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GLUCOSE METABOLISM IN DIFFERENT REGIONS OF THE RAT BRAIN UNDER HYPOKINETIC STRESS INFLUENCE

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GLUCOSE METABOLISM IN DIFFERENT REGIONS OF THE RAT BRAIN UNDER HYPOKINETIC STRESS INFLUENCE[1]

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Introduction

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Glucose exchange in the vertebrate brain, the principal route for aerobic energy yield, is linked with the maintenance of stable concentrations of neurally effective amino acids (cyclic amino acids, glutamate, glutamine, aspartate, gamma-aminobutyrate=GABA [1-3 among others]). Through the incorporation of these amino acids into peptide chains there is a constant transfer of glucose-C to the protein fraction. This is an essential detail of cerebral protein homoeostasis [1,4,5,6]. The quantity and quality of glucose movement within the metabolic sequence $glucose_{plasma} \rightarrow glucose_{brain} \rightarrow$ glycolysis → tricarboxylic acid cycle → amino acids → protein deviate under the influence of the most varying factors, among others due to changes in the excitation state of the central nervous system because of environmental conditions [7,8,9]. Specialized neural functions, such as the neuroendocrine metabolism processes in the hypothalamus, are also reflected gradually in changes of the glucose, amino acid and protein metabolism [10-13].

Within the framework of R. Baumann's basic concept in regard to the etiological significance of psychophysical and socio- or psychoemotional factors in the appearance of defective regulation in the circulatory system and carbohydrate-lipid metabolism [14,15] there have been investigations of the effect on an animal model of environmental stress conditions carried out in this Institute in recent years. In this connection an interest was shown in characterizing the central nervous condition of the test animals by the degree of deviation of glucose exchange along the

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[1] In honor of the 65th birthday of Academy Member Prof. R. Baumann, M.D.

* Numbers in the margin indicate pagination in the foreign text.

above mentioned flux route in integratively as well as autonomically active brain regions.

Materials and Methodology

Male Wistar rats weighing 150-200 g were shut up for several hours and several times a day in small boxes, with a stochastic alternation of rest periods, and thus exposed to considerable stress. The experiment was carried out in the division for Experimental Neuroses and Behavior Research of the Institute [16]. Animals were removed from the immobilization program at various times and given a shot injection in a caudal vein of 50 μCi[U-^{14}C]-D-glucose/100 g body wt and 20 μCi[^{131}I] serum albumin as blood marker. 5.5 min later the animal was killed by heat coagulation of the brain with microwaves (exposure time in MW field 3 sec, rise in brain temperature ab 16°C/s; for apparatus and methodology see [17]). Following decapitation blood samples were taken from the throat vessels , the brain was then totally removed from the skull and prepared, carefully freed of attached vessels and coagula, bisected sagitally and dissected under magnification (diagram Fig. 1). Further treatment of the brain tissue was carried out as follows: Weighing (weights in Table 1), homogenization in 5 parts 0.5 N perchloric acid (wt./vol.), measurement of ^{131}I radioactivity by crystal diffraction and determination of content and ^{14}C radioactivity of /855 the involved metabolites following combined chromatograph-enzymatic chemical isolation procedures (for methodology see [3,18], suitable only for small sample quantities).

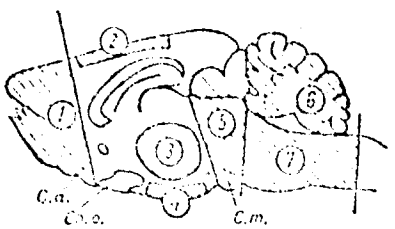

Fig. 1. Diagram of rat brain dissection: C.a. - commissura anterior; Ch.o. - chiasma opticum; C.m. - corpus mamillare

Table 1. Weight of brain samples studied (x̄±s from 48 brains)

Nr.*	a	Hirnregion	mg
1.	b		
2.	c		
3.		Thalamus	79 ± 11
4.		Hypothalamus	31 ± 8
5.	d		111 ± 17
6.	e		115 ± 17
7.			112 ± 22

Key. a. Brain region
b. Frontal pole with remnants of lobus olfactorius
c. parietal cortex
d. mensencephalon
e. cerebellum (gray matter)
f. and
*Numerical symbols as in Figure 1

The individual amino acids were separated from extracts of 3 combined regional samples, the precipitated proteins purified [17] and dissolved in beta-phenylethylamine and the ^{14}C activity measured in the PPO-toluene scintillator.

