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Cerebral excess release of neurotransmitter amino acids subsequent to reduced cerebral glucose metabolism in early-onset dementia of Alzheimer type

Short Note

S. Hoyer and **R. Nitsch**

Department of Pathochemistry and General Neurochemistry, University of Heidelberg, Heidelberg, Federal Republic of Germany

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Summary. A massive cerebral release of amino acids and ammonia was found in early-onset dementia of Alzheimer type. Aspartate and glycine were liberated in high concentrations, whereas glutamate remained rather unchanged. This excess cerebral protein catabolism is due to a 44% reduction in cerebral glucose metabolism. Whereas glutamate and other glucoplastic amino acids may substitute glucose, elevated aspartate may contribute to neuronal damage. The results are discussed with respect to a possible neuronal insulin/insulin receptor deficiency.

Keywords: Dementia of Alzeimer type, amino acids, neurotoxic effect, neuronal damage, insulin receptor.

Introduction

In a recent study, we demonstrated severe abnormalities in cerebral carbohydrate metabolism in early-onset dementia of Alzheimer type (DAT). The cerebral metabolic rate (CMR) of glucose had dropped to 44%, and CMR-lactate increased fourfold as compared to aged-matched controls, whereas cerebral blood flow and the CMRs of oxygen and CO_2 remained unaltered (Hoyer et al., 1988). Moreover, this metabolic perturbation was characterized by a substantial loss of amino acids and ammonia from the brain indicating severe cellular proteolysis.

The present study investigated the manner and extent to which the glucoplastic amino acids (AA) glutamate (Glu), aspartate (Asp), and glycine (Gly) are involved in the above disturbances of glucose and protein metabolism. These three AA are formed from glucose, most abundantly Glu and Asp (Wong and

Tyce, 1983). On the other hand, they can be converted into oxidizable metabolites of glucose breakdown either by alanine aminotransferase or by aspartate aminotransferase. Glu and Asp function as excitatory neurotransmitters (Cotman et al., 1987). In high concentrations, both AA potentiated excitation and caused neuronal death (Rothman, 1984). Gly facilitated excitatory transmission by means of an allosteric activation of the glutamatergic N-Methyl-D-Aspartate (NMDA) receptor in addition to its role as an inhibitory neurotransmitter (Johnson and Ascher, 1987). Here we discuss the role of Asp and Gly which are liberated from DAT brain in high concentrations, and that of Glu, and their probable contribution to neuronal damage.

Subjects and methods

In four out of 20 patients (mean age 46 ± 9 years) clinically diagnosed as having early-onset DAT for a few weeks to 6 months, the concentrations of AA and ammonia were measured by means of the method of Moore and Stein (1954) in arterial blood and mixed cerebral venous blood sampled from the internal jugular bulb. The diagnostic criteria applied to the patients had been detailed elsewhere (Hoyer et al., 1988). In brief, patients' inclusion criteria were severe and persistent impairment of memory and cognition as verified by several subsequent neuropsychiatric examinations, and by the dementia rating scale of Blessed, HAWIE, Raven matrix, Benton, Wiener Determinationsgerät, and the Nuremberg questionaire. Personality changes not of abrupt but rather rapid to insidious onset as well as a more or less rapid progression of symptoms could be found during the observation period. The ischemic score varied between 1 and 4 points. In the EEG, there was a general slowing with a loss of alpha frequencies and the appearance of theta and delta waves without focal disturbance. CT and pneumencephalography, respectively, showed ventricular enlargement. Patients suffering from extracerebral disorders affecting the brain, or head injury, brain tumor, cerebral infection or infarction, endogenous psychosis, seizures and focal abnormalities along with dementia were excluded from the investigation. All patients studied were free of any drug treatment for several weeks prior to the study. The mean age of the four exclusively male DAT patients was 47 ± 4 years. Their results were compared with the data from a group of 15 healthy volunteer medical students (mean a 25 ± 2 years) investigated earlier (Hoyer, 1970; see also Discussion).

