# Selected brain amino acids and ammonium during chronic hypercapnia in conscious rats

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WEYNE, J., F. VAN LEUVEN, H. KAZEMI, AND I. LEUSEN. *Selected brain amino acids and ammonium during chronic hypercapnia in conscious rats.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 44(3): 333–339, 1978. —Glutamic, aspartic, and γ-aminobutyric acid (GABA), glutamine, and ammonium were measured in the brains of unanesthetized normocapnic and hypercapnic (10% $CO_2$; 5 min to 3 wk) rats. Hypercapnia increased glutamine and GABA and decreased glutamic and aspartic acids. Changes occurred within 1 h and were maintained during the observation period of 3 wk. On return to normocapnia amino acid concentrations were almost normal after 1 h. Based on the time course it is concluded that intracerebral hypercapnia is more likely the stimulus for change than acidosis. Ammonium content was unchanged for at least 1 h after the onset of hypercapnia but increased thereafter. Experiments in which glutamine synthesis by brain was impeded by inhibiting the enzyme glutamine synthetase favor the hypothesis that the rise of ammonium content in hypercapnia is initially not seen because of increased glutamine synthesis. The changes observed may have a role in metabolic pH homeostasis of brain tissue and may also be relevant to the modified brain excitability in hypercapnia.

acid-base balance; brain amino acids; hypercapnic encephalopathy

DIFFERENT FUNCTIONS HAVE BEEN assigned to the high concentration of several free amino acids in the brain. They participate in $NH_4^+$ metabolism[1] and $H^+$ homeostasis in the central system (CNS) and they may alter neuronal activity by influencing membrane characteristics and by their possible function as neurotransmitters.

Brain amino acid concentration can be altered both acutely and chronically in response to a number of stimuli (for a review see Ref. 8). In earlier studies in anesthetized rats we reported that acute hypercapnia increases glutamine and decreases glutamic acid content of the brain (29); these changes are similar to those reported by others in dogs (10) and rats (5, 15).

The present study was undertaken to compare the effect of prolonged hypercapnia in conscious rats with data obtained in acute hypercapnia. In addition to glutamic acid and glutamine, aspartic acid, γ-aminobutyric acid (GABA), and $NH_4^+$ were also measured. To evaluate the relationship between the observed changes in glutamic acid, glutamine, and $NH_4^+$, the effect of acute hypercapnia was also investigated when glutamine synthesis was impeded.

Acids such as lactic, pyruvic, and α-ketoglutaric are also modified in acute hypercapnia, but return toward normal values during prolonged hypercapnia (15). A different behavior of the amino acids could be important in the discussion of the possible role of the amino acid changes in pH regulation in the brain and in the pathogenesis of hypercapnic encephalopathy.

## METHODS

### *Materials*

*Animals.* The experiments were performed on conscious adult male rats (age 7–11 mo, body weight 280–300 g) of the inbred laboratory colony. The number of animals in each experimental group is given in the legends to the figures.

*Chemicals.* The ion exchange resins were obtained from Bio-Rad Laboratories, Richmond, Calif. Amino acids and DL-methionine-DL-sulfoximine (MSO) were purchased from Sigma Chemical Co., St. Louis, Mo. Enzymes were obtained from Boehringer, Mannheim, Germany. Other chemicals were obtained from E. Merck, Darmstadt, Germany.

### *Biological Procedures*

The influence of hypercapnia was investigated by keeping the rats in an environmental chamber flushed with $CO_2$-air gas mixture (≃ 10% vol/vol). Control animals were handled in a similar way except that the chamber was flushed with air. Five series of experiments were performed.

*Series I (Effect of short term hypercapnia on brain amino acids).* In this series hypercapnia was imposed from 5 min to 24 h. Under ether anesthesia a polyethylene catheter was introduced in the tail artery and directed toward the aorta. The catheter was tunneled subcutaneously and exteriorised at about 3 cm from the end of the tail. The animals were confined in small individual cages of wire netting. A small perforated disk was pushed over the tail and sutured to the skin to keep the tail's end and the catheter out of the cage. Fixation of the tail limited the movement of rats only to a minor extent. After the rat's recovery from anesthe-

[1] In this paper $NH_4^+$ stands also for the sum of $NH_3$ (ammonia) and $NH_4^+$ (ammonium). $NH_3$, $NH_4^+$, and $H^+$ are in equilibrium according to the reaction $NH_3 + H^+ \rightleftarrows NH_4^+$. The $pK_a$ of this reaction is 9.3; hence in the range of physiological pH values essentially all the material is in the form of $NH_4^+$.

