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Overview

Is Ammonia a Pathogenetic Factor in Alzheimer's Disease?*

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An attempt was made to review experimental evidence in favor of the idea that ammonia plays a role in dementia of the Alzheimer type (DAT). Hyperammonemia causes biochemical and cellular dysfunctions in the brain, which can be found in brains of DAT patients. The most conspicuous among these findings are astrocytosis, impairment of glucose utilization, and a decreased rate of energy metabolism, and the impairment of neurotransmission, with a net increase in excitability and glutamate release. The derangement of lysosomal processing of proteins is another potential site of ammonia action. This aspect is especially important in view of the growing evidence for the role of the endosomal-lysosomal system in the formation of amyloidogenic fragments from β-amyloid precursor protein. Ammonia is not considered a primary factor of the disease. However, since hyperammonemia and release of ammonia from the brains of DAT patients is well supported by published observations, ammonia should be taken into account as a factor that contributes to manifestations and the progression of DAT. If elevated ammonia concentrations turn out to be indeed as important in DAT, as is suggested in this review, rational therapeutic avenues can be envisaged that lead to the amelioration of symptoms and progression of the disease.

KEY WORDS: Alzheimer's Disease; ammonia; hyperammonemia.

INTRODUCTION

The pathological changes in dementia of the Alzheimer type (DAT) include the presence of senile plaques, neurofibrillary tangles and granulovacuolar degeneration in cerebral cortex, amygdala, olfactory tubercle and hippocampus (1,2). Losses of cholinergic (3,4), glutamatergic (5-8), serotonergic (9-12), noradrenergic (9,13-15), dopaminergic (9,16) and somatostatinergic (17), neurons and receptors, respectively, as well as the impairment of the brain GABA system (18) have been observed. Quantitative cell counts have not confirmed astrocyte proliferation, but "reactive astrocytes" are found in neuritic plaques (19,20), and fibrous astrocytes appear to be much more numerous in the cerebral cortex of DAT brains than in the brains of age-matched controls. This has been interpreted as a reactive pathological process involving transformation of protoplasmic to fibrous astrocytes (1).

Numerous hypotheses concerning the pathogenesis of DAT have been published, among which the selective vulnerability of cholinergic neurons in the basal forebrain (21-24) first attracted most attention. Viral agents

* This paper is dedicated to Rudi Vrba, a pioneer of the neurochemistry of ammonia, and a friend, at the occasion of his 68th birthday.

Abbreviations: β-AP, β-amyloid protein; β-APP, β-amyloid precursor protein; CNS, central nervous system; DAT, dementia of the Alzheimer type; GABA, γ-aminobutyrate; MAO, monoamine oxidase; NAD, nicotinamide adenine dinucleotide.

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(25), aluminum (26-28) and other trace element imbalances (29), endogenous colchicine-like factors (30), glutamate dysfunction (31,32), lack of trophic factors, such as nerve growth factor (33) have been proposed among others as etiologic factors.

No evidence for a major gene was found for DAT (34). However, a genetic predisposition is considered to be likely from the high concordance rate for monozygotic and dizygotic twins (35), and an increased frequency of the disease in relatives of affected patients (36). The long arm of chromosome 21 is considered to be the locus of predisposition (37), but the expression of a number of genes encoding for various neuronal and non-neuronal proteins is also changed in DAT brains (38) and may also contribute to the etiology of the disease.

Since the identification of β-amyloid protein (β-AP) as the major constituent of senile plaques, extracellular β-AP deposition is considered to be the central event in the etiology of DAT (39-41). Interest has focused on the mechanisms involved in plaque formation, and the pathologic consequences of β-AP deposition (41-43).

Amyloid defines senile plaques, but other elements may be more specific for DAT. For example, extensive neuritic degeneration was seen only in DAT brains, not in non-demented elderly with senile plaques or in non DAT dementia cases (20).