Statistical analysis was performed by means of the t-test ($p \leqslant 0.05$). Homogeneity of variance was tested by the F-test. In case of different variances, testing for statistically significant differences of the mean values was performed by a modified t-test according to Welch (1947).

Results

As is shown in Table 1, the normal human brain took up ammonia and released a small amount of AA. In DAT, a significant loss of ammonia and AA from the brain was found, so that the cerebral amino-N balance became highly negative whereas it was positive in healthy young adults. In this group, the cerebral arteriovenous differences (avD) of Glu, Asp, Gly were close to zero with a tendency to cerebral release. In DAT, the amount of the release of Asp and Gly increased significantly. In principle, Glu was also released from the brain but the data scattered greatly and fell short of statistical significance. These three AA contributed by around 30% to the overall AA release, being highest in Gly (22%), followed by Asp (4%) and Glu (3%).

Table 1. Cerebral arterio-venous differences of amino acids and ammonia in early-onset DAT and healthy volunteers

	Totality of amino acids	Ammonia	Total balance	Asp	Glu	Gly
	as amino-N in mmol/l × 10^{-4}					
Healthy volunteers n = 15	−33 ± 6	+66 ± 9	+33 ± 7	−1 ± 1	−4 ± 1	−6 ± 2
DAT patients n = 4	−463 ± 35*	−213 ± 24*	−676 ± 24*	−17 ± 13*	−13 ± 12	−102 ± 64*

Values represent means ± SD
* $p \leq 0.05$ vs. healthy volunteers
\+ Cerebral uptake
− Cerebral release

Discussion

It had been demonstrated in an earlier study that neither cerebral blood flow nor the CMRs of glucose, lactate and amino acids changed with advancing age from 21 to 24 years as compared to 55 to 65 years, in healthy subjects (Lying-Tunell et al., 1980). In a previous study, we also found unchanged cerebral blood flow and CMRs-glucose and lactate in healthy subjects aged 44 ± 11 years (Hoyer et al., 1988) as compared to the group of healthy volunteers from whom our control AA data derived. Thus, the comparison of the latter data with those of the DAT patients might be justified. Furthermore, Lying-Tunell et al. (1981) described a significant release of some amino acids from the brain in presenile dementia patients aged 52 to 67 years. It may thus be deduced that the AA changes found in the DAT patients are disease-related and not age-related.

Recent findings from Procter et al. (1988) showed an increased concentration of Asp in antemortem frontal and temporal cortices in DAT, whereas Glu was reduced in temporal cortex but unchanged in frontal cortex. In agreement with these results, the increased Asp release in our DAT patients may reflect a high tissue concentration of Asp, whereas that of Glu may be inferred to be rather unchanged. This constellation may indicate the utilization of Glu and the formation of Asp both in the aspartate aminotransferase reaction. The high concentration of Asp may cause an increased Asp binding to NMDA receptors (Cotman et al., 1987), and therefore an elevated receptor activity. The receptor may additionally be potentiated by the high tissue concentration of Gly (Johnson and Ascher, 1987) the latter supposed from its increased cerebral avD as shown in this investigation.

Activation of the NMDA receptor preferentially localized in pyramidal cells of the hippocampal CA_1 and CA_3 subfields (Monaghan et al., 1983) caused a

significant Ca^{2+} influx into these neurons (Jahr and Stevens, 1987). Hyperactivation of this receptor may maintain an elevated cytosolic Ca^{2+} concentration leading to neuronal death (Rothman et al., 1987). In this respect, it may be of great functional significance that the density of NMDA receptors was found to be highest in the CA_1 subfield of the hippocampus and in layers I to III and Va of the cerebral cortex (Cotman et al., 1987). Morphologically, a similar distribution had been described for neurofibrillary tangles and neuritic plaques: The majority of neurofibrillary tangles was located in the CA_1 subfield of the hippocampus (Hyman et al., 1984) and in cortical layers III and V, mostly in pyramidal cells. Neuritic plaques were most numerously present in cortical layers II and III (Pearson et al., 1985). Indeed, a co-localization of neurofibrillary tangles and glutamatergic neurons was found in the hippocampus in DAT (Maragos et al., 1986).

