Preserved hypocapnic pial arteriolar constriction during hyperammonemia by glutamine synthetase inhibition

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Hirata, Takahiko, Tetsu Kawaguchi, Saul W. Brusilow, Richard J. Traystman, and Raymond C. Koehler. Preserved hypocapnic pial arteriolar constriction during hyperammonemia by glutamine synthetase inhibition. *Am. J. Physiol.* 276 (*Heart Circ. Physiol.* 45): H456–H463, 1999.—Ammonia intoxication, which results in astrocytic edema and glutamine accumulation, blocks cerebral vasodilation during hypercapnia but not during hypoxia. Ammonia's effect on blood flow during hypocapnia is unclear, with some brain regions showing a paradoxical increase in flow. Here, we studied the responses to hypocapnia of pial arterioles not surrounded by astrocytic end feet to avoid mechanical compression by local edema. Blood flow was measured by microspheres in pentobarbital sodium-anesthetized rats equipped with closed cranial windows that permitted intravital microscopy. The normal pial arterial constriction in hypocapnia ($12 \pm 1\%$; mean $\pm$ SE) was blocked ($2 \pm 1\%$) during a 6-h intravenous infusion of ammonium acetate, with some regions (cerebrum, midbrain) showing increased flow during hypocapnia. After pretreatment with methionine sulfoximine (MSO), which inhibits glutamine synthesis, the normal hypocapnic constrictor response was retained in pial arterioles ($11 \pm 2\%$) during hyperammonemia. The increase in the calculated cerebrovascular resistance also was retained. An analog of MSO that does not block glutamine synthesis (buthionine sulfoximine) was ineffective in maintaining hypocapnic reactivity. In a sodium acetate-treated control group, MSO did not alter the pial arteriolar response. Normal vasoconstrictive ability was shown during ammonium infusion in response to U-46619, a thromboxane analog. We conclude that the inhibition of hypocapnic responsivity induced by ammonium is not due to paralysis of the pial arteriolar smooth muscle or to vascular compression by swollen astrocytes but is in some way due to glutamine metabolically produced from the ammonium.

ammonia; carbon dioxide; cerebral circulation; glia; rat

PATIENTS WITH FULMINANT hepatic failure hyperventilate, and their cerebral blood flow (CBF) response to hypercapnia may be impaired (10). Acute increases in plasma ammonia concentration in experimental animals blunt the increase in CBF to hypercapnia in a variety of species (2, 6, 21, 36). Hyperammonemia also causes swelling of astrocytes, including their foot processes surrounding cerebral blood vessels (9, 26, 29, 37). The attenuated CBF response to hypercapnia does not appear to be related simply to a mechanical effect of vascular compression by astrocytes because *1*) the

increase in CBF to hypoxia is intact during acute hyperammonemia (17) and *2*) extraparenchymal pial arterioles not subjected to intraparenchymal astrocytic compression no longer dilate during hypercapnia in hyperammonemic animals (18). The attenuated CBF response during hyperammonemia does not appear to be attributed solely to a direct effect of ammonium ions on vascular smooth muscle because inhibition of glutamine synthetase in the presence of high ammonia levels preserves the increase in CBF and in pial arteriolar diameter during hypercapnia (18, 36).

In the brain, glutamine is synthesized from ammonia and glutamate by glutamine synthetase enriched in glia (7, 30). The brain concentration of glutamine increases in patients with various forms of liver disease and hyperammonemic encephalopathy (24, 32). In experimental hyperammonemia, the use of methionine sulfoximine (MSO) to inhibit glutamine synthetase reduces the watery swelling of astrocytes (39), the increase in tissue water content (18, 35), intracranial hypertension (3, 18, 36), and the increase in extracellular potassium activity associated with astrocyte dysfunction (33). Thus the impaired vascular hypercapnic reactivity may be related to effects of glutamine on cerebral vessels or to other effects of astrocyte dysfunction associated with glutamine accumulation.

Cerebrovascular reactivity to vasoconstrictor stimuli has not been well studied under hyperammonemic conditions. We have previously observed that hypocapnia produces paradoxical increases in CBF in some brain regions during acute hyperammonemia (2) and that this increase is prevented by pretreatment with MSO (36). This paradoxical response did not appear to be related to the increase in tissue water content or intracranial pressure because the decrease in CBF during hypocapnia remained intact in rats with generalized cell swelling induced by hypoosmotic fluid infusion (36). However, it is possible that the selective astrocyte swelling associated with hyperammonemia is ameliorated by hypocapnia, thereby passively permitting an increase in CBF. In this case, the paradoxical increase in CBF would not be attributed to active dilation of arteriolar resistance vessels. Thus it is unclear how much of the abnormal CBF response to hypocapnia is attributed to disturbances in vascular regulation versus effects of astrocyte swelling.