In human brain β-AP is formed from a precursor protein (β-APP), a component of synapses with unknown physiological function (44). It is also a regular constituent of the brains of non-human primates (45). Growing evidence indicates that β-AP deposits are formed due to aberrant processing of β-APP (46). This hypothesis is supported by the occurrence of familial forms of DAT with known mutations in the β-APP, and consequent aberrant processing, for which a model has been proposed (41). The reasons for aberrant β-APP processing in the vast majority of Alzheimer cases is, however, unknown. In human Down's syndrome structurally normal β-APP is overexpressed due to gene duplication (47). The enhanced formation of β-APP in Down's patients appears to be a sufficient reason for the formation of β-AP depositions, which are indistinguishable from those in DAT brains (48). This may be taken as an indication for a restricted capacity of the human brain to process β-APP, and one may conclude that a relatively minor impairment of normal β-APP processing is sufficient for senile plaque formation, especially since the process of plaque formation is very slow.

It has been hypothesized that risk factors in DAT increase with age (49). If this is true, we have to consider consequences of general age-related changes in organ functions as potential contributing factors, among which the impairment of the blood brain barrier function (50) may be of especial importance. Enhanced exposure of the organism to oxygen free radicals, e.g. due to the impairment of catalase and superoxide dismutase, may be another general noxious event, although direct evidence for the validity of this idea with respect to DAT is scarce. But oxygen free radicals have nevertheless been implicated as etiological agents in the process of aging (51), and in several neurodegenerative disorders (52), including DAT (53-55).

Ammonia, a highly toxic agent, is both of environmental (gastrointestinal tract) and endogenous origin. From the known toxicology of ammonia, it appears to be likely that it may contribute in various ways to pathological processes in DAT, but also in other senile dementias. It is the purpose of this paper to discuss the potential involvement of ammonia in the development and manifestations of DAT.

Following the pertinent literature, ammonia and ammonium (salt) will be used synonymously, keeping in mind that at physiological pH (7.4), 1.7% of ammonia (NH_3) is in equilibrium with 98.3% of its protonated form (NH_4^+) (56).

Evidence for Elevated Ammonia Concentrations in Blood and Brain in DAT

There are only a few published observations which directly suggest a role of ammonia in DAT. Since, however, ammonia has not attracted attention in this regard, no systematic studies are available.

a) Fisman et al. (57,58) demonstrated in two reports that postprandial blood ammonia levels were significantly higher in DAT patients than in appropriately matched control subjects. Some DAT patients had triphasic waves on EEG, a waveform suggestive for metabolic encephalopathy (59). The fasting blood ammonia levels in those patients were significantly higher than in the other DAT patients.

b) Branconnier et al. (60) found in patients who met the diagnostic criteria of DAT, but had no liver disease, nor urinary tract infections, 208 ± 136 μg ammonia per 100 ml of plasma. The normal range was 20-94 μg/100 ml; 83% of the patients had blood ammonia concentrations above the normal limits.

c) Hoyer et al. (61) determined arterio-venous differences of ammonia in patients suffering from advanced DAT, and in patients clinically diagnosed as having incipient dementia, in all probability DAT of early onset. Healthy volunteers showed an average ammonia uptake

by the brain of 72 ± 7 $\mu g \cdot kg^{-1} \cdot min^{-1}$. In striking contrast, 27 ± 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of ammonia was released from the brains of patients with advanced DAT. Patients with presumed early-onset DAT released even 256 ± 162 $\mu g \cdot kg^{-1} \cdot min^{-1}$ ammonia into the circulation. These findings suggest excessive ammonia production within the brain, with or without a deficient mechanism of ammonia detoxification.

From the above cited reports it seems evident that in addition to usual age-related impairments of liver function (i.e. reduction of the hepatic detoxification capacity), there are disease-related causes for hyperammonemia in DAT, which are not of gastrointestinal origin.

Sources of Ammonia, and Ammonia Detoxification Mechanisms

Normal ammonia concentrations in vertebrate brains are in the range of 0.1-0.3 $\mu mol \cdot g^{-1}$ wet weight. Concentrations seem to be correlated with the functional state of the brain : reduction in functional activity is associated with a reduced concentration of ammonia, and functional activity, electrical stimulation and many convulsant agents produce elevated brain ammonia levels (56).

