Neurodegenerative Diseases and Schizophrenia as a Hyper or Hypofunction of the NMDA Receptors

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Contents

Abstract

Neurodegenerative diseases, including BSE, Alzheimer’s disease etc. are caused by different mechanisms but may share a final common pathway to neuronal injury due to the overstimulation of glutamate receptors, especially of the N-methyl-D -aspartate (NMDA) receptor subtype. It is generally accepted that the influx of Ca2+ as a result of excessive activation of the NMDA receptor underlies the toxic actions of glutamate in many systems. Also, ammonia intoxication leads to excessive activation of NMDA receptors in brain. On the other hand, Mg2+ competes with Ca2+ at voltage- gated calcium channels both intracellularly and on the cell surface membrane. So, Mg2+ can protect against NMDA- induced neurodegeneration and Ca2+ deficiency can be important about "NMDA hypofunction" in schizophrenia. In addition there can be another example about hypoglutamatergic condition; cannabinoids are known to inhibit Ca2+ channels- glutamate release in schizophrenia, and to inhibit progression of certain neurodegenerative diseases.

There are no scientific references to date in which high intake of crude protein (and potassium) high enough to lead to a state of hyperammonemia (and hypomagnesemia) during the incubation period of the BSE. Therefore there is the first idea of this review; to show the hyperammonemia plus hypomagnesemia"simultaneous" action on the ruminant tissues. So the various clinical symptoms can be observed because the nervous system controlling both voluntary and unvoluntary muscles is affected (Mg and Ca disturbances). If the BSE is involved; a longer- chronic action of corresponding biochemical changes in the blood (CSF) is necessary, to rise irreversible neurodegenerative changes.

Recently was found that elevated manganese in blood was associated with "prion infection" in ruminants. These findings about "manganese theory" act in concert with this "BSE ammonia- magnesium theory". So I will perform some interpretations about this connection and some details will be presented to the Congress, and also second idea of this review; to show that cannabis use can be a proof about the link between the NMDA receptor hyperfunction (neurodegeneration) and hypofunction (schizophrenia).

Introduction

The prion protein (PrP) plays a crucial role in the pathogenesis of transmissible spongiform encephalopathies (TSEs), including Creutzfeldt–Jakob Disease in humans and bovine spongiform encephalopathy (BSE) - scrapie in ruminants.. All are characterized by a pathogenic conformational misfolding in the tertiary structure of PrP, resulting in protein aggregation and severe neurodegeneration. Scientists hypothesize and believe (to date) that prions enter the body through food, thus setting off a chain reaction, converting normal proteins into abnormal ones, creating deposits that cause irreversible brain damage.

However, the PrP also regulates intracellular calcium (Ca^{2+}) homeostasis (HERMS et al.,2000; KORTE et al., 2003; FUHRMAN et al., 2006), which might be of special significance given the intricate relationships between Ca, oxidative stress and long-term neuronal survival. Alterations in Ca^{2+}-dependent K^{+} currents have been observed in hippocampal CA1 pyramidal cells, as well as in Purkinje cells of mice lacking PrP^{C} expression (COLLING et al., 1996; HERMS et al. 2001). Considering these facts together, the question arises whether the function of PrP^{C} in Ca signalling and Ca homeostasis can be directly modulated by oxidative stress (VASSALLO and HERMS, 2003).

Prion diseases have become an important issue in public health and in the scientific world not only due to the possible relationship between BSE and new variant CJD (vCJD) but also due to the unique biological features of the infectious agent. Although the nature of the infectious agent and the pathogenic mechanisms of prion diseases are not fully understood, considerable evidence suggests that an abnormal form (PrP(Sc)) of a host prion protein (PrP(C)) may compose substantial parts of the infectious agent and that various factors such as oxidative stress and Ca cytotoxicity are associated with the pathogenesis of prion diseases (HUR et al, 2002).

The fact that different types of neurons display similar phenotypes with regard to the intracellular free Ca^{2+} concentration indicates that loss of PrP^{C} affects basal mechanisms of Ca^{2+} homeostasis. In general, three mechanisms have to be considered as to how PrP^{C} deficiency might modulate intracellular free Ca^{2+} concentration following depolarization: Firstly, loss of PrP^{C} may modulate Ca^{2+} influx from the extracellular space via voltage gated calcium channels. Secondly, PrP^{C} deficiency may alter Ca^{2+}-induced Ca^{2+} release from intracellular stores, or alter filling of these stores. Thirdly, lack of PrP^{C} may result in enhanced removal of Ca^{2+} from the cytosol, for instance by increased Ca^{2+} buffering within the cytoplasm, extrusion of Ca^{2+} into organelles or through the plasma membrane (FUHRMAN et al., 2006).

Recently, to investigate the role of regular PrP, researchers at the University of Calgary (ZAMPONI et al., 2008) looked at communication among the brain cells of PrP-free mice. When the nerve cells received the messenger glutamate, they went into hyperactive mode, repeatedly firing as if the message had been shouted at them. These overexcited cells were more likely to die because of this overactivation. Normal PrP protein might function to block some N-methyl-D-aspartate (NMDA) receptors and thereby prevent overexcitement of neurons.The researchers also removed magnesium (Mg) from the cells. Without it, the brain cells went into seizure mode.