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sia, individual cages were placed (three at a time) in the environmental chamber. The walls and the floor of the chamber were made of polyurethane to allow for its subsequent filling with liquid nitrogen. The chamber was closed by a Lucite cover and the three arterial cannulas were brought out through the holes in the cover. The chamber (capacity, 13 liters) was flushed (flow 1.5 l/min) with ambient air or with the $CO_2$-air mixture. To prevent accumulation of $NH_3$ from urine a petri dish containing sulfuric acid was placed in the chamber. When the chamber was flushed with air, accumulation of $CO_2$ was prevented by placing a petri dish containing soda lime in the chamber. The animals had free access to water and commercial food.

In six different groups of rats hypercapnia was induced for 5, 10, or 20 min and 1, 3, or 24 h. The animals which were to be exposed to $CO_2$ for less than 24 h were nevertheless kept in the chamber for 24 h and breathed $CO_2$ only at the end of the confinement in the chamber. In a control group the chamber was flushed for 24 h with air. Five minutes before the end of the experiment arterial blood (0.6 ml) was sampled anaerobically, for measurement of $P_{O_2}$, pH, and $P_{CO_2}$, in syringes in which the dead space (30 $\mu$l) was filled with a heparin solution. Dilution of blood with heparin was without effect on measurements as controlled in separate experiments. Blood was not sampled in the animals exposed to hypercapnia for 5 and 10 min.

At the end of the experiment the chamber was opened and the animals killed by filling the chamber rapidly with liquid nitrogen. When all bubbling ceased the frozen animals were removed and stored in liquid nitrogen until further processing (≈15 min).

The procedure used to kill the animals induces anesthesia very quickly. Sudden skin freezing very rapidly produces local anesthesia (cf. human skin surgery) and as the cold wave moves through the tissues, it blocks excitability. When the cold wave attains the brain cortex, general anesthesia is induced when temperature drops to 30–28°C (cf. 14). Separate experiments have shown us that such cortex temperature levels were attained in pentobarbital sodium-anesthetized rats within 10 s. Brain cortex was frozen within 15 s and although it takes ≈90 s to freeze the deepest parts of the brain with this procedure (21), it is unlikely that the amino acid levels were influenced by these time delays. Indeed, fairly identical amino acid contents were reported after decapitation and freeze-blowing (25) where the period of autolysis is respectively longer and shorter than in our conditions. There are also no differences in the brain content of lactic acid and $\alpha$-ketoglutaric acid between areas frozen within 15 and at 90 s (21).

*Series II (Effect of long term hypercapnia on brain amino acids).* In this series two groups of rats were exposed to hypercapnia for 7 and 21 days. To allow comparison with *series I,* this series also included a group exposed to hypercapnia for 1 day. All rats were kept for 20 days in a large Lucite environmental chamber flushed with air or with the same $CO_2$-air mixture as in *series I.* Absorption of $NH_3$ and, when necessary, of $CO_2$ in the chamber was similar to *series I.* The animals had free access to water and food. The chambers were opened three times per week for cleaning during which the rats stayed in room air (≈5 min).

After 20 days the animals were removed from the chamber, the tail artery was cannulated under ether anesthesia, and the rats confined to individual cages and placed in the polyurethane chamber as in *series I.* Between induction of anesthesia and closing of the chamber the animals breathed room air (≈30 min). The chamber was flushed for 24 h with the $CO_2$-air mixture. At the end of the experiment blood was sampled and the animals killed as in *series I.*

*Series III (Effect of acute return to normocapnia on brain amino acids).* A group of rats was kept for 21 days in a hypercapnic atmosphere as in *series II,* after which they breathed room air for 1 h. Arterial blood was sampled and the animals were killed as in *series I* and *II.* A control group where the rats were treated similarly but exposed to air alone was included in this series.

*Series IV (Effect of duration of hypercapnia on brain $NH_4^+$).* The groups of rats were exposed to hypercapnia for 1 and 24 h as in *series I.* This series also included a control group which was not exposed to $CO_2$. The animals were killed at the end of the experiment as in the other series.