A significant amount of the ammonia of the vertebrate organism originates from the gastrointestinal tract. Deficient detoxification due to the impairment of liver function causes hyperammonemia, and since ammonia easily passes the blood brain barrier (56), elevated brain ammonia concentrations are a result. In hepatogenic encephalopathy resulting from acute or chronic liver disease, exogenous (gastrointestinal) ammonia is a key factor in the pathogenesis of the disease (62-64). Bacterial infections of the urinary tract (e.g. the neurogenic bladder) is another potential cause for hyperammonemic encephalopathy (65). Proteins, nucleic acids and hexosamines have long been suggested as sources of cerebral ammonia (66-68). Oxidative deaminations of primary amines (monoamines, diamines and polyamines), glycine catabolism via the glycine cleavage system, deaminations of purines and pyrimidines and glucosamine-6-phosphate, among others, are well known ammonia generating reactions, which may contribute to the steady-state level of brain ammonia (69). The direction of the neural glutamate dehydrogenase-catalyzed reaction appears to be regulated by the intracellular NAD(P)/NAD(P)H-ratio : when the ratio is high, e.g. in the absence of glucose, oxidative deamination of glutamate occurs. In the presence of glucose, when this ratio falls, and 2-oxoglutarate is not rate limiting, reductive amination of 2-oxoglutarate to glutamate seems favored (56).

Hepatic urea formation is the major detoxification reaction of the mammalian organism. In brain, the ATP-dependent formation of glutamine (Figure 1) in glial cells, and its release into the circulation is nearly exclusively responsible for the limitation of ammonia concentrations (56,69). Glutamine synthetase is localized in the soluble and microsomal fractions of glia cells (69).

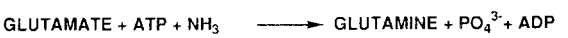

Fig. 1. The glutamine synthetase reaction scheme.

Brain Ammonia Metabolism in DAT

Some observations suggest arguments in favor of the non-physiological accumulation of ammonia in the CNS of DAT patients.

The greatly reduced activity of glutamine synthetase in the brains of aged gerbils (70), the rapid loss of glutamine synthetase during ischemia/reperfusion-induced brain injury (71) and during hyperoxia in neonatal rats (72) are strong indications for the considerable sensitivity of this enzyme against damage by oxygen free radicals. In human brains an age-related gradual reduction of glutamine synthetase activity was observed, with a significantly lower activity of this enzyme in the brains of DAT patients than in age-matched controls (73). In contrast, phosphate-activated glutaminase, the enzyme responsible for intraneuronal liberation of glutamate from glutamine, is not changed in DAT (74). Thus a dysfunction of the key detoxification mechanism in the brains of patients with DAT seems obvious.

Monoamine oxidase B (MAO-B) is mostly extraneuronally localized (75) and represents in the human CNS over 80% of MAO (76). An age-related increase of this enzyme has been demonstrated (77). This increase was more marked in the brains of DAT patients (78,79), and has been related to gliosis involving astrocytes (80). The enhancement of MAO-B activity seems to be due to the presence of more MAO-B molecules, not due to a high-activity isoform of the enzyme (81).

MAO-B deaminates oxidatively numerous endogenous and exogenous primary amines (e.g. dopamine, tyramine, tryptamine, β-phenylethylamine, benzylamine) to form ammonia, hydroperoxide and the aldehyde corresponding to the amine substrate, as is depicted in Figure 2. Since all three reaction products of MAO (and of all oxidative deaminations in general) are noxious agents that need to be inactivated by the mammalian organism, the enzymes and their substrates, but especially the products of these reactions, deserve our special attention as potential pathogenetic factors.

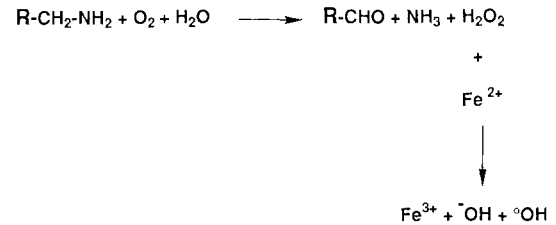

Fig. 2. General scheme of oxidative deaminations of primary amines, and Fe^{2+}-catalyzed radical formation from hydroperoxide.

