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Disturbances of acetyl-CoA, energy and acetylcholine metabolism in some encephalopathies

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Review

Abstract. Acetyl-CoA provision to the synaptoplasmic comparment of cholinergic nerve terminals plays a regulatory role in the synthesis of acetylcholine. The disturbances in glucose utilization and in decarboxylation of the end product of its matabolism pyruvate, are considered to be significant factors causing cholinergic deficits in several diseases of the central nervous system. In this article we review data concerning role of acetyl-CoA in patomechanisms of disturbances of cholinergic metabolism in Alzheimers disease, thiamine deficiency, inherited defects of pyruvate dehydrogenase and diabetes.

Key words: acetylcholine, acetyl-CoA, metabolic encephalopathies, nerve terminals

INTRODUCTION

There are several disorders of the CNS in which a dominant feature is the progressive impairement and subsequent destruction of the cholinergic system. They include Alzheimers disease, dialysis encephalopathy, thiamine deficiency, alcoholism, inherited deficiencies of pyruvate dehydrogenase and Huntington's disease. Disturbances in cholinergic transmission cause in affected individuals a wide range of psychoneurological symptoms such as different forms of mental disability (agnosia, apraxia, aphasia), hyperexcitability (convulsions, ataxia), muscular weakness as well as sensory disturbances. Different ethiologic factors responsible for development of these pathologies may affect various steps of acetylcholine (ACh) metabolism in different groups of cholinergic neurones yielding distinct clinical symptoms for the particular disease.

Ultimate indicators of impairement of cholinergic neurones in these disorders are however autopsy findings which reveal decreases in levels of various specific cholinergic markers such as choline acetyltransferase (CAT), high affinity choline uptake system (HACU) or density of cholinergic receptors in affected areas of the brain. Another typical feature of pathologies that preferentially affect the central cholinergic system is a concomitant decrease of glucose utilization and the suppression of oxidative metabolism in the brain. Irrespective of the latter, if this inhibition is the cause or the consequence of the disease, it should bring about insufficient production of acetyl-CoA. Indirect evidences supporting this claim come from studies on animal brains (Ričny and Tuček 1980, Szutowicz et al. 1981,Gibson et al. 1982, Szutowicz et al. 1994a). In cholinergic neurones acetyl-CoA is utilized not only for energy production but also for ACh synthesis. For this reason cholinergic neurones might be more vulnerable to any limitation of acetyl-CoA provision than other types of brain cells.

Recent data indicate that the opposite situation should be also taken into consideration. The rises in concentrations of glucose and (or) ketoacids in extracellular fluid as well as a decrease in insulin levels taking place in experimental diabetes may exert direct and independent influences on function of cholinergic neurones (Wozniak et al. 1993 for review). The significance of this phenomenon for cholinergic pathology in diabetic encephalopathy in humans has not been so far investigated.

REGULATION OF ACETYLCHOLINE SYNTHESIS

Choline acetyltransferase

Functionally important neurotransmitter pool of ACh is synthesized in cytoplasm of cholinergic nerve terminals in the reaction catalysed by CAT according to equation:

$$\text{choline} + \text{acetyl-CoA} \rightleftharpoons \text{ACh} + \text{CoA}$$

This reaction is reversible but its equilibrium constant equal to about 13 favours ACh fromation (Hersh 1982). In spite of large variations in the activity of cholinergic neurones and consequently in rates of ACh release from their nerve terminals, the level of transmitter in the brain is maintained at a constant level. This indicates that the velocity of ACh synthesis has to be very well adjusted to rate of its loss from neurones (MacIntosh and Collier 1976).

It is commonly accepted that the activity of CAT is high enough to meet these requirements. This view comes from data on CAT activities measured at saturating concentrations of substrates which were found to be far in excess of actual rate of ACh synthesis under *in vivo* and *in vitro* conditions (Fonnum 1969, Benjamin Quastel 1981, Szutowicz et al. 1982b, Schliebs et al. 1989, Szutowicz and Bielarczyk 1991). On the other hand, there are indications that only a small, membrane bound fraction of CAT might participate in ACh synthesis in nerve terminals (Cooper 1994).

In addition, endogenous levels of choline and acetyl-CoA in brain are much below their optimal concentrations for CAT (Hebb 1972, Reynolds and Blass 1975, Tuček 1983). Therefore, the rate of

ACh synthesis under out of equilibrium conditions caused by transmitter release is presumably regulated both by CAT content as well as by its substrates concentrations in cholinergic terminals. In consequence, the depression of even one of these components under pathologic conditions might be sufficient for ACh deficit to develop. The decrease of CAT activity in brain tissue may result from either downregulation of its synthesis in existing neurons or (and) from the loss of cholinergic neurons (Rallet and Smith 1993). In practice CAT deficits caused by the destruction of cholinergic cells may be aggravated by suppression of enzyme expression and (or) substrate supply in the remaining ones.