Determination of the DNS content was made with UV spectrophotometry [19]

The theoretical bases for quantification of the glucose flux have been described [3].

Adhering, even in the state of stress with normal course [18] of identical kinetic patterns, to the stipulation of: 1. the elimination of ^{14}C from the blood plasma and 2. the accumulation of the glucose ^{14}C in the amino acid pool, the incorporated amounts of glucose-C were approximated over

$$\frac{\text{nCi } ^{14}C \text{ in total amino acids} \cdot g^{-1} \text{ regional brain tissue after 5.5 min}}{\text{nCi} \cdot \mu\text{Mol}^{-1} \text{ plasma glucose after 5 min}}$$

The time difference of 0.5 min corrected against the flux time of the ^{14}C populations from the plasma to the brain amino acids. In individual experiments with rats 3 weeks after stress had begun, an identical radioactivity time integral was calculated from initial levels and elimination patterns of the ^{14}C-glucose in the plasma that were the same as the control values and thus there was evidence of the applicability of the above stipulations.

Results

Regional Metabolite Levels and Glucose Flux Rates in Initial State

The regional pattern of the pertinent hydrocarbon and N metabolites can be found in Table 2 together with data for DNS and blood content. Glucose content was relatively large in the parietal cortex and smaller in the di- and mesencephalic structures and in the pons and medulla. Glycogen variation was fairly opposed to this with relatively high amounts in the subcortical regions. Lactate showed no real regional differences. Total amino-N content was greater in the cortex and frontal pole than in the subcortex, where extremely small values were measured in the pons and me-

Table 2. Metabolite content in various regions of rat brain
µ Mol/g regional tissue (initial values*)

	Frontal Pole	Parietal Cortex	Thalamus	Hypo-thalamus	Mesence-phalon	Cerebellum	Pons, Medulla oblongata
Glucose	1.78 ± 0.32	2.00 ± 0.46	1.80 ± 0.36	1.80 ± 0.28	1.45 ± 0.35	1.46 ± 0.19	1.38 ± 0.37
Glycogen	1.80 ± 0.61	2.04 ± 0.49	2.70 ± 0.55	2.71 ± 0.66	2.81 ± 0.47	2.76 ± 0.41	2.60 ± 0.48
Lactate	1.62 ± 0.32	1.64 ± 0.38	1.55 ± 0.31	1.64 ± 0.35	1.67 ± 0.41	1.67 ± 0.28	1.62 ± 0.36
Total amino-N	31.2 ± 1.8	32.4 ± 3.8	27.7 ± 3.3	28.5 ± 3.1	26.7 ± 2.4	27.8 ± 2.9	24.3 ± 2.8
Glutamate	11.0 ± 1.5	12.5 ± 1.2	10.8 ± 0.3	9.8 ± 1.4	9.2 ± 0.3	10.9 ± 1.0	7.9 ± 0.3
Glutamine*	5.8	6.7	7.2	5.9	4.3	3.9	3.2
Aspartate	2.8 ± 0.6	3.4 ± 0.4	2.7 ± 0.3	2.9 ± 0.4	2.7 ± 0.6	2.4 ± 0.3	2.6 ± 0.3
γ-Aminobutyrate	2.5 ± 0.6	1.84 ± 0.5	3.0 ± 0.4	4.8 ± 0.4	3.6 ± 0.4	1.84 ± 0.4	2.3 ± 0.5
DNS (mg)	1.28 ± 0.03	1.21 ± 0.06	1.07 ± 0.03	1.22 ± 0.3	1.10 ± 0.10	4.83 ± 0.36	1.13 ± 0.03
Blood cont. (µl)	35.7 ± 8.5	38.2 ± 8.8	31.0 ± 4.2	22.1 ± 6.5	28.5 ± 4.9	34.8 ± 0.8	28.0 ± 7.1

* $\bar{x} \pm s$ on N = 6

** from extracts collected from 3 regions

Table 3. Flux rates of glucose-C in amino acids for various brain regions
microgram atom C · g^{-1} · min^{-1} (initial values)