In the present study, we evaluated the response to hypocapnia of extraparenchymal pial arterioles not surrounded by swollen astrocyte processes to avoid the mechanical compression by local edema during hyperammonemia. Pial arterioles are thought to comprise ~30% or more of total cerebrovascular resistance (CVR;

see Ref. 11). We measured CBF and calculated CVR to determine if there is concordance between the total CVR response and the pial arteriolar segmental response to hypocapnia. Discordance between the total CVR and extraparenchymal segmental responses can provide insight into disturbances of intraparenchymal vascular resistance. We tested the hypotheses *1*) that acute hyperammonemia blocks both the decrease in pial arteriolar diameter and the increase in CVR during hypocapnia and *2*) that inhibition of glutamine synthetase with MSO preserves hypocapnic vasoconstriction in pial arterioles. Because MSO can potentially inhibit γ-glutamylcysteine synthetase (12), we also evaluated the vascular effect of buthionine sulfoximine (BSO), a selective inhibitor of γ-glutamylcysteine synthetase (13). Finally, to test whether impaired hypocapnic vasoconstriction during hyperammonemia was selective or represented a generalized loss of vasoconstrictor capacity, the constrictor response to the thromboxane analog U-46619 (9,11-dideoxy-11α9α epoxymethano-prostaglandin F_2) was assessed in control and hyperammonemic animals.

METHODS

All procedures were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine. Male Wistar rats (350–450 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg) and were maintained with subsequent injections (20 mg/kg) every 90 min. A tracheotomy was performed for mechanical ventilation with 25–30% O_2. Catheters were placed in two femoral veins, one femoral artery, and the tail artery. The left ventricle was catheterized through the right subclavian artery. Rectal temperature was maintained at ~37°C with a warm water-circulating blanket.

A cranial window was constructed over the parietal cortex (18). Dura was exposed through a 3×4 mm craniotomy inside a 7-mm-diameter plastic ring cemented to the skull. Under a well of artificial cerebrospinal fluid, the dura was retracted, and the ring was sealed with a glass cover-up secured with acrylic. Side ports in the ring were used to monitor pressure and to infuse U-46619. Pial vessels were observed through a microscope video recording system (18). Internal diameter was measured on two to four arterioles per rat. Baseline diameter ranged from 20 to 80 μm. For each intervention, the percent change in diameter was calculated for each arteriole segment. These percent changes were then averaged for each rat, and this average value was used in the statistical analysis (n = number of rats).

Blood flow was measured by injection of ~200,000 microspheres (15 μm diameter) labeled with either [153]Gd, [113]Sn, or [46]Sc (New England Nuclear Life Science Products, Boston, MA). The microspheres were injected in the left ventricle, and a reference sample was withdrawn at a rate of 0.68 ml/min from the femoral artery (36). Heparinized blood from a donor rat was infused in a femoral vein to replace blood simultaneously during the arterial withdrawal. Arterial blood samples and tissue from cerebrum, diencephalon, midbrain, combined medulla and pons, and cerebellum were counted for radioactivity in a gamma scintillation counter, and blood flow was calculated as described previously (36). Cerebral perfusion pressure was calculated as the difference between mean

arterial pressure and pressure in the cranial window, and CVR was calculated as perfusion pressure divided by CBF.

Arterial blood pressure and pressure in the cranial window were monitored continuously. Arterial pH and partial pressure of O_2 (P_{O_2}) and CO_2 (P_{CO_2}) were measured with a Radiometer ABL3 electrode system. Hemoglobin concentration was measured with a Radiometer Hemoximeter OSM3. Plasma osmolarity was measured by freezing-point depression, and plasma ammonia was measured by a cation exchange-visible spectrophotometric technique (4). Samples of freshly dissected cortical gray matter obtained after KCl-induced cardiac arrest were weighed before and after drying to obtain tissue water content.

Rats received an intravenous infusion of either sodium acetate or ammonium acetate at a rate of ~50 μmol·kg^{-1}·min^{-1} (0.1 ml/min). Because sodium acetate causes metabolic alkalosis, hydrochloric acid was added to the infusate 30 min after the start of the infusion to control arterial pH. For each of the two salt infusions, rats were pretreated with either the saline vehicle (3 ml/kg), MSO (150 mg/kg; 0.83 mmol/kg), or BSO (880 mg/kg; 4 mmol/kg). Pretreatments were given as a 1-h continuous intravenous infusion starting ~3 h before baseline measurements were made. This dose of MSO is the same as that previously used to inhibit glutamine accumulation in rats (8, 35). This dose of BSO has been shown by others to decrease brain glutathione concentration within 24 h (22).