Choline supply

The brain's capacity to synthesize endogenous choline is very limited. Choline is transported from the blood through the hematoencephalic barrier to extracellular brain space down its concentrations gradient. All brain cells possess a low affinity, high capacity choline uptake system, which assures the equilibrium between extra and intracellular pools of this metabolite (Tuček 1985, Wurtman 1992). The positive charge of choline and negative membrane potential mean that it may accumulate inside the cells in concentrations several times higher than those present in extracellular space. Subsequently choline is incorporated into structural phospholipids (Wurtman 1992). The avearage concentration of choline in cytosol of brain cells was calculated to be close to 50 μM (Tuček 1985). It is very likely, though not proven, that choline in cholinergic terminals may be present at higher than equilibrium concentrations, since they possess additional HACU system (Yamamura and Snyder 1973, Suszkiw and Pilar 1976). This system utilizes energy of sodium gradient across the nerve ending wall for choline transport (Breer and Knipper 1985). Choline supplied by this system is preferentially utilized for ACh synthesis (Suszkiw and Pilar 1976). It has been assessed that about 50% of choline derived from ACh released to and cleaved in the synaptic cleft is recoverd by cholinergic terminals (Collier and Katz 1974). A small fraction of choline may be also provided by hydrolysis of phosphatidylcholine present in neuroronal membranes (Wurtman 1992). Depolarization of cholinergic teminals is known to activate release of ACh and disinhibit choline transport due to a decrease of intraterminal transmitter concentration (Fig. 1) (Marchbanks 1981, Tuček 1985). On the othe hand, the decrease of the membrane potential was found to inhibit choline uptake (Beach et al. 1980, Scremin and Jenden 1993). However, subsequent poststimulation re or hyperpolarization would lead to a marked activation of choline uptake thereby providing increased amounts of the precursor for quick restoration of the ACh pool in terminals (O'Regan and Collier 1981, Tuček 1985).

The selective vulnerability of cholinergic neurones to energy deficits, found to occur in Alzheimers disease (AD) (Perry 1980, Benson 1983) or thiamine deficiency (Butterworth 1989), was assumed to result from the fact that they need choline not only for membrane building (like rest of cells) but also for ACh synthesis (Wurtman 1992). The sustained depolarization of cholinergic nerve terminals, in states of energy deficits, or during excitotoxic stimulation, would activate ACh release and increased demand for choline to reconstitute the transmitter pool. However, this demand could not be met due to inhibition of choline uptake systems by a low membrane potential (Fig. 1) (O'Regan and Collier 1980). In addition, the capacity of HACU system was reported to be reduced in degenerative brain diseases (Pasqual et al. 1991, Berger et al. 1992, Francis et al. 1994). In result of these aberrations choline produced in synaptic cleft could not be taken up back into cholinergic terminals with sufficient yield. The shortage of exogenous choline for ACh resynthesis could be probably replaced by increased hydrolysis of phosphatidylcholine in terminal membranes (Ulus et al. 1989). If such situation persists the amount of phosphatidylcholine in membranes would decrease leading to shrinkage of cholinergic neurones and ultimately to their death (Wurtman 1992).