Brain region	Frontal Pole	Parietal Cortex	Thalamus	Hypo-thalamus	Mesence-phalon	Cerebellum	Pons, Medulla oblongata
Total amino acids minus Alanine	2.9	3.3	2.6	1.8	2.5	2.7	2.0
Glutamate	2.1	2.2	1.6	1.2	1.6	1.8	1.2
Glutamine	0.3	0.5	0.5	0.2	0.4	0.3	0.2
Aspartate	0.3	0.3	0.3	0.2	0.3	0.3	0.4
γ-Aminobutyrate	0.2	0.3	0.2	0.2	0.3	0.3	0.2

dulla. Glutamate content was relatively high in the cortex and frontal pole and relatively low in the pons and medulla, while GABA in the hypothalamus was a factor 2 higher than in the gray matter of the parietal cortex and cerebellum. Apart from a concentration in the cortex, aspartate showed no regional differences in contrast to glutamine with highest values in the cortex and thalamus and lowest in the mesencephalon, cerebellum and pons and medulla.

After 5 minutes the radioactivity in the total amino acid pool, minus that of the alanine -- subtraction of empirically averaged 10% of the ^{14}C content [18] -- practically equalled the degree of marking of the cyclic amino acids. Table 3 gives approximated flux rates of $C_{glucose}$ in total fraction and cyclic-N-metabolites. 60-70% of the radioactivity was found in the regional glutamate, 10-16% in the glutamine and also 10-18% in the aspartate. Participation of the GABA shunt route in $C_{glucose}$ flux to cyclic amino acids was 10% in the frontal pole, 9% in the parietal

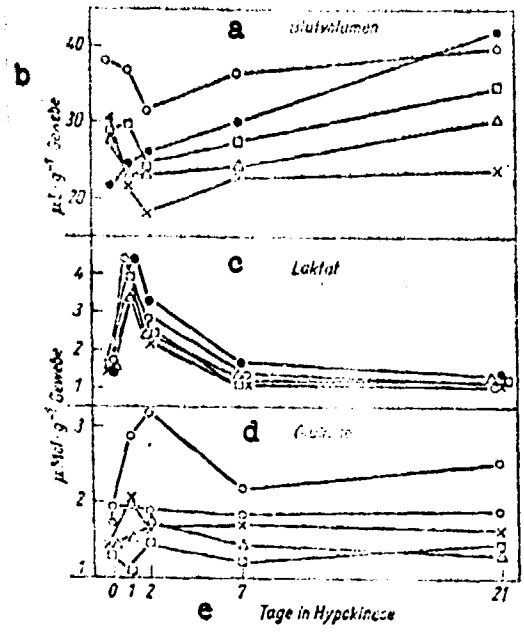

Fig. 2. Blood volume, glucose and lactate content in regions of rat brain during longterm stress due to hypokinesis. As in the following figures, selected brain regions: circle = parietal cortex, thalamus; dot = hypothalamus; triangle = mesencephalon; square = pons and medulla

Key: a - blood volume
b - tissue
c - lactate
d - glucose
e - days in hypokinesis

cortex, 11% in the thalamus, 11% in the hypothalamus, 12% in the mesencephalon, 11% in the cerebellum and 10% in the pons and medulla.

Regional Brain Metabolism in the Hypokinesis Experiment

Metabolite Level

In view of the large time screen a multiphase process was recognizable.

1st week of hypokinesis: following 2 several-hour periods of immobilization there was already a reduction of capillary volumes in the brain areas and an increase in lactate content (Fig. 2); only in the hypothalamus was there an early dilatation of the course of circulation. In this region too there was a rise in the glucose level accompanied only in the thalamus and mesencephalon by a moderate unidirectional movement. There was a significant rise in regional total amino-N content (Fig. 3). At the earliest moment of measurement (after 1 week of stress) there was a reduction in total fractions and individual amino acids (except glutamate in the hypothalamus).

3rd week of hypokinesis: Nothing special about regional glucose and lactate content. Movements of the cyclic amino acids varied: an obvious increase of glutamate and GABA in the hypothalamus with simultaneous decrease of glutamate in the mesencephalon and pons and medulla, as well as a relatively high rise of glutamine in the thalamus.

From weeks 4 to 14: regional levels of glutamate, aspartate and GABA appeared to establish themselves, whereas glutamine stopped at a level lower than the initial value. For glycogen only around the third week of stress was there a determination in all regions of an increase amounting to about 12-20% of the initial value.