In the first experiment, the following five groups were studied: *1*) vehicle pretreatment plus sodium acetate infusion (n = 5); *2*) MSO pretreatment plus sodium acetate (n = 5); *3*) vehicle pretreatment plus ammonium acetate infusion (n = 8); *4*) MSO pretreatment plus ammonium acetate (n = 8); and *5*) BSO pretreatment plus ammonium acetate (n = 5). Hypocapnia was produced for 10 min by increasing minute ventilation for 10 min before infusing sodium or ammonium acetate and again at 6 h of salt infusion. Pial arteriolar diameter changes were measured during each hypocapnic challenge. Blood flow was measured during normocapnia and hypocapnia at 6 h of salt infusion. Blood chemistry was performed at baseline and at 2 and 6 h of salt infusion. Blood gases were also measured during each hypocapnic period.

In the second experiment, rats received either intravenous sodium acetate (n = 5) or ammonium acetate (n = 5). At 6 h of infusion, U-46619 (10^{-8} mol/l) was superfused through the cranial window at a rate of 0.25 ml/min for 5 min, and the diameter response was measured. After a 15-min washout, the diameter response to 10^{-7} mol/l U-46619 was measured.

Statistical comparisons among groups were made with ANOVA and the Newman-Keuls multiple-range test. For measurements repeated over time, comparisons were made with baseline values by repeated-measures ANOVA and Dunnett's test. Comparisons of the percent diameter response with hypocapnia between the first and second hypocapnic challenge were made by paired t-test. Comparisons of the normocapnic and hypocapnic CBF and CVR values at 6 h were made by paired t-test. The level of statistical significance was set at $P < 0.05$ in all tests. Data are expressed as means ± SE.

RESULTS

Infusion of ammonium acetate increased plasma ammonia concentration to ~600 μmol/l by 2 h, and the concentration remained stable through 6 h (Table 1). Values were somewhat greater in groups pretreated

Table 1. *Plasma ammonia concentration and osmolarity during 6-h salt infusion*

		Time, h	
	Baseline	2	6
Plasma ammonia, $\mu mol/l$			
Vehicle + sodium acetate	39 ± 3	27 ± 3	30 ± 5
MSO + sodium acetate	147 ± 15	$109 \pm 11^*$	$88 \pm 7^*$
Vehicle + ammonium acetate	36 ± 3	$546 \pm 27^{*\dagger}$	$561 \pm 24^{*\dagger}$
MSO + ammonium acetate	128 ± 6	$780 \pm 58^{*\dagger}$	$652 \pm 45^{*\dagger}$
BSO + ammonium acetate	63 ± 2	$604 \pm 62^*$	$546 \pm 63^*$
Plasma osmolarity, mosmol/l			
Vehicle + sodium acetate	292 ± 2	292 ± 1	294 ± 3
MSO + sodium acetate	299 ± 1	301 ± 2	$308 \pm 2^*$
Vehicle + ammonium acetate	294 ± 1	$289 \pm 2^{*\dagger}$	$283 \pm 2^{*\dagger}$
MSO + ammonium acetate	296 ± 1	$291 \pm 2^{*\dagger}$	$284 \pm 2^{*\dagger}$
BSO + ammonium acetate	299 ± 4	292 ± 2	$286 \pm 3^*$

Values are means $\pm$ SE. MSO, methionine sulfoximine; BSO, buthionine sulfoximine. $^* P < 0.05$ from baseline; $\dagger P < 0.05$ from corresponding value in sodium acetate group with same pretreatment.

Table 2. *Mean arterial pressure and pressure in cranial window before and during hypocapnic challenges at baseline and at 6 h of salt infusion*

	Baseline		6-h Salt Infusion	
	Normo-capnia	Hypo-capnia	Normo-capnia	Hypo-capnia
Mean arterial pressure, mmHg				
Vehicle + sodium acetate	111 ± 5	120 ± 4	110 ± 3	118 ± 4
MSO + sodium acetate	103 ± 5	117 ± 8	99 ± 9	104 ± 6
Vehicle + ammonium acetate	102 ± 4	97 ± 4	94 ± 4	98 ± 7
MSO + ammonium acetate	101 ± 6	107 ± 8	$86 \pm 7^*$	$98 \pm 6\dagger$
BSO + ammonium acetate	111 ± 6	126 ± 6	100 ± 6	108 ± 5
Cranial window pressure, mmHg				
Vehicle + sodium acetate	8.5 ± 0.4	$7.2 \pm 0.3\dagger$	8.0 ± 0.4	7.4 ± 0.2
MSO + sodium acetate	7.5 ± 0.6	6.9 ± 0.7	7.3 ± 0.8	7.1 ± 0.8
Vehicle + ammonium acetate	8.2 ± 0.6	6.9 ± 0.6	$21.0 \pm 5.8^*$	$18.9 \pm 5.8^*$
MSO + ammonium acetate	8.1 ± 0.4	6.8 ± 0.2	8.4 ± 0.6	8.3 ± 0.6
BSO + ammonium acetate	9.1 ± 0.5	8.2 ± 0.4	$18.7 \pm 2.6^*$	$18.6 \pm 2.9^*$