The physiologic rate in human brain of MAO-B-catalyzed reactions (and of related oxidative deaminations) is not known. In DAT patients the impaired blood-brain barrier (50) may allow the enhanced intrusion of substrates of MAO-B into the brain from the periphery. It is not possible to assess the contribution of these reactions to brain ammonia formation. But it seems not unlikely that the recent reports on the improvement of cognitive functions of DAT patients by treatment with an inactivator of MAO-B (82-85) are due to the reduction of ammonia formation. (In addition to reduced ammonia formation there are of course several other potential explanations for the beneficial effects of MAO-B inhibition in DAT).

Toxic Effects of Ammonia and Alzheimer Pathology

In experimental animals key toxic manifestations of enhanced brain ammonia concentrations are independent of the genesis of the state of hyperammonemia, i.e. the symptoms are much the same after impairment of liver function (e.g. by portacaval shunting), hyperammonemia produced by urease injections, or by inactivation of glutamine synthetase, using methionine sulfoximine (86-88), and resemble pathophysiological observations in the brains of patients with hepatogenic encephalopathy and hereditary defects of urea cycle enzymes (62,63). Based on these facts one should expect to find at least some of the typical pathophysiological consequences of hyperammonemia in DAT.

a) Synaptic Transmission in Ammonia Intoxication. Based on experimental results it was calculated that an increase of ammonia to about 0.5 $\mu mol \cdot g^{-1}$ brain, i.e. a 2–5-fold increase, is sufficient to disturb excitatory and inhibitory synaptic transmission and to initiate the encephalopathy related to acute ammonia intoxication (89). Thus, it seems evident that slowly progressing pathogenetic mechanisms may be initiated even at brain ammonia concentrations only slightly above physiological levels.

Glutamate-mediated excitatory synaptic transmission is decreased by ammonia. Whether this effect is related to a depletion of glutamate in presynaptic terminals is unclear at present (89).

Inhibitory synaptic transmission is also decreased by ammonia, by hyperpolarizing Cl^--dependent inhibitory (e.g. GABAergic) neurons. This effect is related to the inactivation of the extrusion of Cl^- from neurons by ammonia. By the same action ammonia also decreases the hyperpolarizing action of Ca^{2+}- and voltage dependent Cl^--currents (89). Since a large proportion of the GABAergic and other inhibitory neurons control inhibitory inputs, ammonia produces an increase in neuronal excitability by "disinhibition" (90).

The fact that ammonia is capable of interfering with the function of the major excitatory (glutamatergic), and the major inhibitory (GABAergic) neuronal systems of the vertebrate CNS should be sufficient reason to attract our especial interest.

b) Reduced Glucose Utilization. Most conspicuous findings of experimental and human diseases with hyperammonemic states, namely the impairment of brain glucose utilization, with concomitantly decreased rates of energy metabolism (89,91,92) and astrocytic alterations, characterized as "Alzheimer type II gliosis" (93-95) are characteristic for DAT brains as well : In PET studies (8,96,97) cerebral glucose utilization was found to be predominantly reduced in the parieto-temporal cortex. Overall cerebral glucose utilization was found to be diminished by about 50% with normal oxygen consumption in early-onset (98-100), but reduced oxygen consumption in late onset DAT (101). (The impairment of brain energy metabolism in DAT, and of enzymes involved in energy metabolism, has subsequently been reported by several investigators (e.g. see ref. 102-104)).

c) Interference with Glia Function. As has been mentioned before, astrocyte abnormalities are a characteristic of DAT. In a recent paper Frederickson (105) summarized observations supporting the idea that reactive astrocytes may mediate neuropathologic events of DAT, including the facilitation of extracellular depositions of β-AP.