Glutamate and aspartate neurotransmitters produce their effects by interacting with specific receptors on the cell surface, the excitatory amino acid receptors (MONAGHAN et al., 1989). Five receptor subtypes have been identified. The most well characterized excitatory amino acid receptor are subtype is NMDA receptor which is permeable to Ca^{2+} (FAROOQUI and HORROCKS, 1991). Overstimulation of the NMDA receptor as well as other excitatory amino acid receptors results in neurotoxicity and neuronal injury. These receptors are considered as the final common pathway for many acute and chronic neurologic conditions (McDONALD et al., 1988). So an important consequence of NMDA receptor activation is the influx of Ca2+ into neurons. Overstimulation of the NMDA receptor as well as other excitatory amino acid receptors results in neurotoxicity and neuronal injury.. Studies have demonstrated that Mg2+ can protect against NMDA- induced neurodegeneration, brain injury, and convulsions. Mg2+ competes with Ca at voltagegated Ca channels both intracellularly and on the cell surface membrane. Mg2+ is capable of blocking NMDA receptors both intracellularly and extracellularly.

In addition, experiments with sheep, given food heavily contaminated with abnormal (PrPSc) prions, showed that the animals simply digested them, with very few of the prions

surviving. It seems possible that another, unidentified agent might be responsible for the disease. Even more challenging is the suggestion that the abnormal prions might be the consequence of the disease rather than its cause (JEFFREY et al., 2006).

So there can be other alternative "non infectious" hypothesis based on the excessive activation of glutamate receptors, particularly of the NMDA receptor subtype, leads to neuronal degeneration and death. On the other hand a hypofunction of the NMDA receptors can be hypothesized to caused schizophrenia. These hypotheses are presented as the literature review, based also on the author´s experiences about Mg research in ruminants.

I. Neurodegenerative diseases as a hyperfunction of NMDA receptors; presented on the BSE as a example of TSEs

History about BSE and vCJD in the United Kingdom

Identified in 1986, BSE rapidly spread to affect UK herds although the incidence was very limited within individual herds. The source was assumed, on epidemiological grounds, to be from commercial feed which contained rendered animal protein. Certainly, cases dramatically declined subsequent to the time when the ban of meat and meal (MBM), ruminant-derived protein came into force in 1988, establishing this theory beyond reasonable doubt.

The working hypothesis for the origin of BSE is sheep scrapie (WILESMITH et al., 1988; KIMBERLIN, 1993; KIMBERLIN and WILESMITH, 1994; KIMBERLIN, 1996). This hypothesis arises from the original considerations with respect to the reason for only the cattle population in the United Kingdom experiencing a major incidence of BSE (WILESMITH and WELLS, 1991). The risk factors originally identified were fourfold: a large ratio of sheep to cattle population, approximately 4: 1, larger than in any other country. However, there has been no demonstration that feeding cattle meet and bone meal (MBM) produced from sheep has led to the development of BSE since introduction of the MBM is fact; the assumption that the fall in incidence of BSE is due to this ban is no more than an assumption. In addition, the source of the supposedly infected feed, however, was never identified and the disease never reproduced experimentally in this way. Also the limited nature of the infection, localised often to just one animal in a herd, was puzzling, the more especially since this could not be explained by host predisposition, as with sheep to scrapie, since no variant polymorphisms have been identified in cattle.

Later, in 1995, two cases of CJD were reported in young people. By March 1996 that had risen to 10 cases. Neuropathological findings revealed the presence of large amyloid plaques in the brains of these unfortunate people, more akin to Kuru than sporadic CJD, and which was now renamed variant CJD (vCJD). This disease have raised considerable public concern with respect to the unknown extent of the infection in the food chain, the possible transmissibility to humans and most particularly the relationship of BSE to vCJD. The UK government has warned that vCJD, caused by eating beef infected with "mad cow disease" (BSE) could claim many as 250,000 lives. This is double the previous estimate of 136,000 possible deaths. Putting the risk into context, microbiologist and leading CJD expert Dr Stephen Dealler said;" on average people in the UK had eaten 50 meals made from the tissue of an infected animal. At the moment the number of cases of CJD we are seeing are doubling every year. If the double for a long time then the numbers are in millions, if they double for just a few years then the numbers are in thousands…" (HYLAND; November, 2000).

However, it was showed (see statistics), that during last three years (2005- 2007) not thousands- but only 15 people died from vCJD. So the number of vCJD has been in steady decline since 2000 - when 28 people died. It is quite surprising that the one experiment that

would confirm a link between BSE and vCJD has not been carried out. Groups supposedly more at risk such as farmers, vets, abattoir workers and butchers have not shown an increased risk of vCJD... Whatever happened to the great epidemic? Why are the vCJD figures falling?

Initially, the infectious prion was thought to be a modified scrapie prion which had crossed species- this was dismissed by Lord Phillips (2000) on the ground that BSE differed essentially from scrapie in disease-profile, incubation and transmissibility. The report states with confidence that; the BSE agent is not an unmodified form of scrapie! The Report of the BSE Inquiry (2000) concluded BSE probably originated from a novel source early in the 1970s, possibly a cow or other animal that developed disease as a consequence of a gene mutation, rather than rendering of sheep infected with "normal" scrapie. In response, the UK Government asked Professor Horn to lead a small team of scientists to look in greater detail at the origin of BSE by pulling together all scientific understanding, including emerging findings, on the subject. It was concluded; the spread of BSE in cattle to the point where it became an epidemic came about from the use of meat and bone meal (MBM) in cattle feed, see; "Review of the origin of BSE" (DEFRA; July 5, 2001).