Values are means $\pm$ SE. $^* P < 0.05$ from baseline at corresponding CO_2 level; $\dagger P < 0.05$ from preceding normocapnic level.

with MSO compared with vehicle with the same salt infusate. Plasma osmolarity decreased slightly in the three groups during ammonium acetate infusion (Table 1). In all groups, the hypocapnia tests increased arterial pH from ~7.39 to 7.64 by reducing arterial P_{CO_2} from ~40 to 18 mmHg, whereas arterial P_{O_2} was 150–210 mmHg, and hemoglobin concentration was 12–15 g/dl. There were no significant differences in arterial P_{CO_2} among groups.

Mean arterial pressure was unchanged during the 6-h infusion period under normocapnic conditions except in the MSO plus ammonium acetate group where pressure decreased (Table 2). Pressure in the cranial window increased during ammonium acetate infusion in groups pretreated with vehicle and BSO but not MSO. Cortical tissue water content in the ammonium acetate groups pretreated with vehicle and BSO was greater than that in the sodium acetate groups (Table 3).

During the 6-h salt infusion, pial arteriolar diameter was unchanged during normocapnia in the sodium acetate groups (Fig. 1). Diameter increased significantly during normocapnia in the ammonium acetate group pretreated with vehicle but not with MSO. Before salt infusion but after vehicle, MSO, and BSO pretreatments, all groups showed similar vasoconstrictor responses to hypocapnia (Fig. 2). However, after 6 h of ammonium acetate infusion, the vasoconstrictor response to hypocapnia was lost in the groups pretreated with vehicle or BSO. In contrast, the vasoconstrictor response was present in the ammonium acetate group pretreated with MSO, and the response differed significantly from that with vehicle pretreatment. With sodium acetate infusion, the vasoconstrictor response was equivalent with vehicle and MSO pretreatment, and the responses were significantly different from the vehicle plus ammonium acetate group.

In the vehicle plus sodium acetate group, neocortical CBF decreased during the hypocapnic challenge at 6 h of salt infusion (Fig. 3). With ammonium acetate infu-

sion, CBF increased during hypocapnia in the groups pretreated with vehicle and BSO but not in the group pretreated with MSO. CVR increased during hypocapnia in the sodium acetate groups pretreated with vehicle and MSO, whereas there was no significant change in the ammonium acetate groups pretreated with vehicle and BSO (Fig. 4). In the ammonium acetate group pretreated with MSO, CVR increased during hypocapnia.

In medulla plus pons, midbrain, diencephalon, and cerebellum, the decrease in blood flow during hypocapnia was lost in the vehicle plus ammonium acetate group (Fig. 5). Pretreatment with MSO preserved the flow response in medulla plus pons and in diencephalon during hyperammonemia and prevented the increase in midbrain blood flow during hypocapnia.

The ability of pial arterioles to constrict to a non-CO_2 related stimulus was evaluated in separate groups after 6 h of sodium ($n = 5$) or ammonium ($n = 5$) acetate infusion. As in previous groups, baseline diameter

Table 3. *Cortical tissue water content*

	Percent Water Content
Vehicle + sodium acetate	78.9 ± 0.4
MSO + sodium acetate	78.8 ± 0.3
Vehicle + ammonium acetate	$80.8 \pm 0.2^*$
MSO + ammonium acetate	79.9 ± 0.3
BSO + ammonium acetate	$81.0 \pm 0.3^*$

