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Glutamine-dependent inhibition of pial arteriolar dilation to acetylcholine with and without hyperammonemia in the rat

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Abstract

Glutamine has been shown to influence endothelial-dependent relaxation and nitric oxide production in vitro, possibly by limiting arginine availability, but its effects in vivo have not been well studied. Hyperammonemia is a pathophysiological condition in which glutamine is elevated and contributes to depressed CO₂ reactivity of cerebral arterioles. We tested the hypothesis that acute hyperammonemia decreases pial arteriolar dilation to acetylcholine in vivo and that this decrease could be prevented by inhibiting glutamine synthetase with L-methionine-S-sulfoximine (MSO) or by intravenous infusion of L-arginine. Pial arteriolar diameter responses to topical superfusion of acetylcholine were measured in anesthetized rats before and at 6 h of infusion of either sodium or ammonium acetate. Ammonium acetate infusion increased plasma ammonia concentration from ~ 30 to ~ 600 μM and increased cerebral glutamine concentration fourfold. Arteriolar dilation to acetylcholine was intact after infusion of sodium acetate in groups pretreated with vehicle or with MSO plus methionine, which was coadministered to prevent MSO-induced seizures. In contrast, dilation in response to acetylcholine was completely blocked in hyperammonemic groups pretreated with vehicle or methionine alone. However, MSO plus methionine administration before hyperammonemia, which maintained cerebral glutamine concentration at control values, preserved acetylcholine dilation. Intravenous infusion of L-arginine during the last 2 h of the ammonium acetate infusion partially restored dilation to acetylcholine without reducing cerebral glutamine accumulation. Superfusion of 1 or 2 mM L-glutamine through the cranial window for 1 h in the absence of hyperammonemia attenuated acetylcholine dilation but had no effect on endothelial-independent dilation to nitroprusside. We conclude that 1) hyperammonemia reduces acetylcholine-evoked dilation in cerebral arterioles, 2) this reduction depends on increased glutamine rather than ammonium ions, and 3) increasing arginine partially overcomes the inhibitory effect of glutamine.

Keywords

arginine; cerebral circulation; endothelial-dependent dilation; hepatic encephalopathy; hyperammonemia; nitric oxide

Patients with hepatic failure have been reported to have a depressed cerebrovascular response to hypercapnia (9). Depressed cerebral vasodilation to hypercapnia also has been observed in experimental models of acute hyperammonemia in a variety of species, including monkey, dog, cat, and rat (2,6,15,16,33). This depressed vasodilatory response to hypercapnia does not

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represent a generalized vasoparalysis because vasodilation to hypoxia and to arterial hypotension remains intact (14,15).

Hyperammonemia produces substantial increases in brain glutamine concentration (7). Inhibition of glutamine synthetase preserves cerebrovascular reactivity to hypercapnia and hypocapnia during acute hyperammonemia (13,15). Thus the loss of CO₂ reactivity is related to glutamine accumulation rather than to direct vascular effects of ammonium ions. Moreover, intravenous infusion of glutamine in the absence of hyperammonemia decreases hypercapnic vasodilation, although not to the same extent as hyperammonemia (22). Therefore, glutamine can exert a modulatory role in cerebrovascular CO₂ reactivity in vivo.

The mechanism by which glutamine modulates CO₂ reactivity is not precisely known but may be related to decreased nitric oxide (NO) production because infusion of arginine during either hyperammonemia or glutamine infusion improves hypercapnic reactivity (22). In isolated cerebral arteries, NO-dependent vasorelaxation evoked by transmural nerve stimulation is inhibited by glutamine (18). Studies in aortic and venular endothelial cell cultures and isolated aortic rings indicate that elevation of glutamine concentration in the physiological range limits NO production and endothelial-dependent vasorelaxation (1,11,20,31). However, the extent to which glutamine modulates endothelial-dependent vasodilation in vivo has not been well studied.