There scientists say that for many years, in the UK and many other countries (eg USA and continental Europe) dairy cattle were fed protein supplements that contained MBM for the first time at the start of their first lactation, when they were between two and two and a half years of age. During the 1970s feed compounders in the UK began to introduce MBM into the high protein pelleted rations fed to artificially-reared calves from the dairy herd, typically beginning in the first two weeks of life. These considerations imply that the UK was the only country, in which scrapie was endemic, where significant amounts of MBM were fed to very young calves and this practice began in the 1970s. The evidence outlined taken together suggests that cattle may be more susceptible to BSE in the early weeks of their lives. This suggestion is testable by experiment, it was concluded.

Really, is this suggestion testable by experiment? I suppose and believe that during this dietary MBM experiment- scour, or diarrhea may occur in newborn calves. The most effective treatment for scouring calves is administration of fluids. Other treatments may be beneficial, but they are far less important than oral fluid (with a Mg-supplement) and electrolyte replacement. These field experiences were patented by the"Czechoslovak Patent Office" in Prague (HLASNY and HLASNY; 1990, 1992);
Electrolyte replacement for oral application I. Patent No 268881
Electrolyte replacement for oral application II. Patent No 277602

Some details about "ryegrass experiment"; BSE confirmed in cows without the MBM in dairy ration

However, the BSE was tested in dairy cows, see "nutritonal experiment" performed in England; published in Veterinary Record (MOORBY et al., 2000) and in Journal of Dairy Science (MOORBY et al., 2000; DEWHURST et al., 2000).

This experiment was conducted using diets and other conditions typical of northwestern Europe, under well defined conditions of husbandry and nutrition. The effect of altering the amount of protein and energy over the final 6 wk of the dry-period diet and during the first 21 wk of the subsequent lactation was investigated, in 47 dairy cows. Perennial ryegrass silage was used ad libitum; final 6 wk of the dry-period diet and during lactation plus a concentrate with high crude protein (CP) level (22.5%) was fed. Blood samples were taken each week before calving, and during weeks1,3,5,7,13,17, and 21 of lactation.

During lactation daily total dry matter (DM) intake was ca 17.4 kg; the content of CP (N x 6.25) was ca 20% during first 12 weeks, and ca 17.5% of CP in the diet DM, to the 22 wk of the lactation period. So, very high CP concentrations in the diet used, and high levels of

plasma urea-N (38- 43 mM) were found during lactation and also during dry period (30- 36 mM) .

No clinical metabolic disorders were recorded. However, after the collection of the last blood sample (21 wk of lactation), six of the 47 animals (so; 13 per cent!) developed clinical signs of BSE (later histopatologicaly confirmed). Although when they were sampled it was not known that they were incubating the BSE.

My conclusions: long-term dietary crude protein (CP) surpluss, significantly higher than the norm (NRC, 2001; if about daily 30 kg of milk production was recorded; only 15% of CP in DM was needed) during 21 wk of lactation period and mostly in cows during 6 wk of dry period. So, there hyperammonemia plus hypomagnesemia action on the animal tissues (CNS and liver, especially) can be found. If the BSE is involved; a long-chronic action is necessary to rise irreversible neurodegenerative changes. It seems that there is the similarity between the individual susceptibility in the hypomagnesaemic "indicator cows" and the "BSE cows".

In addition, from older australian literature is well known that in cases of protracted ryegrass staggers of sheep and cattle; cerebellar lesions involving Purkinje cell axons... were found (MASON, 1968). In cerebellums with a high density of torpedoes, swollen neuronal elements were sometimes detected in the cerebellar molecular layer. The myelin sheats would appear to remain relatively unaffected about degenerating axons, vacuoles were observed in some torpedoes. It appears that the longer the disturbing syndromes has been present, the greater the likelihood of finding these axonal changes, for example in protracted ryegrass staggers in sheep and cattle. MASON (1968) concluded that the lesions described are not regarded as pathognomonic of protracted ryegrass staggers but probably arise from a number of factors, which may include disturbed neuronal metabolism, neuronal exhaustion and repeated anoxic insults.

In prion diseases cerebellar atrophy is usually severe. The different appearance of the molecular and granular layers in the cerebellum should be noted. The differences are due to different cell types. The granular layer contains mainly Golgi cells and granule cells, whereas the molecular layer contains mainly parallel fibres and dendrites of Purkinje and Golgi cells. Purkinje cell axonal swellings, also termed "torpedoes". Axonal torpedoes are within the granular layer of the cerebellum. Unlike most cerebellar degenerations, there is more pronounced loss of granular neurons than Purkinje cells. In some cases, prion proteins precipitate as amyloid plaques. Gliosis is mostly present in the molecular layer of the cerebellar cortex. Recent data point to synapses as principal targets of abnormal PrP deposition. Moreover, impairment of glomerular synapses and attenuation of parallel fiber pre-synaptic terminals on Purkinje cell dendrites is a cardinal consequence of abnormal PrP metabolism in CJD. Accumulation of synaptic proteins in the soma and axonal torpedoes of Purkinje cells suggests additional impairment of axonal transport (FERRER, 2002).

The begining of the higher dietary protein recommendation including undegradable protein (MBM...) in dairy rations

Several new theoretical protein systems have been proposed during the mid 1970s to the mid 1980s. Four new U.S. systems have been proposed. These systems was called (a) "Burroughs system" (BURROUGHS et al., 1975a; 1975b); (b) "Satter system" (SATTER, 1982); (c) "Chalupa system" (CHALUPA, 1980), (d) "Cornell system" (FOX et al., 1982; Van SOEST et al., 1982) sumarized in "Ruminant Nitrogen Usage" (NRC, 1985). As reported by the USDA, average production per cow in the United States reported in 1975 was 10,360 lbs as compared to 14,213 lbs in 1988.