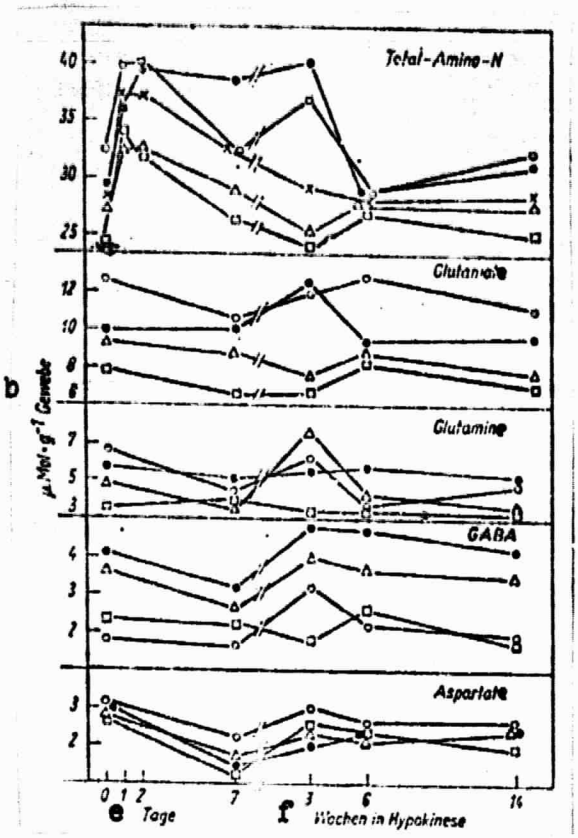

Fig. 3. Change in total amino-N and cyclic amino acids content in regions of rat brain during hypokinesis

Key: b - tissue
e - days
f - weeks in hypokinesis

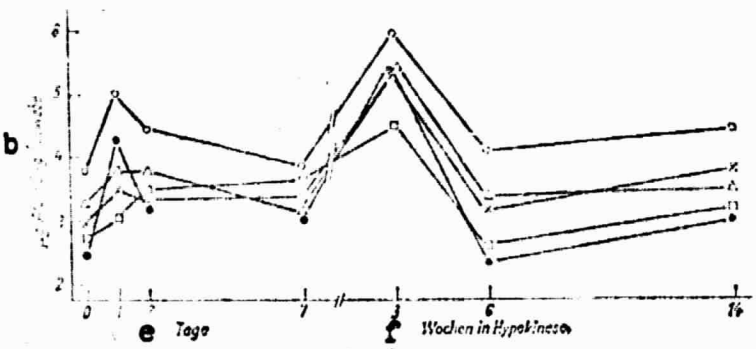

Fig. 4. Flux rates of glucose-C in total amino acids during hypokinesis

Key: b - tissue
e - days
f - weeks in hypokinesis

Glucose Flux

The random sampling pattern shows maxima of the $C_{glucose}$ flux following day 1 of immobilization (Fig. 4). Initially the aerobic glucose exchange rose above the initial value (factors): hypothalamus 1.7, thalamus 1.5, cortex 1.3, mesencephalon 1.3 and pons and medulla 1.1. At the second flux peak the 2.2 hypothalamus factor is likewise largest, followed by thalamus 1.9 and pons-medulla 1.7, mesencephalon 1.6 and cortex 1.5 (cerebellum and frontal pole each between 1.5 and 1.6). Fig. 5 demonstrates the flux changes in the GABA shunt route. In all regions the substrate exchange in this bypath of the tricarboxylic acid cycle already went up after the first week of stress in the cortex and pons and medulla but notably late in the hypothalamus (week 6). /858

Incorporation of Marked Glucose in Glycogen /859

Fig. 6 presents the course of the process for an incorporation period of 5.5 min by means of the quotient

$$\frac{^{14}C \text{ to glycog.} \cdot g^{-1} \text{region.brain tiss.}}{^{14}C \cdot \mu Mol^{-1} \text{ brain glucose}}$$

A general decrease after stress day 2 was followed by a stimulation of the incorporation rate with a maximum between stress weeks 1 and 3.