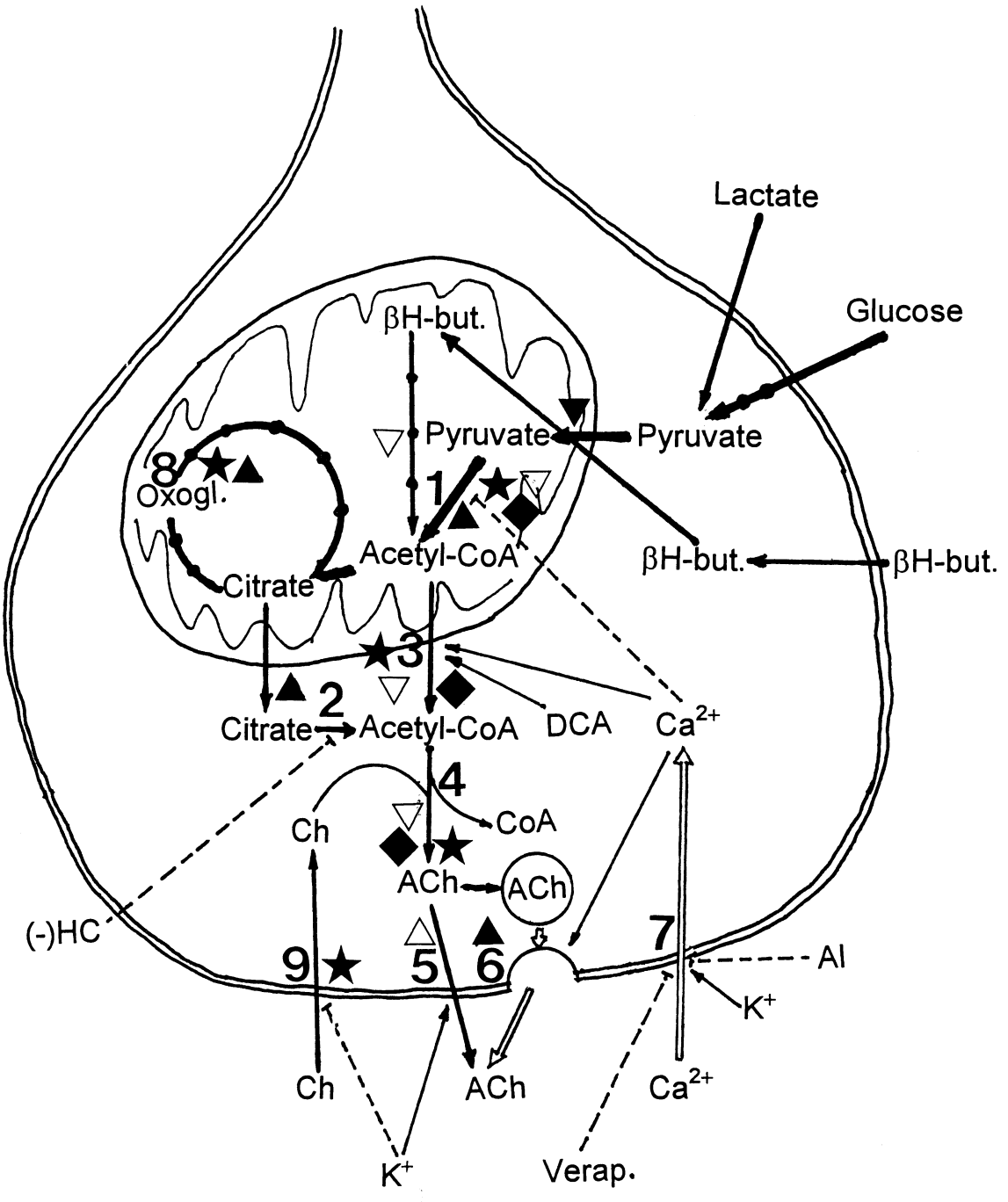

Fig. 1. Putative sites of disturbances in acetyl-CoA metabolism in cholinergic terminals in different encephalopathies. Symbols used: 1, pyruvate dehydrogenase (EC 1.2.4.1.); 2, ATP-citrate lyase (EC 4.1.3.8.); 3, direct transport of acetyl-CoA through the mitochondrial membrane; 4, choline acetyltransferase (EC 2.3.1.6.); 5, Ca-independent ACh release; 6, Ca-dependent (quantal) ACh release; 7, calcium influx; 8, 2-Oxoglutarate dehydrogenase (EC 1.2.4.2.); 9, high affinity choline uptake. Thick and medium thick arrows indicate different enzymatic and transport steps. Thin solid arrows and thin dashed indicators point activatory and inhibitory influences, respectively for cations and drugs. Abbreviations: (-)HC, (-)hydroxycitrate; DCA, dichloroacetate; Verap., verapamil; Oxogl., 2-oxoglutarate; βH-but., β-hydroxybutyrate. Possible sites of metabolic disturbances in different ecephalopaties are marked by: star, Alzheimer's disease; triangle, thiamine deficiency; diamond, inherited pyruvate dehydrogenase deficiency; reversed triangle, diabetes. Open and filled symbols indicate possible activatory and inhibitory influences, respectively.

Acetyl-CoA supply

Acetyl-CoA for ACh synthesis is generated in nerve terminal mitochondria from pyruvate derived from glucose in the pyruvate dehydrogenase reaction (Tuček and Cheng 1970, Lefresne et al. 1973). Under certain physiologic and pathologic coditions β-hydroxybutyrate and lactate may in part substitute glucose as a source of acetyl-CoA for the trasmitter synthesis (Fig. 1) (Gibson and Shimada 1980, Sterling et al. 1981, Izumi et al. 1994, Szutowicz et al. 1994b, Larrabee 1995). There is a strict direct relationship between the rate of pyruvate decarboxylation and the rate of ACh synthesis in brain even though the bulk of mitochondrial production of acetyl-CoA in cholinergic neurones is utilized for energy production and only about 1% is converted to ACh (Lefresne et al. 1973, Gibson et al. 1975). It may due to the fact that intramitochondrial acetyl-CoA does not freely pass a mitochondrial membrane to reach the site of ACh synthesis in cytoplasm. Hence, the trasport of acetyl units from mitochondria to cytoplasm may be a rate limiting factor in ACh synthesis (Szutowicz et al. 1981, Bielarczyk and Szutowicz 1989). Despite of almost 30 years of research, pathways and regulatory mechanisms of the transfer of acetyl groups from intramitochondrial to extramitochondrial compartment of the cholinergic neurone remain unclear. Limited knowledge on this subject may result from the fact that cholinergic neurones form a small fraction (1%) of whole population of brain cells. Therefore one may estimate that cholinergic neurons synthesize only 1% of brain acetyl-CoA, 0,01% of which is utilized for ACh synthesis. Thus any extrapolation of acetyl-CoA estimations in whole brain tissue, to cholinergic compartment must be treated with great caution. Somewhat better proportions between cholinergic and noncholinergic compartment exist in nerve terminals isolated form the brain which were found to contain about a 10% fraction of cholinergic nerve endings (Richardson et al. 1981).

It is pretty well documented that about 30% of acetyl units for ACh synthesis leaves mitochondria of nerve terminals as citrate, which in synaptoplasm is converted back to acetyl-CoA by ATP-citrate lyase (Fig. 1) (Szutowicz et al. 1977, Gibson et al. 1980, Szutowicz et al. 1981, Tuček 1985, 1993 for review). This pathway seems to be very important for the provision of acetyl-CoA in activated cholinergic terminals. Our recent data show that the specific inhibitor of ATP-citrate lyase, (-)hydroxycitrate did not affect cytoplasmic acetyl-CoA content and ACh synthesis in resting terminals while supressing both parameters in depolarized ones in the presence of exogenous Ca^{2+}(Szutowicz et al. 1994a).

Data concerning other pathways are scarse and less clear. Inner mitochondrial membrane posses Ca^{2+}-activated hydrophilic channels which may allow some acetyl-CoA moieties to pass directly to synaptoplasmic compartments (Selwyn 1987). Ričny and Tuček (1983) have shown that isolated glial mitochondria released acetyl-CoA when exposed to nearly physiologic rises of Ca^{2+}. They however did not make clear if cytosolic Ca^{2+} might activate the direct transfer of acetyl-CoA from intraterminal mitochondria in situ, during the depolarization of nerve terminals. Indeed, our later studies have shown that in synaptosomes incubated in high K^+ medium, the exogenous Ca^{2+} decreased acetyl-CoA level in their mitochondria probably due to the stimulation of its passage to synaptoplasm (Fig. 1). On the contrary, inhibition of Ca entry by verapamil, increased acetyl-CoA content in mitochondria but decreased its concentration in synaptoplasm yielding suppression of ACh synthesis (Bielarczyk and Szutowicz 1989, Szutowicz and Bielarczyk 1991). Thus the cooperation of the direct and indirect patways of acetyl group transport through the mitochondrial membrane seems to be necessary to maintain sufficiently high concentration of cytoplasmic acetyl-CoA for depolarization--evoked ACh synthesis.