Two new European systems- the ARC (1980) system in Great Britain and the PDI grele systém in France (VÉRITÉ et al., 1979)- are official proposals within each country.

KAUFMANN (1979) has proposed a system in Germany, and LANDIS (1979) in Switzerland. DANFAER (1979) has outlined many factors in a model of protein utilization from Denmark. All of the new systems consider the dietary intake crude protein to be divided into undegradable dietary protein (UDP) and degradable dietary protein (DRP) fractions.

In 1980 a technical committee of the Agricultural Research Council (ARC) produced "The Nutrient Requirements of Ruminant Livestock". This publication first drew attention of the U.K. feeding industry to the specific need for undegradable dietary protein (UDP) i.e. a dietary source of essential amino acids which escapes degradation in the rumen and so can augment the supply of amido acids. It became clear that animal proteins were a particularly rich source of UDP, not only because they contained a near ideal balance of amino acids but also because they were highly undegradable,; i.e. the majority of the protein in ingredients such as fishmeal and MBM, escaped degradation in the rumen and so could provide the extra amino acids thought necessary for high milk yields in lactating dairy cows, especially. However, the ARC (1980) publication, according to professor John Webster grossly overstimated the requirement of cattle for UDP in the UK (WEBSTER, 1992; ALDERMAN et al.,1993).

Why the BSE epidemic occured in the UK, especially ?

Rainfall and the available water capacity of the soil were major forage yield determinants, with output in the UK ranging from 6000-14000 kg DM/ha under intensive fertilization in 1980s (LEE, 1988). There are notable exceptions such as Benelux, wchich although characterized by the highest pasture yields in Europe, however with a comparatively low share of grassland in total ruminant feed composition (50- 55%) – compared with Ireland (97%), U.K. (83%), France (71%).

In Britain perennial ryegrass is the most important species of sown pastures, but Italian ryegrass (Lolium multiflorum), timothy (Phleum pratense), cocksfoot and the fescues (Festuca spp.) are also common. The composition of the dry matter (DM) of pasture is very variable: for example, the crude protein (CP) may range from as little as 30 g/ kg in very mature herbage to over 300 g/kg in young, heavily- fertilized grass (McDONALD et al., 1988). According to "50 – year review" about the fertilizer applications in the UK (HEMINGWAY, 1999), there was mayby highest nitrogen fertilizers consumption in the world; in England and Wales, especially (1983-1988): This author´s summary indicates that in the UK there was the "intention" to use the high N- fertilization (and K-fertilization) for intensive silage production, especially. The effect of changing patterns of fertilizer applications on the major mineral composition of herbage in relation to the requirements of cattle: „ 50 – year review"; showed that the long time research about the NPK fertilization in the UK has been sumarized. For example in 1983- 1988 period, in England and Wales; higher rates were used for intensive silage production; 201 kg (nitrogen), 15 kg (phosphorus) and 53 kg (potassium) per ha. In contrast, recommended applications (MAFF, 1994) were much higher 340 kg N, 18 kg P and 25 kg K per ha for grazing and 380 kg N, 40 kg P and 260 kg K per ha for intensive silage. Later, nitrogen application rates to grass have progressively declined. Increasing environmental issues and the present interest in organic farming and low input systems indicate that these trends will continue in the UK. Present overall fertilizer use for grazing on dairy farms is about 170 kg N, 10 kg P and 20 kg K per ha (HEMINGWAY, 1999).

In Britain- Ireland, a high intake of grasses in ruminants; available water capacity, high N (and K-fertilization by animal excrements), cool and cold marine climatic region; these circumstances are ideal for the subclinical (chronic) hypomagnesaemia in ruminants. However, in the UK, there was not interest about the Mg-research in ruminants within twenty years period (1976- 1996). In contrast it can be interpreted , acording to the study of HEMINGWAY (1999); that to the mid of 1970s, in the UK there the agronomic Mg research was greatest in the world. Also, according to the DUA and CARE (1995) , there was the greatest "veterinary" Mg-research in ruminants in the world; also unfortunately only to the mid of 1980s. This can show on the real probability about the "abnormal" Mg-deficit in British ruminants from 1950s to 1980s . From both publications, there is evidence about reality of the Mg-deficit also in the next decade years (1985-1995) in British ruminants. However, with the high probability that after significant increase of crude protein in dairy rations (ARC, 1980) in mid- 1980s, it was without equality of oral Mg-supplementation. And after 1993/94 period, there is some evidence about the increase of additional dietary Mg-supplementation in dairy rations (McCOY et al., 1994).

Higher additional dietary Mg-supplementation in dairy cows as an European "phenomenon" at the beginning of 1990s; and decrease of BSE incidence in the UK

Therefore if we will put this "phenomenon" into practice; significantly higher additional dietary Mg-supplementation - can be a cause about the BSE incidence decrease in the UK, after 1993/94 period. Also about the Mg research from Czech Republic (to the end of 1980s) there is well known experiment (HLASNY, 1989) published in Czech scientific journal Veterinary Medicine (Prague), Dec 1989, 34 (12); 717-725. See the abstract (Internet) about the article; "Evaluation of a new mineral feed supplement used in feeding young breeding cattle in the winter season"(http://www.ncbi.nlm.nih.gov/pubmed/2631375?dopt=Abstract);

