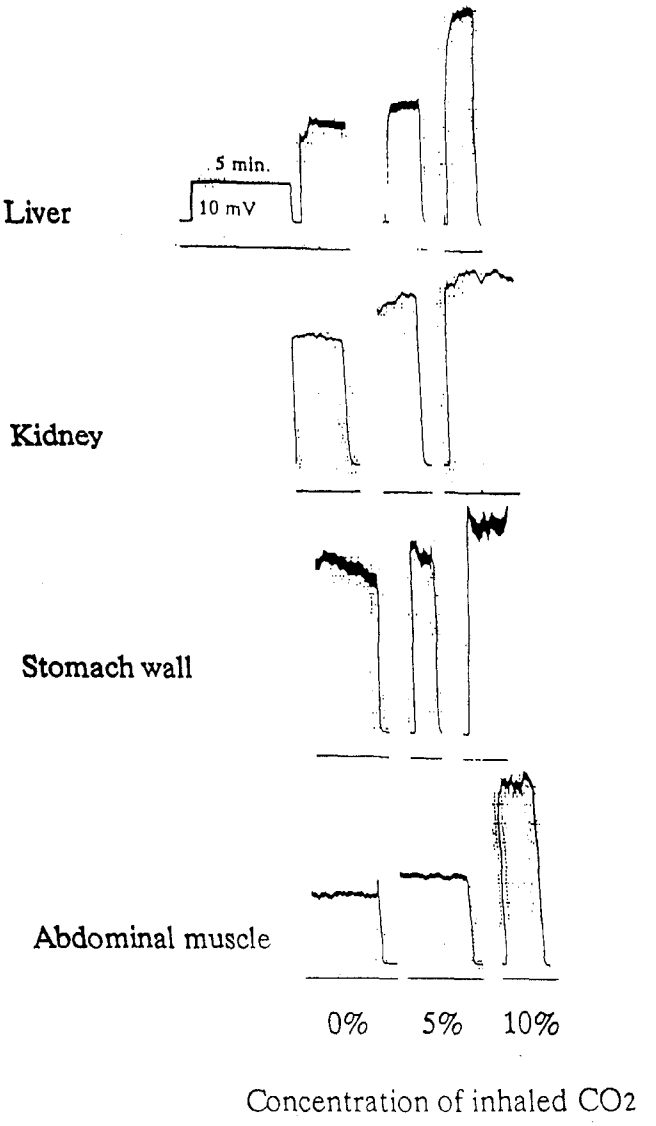

Fig. 1. Change in (regional blood flow) (rBF) in liver, kidney, stomach wall, and abdominal muscle following inhalation of CO_2

No significant changes in SBP, CO, RT, Hb, pH, or BE were observed following administration of AZ (Table 2). The PaO_2 increased significantly. A slight but significant decrease in $PaCO_2$ was observed at the highest cumulative dose of AZ (12 mg·kg^{-1}). The rBF in liver and kidney was increased after the repeated administration of AZ (Figs. 3, 4), although the rBF in the stomach wall and abdominal muscle was unchanged.

Discussion

Hypercarbia has been shown to increase blood flow in tissues and organs [10,11,15,16]. In addition to stimula-

Table 1. Various parameters before and after CO_2 loading

Parameter (FiO_2 0.5)	Control	Inhalation of CO_2 5%	Inhalation of CO_2 10%
SBP (mmHg)	99 ± 4	100 ± 4	97 ± 3
CO (ml/min)	162 ± 10	193 ± 8*	215 ± 3*
RT (°C)	38.0 ± 0.2	37.9 ± 0.2	37.8 ± 0.2
Hb (mg/dl)	13.1 ± 0.3	13.7 ± 0.3	13.5 ± 0.5
$PaCO_2$ (mmHg)	37.8 ± 1.3	54.3 ± 1.8*	72.7 ± 2.0*
PaO_2 (mmHg)	300.1 ± 18.9	356.3 ± 16.0	373.3 ± 14.5
pH	7.401 ± 0.027	7.219 ± 0.026*	7.086 ± 0.028*
BE (mEq/L)	−1.2 ± 0.7	−2.5 ± 0.8	−2.8 ± 0.7

SBP, systolic blood pressure; CO, cardiac output; RT, rectal temperature; Hb, hemoglobin in blood; $PaCO_2$, partial CO_2 pressure in arterial blood; PaO_2, partial O_2 pressure in arterial blood; pH and BE, pH and base excess in arterial blood.
Values are means ± SE; $n = 13$.
* $P < 0.05$, compared with control value.

