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GLUTAMATE AND GLUTAMINE IN THE BRAIN OF THE NEONATAL RAT DURING HYPERCAPNIA

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(2 figures)

In order to study the influence of hypercapnia on the content of glutamate and glutamine in the developing brain, pregnant rats and their offspring were kept in CO_2 rich (6-10 %) atmosphere and the litters were killed at different ages between 4 and 28 days. In the hypercapnic rats the content of both amino acids in the brain increases with age with almost the same time course as in normocapnic rats. At any age the glutamate content is lower in the hypercapnic animals than in control rats, whereas the glutamine content, beyond the first 8 days of life is increased. Both effects are rapidly reversible on return to air breathing. Although the glutamate-glutamine system is in full development, the influence of hypercapnia can be compared to that observed in adult rats. Hypercapnia did not change the glutaminase and the glutamine synthetase activity of the brain.

In different species the morphological, behavioural and functional maturation of the brain during neonatal life is accompanied by a progressive increase in the content of glutamate and of its metabolic derivatives aspartate, γ-aminobutyrate and glutamine (VERNADAKIS & WOODBURY, 1962; BERL & PURPURA, 1963; MIHAILOVIC & KRZALIC, 1964; DRAVID & JILEK, 1965; AGRAWAL *et al.*, 1966; RAMIREZ DE GUGLIELMONE & GOMEZ, 1966; BAYER & MC MURRAY, 1967; PATEL & BALAZS, 1970). Together with the increasing concentrations of these amino-acids an increasing activity of several key enzymes for the control of their metabolism *e.g.* glutamine synthetase (WU, 1964; BERL, 1966) and glutaminase OERIU *et al.*, 1963) was described.

On the other hand it was observed that, at least in adult rats, the glutamine content of the brain markedly increases during acute (50 min) hypercapnia while the glutamate content decreases (VAN LEUVEN & WEYNE, 1972; WEYNE *et al.*, 1973; *cf.* MESSETER & SIESJÖ, 1971*a*). In unanaesthetized rats both modifications fully occur within 1 hour and remain practically unchanged for at least 3 weeks of sustained hypercapnia (WEYNE *et al.*, 1976).

The aim of the present experiments was to investigate the possible influence of hypercapnia upon the contents of glutamine and glutamate in the developing rat brain where these contents are rapidly changing with age. It is known that these contents can be modified by *e.g.* neonatal thyroidectomie (RAMIREZ DE GUGLIELMONE & GOMEZ, 1966). A preliminary communication of the results obtained was published (VAN LEUVEN *et al.*, 1973). Besides the influence of hypercapnia on the levels of glutamine and glutamate, the activity *in vitro* of the enzymes, glutaminase and glutamine synthetase, responsible for their interconversion, was also investigated.

METHODS AND PROCEDURES

White rats of an inbred strain were used. Two groups of pregnant female rats were placed in environmental chambers, approximately one week before the expected day of delivery. The control group was placed in air, the experimental group in a CO_2 rich atmosphere. The CO_2 concentration in the chamber amounted to 6 % but was increased to 10 % 4 days after delivery without harming the animals. Food and water were supplied *ad libitum*.

Experimental and control litters were kept with their mothers until sacrificed (see below). Because of the experimental protocol it is not possible to study littermates in normal and in hypercapnic conditions; however, littermates were killed at different ages whenever this was possible. Littermate controls were not considered necessary because of the genetic homogenity of the strain of our rats.

Determination of glutamine and glutamate.

The brains of the control and experimental litters were studied at 4, 8, 12, 16, 24 and 28 days after birth. The animals were killed

by immersion in liquid nitrogen; the frozen brain (whole skull content) was removed, weighed and immediately homogenized in cold ethanol-water (3 : 1, v : v). Determinations of glutamine and glutamate in individual brains were made with enzymatic methods as described previously (WEYNE *et al.*, 1973) and expressed as μmol/g wet brain weight.

Determination of the activity in vitro *of glutaminase (E.N. 3.5.1.2) and glutamine synthetase (E.N. 6.3.1.2).*

The brains of the control and experimental litters were studied 4 and 24 days after birth. The animals were killed by decapitation, the brains removed, rinsed and homogenized in distilled H_2O. In the case of 4-day-old rats two brains were pooled.

Determination of the activities of glutaminase and glutamine synthetase was done by classical methods (SAYRE & ROBERTS, 1958; WU, 1963), with the only exception that in the glutaminase-assay, the glutamate formed was measured instead of NH_4^+. The results are expressed as units per gram wet brain weight, one unit being the amount of enzyme which synthetizes 1 μmol of reaction product per hour under the condition specified.