A total of 260 tons of a new mineral feed supplement [MKP-C(P)] was used on farms in the Písek district. In the period from January to April, 1988, the mortality of calves decreased by 2.9% in comparison with the same period of 1987 when the Polymin Z or MKP-C supplements had been used. The MKP-C(P) supplement, containing 8 to 10% calcium, 3 to 4% phosphorus, 6 to 7% magnesium and 8 to 10% sodium, was used in all first-calver barns in the 1987/88 winter feeding season. It was administered to about 7300 highly pregnant heifers and first-calvers at rates of 0.15 to 0.2 kg per head and day. It has been concluded from the results of the experimental and farm-scale testing of the MKP-C(P) supplement, containing much more magnesium (by 180%) and less phosphorus (by 46%) than the commercially produced supplements MKP-C and Polymin Z, that the new product is able to meet all the phosphorus requirement, together with a rational management of magnesium, in young breeding cattle. Apart from a higher effect, especially in the compensation of hypomagnesiaemia and metabolic acidosis, the use of the new supplement in first-calver stocks allows to reduce the total financial costs of mineral feed supplements by 20%. The basis for the reduction of costs is the saving of phosphates in the potato-growing areas; this is also associated with a reduction of the supply of cadmium to the cattle feed rations

There was also published (HLASNY, 1989) the similar work in another Czech scientific journal Biol.Chem. Vet. (Prague), May 1989, 31 (2); 157- 171, see the article; "Providement for an optimum supply of sodium and magnesium to the feed rations of dairy cows and high pregnant heifers" (see abstract).

Feed samples were subjected to the laboratory examination and 58 feed rations (FR) and 58 metabolic profile tests were evaluated in 1200 dairy cows and high pregnant heifers, in the winter and summer feeding seasons. It was found that when one kg of soil contains around

180 mg of potassium (K) and when there are about 50% of soils with pH up to 5.5, the plants growing on such soils have lower contents of Mg and Na and the animals utilize both at a lower rate. With the daily forage supply of 200- 300 g of potassium to the FR; it was necessary to exceed the supply of magnesium to the FR by 60- 100 % , with the respect to the Czechoslovak Standard Norm (CSN). As there is a generally high content of potassium in the FR given to lactating cows and heifers, increased supply of sodium and magnesium to the FR, represented by the maximum tolerated values, was presented in the computer-mathematical expression.

These results as a field experiences were also patented by the"Czechoslovak Patent Office" in Prague (HLASNY, 1991); Mineral supplement for breeding cattle. Patent No. 274 171.

These recommendations that much more magnesium (by 180%) is necessary in dairy mineral supplements, really it was commonly realized in Europe, at the beginning of 1990s. This phenomenon about the "European great Mg interest" at the beginning of 1990s, it was also presented at the "3rd European Congress on Magnesium" (March, 1990) in Geneve. there we presented (with Dr.Steidl) above mentioned results from Czechoslovakia, see the article; "Mechanism of the origin of magnesium deficiency in feedstuffs and in nutrients" in Magnesium Research (1990, 3: 48). During 1980s I collaborated with well known Czech neurologist professor Ladislav Steidl (Palacky University, Olomouc), he was a member of the editorial board (1975- 2001) of the International journal "Magnesium research".

Effects of high nitrogen soil fertilization and magnesium deficiency, especially in ruminants

In British veterinary journals (period; 1985-1995), there is only one information (article as survey) about the cow hypomagnesaemia testing (McCOY et al., 1993)- from Northern Ireland. There clotted blood samples submitted under the Brucellosis Eradication Scheme were used for this survey published in The Veterinary Record; 513 dairy and 1266 suckler cow herds were sampled during the grazing season from March to November 1991 (to February 1992- suckler cows). It was found; serum blood Mg below 0.8 mmol/l in 14.1 of dairy and in 33.9% of suckler cows. The peak of hypomagnesaemia incidence; in both dairy and suckler herds occurred in the period from March to June, coinciding with the period of peak milk production. In addition, in 8.2% of suckler cows – the blood Mg below 0.6 mmol/l was found !

Grazing experiments (BARTLETT, 1958) on pastures containing different amounts of clover and similarly finding in the field conditions (HLASNY, 1991) have shown higher serum Mg levels in cows grazing on the clover rich sward, then grass forage. The crude protein intake of ruminants grazing young grass fertilized by nitrogenous fertilizers, was increased by approximately 25-35% in Britain (HEAD and ROOK, 1955). Highly fertilized young herbage is characterized by a high content of crude protein (CP) and a high rate and extent of degradation of CP causing high concentrations of ammonia-N in the rumen (van VUUREN et al., 1986). Lush grass innately has an increased level of crude protein. As this protein is readily fermentable, it leads to increased intraruminal ammonia concentrations up to 30- 70 mmol/l (MARTENS and RAYSSIGUIER, 1980) and to a decrease in the availability and absorption of magnesium(MARTENS et SCHWEIGEL, 2000; URDAZ et al., 2003; FONTENOT et al., 1989).

While non-ruminants absorb Mg primarily from the small intestine, ruminants are able to absorb much of their Mg requirement from the rumen. In fact, the reticulum and rumen can account for up to 80% of the Mg absorption along the entire digestive tract (REMOND, et al, 1996). . Probably, the nutrient having the greatest adverse effect on Mg absorption is an excess of K in the ration, as shown by at least four sheep experiments (GRACE, et al, 1988;

YANO, et al, 1990; DALLEY, et al, 1997; WACIRAPAKORN, et al, 1996). Ammonia absorption from the rumen is linearly related to ruminal ammonia concentration between 3 and 18 mmol/l (BODEKAR et al., 1990) and is normally dotoxified in the liver to urea. It appears that ruminal ammonia may contribute to decreased Mg absorption under the circumstances which may be encountered during grazing. In the rumen of sheep, insoluble quanite ($MgNH_4PO_4$) formation is seen to occur at pH 6.2 – 7.2 with ruminal ammonia concentration in the range of 40 mmol/l and depresses the available amount of magnesium (AXFORD et al., 1982).