Astrocytic damage by ammonia is followed by a decrease of glutamine synthetase activity, as was evidenced from the reduction of the activity of this enzyme by 15% in rats with portacaval shunts (106). However, this decrease in synthetase activity may cause further damage to astrocytes. It is well established that glutamine synthetase is critically involved in the regulation of intracellular ammonia and acid-base balance (56). Any derangement of the function of this enzyme will be followed by the amplification of ammonia toxicity. There-

fore, it is not surprising that an increased intracellular pH, and swelling of astrocytes was observed in hyperammonemic rats (107). pH-Determinations in brains of DAT patients (e.g. by measurement of the chemical shift of the signal of inorganic phosphate by in vivo NMR spectroscopy) should be able to render appropriate evidence for a role of acid-base imbalance in DAT.

Increasing evidence emerges for a role of microglia in DAT pathology (108). These cells are seen in many degenerating cells, and virtually every senile plaque has microglial cells or cell processes in the plaque (20). It is believed that microglia invasion is an indication for the brain's attempt to rid itself of immunogenic proteins. Since β-APP is likely to be formed in microglia (109) these cells may contribute to the formation of β-AP depositions in two ways, by phagocytosis of nerve ending membranes, and by their intrinsic β-APP.

Effects of ammonia on microglia function seem not to have been studied. In view of the potential importance of microglia in DAT pathology this aspect of neurodegeneration needs to be explored.

From the key observations reported in the two preceding sections the scenario schematized in Figure 3 seems evident : Damage of glutamine synthetase (and of other proteins) e.g. by oxygen free radicals could be one of the primary events. The impairment of brain ammonia detoxification is expected to initiate vicious circles which result in the progressively increasing accumulation of ammonia with astrocytosis as a consequence. The impairment of glucose utilization is another potential primary event (98), expected to have similar consequences as the impairment of ammonia detoxification.

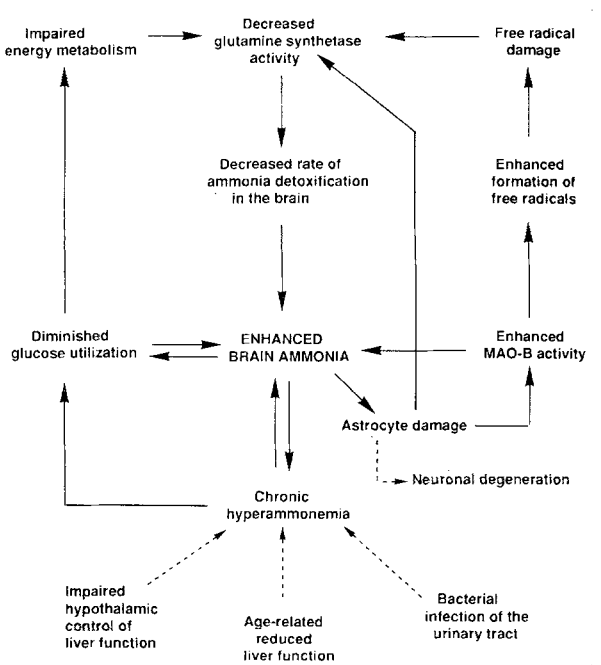

Fig. 3. Positive feedback regulatory cycles in states of enhanced brain ammonia concentrations, and pathologic consequences of hyperammonemia. In addition to enhancement of brain-born ammonia exogenous sources due to impaired liver function or bacterial infections of the urinary tract may contribute to hyperammonemia in aged people.

d) Hyperammonemia and Excitotoxic Amino Acids. Presumably the most conspicuous difference between the amino acid patterns of cirrhotic (62) and DAT patients (74) is the several-fold increase of glutamine in all brain regions of cirrhotics, but no change in the concentration of this amino acid in the brains of DAT patients. Likewise, no increase of glutamine was detected in the CSF of patients with DAT (110), whereas the levels of this amino acid were elevated in the CSF of experimental animals with portal-systemic encephalopathy (111). These findings suggest the inability of the brains of DAT patients to enhance glutamine formation above a certain level, and may be taken as an indication for a considerable sensitivity of DAT brains even to small increases in the rate of ammonia formation. Due to the elevation of ammonia levels, reductive amination of 2-oxoglutarate (catalyzed by glutamate dehydrogenase) may take place, both in DAT and hepatogenic encephalopathy. Presumably, this "extra" glutamate can only be removed from the brain as glutamine in the latter disease not in DAT brains, due to its limited capacity of ammonia detoxification. Glutamate formation from 2-oxoglutarate impairs at the same time energy metabolism, by decreasing the equilibrium concentration of this substrate of the tricarboxylic acid cycle.