*Series V (Effect of MSO on brain amino acids and $NH_4^+$ during hypercapnia).* To examine the relations between the glutamic acid fall and the glutamine rise and between $NH_4^+$ and glutamine in acute hypercapnia, two groups of rats were treated with 80 mg/kg ip MSO, a nonconvulsive dose, to inhibit glutamine synthesis in the brain (12); 24 h later the animals were confined to the polyurethane chamber. For the first group the chamber was flushed for 1 h with air; for the second group it was flushed with a 10% $CO_2$-in-air mixture. In addition, this series included two control groups who received saline injections instead of MSO. The animals were killed as in the other series.

Inhibition of glutamine synthetase was confirmed by measuring, in separate rats, its activity in the brain by a colorimetric method (12). The activity was 33.6 ± 1.3 U/g wet wt in sham-treated rats and 12.3 ± 1.2 U/g wet wt in MSO-treated rats (mean ± SEM, 6 rats in each group, $P < 0.001$).

### *Analytical Methods*

*Blood.* $P_{O_2}$, pH, and $P_{CO_2}$ were measured at 37°C with appropriate electrodes (Radiometer, Copenhagen, Denmark). Plasma [$HCO_3^-$] was calculated from the Siggaard-Andersen nomogram (23).

*Brain.* PREPARATION OF BRAIN SAMPLES. The cerebral hemispheres were weighed in their frozen state, immediately after removal from the skull. They were transferred to a homogenization vial and stored at −20°C until further processing (≈30 min). For each gram of tissue, 5.2 ml ice-cold trichloroacetic acid (10% wt/vol in $H_2O$) was added and the tissue immediately homogenized in a blendor for 2 min at 0°C. The homogenates were centrifuged at 1,500 *g* for 20 min at 0°C. The clear supernatant was neutralized with 1 N NaOH to pH 7.0 ± 0.1. The neutral extract was used for further analysis.

DETERMINATION OF AMINO ACIDS. Amino acids were

separated with a chromatographic method (2). A portion of the neutralized extract was applied to a 0.7 × 25-cm column of AG 1 × 4 (200–400 mesh) in the acetate form. Elution with $H_2O$, 0.05 N, and 0.3 N acetic acid resulted in three fractions: the first was a mixture of neutral and basic amino acids, whereas the second and third fractions contained glutamic acid and aspartic acid, respectively. The first fraction was brought to pH 2.5 with 1 N HCl and fractionated on a 0.7 × 13-cm column of AG 50 W × 4 (200–400 mesh). Elution with 0.10 M sodium citrate buffer pH 3.25 resulted in a fraction containing glutamine, while GABA was eluted from the column with 0.12 M sodium citrate buffer pH 5.30.

The glutamic acid, aspartic acid, and GABA fractions were checked for purity by paper electrophoresis. No ninhydrin positive contaminants were detected and the concentrations of these amino acids were subsequently quantified with the ninhydrin colorimetric method (18).

The glutamine was hydrolyzed (2 N HCl, 100°C, 120 min) and the resulting glutamic acid fluorimetrically determined with an enzymic method (6). A standard mixture of the amino acids was chromatographed in identical circumstances with each run. Recoveries were 99.0 ± 1.5% (range 94–103%) for glutamic acid, 96.0 ± 3.0% (89–99%) for glutamine, 100.0 ± 2.5% (95–104%) for aspartic acid, and 98.0 ± 3.0% (91–105%) for GABA (mean ± SD, $n$ = 16 each).

DETERMINATION OF $NH_4^+$. $NH_4^+$ was measured with an ion-change method (11) adapted to a small-column procedure.

Concentrations are expressed per unit wet brain weight. Results of the experiments were subjected to statistical analysis by the Student $t$-test.

## RESULTS

### *Acid-Base Changes in Arterial Blood During Hypercapnia (Series I and II)*

The mean arterial $P_{CO_2}$, pH, plasma [$HCO_3^-$], and $P_{O_2}$ in one group of control rats are compared to those in rats exposed to $CO_2$ for varying periods of time in Fig. 1. The mean $P_{aCO_2}$ was approximately 80 Torr in all hypercapnic groups, which is about 40 Torr higher than in the control group. Arterial pH was lowest 20 min after the onset of hypercapnia and increased progressively until 7 days of hypercapnia and remained about the same up to 21 days at a value still lower than control. The [$HCO_3^-$] increased progressively in the first 7 days of hypercapnia but remained unchanged thereafter. The mean $P_{aO_2}$ was 95 Torr in the control group and 105 Torr in the hypercapnic groups; this higher value in probably the result of an increased ventilation per unit metabolic rate.