Values are means $\pm$ SE. $^* P < 0.05$ from vehicle + sodium acetate and MSO + sodium acetate groups.
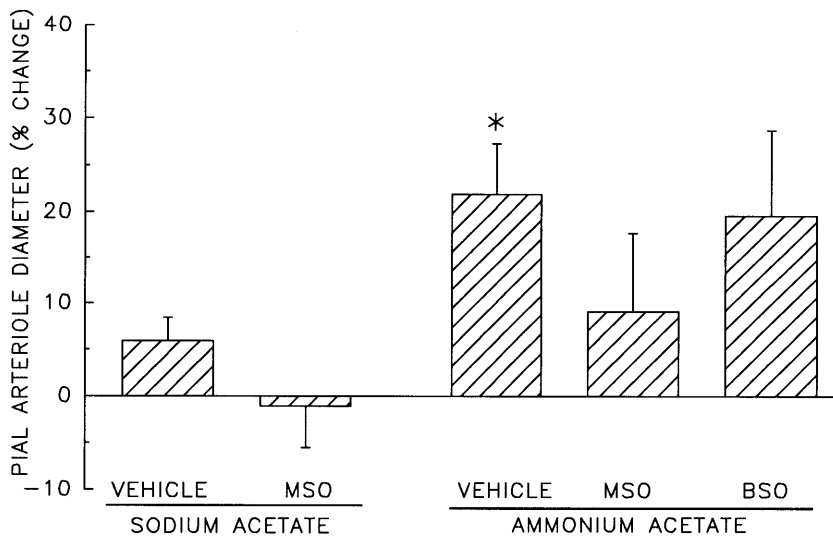
Fig. 1. Pial arteriolar diameter during normocapnia at 6 h of sodium acetate or ammonium acetate infusion expressed as a percent change of normocapnic baseline before salt infusion but after pretreatment with vehicle, methionine sulfoximine (MSO), or buthionine sulfoximine (BSO). Values are means ± SE. * $P < 0.05$ from zero change.

increased $23 \pm 4\%$ with 6 h of ammonium acetate infusion but was unchanged with sodium acetate infusion ($4 \pm 3\%$). With superfusion of 10^{-8} and 10^{-7} mol/l of U-46619, robust constriction occurred in both groups (Fig. 6). Percent constriction was greater in the ammonium acetate group.

DISCUSSION

The major new findings of this study are *1*) acute hyperammonemia abolishes the constrictor response to hypocapnia in extraparenchymal pial arterioles and abolishes the increase in total CVR calculated from perfusion pressure and neocortical blood flow and *2*) pretreatment with the glutamine synthetase inhibitor MSO preserves hypocapnic vasoconstriction in pial arterioles and the increase in CVR during hypocapnia in hyperammonemic animals. The present results also confirm our previous findings that MSO prevents the increase in cortical tissue water content and intracranial pressure associated with acute hyperammonemia (18, 35, 36, 39). Therefore, the alteration in vascular reactivity and other associated pathophysiological changes are related to glutamine accumulation and not solely to ammonium ions.

Hyperammonemia increased basal diameter of pial arterioles without increasing normocapnic CBF above that of the control group. This dilation of extraparenchymal arterioles probably represents an autoregulatory response to increased intracranial pressure and direct compression of intraparenchymal vessels by swollen astrocytes. Autoregulation is intact in this model of hyperammonemia (17). Electron microscopic analysis of fixed tissue sections indicated that capillaries and small venules appeared to be more elliptical in hyperammonemic rats consistent with vascular compression, whereas MSO pretreatment preserved a circular appearance (39). An increase in extracellular potassium activity associated with astrocyte dysfunction (33) may contribute to the increase in basal pial arteriolar diameter required to compensate for an increase in intraparenchymal vascular resistance.

An increase in basal diameter of pial arterioles per se might limit vasodilator capacity but would not be expected to attenuate vasoconstrictor capacity. We found that pial arterioles still constricted to U-46619 during
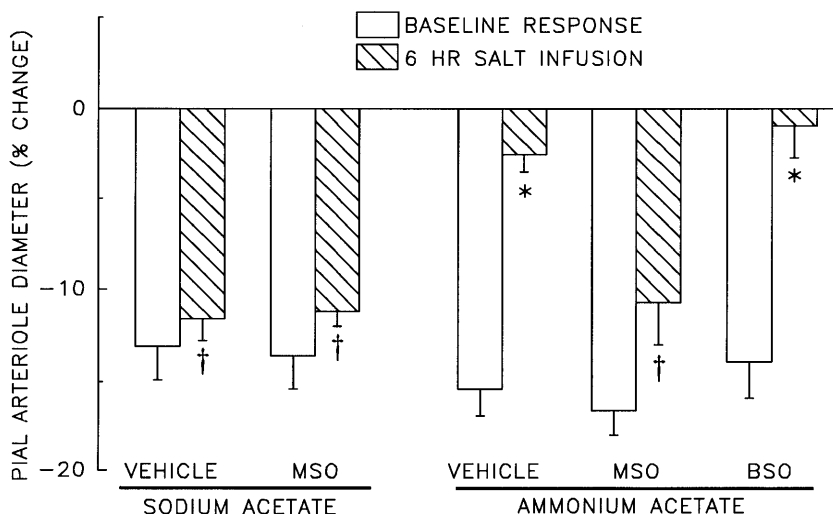