Glutamate concentrations are lower in the brains of DAT patients than in age-matched controls, due to losses of glutamatergic neurons (74), but CSF levels of glutamate are elevated, both in DAT (110) and in portal systemic encephalopathy (111), indicating enhanced extracellular concentrations of this amino acid. Disregarding the mentioned possibility of the enhanced formation of glutamate by reductive amination of 2-oxoglutarate the increase of extracellular glutamate concentrations is most probably a result of the impairment of the uptake of glutamate into perineuronal astrocytes due to the deranged astrocyte function by ammonia. Since it is well established that the neurotoxic effects of glutamate (112) are enhanced by inhibition of uptake sites (113), derangement of glial uptake mechanisms could be a major reason for excitotoxic cell damage in DAT.

The release of aspartate from the brains of patients with early-onset DAT (98) is indicative for a further cause of excitotoxic damage during a certain stage of

the disease. Patients with a mean age of 60 years had normal CSF levels of aspartate (110).

It has already been mentioned that there is evidence for the selective loss of glutamate receptors in cortex and hippocampus of DAT brains (5,7). In this context a recent observation seems of interest : In the cerebellum of hyperammonemic rats a decrease of the number of both, high- and low-affinity binding sites of glutamate was noticed. The decrease was only in the N-methyl-D-aspartate-specific binding sites, without any alterations in the binding sites of kainate or quisqualate. These effects were mimicked when the membrane preparations from normal animals were incubated with ammonium acetate. Binding of muscimol (a GABA receptor agonist) was enhanced under the same experimental conditions (114). These observations underline again the ability of ammonia to affect functions of both glutamatergic and GABAergic neurons.

Excitotoxic mechanisms in the pathogenesis of DAT (31,32,115,116) are especially attractive, since they are able to explain symptoms of cortical disconnection (e.g. aphasia), and memory dysfunction. Ammonia-induced astrocytosis may well have an important contribution to excitotoxic mechanisms.

Potential Consequences of Enhanced Brain Tryptophan Metabolism

The enhanced uptake and turnover of tryptophan in hepatic failure (117) was considered a pathogenetic factor in hepatic encephalopathy (118). But hyperammonemia in the absence of any derangement of liver function also causes the enhancement of tryptophan uptake by the brain (119). The reports concerning the rate of tryptophan and serotonin metabolism in DAT are controversial (84). The following considerations may nevertheless be valid in view of the data which support a role of hyperammonemia in the pathogenesis of DAT.

Kynurenine is a toxic (120) metabolite of tryptophan. It is formed by oxidative cleavage of the pyrrole ring to N-formyl kynurenine and enzymatic removal of the formyl residue (121). Quinolinic acid is formed from kynurenine. It is excitotoxic, similar to glutamate and kainic acid (122). Enhanced kynurenine concentrations in plasma, CSF, and brains of hyperammonemic patients (with liver cirrhosis) have been recognized as a consequence of enhanced tryptophan levels (123). Increased quinolinic acid formation in brain seems not to be a direct consequence of enhanced kynurenine formation. However, quinolinic acid concentrations were found to be elevated in the brains of aged rats (124). Therefore, they may be a consequence of enhanced quinolinic acid formation in liver, and/or enhanced uptake due to the age-related impairment of the blood-brain barrier (50).

Based on the available information it is not difficult to imagine a scenario for aged people with chronic hyperammonemia, as is depicted in Figure 4. In addition to excitotoxic damage generated by quinolinic acid and kynurenine, the impairment of lysosomal proteolysis by both, tryptophan and kynurenine (125) is a most likely direct consequence of chronically elevated brain ammonia concentrations.