Incorporation of Glucose-[^{14}C] in proteins

As a working criterion one may use the quotient

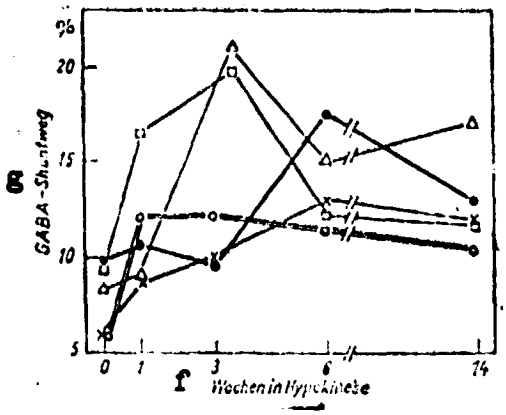

Fig. 5. Flux of glucose-C in GABA shunt route as % of flux of tricarboxylic acid cycle (calculated to include theoretical decarboxylation rates)

Key: f - weeks in hypokinesis
g - shunt route

$$\frac{^{14}C \text{ in proteins}}{^{14}C \text{ in total amino acids}}$$

for each gram of reginal brain tissue and an incorporation time of 5.5 min. Practically /860 speaking, only the utilization of $C_{glucose}$-flux marked amino acids for protein synthesis was involved (alanine, aspartate and glutamate [1, 20]). The selected incorporation time lay within the area of the linear increase of protein marking [21,22]. The symbols in the initial state -- zero point in time in Fig. 7 -- indicate, under normal conditions of animal behavior, a transfer of glucose-^{14}C , in porportional amounts that are regionally about the same, from the amino acid fractions to the protein fractions. Following day 1 of hypokinesis protein marking went down to ca. 50% and after day 2 it reached a minimum of about 20% of the relative marking degree. This determination was likewise made during the one-week spot check without more pronounced regional differences. During the period of stress weeks 2-3 the incorporation rate was higher: for thalamus and hypothalamus it was about 5 times greater and about 3 times for the remaining areas. The persistent activation in the hypothalamus and the pons and medulla was notable (only slight reduction of the quotient in the course of stress week 6).

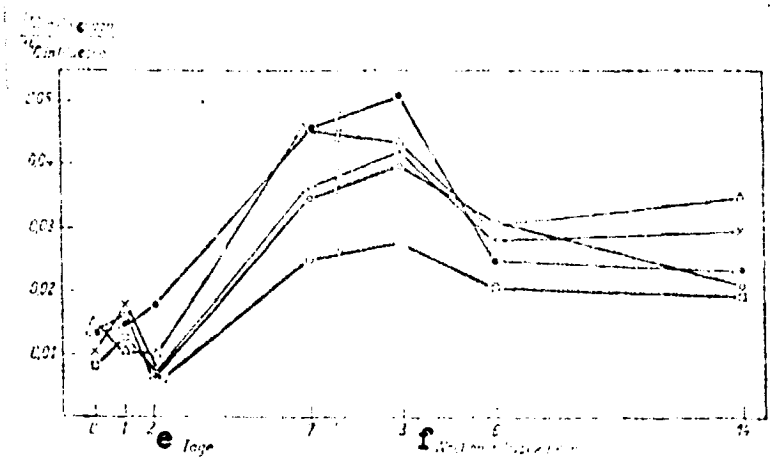

Fig. 6. Incorporation of [^{14}C]-glucose in glycogen in the course of hypokinesis

Key: e - days
f - weeks in hypokinesis

Discussion

When compared with cold fixation [23-25], the regionally arrested metabolite content following rapid heating of the rat brain via heat coagulation of the enzyme proteins seems to give the earliest picture of the native condition. The lactate level,