The severe release of AA from the brain may be subsequent to an abnormality in cerebral glucose metabolism. When the intracellular glucose concentration was reduced due to arterial hypoglycemia, Glu and Asp were partly released from the brain into the extracellular fluid (Butcher et al., 1987), and partly utilized by the brain to substitute glucose (Norber and Siesjö, 1976).

In DAT, the arterial glucose concentration and its supply to the brain was unchanged as compared to normoglycemic controls (Hoyer et al., 1988). On the other hand, the reduction in cerebral glucose utilization in DAT was comparable in extent to that in severe hypoglycemia, and was also accompanied by a cerebral loss of AA. Otherwise, the cerebral excess release of ammonia may indicate an utilization of AA in order to substitute carbohydrate lack in DAT brain (Table 1), so that the overall amount of AA produced by catabolic proteolysis in the brain may be assumed to be higher than that of cerebral release.

The severely disturbed glucose utilization in DAT brain is associated with a more or less consistently reduced activity of phosphofructokinase (Bowen et al., 1979; Sims et al., 1987a) and a reduction in pyruvate dehydrogenase activity (Sorbi et al., 1983) indicating a disturbed glycolytic glucose breakdown and pyruvate oxidation. On the other hand, substrate oxidation in the tricarboxylic acid cycle and formation of ATP appeared to be less or not involved (Sims et al., 1983, 1987b). Such an abnormality may be suggestive of a perturbation of cellular regulation of glucose metabolism.

The regulation of glucose metabolism is mediated by means of insulin binding to its receptor in non-nervous tissue (Kahn, 1985). Although the role of insulin in the brain has not been clarified as yet, it is tempting to assume a similar insulin effect in the brain as in non-neuronal tissue (Hendricks et al., 1983), all the more so since insulin synthesis had been shown to occur in hypothalamus (Young, 1986), and insulin receptors were found throughout the brain (Werther et al., 1987). Therefore, the hypothesis has been advanced that an early metabolic abnormality in the pathogenesis of DAT may be due to a deficiency of the insulin/insulin receptor action producing reduced glucose utilization and subsequent proteolysis in the brain (Hoyer, 1988).

References

Bowen DM, White P, Spillane JA, Goodhardt MJ, Curzon G, Iwangoff P, Meier-Ruge W, Davison AN (1979) Accelerated ageing or selective neuronal loss as an important cause of dementia? Lancet I: 11–14

Butcher SP, Sandberg M, Hagberg H, Hamberger A (1987) Cellular origins of endogenous amino acids released into the extracellular fluid of the rat striatum during severe insulin-induced hypoglycemia. J Neurochem 48: 722–728

Cotman CW, Monaghan DT, Ottersen OP, Storm-Mathisen J (1987) Anatomical organization of excitatory amino acid receptors and their pathways. TINS 10: 273–280

Hendricks SA, Roth J, Rishi S, Becker KL (1983) Insulin in the nervous system. In: Krieger DT, Martin JB, Brownstein MJ (eds) Brain peptides. Wiley, New York, pp 903–939

Hoyer S (1970) Der Aminosäurenstoffwechsel des normalen menschlichen Gehirns. Klin Wochenschr 48: 1239–1243

Hoyer S (1988) Metabolism and circulation in normal and abnormal aging processes of the brain. What are the mechanisms of neuronal degeneration? In: Henderson AS, Henderson JH (eds) Etiology of dementia of Alzheimer's type. Dahlem workshop report LS 43. Wiley, Chichester (in press)

Hoyer S, Oesterreich K, Wagner O (1988) Glucose metabolism as the site of the primary abnormality in early-onset dementia of Alzheimer type? J Neurol 235: 143–148