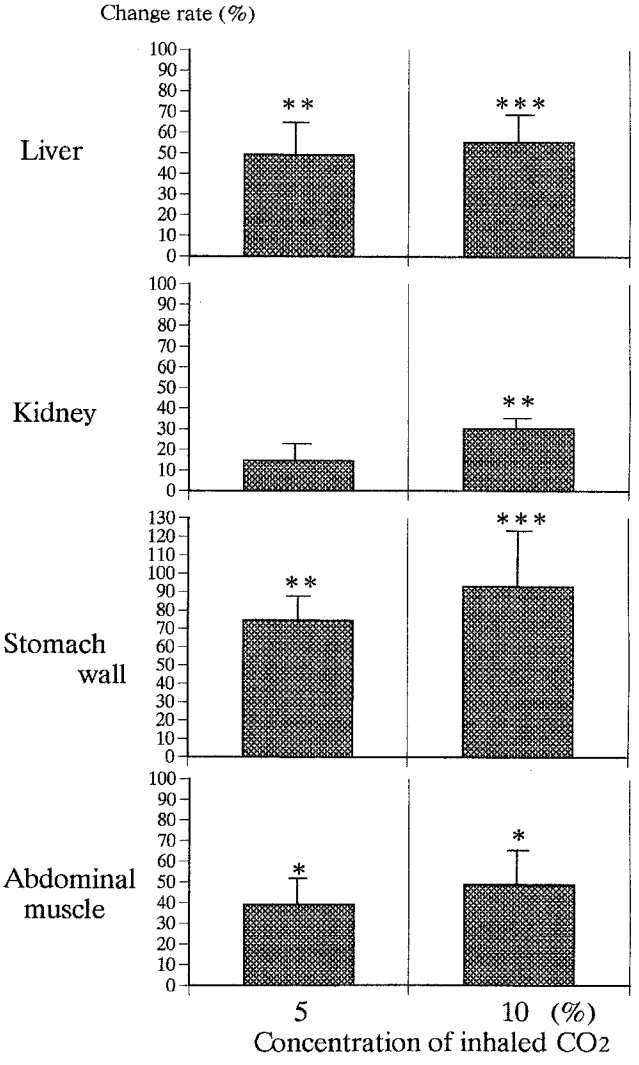

Fig. 2. Percent change in rBF in liver, kidney, stomach wall, and abdominal muscle following inhalation of CO_2 (mean ± SE, $n = 13$). *$P < 0.05$, compared with control value; **$P < 0.01$, compared with control value; ***$P < 0.001$, compared with control value