In the present study, we evaluated whether endogenous increases in brain tissue glutamine concentration associated with hyperammonemia or exogenous increases in glutamine bathing pial arterioles cause impaired vasodilation in vivo to the endothelial-dependent dilator acetylcholine (ACh). We tested the hypotheses that 1) acute hyperammonemia decreases pial arteriolar dilation to ACh, 2) this decrease could be prevented by inhibiting glutamine accumulation with the glutamine synthetase inhibitor L-methionine-S-sulfoximine (MSO) or by intravenous infusion of L-arginine, and 3) abluminal increases in glutamine concentration produced by superfusion of a cranial window with artificial cerebrospinal fluid (CSF) containing elevated L-glutamine attenuates dilation to ACh but not to the endothelial-independent dilator sodium nitroprusside.

METHODS

All procedures were approved by the Institutional Animal Care and Use Committee. Male Wistar rats (~350 g) were anesthetized with pentobarbital (65 mg/kg ip). Anesthesia was maintained throughout the experiment by additional intraperitoneal injections of pentobarbital (20 mg/kg every 90 min). The lungs were mechanically ventilated with O₂-enriched air to maintain the arterial partial pressure of O₂ in the range of 140–190 Torr and the arterial partial pressure of CO₂ (PaCO₂) in the range of 35–45 Torr. A femoral artery and tail artery were catheterized for monitoring arterial blood pressure and sampling arterial blood. Rectal temperature was maintained at ~ 37°C with a warming blanket.

A closed cranial window was constructed over the parietal cortex for measuring pial arteriolar diameter by intravital microscopy (15). A plastic ring was cemented to the skull around a ~ 4-mm craniotomy. The dura mater was carefully incised and retracted. A glass coverslip was cemented to the plastic ring to seal the window filled with artificial CSF containing (in mM) 151 Na⁺, 3 K⁺, 1.3 Ca²⁺, 0.6 Mg²⁺, 134 Cl⁻, 24.6 HCO₃⁻, 6 urea, and 3.7 glucose (19). The window was equipped with an inflow port and an outflow port for superfusing artificial CSF and ACh, with a port for measuring fluid pressure, and a thermistor for monitoring fluid temperature. The diameter of pial arterioles was measured with a microscope equipped with a video recording system.

The first experimental protocol involved measuring pial arteriolar responses to ACh before and after a 6-h infusion of sodium acetate (NaAc) or ammonium acetate (NH₄Ac). Baseline measurements of pial arteriolar diameter and arterial blood analysis were made 45 min after completion of the surgical procedures. The cranial window was superfused with artificial CSF for 3 min followed by 30 μ M ACh for 5 min at a rate of 250 μ l/min. After the change in diameter was recorded and the ACh was washed out, a continuous intravenous infusion of either NaAc or NH₄Ac was started (50 μ mol·kg⁻¹·min⁻¹, 0.1 ml/min). Arterial blood analysis was repeated at 2 and 6 h of infusion, and changes in baseline diameter were measured hourly. At 6 h of infusion, the change in diameter during superfusing of 30 μ M ACh was measured.

To inhibit glutamine synthesis, 150 mg/kg (0.83 mmol/kg) of MSO (Sigma, St. Louis, MO) was infused intravenously 3 h before the baseline measurements were obtained. Control groups received saline vehicle (3 ml/kg). MSO can induce seizures, but seizures are prevented by coadministration of L-methionine without altering the effect of MSO on glutamine synthetase activity (27). Groups receiving MSO also received an intraperitoneal injection of 6.7 mmol/kg of L-methionine. To control for a possible influence of L-methionine on the ACh response during hyperammonemia, a group receiving NH₄Ac was pretreated with L-methionine alone. To test whether L-arginine could reverse the effect of hyperammonemia on the ACh response, 2 mmol·kg⁻¹·h⁻¹ of L-arginine was infused intravenously for the last 2 h of NH₄Ac infusion. This infusion rate of L-arginine has been shown to restore hypercapnic dilation during NH₄Ac infusion (22). Thus six groups of rats were studied in the first experiment: 1) vehicle pretreatment and NaAc infusion ($n = 8$); 2) MSO/methionine pretreatment and NaAc infusion ($n = 8$); 3) vehicle pretreatment and NH₄Ac infusion ($n = 8$); 4) L-methionine pretreatment and NH₄Ac infusion ($n = 6$); 5) MSO/methionine pretreatment and NH₄Ac infusion ($n = 8$); and 6) vehicle pretreatment and NH₄Ac infusion with subsequent L-arginine infusion ($n = 8$).