Acetylcarnitine (ACT) is another putative carrier for acetyl-CoA. ACT and carnitine alone were found to increase ACh synthesis from glucose both under *in vivo* and *in vitro* conditions (Gibson and Shimada 1980, Doležal and Tuček 1981). More in-

direct evidences show that carnitine may prevent depletion of ACh in brains of quinuclidynyl benzylate stimulated animals (Ričny et al. 1992). It has been also reported that L-ACT alleviated memory deficits in poeple with Alzheimer's disease (Pettegrew et al. 1995). The latter effect could be however brought about by the ability of carnitine to increase supply of acetyl-CoA for the synthesis of structural lipids in the brain (Aureli et al. 1990, Carta et al. 1993).

Data presented in the following pargraphs demonstrate that acetyl-CoA provision to the cytoplasm of cholinergic neurones may be the step of ACh metabolism, which is potentially susceptible to different pathologic insults. Thereby it ought to be considered as a significant factor in patomechanism of cholinergic pathologies.

ACETYL-CoA IN PATHOLOGIES OF CHOLINERGIC SYSTEM

Alzheimers disease and dialysis encephalopathy

The destruction of cholinergic neurones in AD and dialysis encephalopathy is well documented. Several reports have been showing marked reductions of cholinergic markers like CAT activity, its immunostaining or mRNA content, as well as HACU activity and ACh synthesis in susceptible brain areas (McGeer and McGeer 1981 for review, Bowen 1983, Pascual et al. 1991, Francis et al. 1994). These cholinergic deficiencies colocalized with typical morphological lesions - amyloid plaques and neurofibrillary tangles. Highest decreases in values of cholinergic markers varying from 50 up to 90%, against age matched controls, were observed in brain areas responsible for learning and memory storage including: septum, hippocampus, temporal and parietai cortex, as well as in olphactory bulbs (McGeer and McGeer 1981, Reinikainen et al. 1988, Talamo et al. 1989). The degree of decrease of cholinergic markers in these ereas corresponded with the degree of dementia immediately before death (Perry 1986, Bierer et al. 1995).

Another intriguing finding was inhibition of glucose uptake and its oxidative metabolism, probably due to reduction of glucose transporter in AD brains (Benson 1983, Bowen 1983, Kalaria and Harik 1989). These findings remained in accord with 30 - 80% reductions of pyruvate and oxoglutarate dehydrogenase (PDH, OGDH) activites found in sites overlaping those with cholinergic deficits (Perry et al. 1980, Sheu et al. 1985, Butterworth and Besnard 1990, Mastrogiacomo et al. 1993). These findings may be indices of marked impairement of acetyl-CoA and energy production in AD brains. In addition, a significant decrease of ATP-citrate lyase activity was also detected which was concordant with preferential localization of this enzyme in cholinergic neurones, shown in animal studies (Perry et al.1980, Szutowicz et al. 1982a,b). On the other hand, even complete destruction of cholinergic neurones should not bring about so profound reductions in PDH and OGDH activities since their distribution in normal brain does not match distribution of cholinergic innervation (Fonnum 1969, Szutowicz et al. 1982b, Butterworth et al. 1985, 1986, Mastrogiacomo et al. 1993). This discrepancy might be explained by immunochemical data showing no substantial changes in the amount of PDH protein in AD brains and suggesting the existence of an endogenous inihibitor or inactivatory modification of the PDH complex (Sheu et al. 1985). The nature of this phenomenon is not known. It however indicates that disturbances of energy metabolism in AD brain are not confined to cholinergic neurones only.

The preferential damage of the cholinergic cells in the course of AD may result from their particular vulnerability to decreased provision of both acetyl-CoA and choline, which are utilized by them not only for energy production and synthesis of structural lipids but also for synthesis of ACh (Tuček and Cheng 1970, Lefresne et al. 1973, Wurtman 1992). Among possibilities that need to be tested to explain this phenomenon one may quote phosphorylation or other modifications of PDH complex proteins, or its inhibition by compouds accumulated in or produced by cells in the course of the disease (Giulian

et al. 1995). Recent reports evidence that deposits of β-amyloid peptide could induce formation of high conductance Ca^{2+} channels in cells membranes and (or) reactive oxygen species (Arispe et al. 1993, Hartmann et al. 1993, Mattson and Goodman 1995). They would trigger mechanisms, leading to neuronal cells death. The supposition coming from animal studies (Calligan et al. 1995) that neuronal loss in AD might result from thiamine deficit has no support in relevant clinical data.

Aluminum is widely tested although still a controversial factor in patomechanism of AD (Meiri et al. 1993). The cation has been found to accumulate in high concentrations in senile plaques as well as in neurones bearing neurofibrillary tangles (Cooper et al. 1978, Meiri et al. 1993 for review). The local Al level might be high enough to exert multiple effects on the neuronal functions due to its interaction with Ca-dependent processes (Koenig and Jope 1987, Wakui et al. 1990, Wood et al. 1990). However, Al accumulation in AD brain lesions and its neurotoxic influence may be two independent events. Such thesis is substantiated by studies on brains of Parkinson's and dialysis encephalopaty victims. They have shown that loss of cholinergic neurones and excessive accumulation of Al in these brains were not accompanied by morphological lesions seen in AD (Perry et al. 1985, Yasui et al. 1992, Meiri et al. 1993).