RESULTS

No significant difference was noted in pups per litter and in mortality rate between normal and hypercapnic litters. Furthermore, for the first 4 days after birth no significant difference was noted in brain and body weight (Table I). Thereafter, body weight in the hypercapnic rats showed a slower increase with age and had a lower value than the control animals during the entire experimental period. Beyond eight days after birth brain weight was also significantly lower in the hypercapnic animals. Whereas the brain to body weight ratio in normal rats gradually decreased with age, it initially increased in the hypercapnic litters to 12 days after birth. Thereafter the ratio decreased but a lower value than at the age of 4 days was observed only at the very end of the experimental period.

Glutamine and glutamate content of the brain.

In Figure 1 the brain levels of glutamate are plotted in function of age (mean ± S.E.M.). In the control rats the glutamate level

TABLE I. *Body and brain weights and ratio of brain to body weight of rats during postnatal development. Influence of hypercapnia.* Each value is the average of 6 rats ± S.E.M.

Age (days)	Control			Hypercapnia		
	Body weight (g)	Brain weight (g)	Brain / Body	Body weight (g)	Brain weight (g)	Brain / Body
4	10.0 ± 0.0	0.504 ± 0.037	0.050 ± 0.004	10.2 ± 0.4	0.444 ± 0.045	0.044 ± 0.004
8	15.0 ± 0.4	0.668 ± 0.045	0.049 ± 0.004	13.2 ± 0.3	0.617 ± 0.035	0.048 ± 0.003
12	21.8 ± 0.5	0.836 ± 0.051	0.038 ± 0.002	9.8 ± 0.5	0.578 ± 0.010	0.059 ± 0.003
16	33.5 ± 0.8	1.206 ± 0.066	0.036 ± 0.002	21.5 ± 0.4	0.972 ± 0.032	0.045 ± 0.002
20	32.5 ± 1.2	1.109 ± 0.032	0.034 ± 0.002	22.8 ± 0.5	0.955 ± 0.042	0.042 ± 0.002
24	77.6 ± 4.0	1.335 ± 0.060	0.018 ± 0.001	22.2 ± 1.2	1.017 ± 0.035	0.046 ± 0.003
28	66.0 ± 6.0	1.238 ± 0.014	0.019 ± 0.001	41.0 ± 3.0	1.119 ± 0.026	0.028 ± 0.002

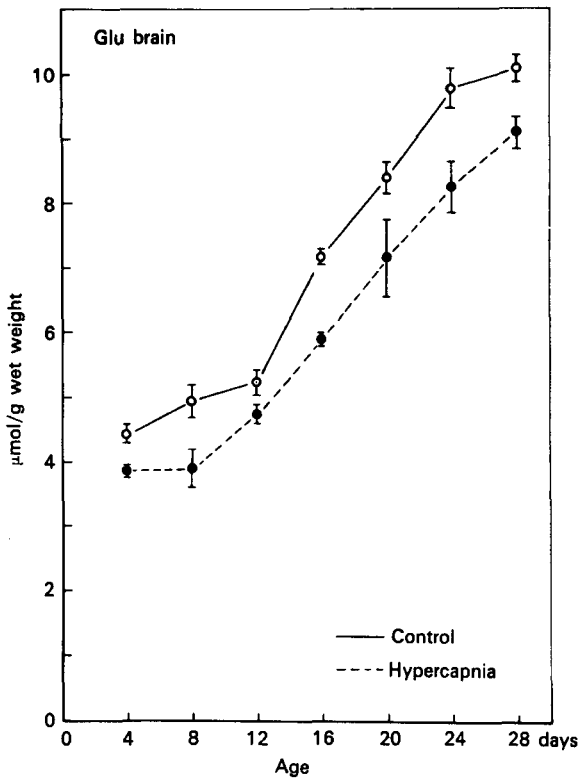

FIG. 1. *Glutamate content of the brain of the rat during postnatal development. Influence of hypercapnia.* Each value is the average of 6 rats, vertical bars indicate S.E.M.

showed the well known increase with age, augmenting progressively from about 4.5 to about 10.2 µmol/g brain between the 4th and 28th day after birth. In the hypercapnic rats the concentration of glutamate also increased with age and the time course of this increase was almost identical as in the control litters. Over the entire experimental period, however, the glutamate level remained about 1 µmol/g lower than in normal rats of the same age.