Arterial blood analysis included measurements of plasma ammonium concentration by a cation-exchange and spectrophotometer analysis previously described (3), plasma osmolarity by freezing-point depression (Advanced Instruments, Norwood, MA), hemoglobin concentration (OSM3 Hemoximeter, Radiometer, Copenhagen), and pH, PaCO₂, and arterial partial pressure of O₂ (Chiron Diagnostics Blood Gas Analyzer, Halstead, Essex, UK). At the end of the experiment, the cerebral hemispheres were rapidly harvested and frozen in subgroups ($n = 5-6$). The cerebral tissue concentration of glutamine, glutamate, and arginine was measured by high-performance liquid chromatography with fluorescent detection as described (17,32).

In the second experimental protocol, the dilator response to ACh was measured in the presence of exogenously increased glutamine in CSF surrounding pial arterioles without hyperammonemia. In four groups of rats, the cranial window was continuously superfused for 1 h at a rate of 0.1 ml/min with artificial CSF containing either 0 ($n = 7$), 0.3 ($n = 6$), 1 ($n = 7$), or 2 mM ($n = 7$) L-glutamine. The 0 and 0.3 mM concentrations were chosen to bracket the normal glutamine concentration of 0.25 mM previously measured in the CSF sampled from rat cranial windows (22). The concentration of glutamine was measured in the effluent CSF collected between 50 and 60 min of superfusion. At 60 min of glutamine superfusion, 10 μ M ACh was added to the glutamine-containing superfusate, and the percent change in pial arteriolar diameter was measured. The dilator response to 3 μ M sodium nitroprusside superfusion was also tested in the presence of the different glutamine concentrations.

Biochemical and arterial blood gas measurements were compared among groups by one-way ANOVA and the Newman-Keuls multiple range test. In the first experiment, percent changes in baseline pial arteriolar diameter during salt infusion and tissue amino acid concentration were compared among groups by ANOVA and the Newman-Keuls multiple range test. Within each group, the percent changes in pial arteriolar diameter, measured at 5 min of ACh

superfusion, were compared before and at 6 h of salt infusion by paired *t*-test with *n* equaling the number of rats per group. Arterial blood measurements and mean arterial blood pressure at 6 h of salt infusion were also compared with baseline values by paired *t*-test. In the second experiment, the change in baseline diameter during glutamine superfusion was analyzed by paired *t*-test. The percent dilation to ACh and nitroprusside was compared among the four groups with different glutamine concentrations by one-way ANOVA and the Newman-Keuls multiple range test. The significance level was set at 0.05 for all tests. All values are expressed as means $\pm$ SD.

RESULTS

Hyperammonemia experiment

Infusion of NH₄Ac increased plasma ammonium concentration from 36 ± 11 to 483 ± 120 μ M by 2 h and to 552 ± 182 μ M by 6 h of infusion. Similar increases were observed in the other groups infused with NH₄Ac (Table 1). Pretreatment with MSO/methionine modestly elevated plasma ammonium before salt infusion. Plasma osmolarity was unchanged during the salt infusion. Arterial pH was slightly lower in the groups treated with MSO/methionine or L-arginine. No differences were present in PaCO₂ or hemoglobin concentration among groups, although PaCO₂ increased slightly and hemoglobin decreased by 10–15% at the end of the salt infusion in some of the groups (Table 1).

The cerebral tissue concentration of glutamine was reduced by $\sim 50\%$ in the NaAc group pretreated with MSO/methionine compared with vehicle (Fig. 1). Infusion of NH₄Ac increased cerebral glutamine concentration fourfold in the group pretreated with vehicle and twofold in the group pretreated with methionine. Coinfusion of L-arginine during the last 2 h of NH₄Ac infusion did not attenuate the increase in tissue glutamine concentration. In contrast, tissue glutamine concentration in the NH₄Ac group pretreated with MSO/methionine was similar to that in the NaAc group pretreated with vehicle.