### *Brain Amino Acids During Hypercapnia (Series I and II)*

The mean values for brain glutamic acid, glutamine, GABA, and aspartic acid during varying periods of hypercapnia are depicted in Fig. 2. During the first 5 min of hypercapnia practically no changes were observed. By 1 h glutamic acid had decreased by 2 mmol/kg or 20%, and remained essentially constant afterward. Brain glutamine increased during the first 20 min of hypercapnia by 1.2 mmol/kg or 33%. Between 20 min and 3 h of hypercapnia, glutamine was constant. After 7 days it increased by 2.2 mmol/kg or 65% and remained at this level till the 21st day. GABA increased by 0.42 mmol/kg or 23% after 20 min of hypercapnia and remained constant afterwards. Although the mean concentration after 1 day of hypercapnia in *Series II* is 0.17 mmol/kg or 7% ($P < 0.001$) lower than in *series I*, the GABA concentration remained essentially constant between 1 and 21 days of hypercapnia. Brain aspartic acid content decreased during the 1st h of hypercapnia by 0.56 mmol/kg or 31% and remained essentially constant afterward.

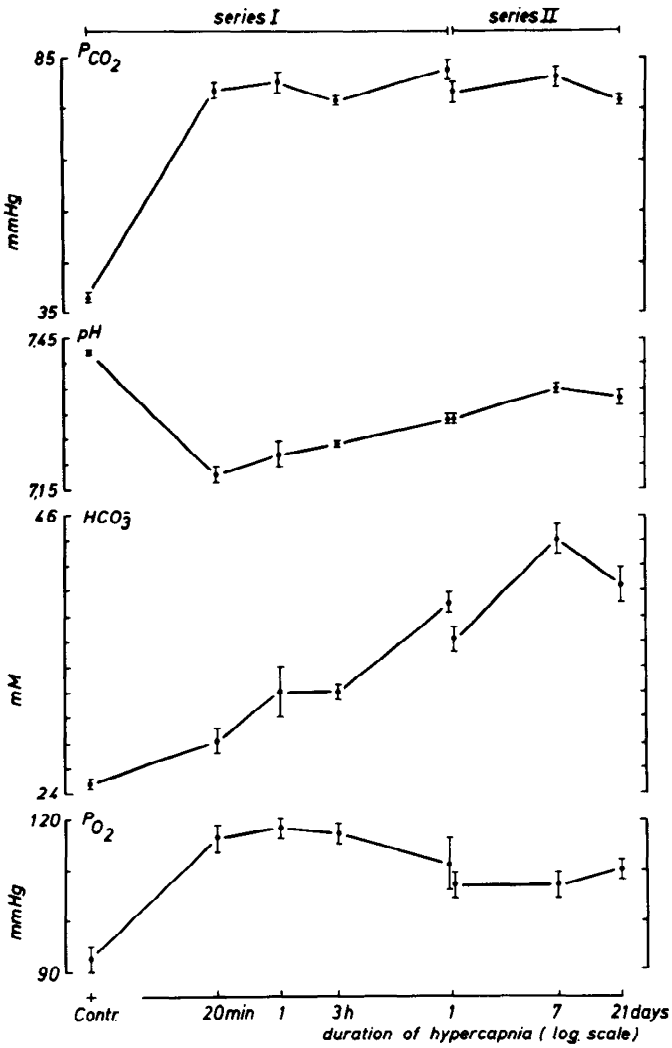


FIG. 1. Blood gases and acid-base balance. Carbon dioxide tension, pH, plasma $HCO_3^-$ concentration, and oxygen tension in the arterial blood of unanesthetized rats in normocapnia (Contr) and in hypercapnia for different periods of time (log scale). Means ± SE in different groups of rats for each point ($n$ = 6 in each group).