Figure 2 represents the glutamine levels in the brain in function of age (mean ± S.E.M.). In the control rats the glutamine concentration showed a moderate increase from about 2.2 to about 3.5 µmol/g brain between the 4th and 28th day after birth; the results suggest that most of this increase occurred between the 8th and 16th day. In the hypercapnic animals the glutamine level of the brain showed a much more pronounced increase with matu-

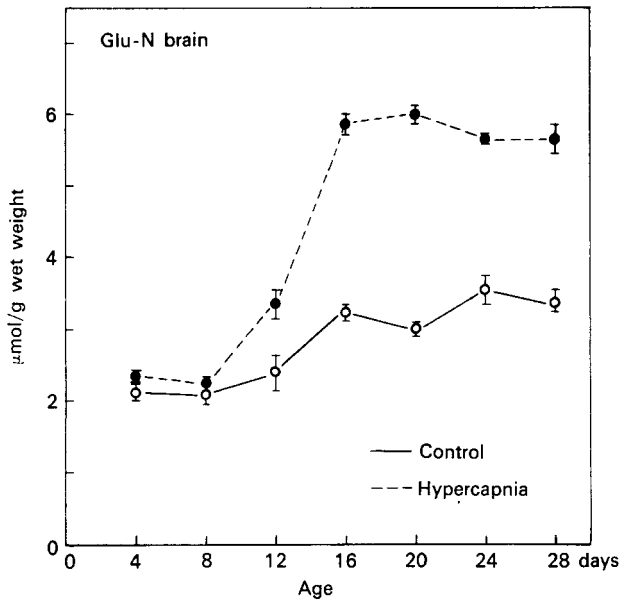

FIG. 2. *Glutamine content of the brain of the rat during postnatal development. Influence of hypercapnia.* Each value is the average of 6 rats, vertical bars indicate S.E.M.

ration from 2.3 to about 6.0 µmol/g. In hypercapnia the increase completely occurred between the 8th and 16th day after birth. As a result the glutamine level between the 16th and 28th day was 2 to 3 µmol/g brain higher during hypercapnia than in normal conditions.

The concentration differences induced by hypercapnia were completely reversible after 1 h of air breathing (Table II).

Enzyme activity of the brain.

The activity of glutamine synthetase and glutaminase in control and experimental litters at 4 and 24 days after birth is represented in Table III (mean ± S.E.M.). In the control litters both enzymes showed the well known increasing activity with age. In the hypercapnic litters too the activity of the two enzymes increased with age. There was no difference in the activity of both enzymes between control and hypercapnic litters at 4 and 24 days after birth.

TABLE II. *Glutamate and glutamine content in the brain of 24-day-old rat.* Influence of hypercapnia and of air breathing during 1 hour after hypercapnia. Each value is the average of 6 rats ± S.E.M.

	Glutamate (μmol/g)	Glutamine (μmol/g)
24 days air	9.81 ± 0.29	3.56 ± 0.18
24 days CO_2	8.24 ± 0.42 ($P < 0.02$)	5.65 ± 0.07 ($P < 0.001$)
24 days CO_2 + 1 hour air	10.20 ± 0.14 ($P > 0.2$)	3.48 ± 0.15 ($P > 0.7$)

TABLE III. *Activity of glutamine synthetase and glutaminase in the rat brain during postnatal development. Influence of hypercapnia.* Each value is the average of 8 determinations ± S.E.M. on 16 (4 days) and 8 (24 days) rats.

Age (days)		Glutamine synthetase (U/g)	Glutaminase (U/g)
4	Control	12.76 ± 0.67	22.75 ± 1.39
	Hypercapnia	13.09 ± 0.48	23.76 ± 0.98
24	Control	31.24 ± 0.86	62.00 ± 1.53
	Hypercapnia	32.43 ± 0.75	63.36 ± 1.53

DISCUSSION

The results indicate that neonatal development of the rat is delayed during hypercapnia. Although not different during the first 4 to 8 days after birth, the body and the brain weight in the hypercapnic litters are definitely lower during the further experimental period. The increase in body weight with age is more delayed in hypercapnia than the increase in brain weight, and the brain to body weight ratio does not decrease immediately after birth as observed in normal rats. A progressive decrease in this ratio is only observed beyond the 12th day after birth.

Our results in control rats confirm the observations of other studies that the glutamate level in the brain of the rat, as in the cat (BERL & PURPURA, 1963; MIHAILOVIC & KRZALIC, 1964), progressively increases after birth and reaches the adult level

between the 20th and 30th day (VERNADAKIS & WOODBURY, 1962; DRAVID & JILEK, 1965; AGRAWAL *et al.*, 1966; RAMIREZ DE GUGLIELMONE & GOMEZ, 1966; BAYER & MC MURRAY, 1967; PATEL & BALAZS, 1970).