Cerebral glutamate concentration was not significantly changed in the hyperammonemic groups pretreated with vehicle or coadministered L-arginine, but decreases occurred in the hyperammonemic groups pretreated with methionine or MSO/methionine (Fig. 1). Analysis of cerebral arginine concentration by ANOVA indicated a significant intergroup effect, but the only difference indicated by the Newman-Keuls multiple range test was a greater arginine concentration in the NH₄Ac group receiving L-arginine compared with the NH₄Ac group pretreated with L-methionine. In a separate group of nonhyperammonemic rats (*n* = 5), infusion of L-arginine at a rate of $2 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for 2 h was found to increase plasma arginine concentration sevenfold (98 ± 20 to 740 ± 569 μ M).

Mean arterial blood pressure was maintained at an average of 95–110 mmHg during the NaAc infusion and during the NH₄Ac infusion with vehicle pretreatment (Fig. 2). However, during the NH₄Ac infusion, mean arterial pressure decreased to 73–75 mmHg in groups pretreated with L-methionine and MSO/methionine and to 84 mmHg in the group with L-arginine coinfusion. Intracranial pressure measured in the closed cranial window increased during NH₄Ac infusion (Fig. 2). The increase was markedly attenuated in the group pretreated with MSO/methionine but not in the groups treated with L-methionine or L-arginine. Cerebral perfusion pressure, calculated as the difference between arterial blood pressure and intracranial pressure, decreased at 6 h of NH₄Ac infusion in the groups treated with L-methionine, MSO/methionine, and arginine, and the levels were significantly lower than that of the NH₄Ac group treated with vehicle (Fig. 2).

Baseline diameter of pial arterioles was not significantly changed over the 6-h infusion of NaAc in the group pretreated with vehicle but increased in the NaAc group pretreated with MSO/

methionine (Fig. 3). Similar increases occurred in the NH_4Ac -infused groups pretreated with vehicle, MSO/methionine, and coinfusion of L-arginine. The increase in baseline diameter was enhanced by 6 h of NH_4Ac infusion in the group pretreated with L-methionine. The latter group had the lowest cerebral perfusion pressure (58 $\pm$ 15 mmHg).

The pial arteriolar dilator response to ACh after 6 h of NaAc infusion was similar to the dilator response before the infusion was started in both the group pretreated with vehicle and the group pretreated with MSO/methionine (Fig. 4). In contrast, the ACh dilator response was completely abolished by 6 h of NH_4Ac infusion in the group pretreated with vehicle. Also, no significant dilation was observed in the NH_4Ac group pretreated with L-methionine alone. However, pretreatment with MSO/methionine preserved ACh dilation during NH_4Ac infusion at the baseline ACh response. Moreover, in the hyperammonemic group receiving L-arginine during the last 2 h of NH_4Ac infusion, significant dilation to ACh occurred, although the response was less than the baseline ACh response.

Glutamine superfusion experiment

Superfusion of the cranial window with artificial CSF containing 0, 0.3, 1, and 2 mM L-glutamine for 1 h resulted in graded increases in glutamine concentrations in the CSF effluent (Table 2). Baseline diameter of pial arterioles remained unchanged during the 1-h superfusion period (Table 2). There were no differences among groups in mean arterial blood pressure or PaCO_2 . The percent dilation to 10 μM ACh was influenced by the glutamine concentration as assessed by ANOVA ($P < 0.01$). The ACh response was significantly reduced in the group superfused with 1 mM L-glutamine compared with the group superfused with 0.3 mM L-glutamine (Fig. 5). The difference between the groups superfused with 0 and 1 mM glutamine was of marginal significance ($P < 0.06$), as determined by the Newman-Keuls multiple range test. Furthermore, the response to ACh in the group superfused with 2 mM L-glutamine was significantly reduced compared with the groups superfused with either 0 or 0.3 mM L-glutamine. In contrast, the dilator response to sodium nitroprusside was not significantly different among the groups superfused with the different L-glutamine concentrations (Fig. 6).