### *Brain Amino Acids After Acute Return to Normocapnia (Series III).*

Mean $P_{aCO_2}$ and brain content of the four amino acids in a group of rats that were kept for 1 h in ambient air after 3 wk of hypercapnia are shown in Fig. 3. Control values are from a group of rats that

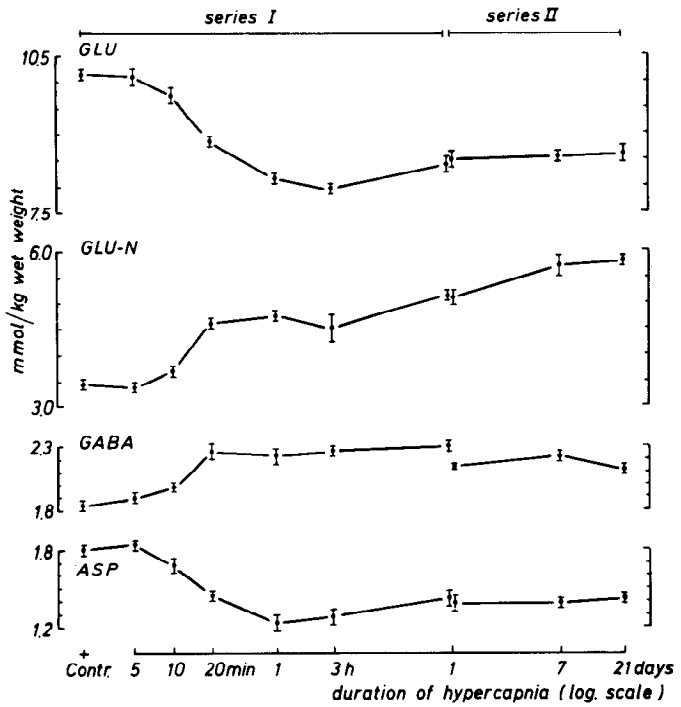


FIG. 2. Amino acids in brain. Concentration of glutamic acid (GLU), glutamine (GLU-N), γ-aminobutyric acid (GABA), and aspartic acid (ASP) in brain of unanesthetized rats. Same animals as in Fig. 1 except for the 5- and 10-min groups. See Fig. 1.

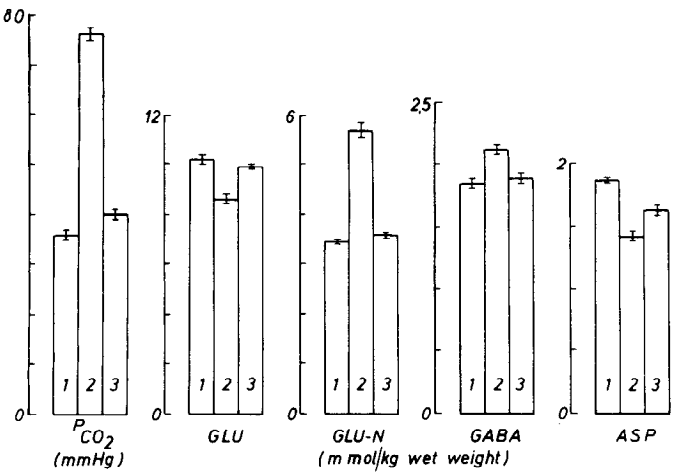


FIG. 3. Arterial $P_{CO_2}$ and amino acids in brain. Means ± SE in 3 groups of unanesthetized rats: *1*) rats breathing room air ($n$ = 6); *2*) rats maintained 3 wk in hypercapnia ($n$ = 6)—same group as in Figs. 1 and 2; and *3*) rats maintained 1 h in room air after 3 wk of hypercapnia ($n$ = 8).

were kept in the chamber on room air. For comparison values from rats after 3 wk of hypercapnia (*series II*) are also shown. Mean $P_{aCO_2}$ in the hypercapnic rats after being on room air for 1 h dropped but was still 4 Torr higher than in the control air-breathing group ($P < 0.05$) and 7 Torr higher than in the control group of *series I* and *II* ($P < 0.001$). The changes in brain amino acid concentration were rapidly reversible in association with the fall in $P_{aCO_2}$. Concentrations of glutamic acid, glutamine, and GABA after 1 h normocapnia were not statistically different from control values. Aspartic acid concentration after 1 h normocapnia was clearly higher than the level after 3 wk of hypercapnia ($P < 0.01$), but still lower than the control value ($P < 0.001$).