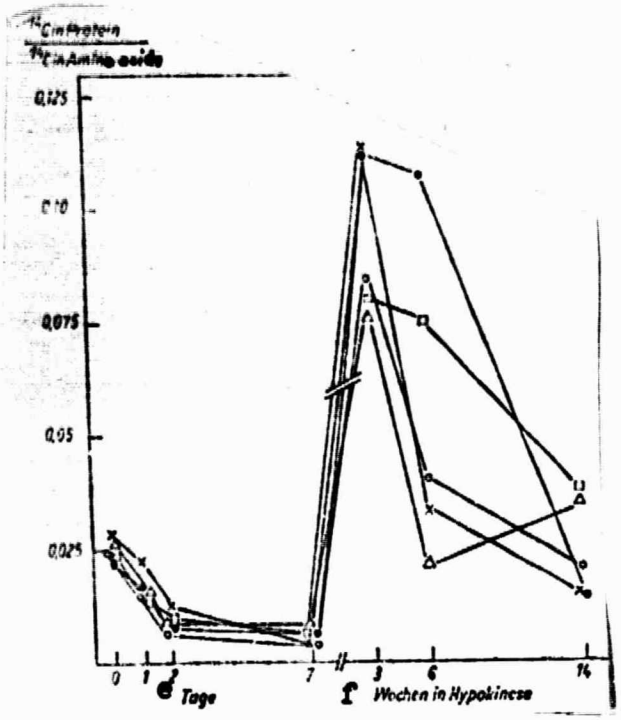

Fig. 7. Protein synthesis from cyclic amino acids during hypokinesis
Key: e - days
f - weeks in hypokinesis

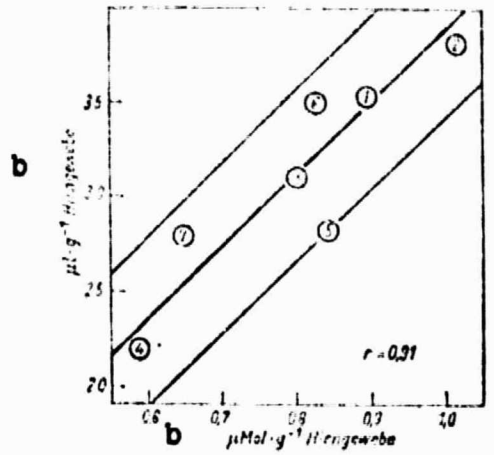

Fig. 8. Relationship between regional capillary volume (blood content, ordinate) and aerobic glucose exchange (abscissa) initially. Glucose exchange calculated to include theoretical decarboxylation rates. Numbers correspond to brain regions as in Fig. 1 and Table 1.
Key: b - brain tissue

indicating a post mortem metabolic dribble, occurred in all brain areas at the level of the "freeze stop" value. [26,27], as happens in the mouse brain under optimal geometric sampling and apparatus conditions [28]. Specific findings on glucose deficit did not bear out a previously described enhancement in phylogenetic younger portions of the brain (dog brain [28] presumable an artifact due to layer cooling of brain tissue [29]). The regional pattern of the cyclic amino acids is in accord with the known literature data [30-33].

The capillary volumes determined by /861
the isotope method bear the following relationship to other vascular and circulatory parameters: 1. a regional variation from the histometrically measured capillary length [34] and 2. a statistically significant relationship with blood perfusion in comparable or identical brain areas (regional value of blood flow [35]; correlation coefficient for 7 areas $r = 0.84$); i.e. the capillary volumes are representative of the circulatory active capillary cross section.

Marking of the amino acids is a quantitative measurement of the entry of glucose-C into the tricarboxylic acid cycle less the losses through metabolic decarboxylation. Now the statistical comparison of vascularization and glucose exchange in Fig. 8 indicates a narrow covariation of local blood supply and aerobic metabolic activity and affirms on the basis of direct flux data a parallelism between re-

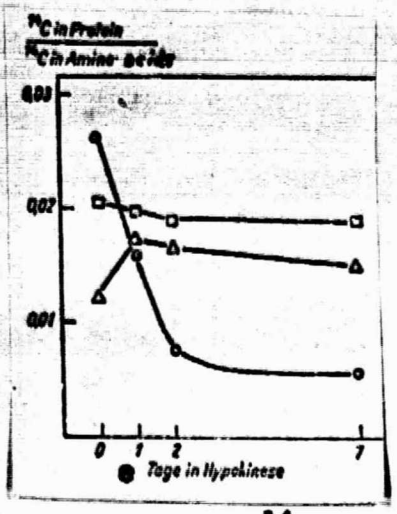

Fig. 9. Comparison of ^{14}C incorporation from [^{14}C]-glucose (circle), [^{14}C]-lactate (triangle) and [^{14}C]-bicarbonate (square) via cyclic amino acids into protein. Mean values of quotients for cortex, thalamus, hypothalamus, mesencephalon and pons-medulla

Key: e - days in hypokinesis

gional blood supply and metabolism [36] which is a conclusion from autoradiographic findings. The metabolic activity gradient cortex and frontal brain > cerebellum > di- and mesencephalon > pons and medulla is the same as the gradient for declining sensitivity to O_2 deficit and clearly indicates the assumed connection between regional metabolic intensity, vascularization and hypoxia resistance [37].