DISCUSSION

The major findings of this study are 1) acute hyperammonemia in rats sufficient to increase fourfold the cortical glutamine concentration abolished pial arteriolar dilation to ACh; 2) preventing the increase in tissue glutamine by inhibiting glutamine synthetase preserved the dilatory response to ACh; 3) intravenous infusion of L-arginine during hyperammonemia partially restored ACh dilation; and 4) elevation of CSF glutamine concentration in the absence of hyperammonemia attenuated dilation to ACh but not to the endothelial-independent dilator nitroprusside. These results indicate that glutamine accumulation in the pathophysiological range, such as that associated with hyperammonemia, is sufficient to impair an endothelial-dependent response in cerebral resistance blood vessels in vivo.

Neither ammonia nor glutamine directly inhibit NO synthase (NOS) enzymatic activity when substrate and cofactors are present (20). Arginine availability for NOS activity is determined by recycling of citrulline to arginine (5,12), cleavage of peptides (12), cellular uptake of arginine and citrulline (37), and metabolism by arginase (39), which has a higher K_m for arginine than NOS (4). Glutamine has been reported to limit recycling of citrulline to arginine by inhibiting arginosuccinate synthetase activity in aortic endothelial cells (29), although others report that the primary effect of glutamine is on citrulline uptake (20,37). Physiological concentrations of glutamine can inhibit release of endothelial-derived relaxing factor in cultured cells (11) and isolated aorta (31), but the inhibitory effect of glutamine on agonist-evoked NO production appears to be independent of arginine availability (1). Alternative mechanisms include glutamine metabolism to glucosamine, which decreases pentose cycle

activity and depletes NADPH necessary for NOS activity (36). In addition to endothelial cells, perivascular nerves surrounding cerebral arteries can recycle citrulline to arginine, and glutamine attenuates arterial relaxation evoked by transmural nerve stimulation (5,18). These effects of glutamine were attributed, in part, to inhibition of citrulline uptake by glutamine. The results of the present study extend these previous in vitro studies by demonstrating that pathophysiological accumulation of glutamine during hyperammonemia is capable of blocking ACh-evoked dilation in vivo and that exogenous application of glutamine can attenuate ACh-evoked dilation. Furthermore, the finding that hyperammonemia in the absence of glutamine accumulation did not block ACh dilation indicates that ammonium ions do not block this NO-dependent response, consistent with the lack of effect of ammonia on arginine synthesis (29) and on NOS-dependent neurogenic vasorelaxation (18). Infusion of L-arginine did not fully restore dilation to ACh during hyperammonemia. Although L-arginine infusion did not significantly increase the overall tissue concentration of L-arginine, this dose of L-arginine increased plasma arginine concentration sevenfold and, therefore, was presumed to increase L-arginine availability within the endothelium. Nonetheless, one possible explanation for the incomplete restoration of the ACh response is that the infusion did not increase arginine concentration sufficiently in the subcellular compartment where NOS is localized and that higher infusion doses are required to fully restore the dilator response.

Hyperammonemia produced two- to fourfold increases in cerebral glutamine concentration. Glutamine synthetase is enriched in astrocytes (21), and much of the increase in glutamine is presumed to occur within astrocytes. However, hyperammonemia also produces increases in interstitial fluid, CSF, and plasma concentrations of glutamine (23,34). Therefore, the endothelium is expected to be exposed to increased glutamine during hyperammonemia.

The glutamine concentration was previously determined to be 0.25 mM in the CSF sampled from the cranial window of the rat (22). When the CSF concentration of glutamine was increased above 1 mM for 1 h without hyperammonemia, the ACh-evoked dilation was attenuated but was not reduced to the same extent as with hyperammonemia. Perhaps longer exposure to elevated glutamine is necessary to completely block the ACh response. Alternatively, other effects of hyperammonemia related to glutamine accumulation may have contributed indirectly to the impaired response.