Lysosomes, β-APP Processing, and Ammonia

Several lines of evidence suggest that β-APP is processed by the endosomal-lysosomal system :

a) Using an antibody, β-APP was localized to lysosomes (126-129).

b) Lysosomal proteinase antigens are prominently localized within senile plaques (130).

c) The degradation but not the secretion of β-APP by PC12 cells was impaired by inhibitors of lysosomal

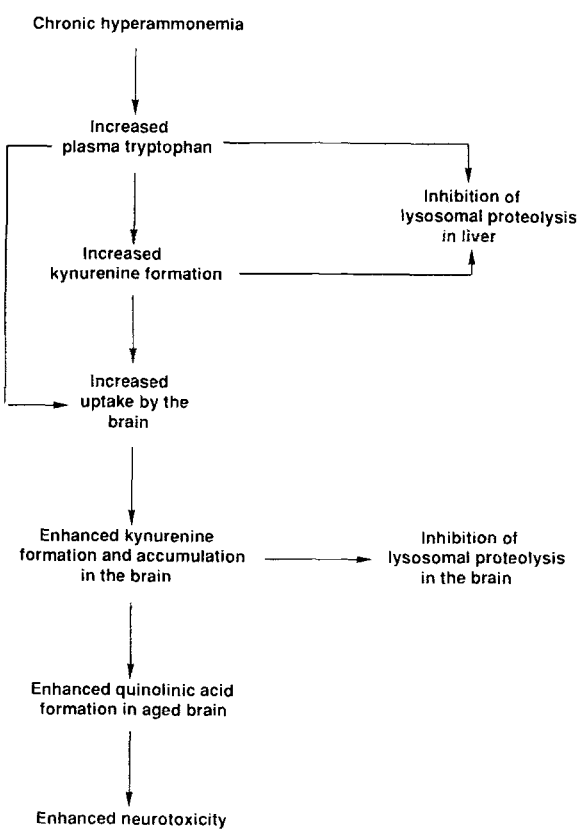

Fig. 4. Potential pathologic consequences of enhanced tryptophan metabolism along the kynurenine-quinolinic acid pathway.

function (i.e. ammonium chloride, leupeptine and chloroquine), (127,131).

d) Rather convincing evidence for the lysosomal processing of β-APP into potentially amyloidogenic fragments was published by Golde et al. (132). These authors produced deletion mutants of CEP4β cells that produce the normal set of carboxyl-terminal derivatives of β-APP, and shortened secreted derivatives were analyzed. It was shown that secretase cleaved β-APP at a single site within the β-AP region, and generated one secreted derivative and one non-amyloidogenic carboxyl-terminal fragment. In contrast, a complex set of carboxyl-terminal derivatives was produced by the endosomal-lysosomal system that included potentially amyloidogenic forms. Exposure of the cells to 50 mM ammonium chloride reduced the entire set of 8-12 kD carboxyl-terminal derivatives and almost abolished the two largest forms; at the same time it augmented the cell content of the full length β-APP. Treatment with ammonium chloride had, however, no effect on secretase cleavage. Thus, it appears that β-APP may be internalized from the cell surface and targeted to lysosomes, where an array of potentially amyloidogenic carboxyl-terminal fragments are generated.

Ammonia (and other weak bases) are known to interfere with lysosomal proteolysis due to elevation of the intralysosomal pH (133). It has been shown for example that human glial cells in culture, if exposed to glycosaminoglycans and ammonium acetate, assume the appearance of cells of patients with mucopolysaccharidosis (134). In hyperammonemic rats hepatic lysosomal proteolysis is diminished (135). It has been mentioned that in the brains of hyperammonemic rats the intracellular pH is increased (107). A number of studies suggested that the transport of ammonia follows the pH gradient, due to the higher permeability of membranes for NH_3 than for NH_4^+ (56). Owing to their low pH, lysosomes are the organelles in which ammonia will preferentially accumulate, even at moderately elevated intracellular ammonia concentrations. From the above mentioned observations the impairment of lysosomal proteolysis in states of hyperammonemia is to be expected, and the gradual accumulation of various proteins, including β-APP. This does, however, not imply that hydrolytic cleavage of all proteins will be equally affected. On the contrary, it is more likely that some of the lysosomal hydrolases are more sensitive than others to the ammonia induced changes of their environment, and the impairment of the cleavage of the selected proteins may be more important than that of others.