Adequate amounts of fermentable carbohydrates are important in maintaining serum magnesium levels, since magnesium solubility and the absorptive surface area of rumen papilla both improve with availability of short chain fatty acids and lowered rumen pH (MARTENS et SCHWEIGEL, 2000). Lush pastures often high in nonprotein nitrogen are relatively low in readily fermentable carbohydrates. The ability of the ruminal microbes to incorporate the nonprotein nitrogen into microbial protein is exceeded and ammonia and ammonium ion build up in the rumen increasing ruminal pH. Magnesium solubility declines sharply as ruminal pH rises above 6.5. Grazing animals tend to have higher ruminal pH because the high content of nitrogen and potassium positively correlate, in ryegrass especially (HLÁSNÝ, 1990). It was found that the effect of ammonium ions on magnesium absorption was greater in the bovine than the ovine rumen (GABEL and MARTENS, 1986). It has been found that only a small proportion of any flock or herd will suffer clinical hypomagnesaemia (BUTLER, 1963).

A genetic factor for magnesium absorption has also been suggested. However, the phenomenon of individual susceptibility to grass tetany within a dairy herd of similar animals, the so-called indicator cows, still requires a convincing explanation (DUA and CARE, 1995). Magnesium is a nutrient required for all animals, but it is especially critical for ruminants. A physiological deficiency of Mg results in hypomagnesemic tetany. Typically, only female ruminants are affected, and the disturbance usually occurs during the early stages of lactation (FONTENOT et al., 1989). In addition, the experiments demonstrated that younger cows are better able to mobilize Mg from the body reserves than older cows (Van MOSEL, et al, 1990).

Recommendations about lower protein intake in dairy cows as a "phenomenon" at the 2001/2002 period; and later steady decrease of BSE incidence in states with high producing dairy cows

At the beginning of 1980s, for more productive high yielding dairy cows more of protein was required. So two new European systems- the ARC (1980) system in the Great Britain and the PDI grele system in France (VÉRITÉ et al., 1979)- were official proposals within each country. Higher protein intake also according to the NRC (1985; Ruminant Nitrogen Usage) and NRC (1989) was recommended.

However, improvements in the research about nitrogen metabolism changed the view on the protein intake in high yielding dairy cows, to the end of 1990s. The previous edition of NRC (1989) was completed and replaced (NRC; January 2001; National Research Council; Nutrient Requirements of Dairy Cattle, 7th Revised Edition) . There are changes about the lowering of protein requirements; in early lactation especially.

During early lactation (0-70 days postpartum) milk production increases rapidly, peaking at 4 to 6 weeks after calving. Protein content is critical during early lactation; rations may need to contain 19% of more crude protein (ENSMINGER et al., 1990). For example, the same high protein level is recommended in turkey- in animals with highest protein requirements from the all domestic animals (NRC,1994). In growing young turkeys (age; 11

to 14 weeks) there is the recommendation 19 percent of protein of the diet (90% dry matter). Almost the same situation is in "monogastric" young rapidly growing pigs allowed ad libitum diet of 90% dry matter (NRC, 1998)- average weight in range 15 kg (20.9% of crude protein) and 35 kg (18% of CP) .

Almost the same high protein recommendations (18.8 % of dry matter) are from McCULLOUGH (1994) recommended to dairy rations of high producing "supercows"- however, during the "all lactation". The similar conditions were recorded in above mentioned experiment (MOORBY et al., 2000), when 13 per cent of experimental cows, the clinical signs of BSE developed ! There during first 12 wk of lactation the content of protein was ca 20%, and during next 11 wk 17.5% in the dry matter of dairy ration.

On the other hand, according to the NRC (1989) this high protein level it was recommended only during first three weeks (0-21 days postpartum) after calving. So above mentioned recommendation were "overdosed" in dairy practice. The research during 1990s resulted to decrease of protein content in dairy rations; see the comparison of the NRC (1989 and 2001);

Dairy cow: 600-680 kg body weight				
	Lactation			Early lactation
Milk yield (kg/day)	35	45	55	35
Degradable protein – "DP" (%):				
NRC,1989	9,7	10,4	10,4	9,7
NRC,2001	9,7	9,8	9,8	10,3
Undegradable protein- "UDP" (%):				
NRC,1989	5,7	6,0	6,3	7,2
NRC,2001	5,5	6,2	6,9	5,6
Crude protein – "CP"- (%):				
NRC,1989	16,0	17,0	17,5	**19,0**
NRC,2001	15,2	16,0	16,7	**15,9**

Therefore if we will put into practice ; the recommendation from the NRC (January 2001) about the significant decrease of crude protein in the early lactation - this "phenomenon" can be a cause about the BSE incidence decrease in the western Europe (UK, Ireland,France, Netherlads, Switzerland…) especially, after 2001/2002 period. There were high producing dairy cows in 1990s, compared with the eastern Europe countries..

Nervous diseases and connections with nutrition in ruminants; as an overstimulation of NMDA receptor and excessive Ca entry into cells

The studies and findings reviewed above show that the surpluss of nitrogen and potassium in dairy cattle ration especially; can have the association with hyperammonemia complicated with the chronic subclinical hypomagnesiemia, and the neurodegeneration can be involved. On the other hand, despite extensive BSE research, there was much that was unanswered or mainly speculative; see The BSE Inquiry (October 2000). Therefore I reviewed more of literature sources about dairy cow nutrition etc. and described a Czech alternative "ammonia-magnesium" BSE theory (March, 2001)- in the Bulletin of Research Institute of Cattle Breeding in Rapotín (see Fig 1).