Hyman BT, van Hoesen GW, Damasio AR, Barnes CL (1984) Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. Science 225: 1168–1170

Jahr CE, Stevens CD (1987) Glutamate activates multiple single channel conductances in hippocampal neurons. Nature 325: 522–525

Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325: 525–531

Kahn CR (1985) The molecular mechanism of insulin action. Ann Rev Med 36: 429–451

Lying-Tunell U, Lindblad BS, Malmlund HO, Persson B (1980) Cerebral blood flow and metabolic rate of oxygen, glucose, lactate, pyruvate, ketone bodies and amino acids. I. Young and old normal subjects. Acta Neurol Scand 62: 265–275

Lying-Tunell U, Lindblad BS, Malmlund HO, Persson B (1981) Cerebral blood flow and metabolic rate of oxygen, glucose, lactate, pyruvate, ketone bodies and amino acids. II. Presenile dementia and normal-pressure hydrocephalus. Acta Neurol Scand 63: 337–350

Maragos WF, Debowey DL, Reiner A, Rustioni A, Penney JB, Young AB (1986) Co-localization of congo red-stained neurofibrillary tangles in glutamate immunoreactive neurons in the hippocampus. Soc Neurosci Abstr 12: 442

Monaghan DT, Holets VR, Toy DW, Cotman CW (1983) Anatomical distributions of four pharmacologically distinct ^{3}H-L-glutamate binding sites. Nature 306: 176–179

Moore S, Stein WH (1954) A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem 211: 907–913

Norberg K, Siesjö BK (1976) Oxidative metabolism of the cerebral cortex of the rat in severe insulin-induced hypoglycaemia. J Neurochem 26: 345–352

Pearson RCA, Esiri MM, Hiorns RW, Wilcock GK, Powell TPS (1985) Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. Proc Natl Acad Sci USA 82: 4531–4534

Procter AW, Palmer AM, Francis PT, Low SL, Neary D, Murphey E, Doshi R, Bowen DM (1988) Evidence of glutamatergic denervation and possible abnormal metabolism in Alzheimer's disease. J Neurochem 50: 790–802

Rothman S (1984) Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. J Neurosci 4: 1884–1891

Rothman SM, Thurston JH, Hauhart RE (1987) Delayed neurotoxicity of excitatory amino acids in vitro. Neuroscience 22: 471–480

Sims NR, Bowen DM, Neary D, Davison AN (1983) Metabolic processes in Alzheimer's disease: Adenine nucleotide content and production of $^{14}CO_2$ from (U^{14}-C) glucose in vitro human neocortex. J Neurochem 41: 1329–1334

Sims NR, Blass JP, Murphy C, Bowen DM, Neary D (1987a) Phosphofructokinase activity in the brain in Alzheimer's disease. Ann Neurol 21: 509–510

Sims NR, Finegan JM, Blass JP, Bowen DM, Neary D (1987b) Mitochondrial function in brain tissue in primary degenerative dementia. Brain Res 436: 30–38

Sorbi S, Bird ED, Blass JP (1983) Decreased pyruvate dehydrogenase complex activity in Huntington and Alzheimer brain. Ann Neurol 13: 72–78

Welch BL (1947) The generalization of student's problem when several different variances are involved. Biometrica 34: 28–35

Werther GA, Hogg A, Oldfield BJ, McKinley MJ, Figdor R, Allen AM, Mendelsohn FAO (1987) Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry. Endocrinology 121: 1562–1570

Wong KL, Tyce GM (1983) Glucose and amino acid metabolism in rat brain during sustained hypoglycemia. Neurochem Res 8: 401–415

Young WS (1986) Periventricular hypothalamic cells in the rat brain contain insulin mRNA. Neuropeptides 8: 93–97

Authors' address: S. Hoyer, M.D., Department of Pathochemistry and General Neurochemistry, University of Heidelberg, Im Neuenheimer Feld 220/221, D-6900 Heidelberg, Federal Republic of Germany.

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