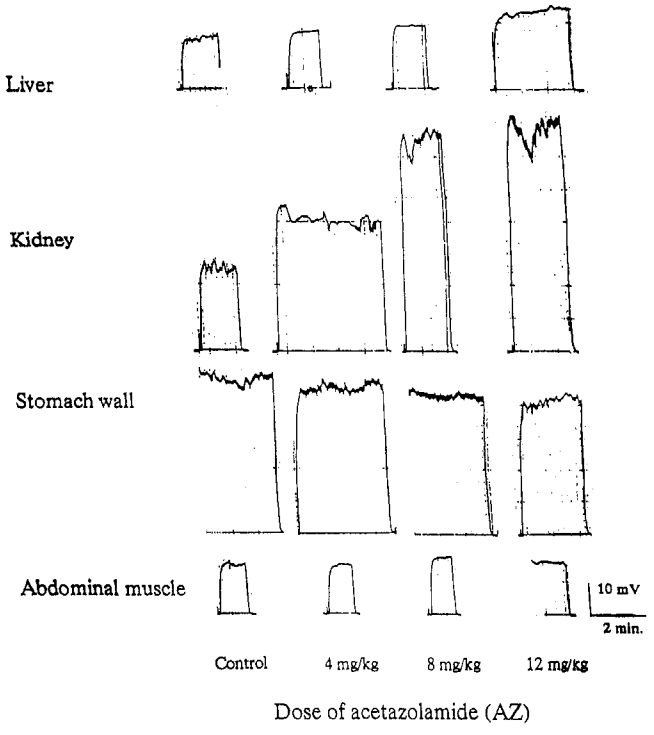

Fig. 3. Changes in rBF in liver, kidney, stomach wall, and abdominal muscle following acetazolamide administration

tion of the sympathetic nervous system and the direct vasodilator effect of CO_2, the increase in blood flow to the organs produced by CO_2 inhalation has been attributed to the distribution of α-receptors on the vessels [10,11]. Vessels with few α-receptors can remain dilated in the presence of increased levels of catecholamines.

In the present study CO_2 inhalation decreased the pH and increased the $PaCO_2$ and cardiac output. The increase in rBF in the liver, kidney, stomach wall, and abdominal musle caused by CO_2 inhalation could be

Table 2. Various parameters before and after administration of acetazolamide

Parameter (room air)	Control	Results, by dose of acetazolamide		
		4mg/kg	8mg/kg	12mg/kg
SBP (mmHg)	96 ± 3	96 ± 2	96 ± 2	99 ± 3
CO (ml/min)	158 ± 6	161 ± 6	163 ± 3	166 ± 12
RT (°C)	38.2 ± 0.1	38.3 ± 0.2	38.3 ± 0.2	38.2 ± 0.2
Hb (mg/dl)	13.7 ± 0.3	13.2 ± 0.3	12.9 ± 0.3	15.3 ± 0.6
$PaCO_2$ (mmHg)	35.5 ± 1.7	35.3 ± 1.1	35.0 ± 1.0	34.0 ± 1.2*
PaO_2 (mmHg)	91.4 ± 2.7	100.8 ± 4.0	110.2 ± 5.9	110.7 ± 5.2*
pH	7.434 ± 0.028	7.429 ± 0.030	7.423 ± 0.029	7.432 ± 0.022
BE (mEq/L)	−0.9 ± 0.5	−1.3 ± 0.4	−1.8 ± 0.3	−2.0 ± 0.5

SBP, systolic blood pressure; CO, cardiac output; RT, rectal temperature; Hb, hemoglobin in blood; $PaCO_2$, partial CO_2 pressure in arterial blood; PaO_2, partial O_2 pressure in arterial blood; pH and BE, pH and base excess in arterial blood.
Values are means ± SE; $n = 12$.
* $P < 0.05$, compared with control value.