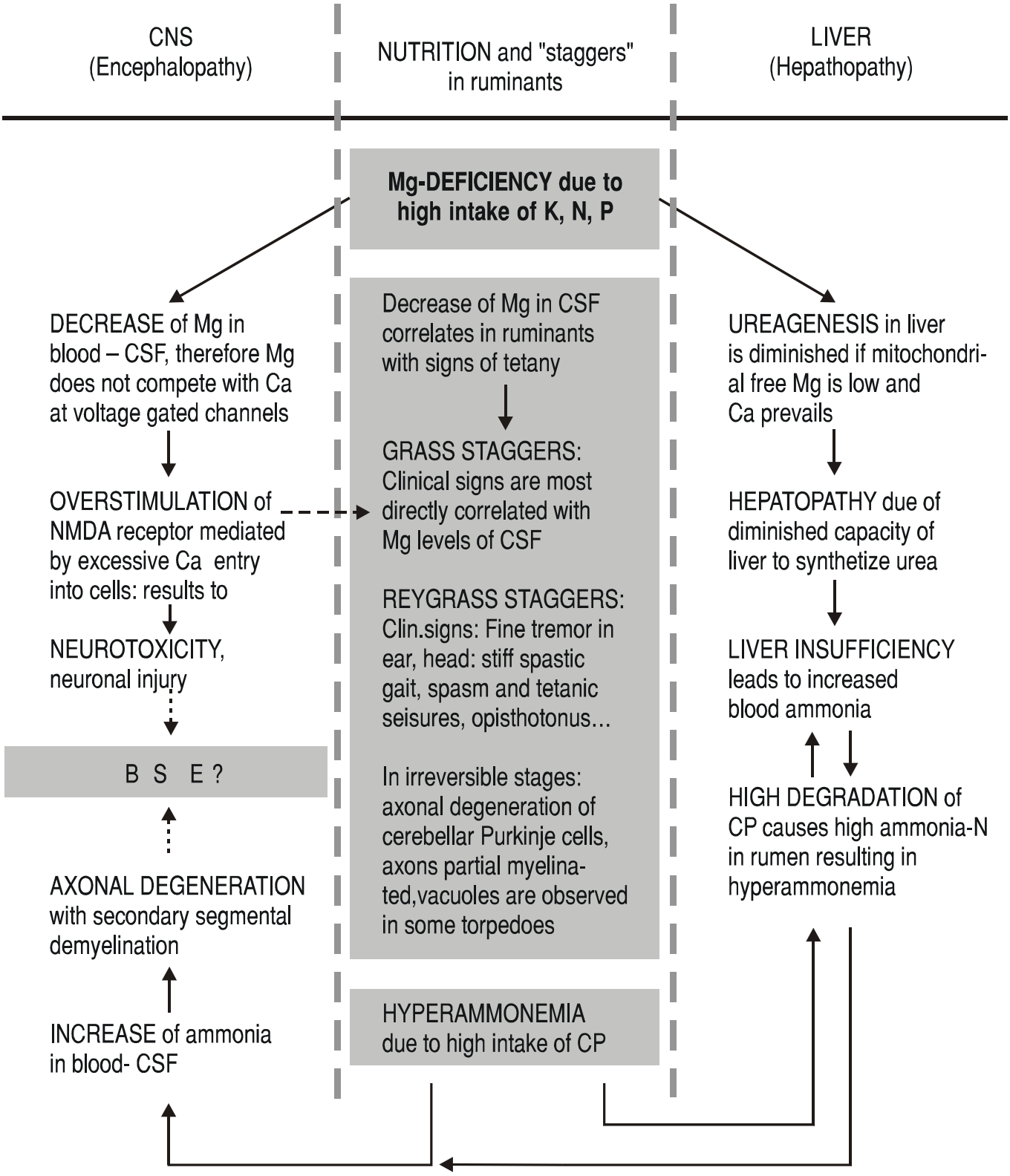
This alternative theory was introduced according to the well- known facts, that; over the past 50 years yields of many crops have increased roughly in proportion to the increase in nitrogen and potassium fertilizer application- peaked in the mid- 1980s, especially in the UK. In addition, the epidemiological studies show, that BSE occurence to this date was mostly in "western countries" where significantly higher NPK fertilization was applied. And also there high protein content in dairy rations was recommended, because high milk production was recorded. This alternative theory is supported by more comprehensive relationships in connection with the situation in the United Kingdom and recent scientific findings; see following review of the literature sources;

The PrPc (normal; prion protein cellular) is a naturally occurring protein found in cells of central nervous system and other tissues. When the PrPc molecule refolds into an aberrant shape it becomes pathogenic and causes Transmissible Spongiform Encephalopathy (TSE). The disease- associated form of the normally occurring prion protein is designated as protease resistant protein (PrP^{Res}) or PrP Scrapie (PrP^{Sc}). PrPc is a cell membrane glycoprotein particularly abundant in the synapses. Prion diseases are characterized by the replacement of the normal PrPc by a protease-resistant, sheet-containing isoform that is pathogenic. A prion is a unique particle that contains no nucleic acid and differs from bacteria, viruses, fungi, viroids and plasmids. Prions are resistant to inactivation by most procedures, such as heat or ionizing ultraviolet radiation, that destroy most biological agents. Prions also are resistant to enzymes such as protease that quickly break down normal proteins. Pathology, in prion diseases, develops only in the brain. No other organ is affected. Early on, neurons develop intracytoplasmic vacuoles. As the disease progresses, vacuolization becomes more pronounced and, microscopically, the cortical neuropil develops a spongy appearance, hence the term spongiform encephalopathy. Advanced cases show neuron loss, gliosis (astrocytosis), and brain atrophy. So as a result prions multiply, are not broken down by proteases and accumulate in brain tissue, where damage results by one of two mechanisms: (1) accumulation of the abnormal form of the protein itself causes the damage ("vacuolization");