### *$NH_4^+$ in the Brain During Hypercapnia (Series IV and V)*

In control air-breathing rats the mean $NH_4^+$ content of the brain was 0.185 and 0.203 mmol/kg, respectively, in *series IV* (Table 1) and *series V* (Fig. 4) ($P > 0.1$). In both series, 1 h of hypercapnia had no effect on brain $NH_4^+$ (Table 1 and Fig. 4) but 1 day of hypercapnia increased brain $NH_4^+$ by 36% ($P < 0.001$).

### *Influence of MSO on the $NH_4^+$ and Amino Acid Contents in the Brain During Acute Hypercapnia (Series V)*

*Effect of MSO during normocapnia.* With administration of MSO there was decreased concentration of glutamic acid (2.9 mmol/kg or 28%; $P < 0.001$), glutamine (1.7 mmol/kg or 52%; $P < 0.001$) and aspartic acid (0.9 mmol/kg or 49%; $P < 0.001$) in the brain (Fig. 4). Brain GABA content was unaltered while brain $NH_4^+$ was clearly increased (0.09 mmol/kg or 45%; $P < 0.001$).

*Effect of MSO on the changes during acute hypercapnia.* Whereas in normal rats the concentration of $NH_4^+$ in the brain was not increased after 1 h of hypercapnia (see above), there was an increase of 0.06 mmol/kg or 20% ($P < 0.02$) in rats treated with MSO (Fig. 4).

On the other hand, after MSO administration there was no significant change in glutamine and aspartic acid concentration during acute hypercapnia. Compared to the normocapnic control value in each series, hypercapnia in the MSO-treated rats provoked an insignificant increase in glutamine (0.25 mmol/kg or 16%; $P >$

TABLE 1. *$NH_4^+$ in the brain of unanesthetized rats*

| Condition | $NH_4^+$, mmol/kg |
|---|---|
| Control, room air | 0.185 ± 0.007 |
| 1 h 10% $CO_2$ in air | 0.174 ± 0.004* |
| 24 h 10% $CO_2$ in air | 0.252 ± 0.014† |

Values are means ± SE; 12 rats in each group. * No significant change. † $P < 0.001$.

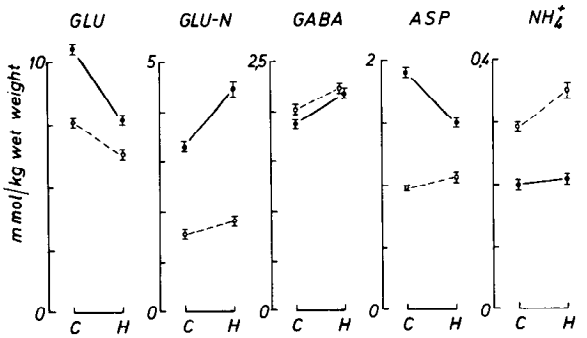


FIG. 4. Amino acids and ammonium in brain. Means ± SE in different groups of unanesthetized rats during normocapnia (C) and after 1 h of hypercapnia (H). Results obtained in 2 groups of rats ($n$ = 8 each) treated with methionine sulfoximine (80 mg/kg ip) 24 h before the experiment (*open symbols* and *interrupted lines*) and in 2 groups ($n$ = 6 each) of sham-treated (NaCl 0.9%) rats (*black symbols* and *full lines*).

0.1) whereas in normal rats the increase amounted to 1.2 mmol/kg or 35% ($P < 0.001$). There was an insignificant increase in aspartic acid (0.10 mmol/kg or 10%; $P > 0.05$) after 1 h of hypercapnia in MSO-treated rats, while in normal rats there was a decrease by 0.40 mmol/kg or 21% ($P < 0.001$).

The decrease in brain glutamic acid concentration and the increase in GABA observed during acute hypercapnia were maintained in MSO-treated rats. Brain glutamic acid decreased by 2.8 mmol/kg or 27% in normal rats ($P < 0.001$) and by 1.3 mmol/kg or 17% in MSO-treated rats ($P < 0.001$). GABA increased by 0.29 mmol/kg or 16% ($P < 0.01$) in normal rats and by 0.19 mmol/kg or 9% ($P < 0.02$) in MSO-treated rats.