Fig. 2. Percent change in pial arteriolar diameter during 10 min of hypocapnia. Baseline hypocapnic response was measured 3 h after administration of vehicle, MSO, or BSO but before salt infusion. Second hypocapnic response was measured at 6 h of sodium acetate or ammonium acetate infusion. Values are means ± SE. * $P < 0.05$ from baseline response in same group. † $P < 0.05$ from response in ammonium acetate group pretreated with vehicle.
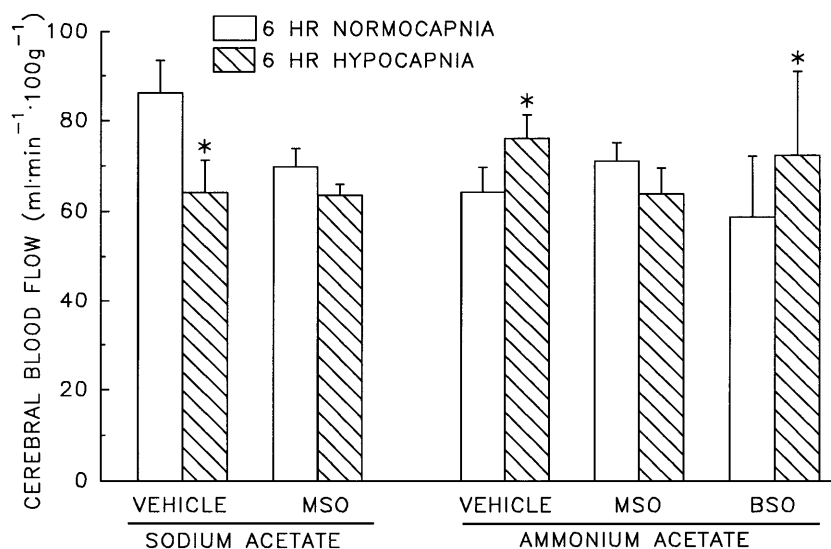
Fig. 3. Blood flow to cerebrum during normocapnia and hypocapnia after 6 h of infusion of sodium acetate or ammonium acetate in groups pretreated with vehicle, MSO, or BSO. Values are means ± SE. *$P < 0.05$ from normocapnic value within same group.

acute hyperammonemia, thereby indicating intact vasoconstrictor capacity. Indeed, hyperammonemia augmented the constrictor response to U-46619 possibly because of the increase in basal diameter. Furthermore, we have previously found that CBF decreased during hypocapnia in rats in which tissue water content was markedly increased by a reduction in plasma osmolarity (36). Thus nonspecific cell swelling does not inhibit hypocapnic vasoconstriction. Therefore, the present results demonstrating selective loss of hypocapnic vasoconstriction together with previous results demonstrating selective loss of hypercapnic vasodilation (18) indicate that hyperammonemia produced a generalized impairment of CO_2 reactivity of pial arterioles.

Pretreatment with MSO preserved hypocapnic vasoconstriction, whereas BSO pretreatment was ineffective. Likewise, MSO but not BSO sustained hypercapnic vasodilation (18) and attenuated watery swelling of astrocytes (39). Thus we attribute the effect of MSO to glutamine synthetase inhibition rather than to γ-glutamylcysteine synthetase inhibition (12). Inhibition of the latter would decrease glutathione, but decreased

glutathione would not be anticipated to restore CO_2 reactivity. Moreover, glutathione concentration was not observed to decrease in brain after MSO administration (31). In addition, MSO does not appear to act directly on the cerebral vasculature to increase CO_2 reactivity because MSO did not potentiate hypocapnic vasoconstriction in the sodium acetate control group. In the same experimental model of acute hyperammonemia, MSO prevented a threefold increase in tissue glutamine concentration (35) and in extracellular potassium activity (33). Thus MSO likely acts by preventing glutamine accumulation or astrocyte dysfunction associated with glutamine accumulation. Astrocyte dysfunction could account for the increase in extracellular potassium activity or could alter the release of other vasoactive substances that interfere with CO_2 reactivity. Our observations of congruent changes in pial arteriolar diameter and total CVR suggest that the vasoactive substance responsible for interfering with CO_2 activity acts equivalently on intraparenchymal and extraparenchymal arterioles and that the substance is capable of diffusing to the pial surface.
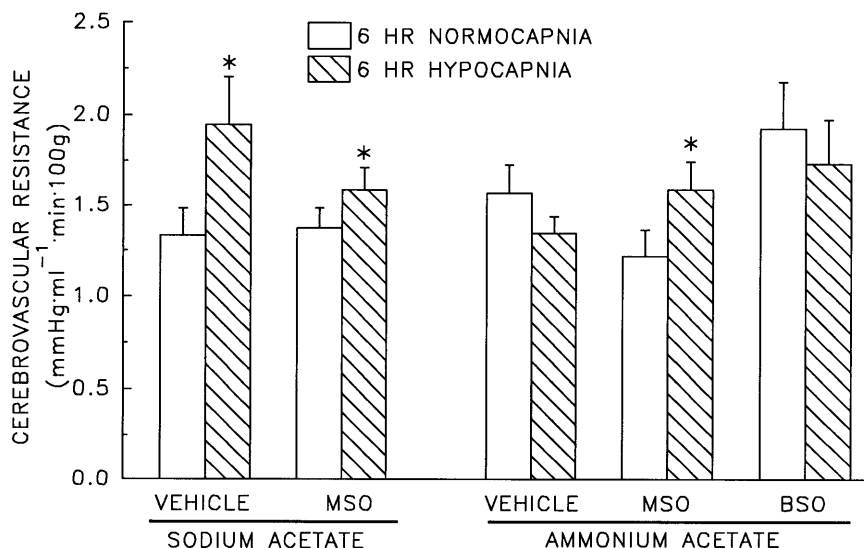