In this regard, previous studies of this acute model of hyperammonemia have demonstrated that astrocyte swelling and increases in water content and ICP are ameliorated by MSO (32, 33,35). The present results showing that ICP measured in the cranial window increased in the hyperammonemic groups treated with vehicle, L-methionine, or L-arginine and that the increase was blunted by MSO/methionine treatment also support these earlier findings that cerebral edema is related to glutamine accumulation in astrocytes enriched in glutamine synthetase. Although the pial arteriolar response to ACh is endothelial dependent (25), loss of astrocyte function can impair endothelial-dependent responses under specific circumstances (38). Therefore, one needs to consider the possibilities that 1) astrocyte dysfunction associated with glutamine-dependent swelling might have secondary effects on endothelial-dependent responses distinct from direct effects of glutamine on the endothelium, and 2) increases in ICP may limit vasodilation.

The first possibility is supported by observations that extracellular K^+ activity, which is known to be regulated by astrocytes, increases to 12 mM in this model of hyperammonemia and that this increase is prevented by MSO treatment (30). Elevated extracellular K^+ activity might interfere with ACh vasodilation. In this case, part of the restoration of ACh dilation by MSO may be attributed to restoring astrocyte regulation of extracellular K^+ and part may be attributed to preventing increases in glutamine that affect endothelial function.

The second possibility, that mechanical effects of ICP will reduce cerebral perfusion pressure and increase baseline arteriolar diameter to an extent that limits ACh-evoked dilation, is not fully supported by the data. Increases in baseline diameter occurred over the 6-h salt infusion in all groups except the control group receiving NaAc after vehicle pretreatment. Similar increases in diameter have been noted previously in hyperammonemic rats (13,15). In the case of hyperammonemic rats treated with methionine, MSO/methionine, or arginine, the decrease in cerebral perfusion pressure in these groups probably contributed to the increase in baseline pial arteriolar diameter as an autoregulatory response. However, the dilator response to ACh was restored in the MSO/methionine group with NH₄Ac, which had a lower cerebral perfusion pressure (67 ± 6 mmHg) than the vehicle group with NH₄Ac (84 ± 14 mmHg). Therefore, the low perfusion pressure was not completely responsible for the impaired dilatory response in the other groups. Likewise, the ACh response was partially restored in the L-arginine-transfused group in which the cerebral perfusion pressure was also low (68 ± 10 mmHg).

Increases in glutamine and extracellular K⁺ activity associated with acute hyperammonemia do not affect all aspects of cerebral vascular reactivity. For example, pial arterioles still dilate in response to hypoxia (15) and constrict in response to the thromboxane agonist U46619 (13). The cerebral blood flow response to hypoxia is intact, and blood flow remains well autoregulated during arterial hypotension (14). Thus the effects on ACh reactivity are specific. However, hyperammonemia also reduces CO₂ reactivity and, like ACh reactivity, this reduction depends on increased glutamine and is partially restored by L-arginine infusion (15,22). Because the CO₂ response is modulated by neuronal NOS activity and the ACh response is dependent on endothelial NOS activity, some commonalities may exist in the effect of glutamine on these responses. However, differences also exist in that the CO₂ response is determined primarily by extracellular pH and the role of neuronally derived NO is that of a modulator in which neuronal NOS inhibition can attenuate but not abolish CO₂ reactivity. Thus factors in addition to suppressing NOS activity by glutamine may come into play in completely abolishing CO₂ reactivity in this model.

In addition to inhibiting glutamine synthetase, MSO increases methylation flux and consequently decreases S-adenosylmethionine (28). Seizures associated with MSO have been attributed to altered methylation because coadministration of L-methionine prevented the decrease in S-adenosylmethionine (26) and in the occurrence of seizures (10) without decreasing glutamine synthetase activity (27). In our experiments, L-methionine was coadministered with MSO to avoid the potential confounding influence of seizure activity. Our observation that the increase in cerebral glutamine concentration during hyperammonemia was prevented when L-methionine was coadministered with MSO indicates that MSO was still effective in reducing glutamine synthesis and accumulation. Increasing S-adenosylmethionine by injection of L-methionine can also lead to the formation of homocysteine, which, in turn, is capable of attenuating cerebrovascular dilation to ACh by a superoxide-dependent mechanism (8,40). Therefore, the improved ACh response in hyperammonemic rats pretreated with MSO/methionine, compared with vehicle treatment, cannot be readily explained by a secondary effect of L-methionine on increasing homocysteine. Nevertheless, the dilator response to ACh was moderately reduced 3 h after MSO/methionine treatment (before the NH₄Ac infusion began). This lower baseline ACh response may have been the result of increased homocysteine.