## DISCUSSION

Decrease of brain glutamic acid and increase of glutamine have been reported in acute hypercapnia in anesthetized rats and dogs (10, 29) and the time course of the changes of these amino acids and also of aspartic acid and GABA during relatively short (from 2 min to 3 h) periods of hypercapnia was recently described in anesthetized rats (4, 5). Almost no data are available on the effect of chronic hypercapnia, nor how anesthesia may influence levels of free amino acids. Increased brain glutamine levels during chronic hypercapnia were reported in rats (9) while brain glutamic acid remained low between 15 min and 3 days (15). The present experiments extend that observation and also show that the other amino acids do not return to normal levels during 3 wk of hypercapnia.

The increase in glutamine and the decrease in glutamic acid are quantitatively the most important changes and very similar in the beginning of hypercapnia (Fig. 2), suggesting interconversion of glutamic acid into glutamine. The MSO experiments however indicate that the glutamic acid fall is not necessarily related to the rise in glutamine. The changes in GABA and aspartic acid also indicate that hypercapnia has a greater influence than merely enhancing interconversion of glutamic acid into glutamine. Possibly in hypercapnia glutamic acid is used as a fuel for respiration in the tricarboxylic acid cycle (17).

The amino acid concentrations studied do not show normalization during three weeks of hypercapnia. In this respect they differ markedly from lactic, pyruvic, and $\alpha$-ketoglutaric acid whose concentrations in brain, after a decrease to 35–55% of the control level at 45 min of hypercapnia, return to 80–90% of control level when hypercapnia is maintained during 3 days (15).

Since it is known that intracellular pH in the brain also progressively normalizes during sustained hypercapnia (1, 16), the persistence of the amino acid changes favors the concept that hypercapnia rather than acidosis is the stimulus for the observed effects.

### *What Biochemical Pathways Might be Involved?*

$CO_2$ fixation at the pyruvic acid-oxaloacetic acid level (19, 26) and rapid exchange between tricarboxylic acid (TCA) cycle and amino acid metabolism at the $\alpha$-ketoglutaric acid-glutamic acid level are typical features of brain metabolism (Fig. 5). The brain continuously synthesizes glutamine (7), in which $NH_3$ availability plays an important role. In $NH_3$ intoxication increased amidation of glutamic acid results in increased glutamine content (26). In hypercapnia also amidation of glutamic acid is enhanced (30) and indications of increased $NH_3$ availability have been found. Brain $NH_4^+$ content is increased in acute severe hypercapnia (4, 17) and also in prolonged mild hypercapnia (Table 1). In acute mild hypercapnia no significant increase of $NH_4^+$ (Table 1, cf. 7) was found, but increased $NH_3$ generation need not necessarily increase the $NH_4^+$ content provided the capacity of the amidation reaction is sufficient. This concept finds support in the MSO experiments which show that mild acute hypercapnia causes a rise of $NH_4^+$ content when amidation of glutamic acid is impeded.

The increased ammoniagenesis during hypercapnia most probably occurs in the CNS itself as glutamine in the CNS starts to rise when the $NH_4^+$ content in the arterial blood is unchanged (10). $NH_3$ is probably derived from free amino acids either present initially or released by proteolysis during hypercapnia (17). Glutamic acid is one of the candidates although it normally enters the TCA cycle through initial transamination