In our experiments the glutamine level in the brain of the control litters also increased between the 4th and 28th day after birth. This increase is, however, less marked than for glutamate. A small increase was also observed in other studies (VERNADAKIS & WOODBURY, 1962; DRAVID & JILEK, 1965; AGRAWAL *et al.*, 1966; BAYER & MC MURRAY, 1967; PATEL & BALAZS, 1970). According to some authors (AGRAWAL *et al.*, 1966; BAYER & MC MURRAY, 1967) the glutamine level in the brain initially decreases during the first postnatal week, and then shows a secondary increase toward the adult level. Other studies, however, reported a progressive increase of the glutamine level starting from a low value at birth (VERNADAKIS & WOODBURY, 1962; DRAVID & JILEK, 1965; PATEL & BALAZS, 1970). Still other authors reported in the cat (BERL & PURPURA, 1963; MIHAILOVIC & KRZALIC, 1964) and in the rat (WU, 1964) almost unchanged glutamine levels during the first 30 days after birth. That in the different studies the glutamine level, in contrast with glutamate, did not always show an increase with age might be related to differences in the brain samples. In extensive regional studies in the cat a postnatal increase in glutamate was observed in all regions studied whereas the changes in glutamine differed in different parts (BERL & PURPURA, 1966).

Our results and also some other observations (PATEL & BALAZS, 1970) suggest that the glutamine increase with age can be divided in 3 phases. Between the 4th and 8th day after birth the glutamine level stays at a constant low level, it increases markedly between the 8th and 16th day, and it remains at that level between the 16th and 28th day after birth.

Our results during hypercapnia indicate that the glutamate and glutamine levels in the rat brain also increase progressively between the 4th and 28th day after birth in these conditions. There is, however, a well-marked influence of the hypercapnia on the absolute value of both amino acids.

During the whole experimental period the glutamate level is systematically lower at each age in the hypercapnic animals; the mean difference amounts to about 1 μmol/g brain.

During hypercapnia three phases could also be discerned in the glutamine increase with age. Between the 4th and 8th day after birth hypercapnia has almost no influence on the glutamine level. Between the 16th and 28th day the glutamine level is systematically higher at each age in the hypercapnic animals than in the controls; the difference amounts to 2-3 μmol/g brain. Between the 8th and 16th day the increase of the brain glutamine level is particularly activated in the hypercapnic rats.

On a qualitative basis the influence of hypercapnia on the glutamine and glutamate levels of the brain in developing rats, except in the first 8 days after birth, can be well compared to that described in adult rats (VAN LEUVEN & WEYNE, 1972; WEYNE *et al.*, 1973 & 1976).

During the first 8 days after birth hypercapnia has almost no influence on the glutamine level but a pronounced influence on the glutamate level. This indicates that the glutamate decrease and the glutamine increase are not necessarily a coupled phenomenon. In adult rats uncoupling of the glutamine increase and the glutamate decrease in hypercapnia is observed after inhibition of the glutamine synthetase activity (WEYNE *et al.*, 1976).

Our experiments do not indicate how hypercapnia influences the levels of glutamine and glutamate in the brain. As the effect of hypercapnia is completely reversible within 1 h, it is not related to the delayed development but to a more direct interference with brain metabolism. Hypercapnia does not change the activity of the two enzymes regulating the interconversion of glutamine into glutamate. Such measurements *in vitro*, however, give only restricted information about the enzyme-activity *in vivo*. From previous experiments we concluded that hypercapnia increases production of NH_3 in the brain, which in turn, increases the glutamine concentration (WEYNE *et al.*, 1976). Probably other factors are also involved as hypercapnia also influences the α-ketoglutarate (MESSETER & SIESJÖ, 1971*a*), GABA and aspartate concentrations in brain (FOLBERGROVA *et al.*, 1972; WEYNE *et al.*, 1976).

Although hypercapnia produces intracerebral acidosis the effects on brain amino acids is probably not acidosis-dependent. In adult rats hypercapnia disturbs the concentration of different brain amino acids, including glutamine and glutamate, within 1 h and the effect is maintained for several weeks (WEYNE *et al.*, 1976). On the other hand intracellular pH in the brain, after the initial

acidotic shift, normalizes within a few hours of hypercapnia (MESSETER & SIESJÖ, 1971*b*; ARIEFF *et al.*, 1976). Hypercapnia then, rather than acidosis, must be the stimulus for change of the brain amino acids (*cf.* WEYNE *et al.*, 1973 & 1976). The present experiments indicate that in young rats too the effect of hypercapnia on brain glutamine and glutamate is maintained for weeks and to all probability is also not acidosis-dependent.

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