A notable point is the incongruence /862 of regional glucose exchange and cell thickness (DNS content [38]): the cortex/cerebellum relationship for the flux ratio is 1:0.8 and for the DNS content the ratio is 1:4; moreover metabolism in brain areas of fairly equal cell thickness differed by a factor of 1.8 (comparison cortex:hypothalamus). There was no data in the literature on the comparison of aerobic glucose exchanges in individual brain areas.

Among the regional metabolite levels studied only glutamate bore a direct relationship to the locally corresponding glucose flux (glutamate content:flux of glucose-C to glutamate in 7 brain regions, correlation coefficient $r = 0.91$). This kind of level behavior is explained by the function of this amino acid as the primary collection basin for the influx of 60-70% of the glucose-C incorporated in the amino acid pool. The substrate flux in the GABA shunt was 5-10% of the exchange in the tricarboxylic acid cycle (as is also the case for the total mouse brain [39,40]) and was not larger in the hypothalamus region with a high GABA level.

On the wide-meshed random sampling screen of the stress experiments there was noted a biphasic course taken by metabolic events. Initial stimulation of aerobic glucose exchange at the outset of the longterm stress period followed a course in harmony with a decrease in capillary volumes directed at substratum surplus demand and this resulted in functional hypoxia for all brain areas (ubiquitous lactate increase). The assumed reason for diminution of vasal volume is vasoconstriction via extracerebral mechanisms. This view is favored by the mobilization of the renin-angiotensin system by stimulation of brain structures [41-43] as well as during stress [44,58-60] and the

great sensitivity of the brain vessels to this humoral factor [45]. Likewise during the first stress days there was a rise of glucose consent in the thalamus and particularly in the hypothalamus, whereas in the plasma it was constant. This regionally selective hexose accumulation can be explained only by a local activation of the glucose transport plasma → brain tissue or by a corresponding increase in the distribution area for glucose in the brain tissue (intracellular accumulation [18]). This finding, interesting in respect to the stimulation or depression of glucose-sensitive neurons in the hypothalamus [46-49] and affecting the nutritional behavior of the test animals was not supplemented in the present experimental series by any special studies. In the course of stress regional vascular volumes increased, most of all in the hypothalamus (Factor 2), less so in the cortex. In view of the protracted period the explanation is not found in vasodilatation but in capillary neoplasm (as in continuous O_2 deficit [50] or functional loading of brain areas [51,52]). /863

The second maximum for glucose metabolism with generally higher flux rates was reached around stress week 3. At this temporal point there was a distinct increase in the utilization of cyclic amino acids for protein synthesis. Testing for higher nerve activity through conditioned reflex defense methods in parallel experiments showed a distinct improvement in the test animals' ability to function [16]. This agrees strikingly with the established association between memory function and protein synthesis [53-55].

The sharp reduction of glucose-C incorporation in protein during stress week 1 seems significant. We may assume that the ubiquitous decrease of cyclic amino acids in the brain, presumably a symptom of tissue hypoxia (see [56]), is caused by a decrease of glucose flux to a precursor compartment of protein synthesis in favor of a rise in the oxydative transfer in the "energy cycle" (compartmentalization of cerebral intermediary metabolism [57]). For more accurate characterization we conducted addi- /864
tional experiments to study, during the critical stress phase, the incorporation of lactate-^{14}C and bicarbonate-^{14}C from the plasma into the brain proteins via the cerebral amino acid pool (representing the application of 75 µCi [U-^{14}C]-D-lactate or 150 µCi [^{14}C]-bicarbonate per rat, otherwise as described for glucose). The marked carbon of both precursors appears likewise only in the cyclic amino acids. Fig. 9 presents the precursor-substrate relationships. In contrast to glucose as a ^{14}C carrier, the course taken by lactate and bicarbonate -- introduced into the cerebral intermediary transfer by CO_2 fixation -- does not deviate essentially from the initial value.

Incorporation of cyclic amino acids into proteins was thus decreased in a precursor pool fed by glucose flux. This confirms the hypothesis of various amino acid compartments with varying activity patterns during stress.

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