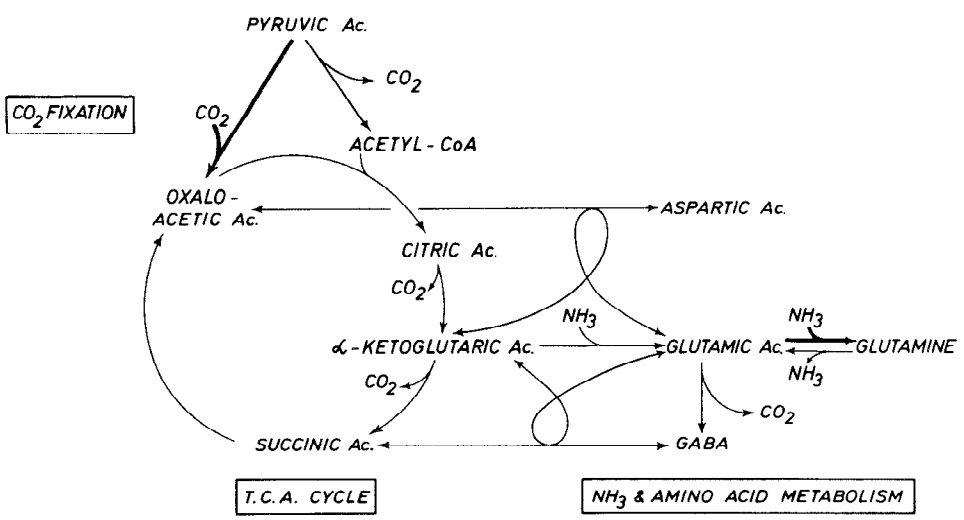


FIG. 5. Tricarboxylic acid cycle (nonrelevant intermediates are omitted) and its relations to amino acid metabolism and $CO_2$ fixation. In hypercapnia amidation of glutamine acid and $CO_2$ fixation are enhanced (*thick lines*).

(24), but in hypercapnia the fall in $\alpha$-ketoglutaric acid (4, 5) might induce deamination (27).

In addition to the amidation reaction, $CO_2$ fixation is also increased in hypercapnia (20); nevertheless the brain contents of all metabolites between oxaloacetic acid and glutamic acid decrease (4, 5). Such increased $CO_2$ fixation prevents depletion of TCA cycle intermediates and glutamic acid in $NH_3$ intoxication (26); in hypercapnia the mechanism is only partially effective.

### *Possible Consequences of the Changes in the Amino Acids*

Factors controlling intracellular pH ($pH_i$) in the brain in hypercapnia were reviewed recently (22). They include the following. *1*) Physicochemical buffering, which in hypercapnia depends on the existence of non-$HCO_3^-$ buffers; intracellular [$HCO_3^-$] increase goes hand in hand with an equimolar decrease in the nonbicarbonate buffer content and the concentration of the buffer base (sum $HCO_3^-$ and non-$HCO_3^-$ buffers) remains constant. *2*) Consumption of nonbuffer acids, which increases the concentration of the buffer base and to a smaller extent of $HCO_3^-$. *3*) Addition of $HCO_3^-$ from other compartments by "transmembrane flux."

That changes in the production of acids during $P_{CO_2}$ alterations contribute to regulation of brain [$HCO_3^-$] was first described in acute hypocapnia where the fall in [$HCO_3^-$] exceeded physicochemical buffering. The effect was explained by the rise of lactic acid in brain (13, 28). In acute hypercapnia too the [$HCO_3^-$] rise in brain exceeds physicochemical buffering due to decrease of acids, mainly glutamic acid (22).

The effect of the amino acid changes on the buffer base in the present experiments is determined by the change in the sum of the concentrations of glutamic and aspartic acids (GABA and glutamine are zwitterions with practically no net charge in the physiological pH range). The mean value decreased by 0.5, 1.6, 2.6, and 2.7 mmol/kg, respectively, at 10 min, 20 min, 1 h, and 3 h after onset of hypercapnia, which is in good agreement with Siesjö's data at 45 min (22). As brain $pH_i$ is normal again at 3 h but not at 1 h of hypercapnia (1), and the acid fall at 3 h equals that at 1 h, complete normalization of $pH_i$ depends upon other mechanisms. Between 1 and 3 wk of hypercapnia the fall of glutamic and aspartic acid still amounts to 2.0 mmol/kg. Glutamic and aspartic acids thus contribute to regulation of $pH_i$ in acute hypercapnia by their fall in concentration, which actively increases buffer base and [$HCO_3^-$], and in chronic hypercapnia by their steady low concentration which maintains passively the high [$HCO_3^-$] and buffer base.

Decreased brain excitability (31) without changes in oxygen consumption (17) or phosphorylation energy state (3, 17) has been described in acute hypercapnia with $P_{CO_2}$'s comparable to those in the present experiments. Changes in the brain content of certain amino acids which are possible neurotransmitters (increase of GaBA, inhibitory, and decrease of glutamic and aspartic acids, excitatory) may be important for decreased brain activity in hypercapnic encephalopathy (for further discussion see ref. 30). Long-term experiments where excitability, amino acid metabolism, and acid base balance are simultaneously investigated are needed to substantiate this possibility.

H. Kazemi was on leave of absence from the Medical Services (Pulmonary Unit), Massachusetts General Hospital and Department of Medicine, Harvard Medical School, Boston, U.S.A.

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