The Al may be transported into brain cells even under physiologic conditions using transferrin and its surface receptors (Shi and Huang 1990, Martin 1992, Walton et al. 1995). Excessive accumulation of Al in AD neurones could be presumably promoted by its binding to hyperfosforylated tau neurofibrillary protein and other abberant structures of pathologically changed neurones (Scott et al. 1993, Shin et al. 1994). Concentration of Al in these lesions was reported to reach very high 0.25 mM concentrations (Meiri et al. 1993). The Al entry to all brain cells could be also facilitated by the decrease of tissue pH caused by local hypoxia due to insuffiencies in brain microcirculation, common in aged poeple (Shi and Haug 1990). Inhibitory effects of Al on oxidative metabolism are thought to be due to its interference with Ca entry to cytoplasm, and competition with Ca and Mg for nucleotide and enzyme binding sites (Johnson and Jope 1986, Martin 1992, Meiri et al. 1993). In addition, inhibitory complexes of Al are very stable; their dissociation rates were found to be 1,000 times slower that those of Ca (Martin 1992).

There are indications that Al might exert a direct effect impairing function of cholinergic terminals (Lai et al. 1980, Johnson and Jope 1986, Meiri et al. 1993). We have found that 0.25 mM Al only slightly inhibited the pyruvate consumption in resting nerve endings, but had no effect in those activated by addition of Ca (Szutowicz et al. 1993). Hence this finding would not explain the mechanism of marked suppression of PDH activity in AD brains (Sheu et al. 1985). On the other hand, in K-Ca-activated synaptosomes, Al increased acetyl-CoA content in their mitochondria, reduced its level in synaptoplasm and inhibited ACh synthesis, respectively (Table I, Fig. 1) (Szutowicz et al. 1993). Aluminum partially reversed suppressory effects of verapamil on the level of acetyl-CoA in synaptoplasm as well as on ACh synthesis. It indicates that Al presumably interacts with the verapamil binding site on plasma membrane calcium channels (Fig. 1) (Szutowicz et al. 1993). Interaction of Al with plasma membranes as a sole source of these metabolic changes is supported by neuronal culture studies showing no entry of Al into the cytoplasm at physiologic pH and a relatively short incubation time (Shi and Haug 1990). This finding substantiates the hypothesis, that besides widely recognized pathogenic mechanisms in AD brain, Al might be an additional factor aggravating existing cholinergic deficits by the inhibition of Ca-dependent acetyl-CoA transport to cytoplasm of nerve terminals of remaining cholinergic neurones (Fig. 1).

Thiamine deficiency

Thiamine deficiency in humans and animals leads to progressive and potentially fatal, if not treated, encephalopathy. Clinical symptoms of Wernicke-Korsakoff syndrome in humans, or pyri-

TABLE I

Relationships between rates of acetylcholine synthesis and acetyl-CoA contents in brain compartments

Animals	Incubation conditions Preparation	Acetyl-CoA Content	Acetylcholine Content	 Synthesis	Reference
Thiamine deficient diet	- Whole brain	67	64		Heinrich et al. 1973
Thiamine deficient diet	- Whole brain	118	109		Reynolds, Blass 1975
Normal	0.5 mM glucose* 10.0 mM glucose* Brain slices	34 192	52 184	31 21	Ričny, Tuček, 1981
Normal	2.5 mM pyruvate, 2.5 mM L-malate 0.25 mM 3-bromopyruvate Synaptosomes Synaptoplasm	 11 16		 33	Bielarczyk, Szutowicz 1989
Normal	2.5 mM pyruvate, 2.5 mM L-malate 0.25 mM $AlCl_3$ Synaptosomes Synaptoplasm	 100 71		 79 (36) [138]	Szutowicz et al. 1993
Normal	2.5 mM pyruvate, 2.5 mM L-malate 1.0 mM (-)hydroxycitrate Synaptosomes Synaptoplasm 1.0 mM (-)hydroxycitrate, 0.5 mM dichloroacetate Synaptosomes Synaptoplasm	 90 73 106 102		 71 (27) [118] 102 (97) [108]	Szutowicz et al. 1994a
Streptozotocin diabetes Nontreated Insulin treated	2.5 mM pyruvate, 2.5 mM L-malate 2.5 mM β-hydroxybutyrate Synaptosomes Synaptosomes	 129 149		 142(133) [150] 149(172) [126]	Szutowicz et al. 1994b
Thiamine deficient diet, pyrithiamine	2.5 mM pyruvate, 2.5 mM L-malate Synaptosomes Synaptoplasm	 82 77		 93 (49) [135]	Kisielevski et al. 1995

Relative values presented here were calculated from original data as a percentage of respective controls. ACh data concern its synthesis/release at different incubation conditions: no brackets, ~ 30 mM KCl and 1 mM $CaCl_2$, , maximal ACh synthesis; square brackets, ~30 mM KCl, no $CaCl_2$, ACh synthesis linked with its nonquantal release; round brackets, Ca-evoked ACh synthesis -linked with its quantal release (Fig. 1) (Thesleff and Molgó 1983). *Data refer to values at 2.0 mM glucose.