In conclusion, the results of the present experiment indicate that cerebrovascular dilation to ACh becomes impaired during acute hyperammonemia. This impairment is related to glutamine accumulation rather than to ammonium ions because preventing glutamine accumulation during hyperammonemia restored ACh vasodilation and elevation of glutamine alone attenuated ACh vasodilation. Therefore, these results represent the first findings that pathophysiological alterations in glutamine concentration can influence an endothelial-

dependent response in vivo. Improved vascular function with L-arginine infusion may help explain an early report showing that arginine administration to rats receiving lethal injections of ammonium sulfate reduced mortality (24).

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Fig. 1.

Cerebral tissue concentration ($\pm$ SD) of glutamine (*top*), glutamate (*middle*), and arginine (*bottom*) after a 6-h infusion of sodium acetate in groups pretreated with vehicle ($n = 5$) and L-methionine-*S*-sulfoximine (MSO) + L-methionine (Met; $n = 6$) and after a 6-h infusion of ammonium acetate in groups pretreated with vehicle ($n = 5$), Met ($n = 6$), MSO/Met ($n = 6$), and L-arginine (Arg; $n = 5$). * $P < 0.05$ from vehicle-sodium acetate group; + $P < 0.05$ from MSO/Met-ammonium acetate group. Arginine concentration in the L-arginine group was greater than in the Met-ammonium acetate group but was not significant from the other groups.

Fig. 2.

Mean arterial blood pressure (*top*), intracranial pressure (*middle*), and cerebral perfusion pressure (*bottom*) ($\pm$ SD) during ACh superfusion before (0 h) and at 6 h of infusion of sodium acetate in groups pretreated with vehicle ($n = 8$) and MSO/Met ($n = 8$) and after 6 h of infusion of ammonium acetate in groups pretreated with vehicle ($n = 8$), Met ($n = 6$), MSO/Met ($n = 8$), and Arg ($n = 8$). * $P < 0.05$ from 0-h value within group; + $P < 0.05$ from vehicle-ammonium acetate group at same time point.

Fig. 3.

Percent change in baseline pial arteriolar diameter (3SD) after 6 h of infusion of sodium acetate in groups pretreated with vehicle ($n = 8$) and MSO/Met ($n = 8$) and after 6 h infusion of ammonium acetate in groups pretreated with vehicle ($n = 8$), Met ($n = 6$), MSO/Met ($n = 8$), and Arg ($n = 8$). * $P < 0.05$ from vehicle-sodium acetate group; + $P < 0.05$ from vehicle-ammonium acetate group.

Fig. 4.

Percent change in pial arteriolar diameter ($\pm$ SD) during 30 M ACh superfusion before (0 h) and at 6 h of infusion of sodium acetate in groups pretreated with vehicle ($n = 8$) and MSO/Met ($n = 8$) and after 6 h of infusion of ammonium acetate in groups pretreated with vehicle ($n = 8$), Met ($n = 6$), MSO/Met ($n = 8$), and Arg ($n = 8$). * $P < 0.05$ from 0-h value within group; + $P < 0.05$ from vehicle-ammonium acetate group at same time point.

Fig. 5.

Percent change in pial arteriolar diameter ($\pm$ SD) during 10 μ M ACh superfusion vs. glutamine concentration in outflow of cranial window after 1 h of superfusion with 0 ($n = 7$), 0.3 ($n = 6$), 1 ($n = 7$), and 2 mM ($n = 7$) glutamine in inflow. * $P < 0.05$ from diameter at 0 mM inflow glutamine; $P < 0.05$ from diameter at 0.3 mM inflow glutamine.

Fig. 6. Percent change in pial arteriolar diameter ($\pm\text{SD}$) during 3 μM sodium nitroprusside superfusion vs. glutamine concentration in outflow of cranial window after 1 h of superfusion with 0 ($n = 7$), 0.3 ($n = 6$), 1 ($n = 7$), and 2 mM ($n = 7$) glutamine in inflow. There was no significant effect of glutamine.