Fig. 4. Cerebrovascular resistance during normocapnia and hypocapnia after 6 h of infusion of sodium acetate or ammonium acetate in groups pretreated with vehicle, MSO, or BSO. Values are means ± SE. *$P < 0.05$ from normocapnic value within same group.
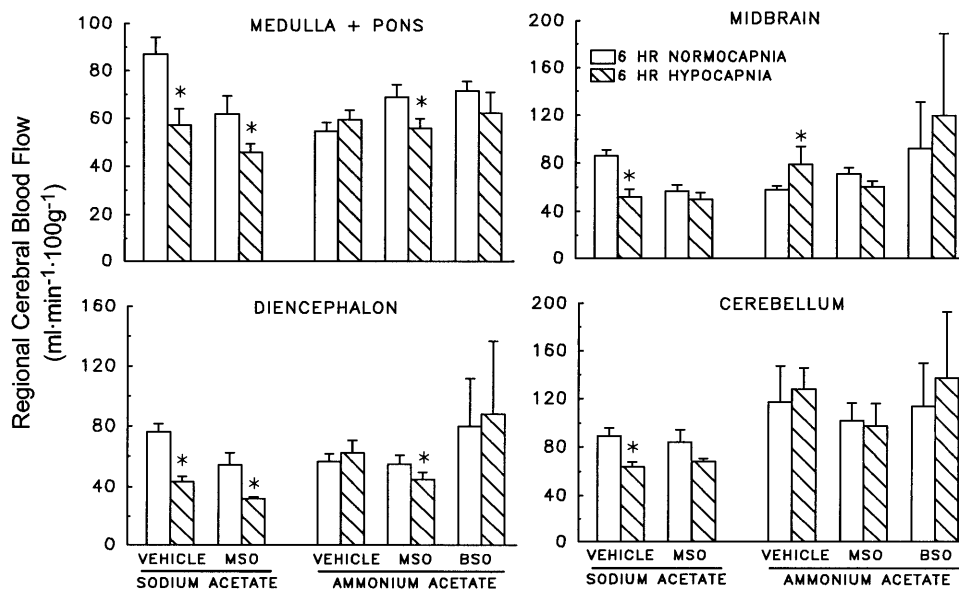
Fig. 5. Regional blood flow to combined medulla plus pons, midbrain, diencephalon, and cerebellum during normocapnia and hypocapnia after 6 h of infusion of sodium acetate or ammonium acetate in groups pretreated with vehicle, MSO, or BSO. Values are means ± SE. *$P < 0.05$ from normocapnic value within same group.

Glutamine is not considered to exert direct effects on vascular smooth muscle contraction, but it can limit the generation of endothelial-derived relaxing factor from aortic endothelial cells (1, 15, 34, 40) and the relaxation of isolated cerebral vessels evoked by transmural nerve stimulation (27). Basal levels of nitric oxide may be required for full expression of CO_2 reactivity (19, 20). Thus one possibility is that the increase in glutamine over a 6-h period suppresses basal nitric oxide production sufficient to impair CO_2 reactivity. Whether glutamine accumulation alone is capable of completely abolishing CO_2 reactivity is unclear. Changes in extracellular potassium activity can modify the response of pial arterioles to extracellular pH changes thought to mediate CO_2 reactivity (25). However, the 12 mmol/l concentration of potassium observed in this model (33) would not be expected to completely suppress the response to pH changes. The present results indicate that ammonium ions by themselves do not cause the loss of CO_2 reactivity, but it is conceivable that the increase in glutamine, together with an increase in potassium or ammonium ions, causes a complete suppression of cerebrovascular CO_2 reactivity.