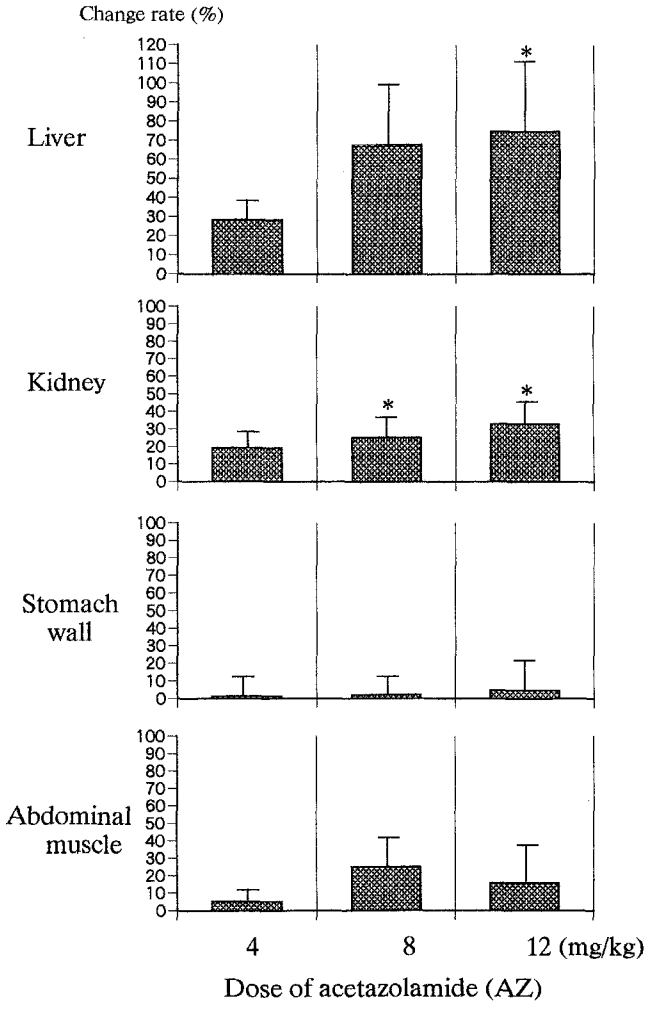

Fig. 4. Percent change in rBF in liver, kidney, stomach wall, and abdominal muscle following acetazolamide administration (mean ± SE, $n = 12$). *$P < 0.05$, compared with control value

attributed to the increase in cardiac output secondary to the decrease in pH. Okazaki et al. [9] conducted a similar study in dogs involving CO_2 inhalation and reported increased flow in the hepatic artery and portal vein, decreased flow in the femoral artery, and no change in cardiac output or blood flow in the kidney. The apparent difference in organ blood flow in the present study in rabbits could be attributed to a difference in the effect on cardiac output in this species.

The rBF was increased in the liver and kidney in response to the inhibition of CA without a corresponding decrease in pH or an increase in $PaCO_2$ or cardiac output. An increase in PO_2 and a slight decrease in PCO_2 were observed at the highest cumulative dose of AZ. Consistent with data from the present study, previous studies have reported an apparent increase in blood flow in the brain and kidney induced by AZ [17–21]. The organ selectivity and the absence of meaningful systemic effects in the present study argue in favor of a local effect. Because the organs affected correspond to the distribution of CA activity, it is reasonable to hypothesize that the AZ-induced increases in rBF are due to the local accumulation of CO_2 by organs with high levels of CA activity.

The inhibition of CA by AZ is reported to cause CO_2 retention in organs and tissues, whereas $PaCO_2$ remains within the normal range [4]. CO_2 retention appears to be a key factor that affects blood flow during the inhalation of CO_2 gas. Inhibition of CA can also be considered to result in retention of CO_2, which can stimulate the respiratory center to induce an increase in PaO_2 and a slight decrease in $PaCO_2$. Differences in rBF among organs may result from factors that affect the differential sensitivity of the vasculature to the accumulation of CO_2, reflected by the activity of CA in each organ. CA may be involved in the control of vasodilation in

response to the mild local acidosis induced by CO_2 retention. Inhibition of CA may result in a "steal phenomenon" in blood vessels. This phenomenon has been reported in studies of normal and pathological arteries following administration of AZ to patients with moyamoya disease, which results in progressive obliteration of the intracranial carotid arteries and the development of an extensive vascular network with dilatation of many small branches beyond the stenosis [22].

Other mechanisms that have been proposed to explain the increase in blood flow in organs following administration of AZ include an increase in cardiac output produced by an increase in intravascular volume and the action of the heart or by the induction of vasodilation (decreased vascular resistance) produced by prostaglandin I_2 (PGI_2), CO_2, and nitrous oxide (NO) [23–25]. Although cardiac output was unchanged following administration of AZ in the present study, the influence of such factors as catecholamines, PGI_2, CO_2, and NO may have been related to vasodilation and the observed increase in blood flow in the liver and kidney.

Results of this study indicate that following administration of AZ blood flow was increased in tissues and organs that contain large amounts of CA. Differential retention of CO_2 is implicated as a possible mechanism to explain the selectivity of the effect. The administration of AZ may thus be useful for maintaining the blood flow and oxygen supply to the liver and kidney in patients with serious complications of hypoxia, such as the postshock condition.

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