thiamine encephalopathy in rats such as memory loss, ophtalmoplegia, ataxia and convulsions are thought to be signs of imairement of cholinergic system. Most of the metabolic studies on thiamine deficient rats shows consistently 10-60% decrease of ACh synthesis in homogenates and slices from different regions of the brain as well as in cervical ganglia (Gibson et al. 1982 for review). This suppression became more evident when ACh metabolism was stimulated by depolarization (Gibson et al. 1984). On the other hand, several studies showed an unchanged or relatively slightly decreased ACh concentration in affected brains (Gibson et al. 1982, Platakis et al. 1982). The explanation for this apparent discrepancy is that in any dynamic system the rate of synthesis tends to readjust to maintain homeostasis. Under physiologic conditions the increasing firing frequency of the synapse is known to activate the rate of ACh release severalfold with no effect upon the level (McIntosh and Collier 1976, Tuček 1993). In TD animals this adjustment mechanism may be sufficient only under resting conditions (Sacci et al. 1978). If animals were killed during such a functional status of cholinergic neurones, no change of ACh content was recorded. The significance of cholinergic mechanism in TD is also supported by studies with acetylcholinesterase inhibitors which alleviated some symptoms of this pathology as well as the mortality of experimental animals (Blass et al. 1981, Gibson et al. 1982).

On the contrary, the structural integrity of cholinergic neurones apears to be well preserved in TD. Activities of CAT, acetylcholinesterase as well as HACU system were found not to be decreased (Gibson et al. 1982, Plaitakis et al. 1982, Thompson et al. 1985, Kisielevski et al. 1995). Hence, the inhibition of ACh synthesis has been repeatedly explained by limitations of acetyl-CoA and energy production caused by inhibition of pyruvate oxidation and energy metabolism in TD brain. In fact, several concordant reports show that activites of OGDH in whole and several regions of TD brains are decreased from 20 to about 50%. These decreases were found to be much greater in TD caused by joint application of thiamine free diet and pyrithiamine (thiamine antagonist) than by the diet alone (Butterworth 1986 for review). It corresponded well to more severe clinical symptoms and more severe neuropathological brain damages taking place in the former experimental group. On the contrary, activities of PDH were much less or even not affected by these treatments. What is even more supprising, the greater and more widely spread decreases in PDH activity were found in rats made deficient by simple feeding with thiamine free diet than in those treated additionally with pyrithiamine (Butterworth 1986). Irrespective of inconsitecies in these enzymatic studies, the existence of inhibition of pyruvate and oxoglutarate oxidation as well as decrease in high energy phosphates levels was reported in both experimental models (Aikawa et al. 1984, Butterworth 1989, Kisielevski et al. 1995).

One has to stress that the assumption that these enzymatic changes might bring about a shortage of acetyl-CoA in the cholineregic neurones was not substantiated by adequate experimental data. The reports on the levels of acetyl-CoA as an indirect indicator of its availability are scarse. They show either its decrease or no change of its level in TD brain (Table I) (Heinrich et al. 1973, Reynolds and Blass 1975).

Moreover, it has appeared that prolonged tea consumption which evoked moderate thiamine deprivation with no signs of neural pathology caused much greater suppression of PDH activity and ACh synthesis than pyrithiamine encephalopathy (Ruenwongsa and Pattavibag 1982, 1984). Reduced activities of thiamine dependent enzymes were also found in brains of alcoholics who died with no signs of Wernicke encephalopathy (Lavole and Butterworth 1995). These findings indicate that limitation of the acetyl-CoA supply in the TD brain may be only one of several factors needed to combine to evoke encephalpathy.

In addition, data described above concern whole brain preparations containing a great variety of glial and neuronal cells. Vulnerability of different groups of cells in respective brain regions to TD may vary markedly. For example, histopathologic data indicate that glial cells but not neurones may

be the site of early lesions in TD (Robertson et al. 1968). Enzymologic and metabolic studies of more (pons, nucleus vestibularis) and less TD-sensitive regions (cortex, nucleus cochlearis) have no potential to solve this problem (Butterworth 1986, Gibson et al. 1989). Therefore, the evaluation of the significance of eventual acetyl-CoA shortage in development of cholinergic deficits needs studies taking into consideration specific features of its metabolism in nerve terminal compartment.

Our studies performed on isolated cortical synaptosomes and nonsynaptosomal (mainly glial) mitochondria fractions showed in boh of them a decrease of PDH activity and pyruvate consumption. The inhibition was slightly higher in isolated mitochondria: 23-45% vs. 19-30%, depending on assay conditions (Kisielevski et al. 1995). On the contrary, the decrease in OGDH activity appeared to be much higher in synaptosomal fraction (78% vs. 35%). These data suggest that the regional differences in sensitivity of TPP-dependent enzymes to TD, reported elswhere (Butterworth 1986), may result from variable proportions between glial and neuronal cells in various brain regions. No changes in synaptosomal CAT activity were found, indicating good preservation of cholinergic terminals in TD (Plaitakis et al. 1982, Kisielevski et al. 1995).

We also studied the effect of TD on acetyl-CoA content in mitochondrial and synaptoplasmic compartments of nerve terminals to test the hypothesis of its involvement in mechanism of ACh deficiency in this pathology. Synaptosomes were incubated with pyruvate in the presence or absence of L-malate to assure condensation of acetyl-CoA to citrate or one step utilization of pyruvate, respectively. In former conditions both ATP-citrate lyase and the direct pathway of provision of synaptoplasmic acetyl-CoA were found to be operative in nerve terminals (Szutowicz et al. 1977, 1981, 1994a, Bielarczyk and Szutowicz 1989). With pyruvate as a sole substrate only the direct trasport of acetyl-CoA was assumed to take place (Kisielevski et al. 1995). High $[K^+]$ was used to depolarize synaptosomes in the absence or presence of Ca^{2+} to examine ACh synthesis under conditions of resting and its activated (quantal) release, respectively (Lefresne et al. 1973).