The recent observation of the enhanced proteolysis of MAP-2 (a protein, controlling together with tau protein the polymerization of microtubules) in hyperammonemic rat brain (136) is in apparent disagreement with the above assumption, and points at another aspect of proteolytic activity in hyperammonemia : From the fact that lysosomal hydrolases of different classes have been localized in extralysosomal compartments (e.g. in perikarya and proximal dendrites of many cortical neurons), and in senile plaques (137,138), it is tempting to speculate that the fragile lysosomes are disrupted due to ammonia accumulation and metabolic derangements following abnormal ammonia accumulation, so that lysosomal hydrolases are able to exert detrimental effects in various parts of the cells to which they normally do not have access. This idea is further supported, albeit indirectly, by the observation that β-glucuronidase (a lysosomal enzyme) activity measured post-mortem in DAT brains, and the metabolic rates for glucose, determined pre-mortem by PET in the same patients, were inversely correlated (8). The invasion of microglia into cortical and other brain areas with prevailing neuronal degeneration is a potential source of lysosomal enzymes, since, as has been mentioned, microglia are an invariable component of senile plaques (20).

CONCLUSIONS

It is not generally recognized that there is considerable evidence in favor of the idea that hyperammonemia may be a common feature of DAT. Although age-related reduction of liver function could contribute, excessive ammonia formation by hydrolytic processes and oxidative deaminations, together with the impairment of the physiological detoxification mechanisms are more likely causes of DAT hyperammonemia.

Although considerably different with respect to disease progression and symptoms, hepatogenic hyperammonemias are the cause for pathologic alterations of the brain that are also found in DAT. The derangement of astrocyte metabolism and astrocytosis, the decrease in the rates of glucose utilization and energy metabolism, and the enhanced release of excitotoxic amino acids are perhaps the most important ones. Those hypotheses concerning the etiology of DAT which are centered around astrocyte dysfunction and excitotoxic mechanisms are, therefore, of great interest in connection with hyperammonemia.

A major difference between brain-born hyperammonemia, as is suggested for DAT, and hyperammonemia generated by liver dysfunction is that in the latter not only ammonia, but aliphatic amines, mercaptans and other toxins generated in the gastrointestinal tract, may

enter the brain and contribute to the pathophysiology of the disease (64,139).

It was not the purpose of this paper to generate an "ammonia hypothesis of DAT", but it was intended to attract attention to the possibility that ammonia is an important factor which may affect manifestations and progression of DAT by interfering with basic functions of the brain, such as neurotransmission and proteolysis.

No attempts were made in this review to connect hyperammonemia to regional aspects of neuronal degeneration, to the selective vulnerability of cholinergic and other neurons, or to behavioral and cognitive abnormalities in DAT, in order to avoid overinterpretation of the scarce data. But, I have no doubt that once a role of ammonia in the pathophysiological manifestations of DAT has attracted more general attention, refined and specifically designed experiments will soon provide answers to those open questions that are needed to allow us to draw a more detailed picture of the role that ammonia might play in the etiology of DAT.

A considerable effort is presently put on the identification of targets which may allow the development of preventive therapies for DAT patients. As has been pointed out, the effects of ammonia on brain metabolism are expected to be such that progressive amplifications of initially mild functional derangements may occur. If this is true, prevention, or amelioration of hyperammonemia may favourably influence symptoms of the disease, and decrease the rate of its progression. Therapeutic efforts in hepatogenic encephalopathy, suitable to antagonize central toxicity of ammonia may, therefore, prove beneficial for patients suffering from DAT.

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