In hyperammonemic rats, hypocapnia produced a paradoxical increase in CBF without a significant increase in pial arteriolar diameter or arterial blood pressure. Because glia are enriched in carbonic anhydrase (5) and because bicarbonate ion transport may be important in cell volume regulation, it is possible that hypocapnia reduces the cell volume of swollen astrocyte processes and passively permits a paradoxical increase in CBF. However, we did not detect a significant decrease in pressure under the cranial window during hypocapnia in this experiment or in cisterna magna pressure in a previous experiment (36). Thus, if brief hypocapnia reduces cell volume in hyperammonemic rats, the reduction is probably small or highly localized. Nevertheless, a localized reduction of swelling of astrocyte foot processes during hypocapnia could decom-

press capillaries and venules. Therefore, we cannot exclude a small mechanical effect contributing to the paradoxical increase in CBF during hypocapnia in addition to the primary loss of vascular reactivity indicated by the lack of change in pial arteriolar diameter.

Blood flow in brain regions other than the neocortex also failed to decrease during hypocapnia, indicating that the loss of CO_2 reactivity extends beyond cortical pial arterioles. The paradoxical increase in CBF was most prominent in the midbrain, which is consistent
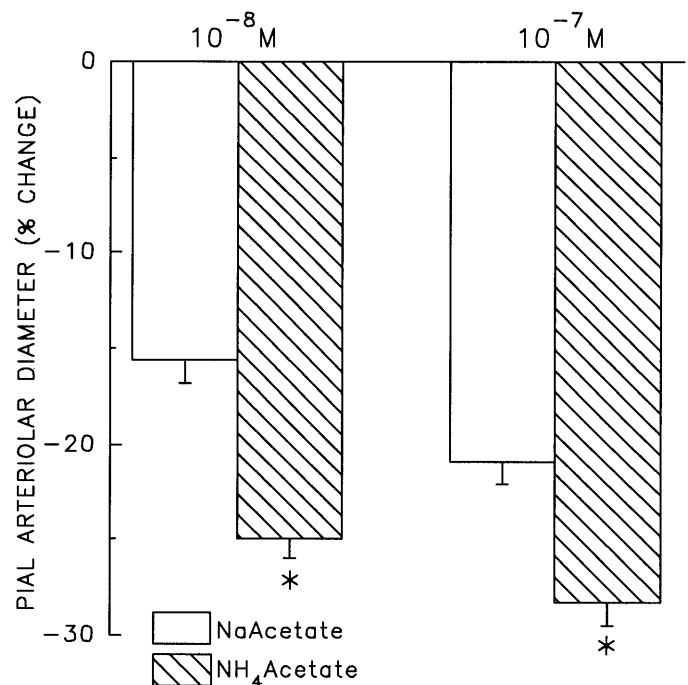

Fig. 6. Percent change in pial arteriolar diameter during superfusion of 10^{-8} and 10^{-7} mmol/l of U-46619 at 6 h of intravenous infusion of sodium acetate or ammonium acetate. Values are means ± SE. *$P < 0.05$ from response in sodium acetate group.

with our previous studies in rats (36) and dogs (2). The midbrain may be more sensitive to metabolic alterations during hyperammonemia (28), and respiratory alkalosis may increase diffusion of free ammonia across the blood-brain barrier. In support of this possibility, metabolic alkalemia selectively increased midbrain blood flow in hyperammonemic dogs (38). Thus another consideration in interpreting the paradoxical increase in CBF is that hypocapnia may suddenly increase the ammonia load seen by perivascular astrocytes and produce local metabolic changes that lead to active dilation of intraparenchymal arterioles. If so, this local metabolic change is not associated with significant changes in global O_2 consumption (2) or ATP concentration (16).

In the present study, most physiological parameters were well matched between vehicle and MSO treatment groups. However, hypocapnia produced a 12-mmHg increase in arterial pressure in the hyperammonemic group pretreated with MSO. This increase in pressure may produce an autoregulatory constriction of pial arterioles. However, the magnitude of the increase in arterial pressure is probably too small to fully account for the 11% decrease in arteriolar diameter. Increases in arterial pressure of at least 30 mmHg are required to reduce a diameter of 5–10% (14, 23). Hence, we believe that vasoconstriction seen in this group is related largely to hypocapnia rather than to the increase in arterial pressure.

In summary, the present results demonstrate that acute hyperammonemia causes a loss of hypocapnic vasoconstriction of pial arterioles and that the lack of a decrease in CBF is primarily attributed to a loss of active vasoregulation rather than mechanical effects of tissue swelling. Preservation of hypocapnic constriction by MSO with elevated ammonia implicates glutamine accumulation rather than ammonium ions in blunting CO_2 reactivity. The mechanism may involve effects of glutamine on the vasculature or secondary effects of glutamine metabolism in astrocytes, causing alterations in astrocyte function. In either case, the present study in conjunction with previous work in this model suggests that selective astrocytic pathology may interfere with cerebrovascular regulation in a manner that is relatively selective for CO_2 reactivity.

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