The acetyl-CoA contents in TD synaptosomal mitochondria and cytoplasmic fractions were decreased by 23%, when they were incubated with pyruvate in the presence of L-malate (Table I). In the absence of L-malate the content of acetyl-CoA in mitochondria increased by 50% but that in synaptoplasm decreased by 45% (Kisielevski et al. 1995), probably due to the limitation of citrate formation, which is known to transport a significant fraction of acetyl units from mitochondria to synaptoplasm (Szutowicz et al. 1981, Tuček 1985). Under these conditions TD had no effect on acetyl-CoA contents and its compartmentalization in synaptosomes (Kisielevski et al. 1995). From these data one may conclude that TD inhibits indirect transport of acetyl-CoA by ATP-citrate lyase pathway not affecting its direct flux through the mitochondrial membrane (Fig. 1).

The changes in ACh synthesis by nerve terminals of TD brain were apparently not related to changes in synaptoplasmic acetyl-CoA level or its synthesis in mitochondria (see above). Under resting conditions and in the presence of L-malate, ACh synthesis in TD synaptosomes was 35-60% higher than that in controls. However, in TD terminals Ca-evoked ACh synthesis was much lower than that in controls (Tab. I). The rate of citrate-independent ACh synthesis corresponded to 50% of total transmitter synthesis. The synthesis of this fraction of ACh was increased in TD, both in resting and Ca-activated conditions. The increase of Ca-independent release of ACh in TD nerve terminals was likely to be caused by energy deficits resulting from decreased oxidation of pyruvate/oxoglutarate and (or) from depletion of thiamine triphosphate from their membranes (Dreyfus 1985, Hazell et al. 1993). Thiamine triphosphate has been shown to be involved in sodium-gating mechanisms of excitable membranes (Dreyfus 1985). Deficient terminals would be unable to accumulate ACh in synaptic vesicles and maintain membrane potential. It would increase a nonquantal release and compensatory synthesis of ACh (Fig. 1). It might explain symp-

toms of neuronal hyperexcitability and transmitter depletion in brains of TD animals *in vivo* (Gibson et al. 1982, Plaitakis 1982, Butterworth 1989). On the contrary, the Ca and citrate-dependent quantal release of ACh would be markedly reduced (Fig. 1) (Kisielevski et al. 1995). The inhibition of provision of cytoplasmic acetyl-CoA *via* ATP-citrate lyase pathway could be compensated in part by increased direct transport of acetyl-CoA from mitochondria. It could be facilitated by marked increase of intramitochondrial acetyl-CoA, due to inhibition of oxoglutarate decarboxylation yielding insufficient production of oxaloacetate (Fig. 1) (Butterworth et al. 1986). Such supposition is supported by data showing low levels of aspartate in TD brains (Hazell et al. 1993). The data presented provide a circumstantial evidence that functional cholinergic deficit in TD is likely to be caused rather by reduction of ACh pool available for quantal release than by decreased provision of synaptoplasmic acetyl-CoA.

Inherited PDH deficiences

The genetic disturbances leading to insufficient expression or synthesis of aberrant PDH protein(s) bring about limitation of production of acetyl-CoA in all tissues of affected individual. The clinical symptoms include lactic acidosis and multiple morphologic and functional abnormalities of the central nervous system (microcephalia, seizures, mental retardation, ataxia). The severity of these symptoms was found to be strightly related to the degree of the enzyme deficiency (Kark and Rodriguez-Budelli 1979, Butterworth 1985). Although, no direct observations have been made, some evidences indicated that ACh metabolism may be impaired in these conditions (Gibson et al. 1982).

In animal studies variety of unrelated compounds like barbiturates, haloperidol, branched chain ketoacids and bromopyruvate were reported to bring about the inhibition of PDH activity, which was accompanied by a proportional suppression of ACh synthesis (Lefresne et al. 1973, Gibson et al. 1982, Bielarczyk and Szutowicz 1989). We have found that bromopyruvate caused a marked decrease of acetyl-CoA in nerve terminal cytoplasm (Bielarczyk and Szutowicz 1989). Hence, it was possible to link directly drop of synaptoplasmic acetyl-CoA with inhibition of neurotransmitter synthesis. The dichloroacetate (DCA), a potent inhibitor of PDH kinase was used to reduce existing enzyme deficits with hope to increase content of active dephospo form of the enzyme (Whitehouse et al. 1974, Kuroda et al. 1987, Stackpole 1989).

In accord with this assumption remained improvement of clinical status and decrease of lactic acidemia observed in patients after application of DCA (Ito et al. 1992). These effects could be due to the action of this compound on periferal tissues where a marked fraction of PDH occurs in inactive phosphorylated form. Moreover, PDH activity in cultured fibroblasts from most of PDH deficient individuals failed to respond with activaton to the application of DCA (Kuroda et al. 1987). Also the effect of DCA on pyruvate oxidation in the brain appeared to be negligible since PDH in this tissue was found to be almost completely dephosposhorylated (Shaffer and Olson 1980, Szutowicz et al. 1994a). These discrepancies point out the existence of additional sites of DCA action on pyruvate metabolism. Our studies have shown that DCA might overcome inhibition of ACh synthesis by the activation of direct, Ca-dependent transport of acetyl-CoA from mitochondria to synaptosomal cytoplasm (Fig. 1, Table I) (Szutowicz et al. 1994a).

Diabetic encephalopathy

Diabetic encephalopathy is thought to develop mainly due to arteriosclerotic changes in brain vessels. There are however indications that diabetes may exert direct effects on metabolism of the brain that might contribute to the development of this pathology. It may be clinically manifested as motor seizures, nonketotic hyperglycemic coma or ketotic coma.