Table 1
Arterial blood analysis before and at 6 h of sodium acetate or ammonium acetate infusion

	Sodium Acetate		Ammonium Acetate		
	Vehicle	MSO + methionine	Vehicle	Methionine	MSO + methionine
Ammonium, μM					
0 h	35 $\pm$ 15	127 $\pm$ 30 [†]	34 $\pm$ 10	26 $\pm$ 10	159 $\pm$ 58 [†]
6 h	45 $\pm$ 33	92 $\pm$ 35 [*]	563 $\pm$ 165 ^{*,†}	625 $\pm$ 94 ^{*,†}	607 $\pm$ 220 ^{*,†}
Osmolarity, mosmol/l					
0 h	305 $\pm$ 15	311 $\pm$ 11	307 $\pm$ 16	313 $\pm$ 17	299 $\pm$ 22
6 h	309 $\pm$ 11	314 $\pm$ 6	306 $\pm$ 13	301 $\pm$ 9	302 $\pm$ 16
pH					
0 h	7.42 $\pm$ 0.03	7.35 $\pm$ 0.02 [†]	7.40 $\pm$ 0.04	7.40 $\pm$ 0.04	7.35 $\pm$ 0.02 [†]
6 h	7.43 $\pm$ 0.04	7.36 $\pm$ 0.07 [†]	7.44 $\pm$ 0.04 [*]	7.34 $\pm$ 0.06 ^{*,†}	7.30 $\pm$ 0.03 ^{*,†}
Pco ₂ , Torr					
0 h	39 $\pm$ 2	39 $\pm$ 2	38 $\pm$ 2	38 $\pm$ 2	41 $\pm$ 4
6 h	40 $\pm$ 3	43 $\pm$ 3 [*]	41 $\pm$ 3 [*]	39 $\pm$ 3	43 $\pm$ 4
Hemoglobin, g/dl					
0 h	13.6 $\pm$ 2.0	13.6 $\pm$ 1.3	12.4 $\pm$ 1.6	13.2 $\pm$ 2.0	13.4 $\pm$ 1.9 [*]
6 h	11.9 $\pm$ 1.1	11.2 $\pm$ 1.1	11.9 $\pm$ 1.0	12.8 $\pm$ 0.9	10.6 $\pm$ 1.1
Arginine					
					35 $\pm$ 9
					665 $\pm$ 222 ^{*,†}
					307 $\pm$ 17
					303 $\pm$ 18
					7.38 $\pm$ 0.01
					7.34 $\pm$ 0.07 [†]
					39 $\pm$ 3
					38 $\pm$ 3
					14.5 $\pm$ 1.2
					12.2 $\pm$ 1.3 [*]

Values are means $\pm$ SD. MSO, L-methionine-S-sulfoximine.

^{*} $P < 0.05$ from corresponding value at 0 h by paired t -test.

[†] $P < 0.05$ from corresponding value in vehicle + sodium acetate group by ANOVA and Newman-Keuls test.

Table 2

Effects of glutamine superfusion in cranial window

	Inflow Glutamine Concentration, mM			
	0	0.3	1	2
Outflow glutamine, μ M	240 $\pm$ 35	547 $\pm$ 23	1325 $\pm$ 298	1986 $\pm$ 87
Baseline arteriolar diameter, %change	-0.1 $\pm$ 3.9	-2.6 $\pm$ 2.9	4.2 $\pm$ 5.8	-0.4 $\pm$ 3.1
Arterial Pco ₂ , Torr	39 $\pm$ 2	39 $\pm$ 3	39 $\pm$ 1	38 $\pm$ 3
MABP at 60 min of glutamine superfusion, mmHg	104 $\pm$ 12	109 $\pm$ 10	114 $\pm$ 13	113 $\pm$ 9
MABP during acetylcholine superfusion, mmHg	105 $\pm$ 15	113 $\pm$ 9	115 $\pm$ 12	116 $\pm$ 8

Values are means $\pm$ SD. MABP, mean arterial blood pressure.