The presence of high concentration of (Glut1) glucose transporter on the blood-brain barrier makes the brain an insulin independent organ in terms of supply of this energy subtrate (Wozniak et

al. 1993, Maher et al. 1994). The expression of this carrier in vascular epithelium and glial cells was found to be reciprocally regulated by glucose concentration in the blood (Walker et al. 1988, Wozniak et al. 1993). This feed back mechanism is thought to keep the rate of glucose supply to brain cells on a stable level. It may be however insufficient when changes in the glycaemia level are too fast. Neuronal cells possess predominately Glut3 transporters which however do not seem to be regulated by glycaemia (Maher and Simpson 1994, Maher et al. 1994). Hence, hyperglycemia, hyperosmia and ketonemia should be considered as substantial factors giving rise to a broad range of short term and adaptative metabolic responses in diabetic brain. Also a deficiency of systemic insulin, which functions in the brain as a neurotropic factor and peptidergic neurotransmitter, was reported to affect several transmitter systems including the cholinergic one (Amir and Shechter 1988, Clarke et al. 1988, Wozniak et al. 1993).

There is a general agreement that insulin may exert inhibitory influence on cholinergic neurones. Insulin caused reversible suppression of CAT expression, while activating glutamate decarboxylase in primary cultures of rat striatal neurones (Brass et al. 1992). Streptozotocin diabetes increased activity of CAT in central and peripheral nervous system (Wahba and Soliman 1988, Wahba et al. 1992). Streptozotocin diabetes increased efflux of ACh from parasympatethic neurones of the heart (Uccioli et al. 1993). These effects are thought to be mediated by specific high affinity insulin surface receptors, present in neurones at reasonably high densities (Unger et al. 1991).

Insulin may also modify the provision of energy metabolites for synthesis of acetyl-CoA in brain. It caused slight but significant activation of Glut1 transporter in glial cells and blood-brain barrier (Partridge et al. 1990, Maher et al. 1994). In streptozotocin-diabetic rats insulin activated β-hydroxybutyrate uptake by the brain (McCall et al. 1982, Werner et al. 1989). Another data demonstrate that levels of glicaemia and ketonemia may directly modify an ACh metabolism. Hyperglyceamia increased scopolamine-induced efflux of ACh in hippocampus (Durkin et al. 1991) and reversed depressive effect of quinuclidynyl bezilate on striatal ACh content (Ričny et al. 1992). Ketosis caused by high fat diet or diabetes increased participation of β-hydroxybutyrate in ACh synthesis (Gibson and Shimada 1980, Sterling et al. 1981, Szutowicz et al. 1994b). It has been shown that acetyl-CoA and ACh levels in brain slices varied in direct relation with the wide range of glucose concentrations (from 0.5 to 10 mM) explaining mechanism of cholinomimetic effects of *in vivo* hyperglycemia (Table I) (Ričny and Tuček 1980). On the other hand, adaptative, inverse changes of Glut1 transporter expression ought to readjust glucose homeostasis in the brain in long term perspective (Walker et al. 1988, Maher et al. 1994). However more direct data on relationship between acetyl-CoA and ACh synthesis in diabetic brain are not available.

Our studies have shown that nerve terminals isolated from brains of streptozotocin diabetic rats utilized pyruvate, and β-hydroxybutyrate about 50% faster than the control ones (Szutowicz et al. 1994b). This change was accompanied by a significant increase of synaptosomal acetyl-CoA and 50% increase of depolarization-activated ACh synthesis (Table I). Insulin treatment of diabetic animals brought ACh synthesis back to control values, without affecting the increased level of acetyl-CoA (Table I) (Szutowicz et al. 1994b). Ketonemia coexisting with hyperglycemia probably did not cause excessive production of acetyl-CoA in neurones, since pyruvate and β-hydroxybutyrate reciprocally inhibited utilization of each other (Szutowicz et al. 1994b). The inhibitory interactions in diabetic as well as physiological ketotic states were found to be much stronger than those in control conditions. This mechanism could prevent rises in the acetyl-CoA level and possibly excessive ACh synthesis in these states (Kenna et al. 1994). However insulin application to diabetic-ketotic rats increased β-hydroxybutyrate utilization in nerve terminals, yielding elevation of acetyl-CoA level and ACh synthesis (Table I) (McCall et al. 1982, Szutowicz et al. 1994b). Hence the excessive accumulation, syn-

thesis and release of ACh might be presumably the case in diabetic brain. This cholinergic hyperactivity is likely to be caused by increased accumulation of acetyl-CoA in nerve terminals resulting from the adaptative activation of the utilization of energy substrates in this compartment by existing hyperglycemia and ketonemia. The participation of hypoinsulinemia in this disfunction can not be excluded since exogenous insulin tended to normalize some of disturbed metabolic parameters. The significance of this apparent change in ACh metabolism for diabetic neuropathology remains to be elucidated.

This review indicates that disturbances in acetyl-CoA metabolism play a key role in several pathologies of the central nervous system in which deficiencies or destruction of the cholinergic system are a dominant feature. Particular susceptibility of cholinergic neurones to the shortages of acetyl-CoA might result from the fact that they, unlike other types of neurones, utilize this intermediate not only for energy production and structural lipid synthesis but also for ACh synthesis. In addition, choline which in noncholinergic neurones is a substrate for lipid synthesis, in cholinergic ones serves additionally as a substrate for ACh formation. The identification of sites of these metabolic lesions in nerve terminal compartment of cholinergic neurones should be considered an important task for understanding their preferential vulnerability in several pathologies of the nervous system.

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