(2) the loss of function of normal protein results in cell death ("astrocytosis"). These both mechanisms can be involved by hyperammonemia and hypomagnesemia (FERRER, 2002).

Fig. 1

Nervous diseases and connections with nutrition in ruminants



CNS: Central nervous system • CSF: Cerebrospinal fluid • CP: Crude protein
NMDA: "N-Methyl-D-Aspartate" receptor.

MULLER et al.(1993) incubated rat cortical cells with the scrapie prion protein (PrP^{Sc}). At concentrations of 3 ng/ml of PrP^{Sc} and higher, the viability of the cells decreased significantly

after a 12-h incubation period. PrPSc did not affect the viability of astrocytes. Drugs known to block NMDA receptor channels, such as memantine, prevented the effect of PrP^{Sc}. They concluded that antagonists of the NMDA receptor – channel complex (i) abolish the PrP^{Sc} – induced neuronal injury in vitro, and (ii) display no influence on the usynthesis and/or the processing of PrionSc. Later it was found that cellular prion protein (PrPc) is associated with regulation of intracellular free Ca levels through an interaction with voltage-sensitive Ca channels (WHATLEY et al., 1995).

The infectious prion protein (PrP^{Sc}) is the etiological agent of transmissible neurodegenerative conditions such as scrapie or CJD. Its fragment 106-126 (PrP 106-126) has been reported to maintain most of the pathological features of PrP^{Sc}. This prion protein fragment directly stimulates the proliferation of astrocytes via an increase in intracellular Ca2+ through the L-type voltage-sensitive Ca channels (FLORIO et al., 1996). However, there are two protein syntetic fragments; beta-amyloid 25-35 (betaA 25-35) and PrP 106-126. The toxicity of both peptides involves Ca2+ uptake through voltage-sensitive Ca2+ channels but only PrP 106-126 toxicity involves the activity of NMDA receptors (BROWN, D.R. et al., 1997).

Studies of the intracellular free Ca concentration revealed an alteration of the maximal increase of intracellular Ca concentration with depolarization in the prion protein-deficient mice ($Prnp^{0/0}$) mouse Purkinje cells. These data provide strong evidence (HERMS et al.,2001) that Ca^{2+}-activated K^+ currents in $Prnp^{0/0}$ mice are reduced due to an alteration of intracellular Ca homeostasis.

Reactive oxygen species (ROS), such as H_2O_2 and superoxide anion, are known to act as second messengers in signal transduction. Exogenous ROS can stimulate downstream intracellular signalling systems, including intracellular Ca (MAHER and SCHUBERT, 2000). There is a growing body of evidence that the function of PrP^C is linked to a neuroprotective response to ROS, and to regulation of intracellular Ca homeostasis. In particular, cross-linking of PrP^C can induce a transient Ca response (STUERMER et al., 2004). An increase in intracellular Ca $[Ca^{2+}]_i$ is a well characterized response to extracellular H_2O_2 (CROSSTHWAITE et al., 2002), and is the consequence of release of Ca from endoplasmic reticulum stores (RICE et al., 1992; SU et al., 1999).

A role for PrP^C in enhancing Ca^{2+} influx through voltage gated Ca^2 channels (VGCCs) was initially suggested based on microfluorimetric measurements on synaptosomal preparations (WHATLEY et al., 1995). However, more recent patch-clamp studies on cerebellar granule cells revealed that the application of recombinant PrP depresses, rather than enhances, the Ca^{2+} influx through L-type VGCCs (KORTE et al., 2003). Interestingly, a depression of VGCCs was also observed in cell lines infected with PrP^{Sc} (SANDBERG et al., 2004) Whether this is due to a loss of function of PrP^C in modulating VGCCs or caused by a different independent mechanism is a matter of speculation.

Normal PrPc is localized in synaptic membranes, and PrP-deficient mice show defects in neural transmission, including a reduced slow after hyperpolarization (AHP) following trains of action potentials. POWEL et al. (2008) explored mechanisms that might reduce the slow AHP, including dysfunction of Ca-dependent potassium channels, voltage-gated Ca channels, and Ca homeostasis. They found no defect in potassium or Ca channel function. Instead, they found an increase in Ca buffering and extrusion rate. These effects were mediated in part by an increase in Ca uptake into the endoplasmic reticulum via the sarco/endoplasmic reticulum pump Ca -ATPase (SERCA). How PrP interacts with SERCA and whether abnormal Ca buffering leads to prion disease still need to be explored. These data implicate sarcoplasmic/endoplasmic reticulum Ca ATPase in the enhanced Ca^{2+} buffering, and extrusion into the endoplasmic reticulum, which contains substantial amounts of PrP in wild-type mice. Altered Ca^{2+} homeostasis can explain several phenotypes identified in PrP-deficient mice.

Hyperammonenia and neuronal toxicity

Ammonia toxicity is mediated by the NMDA type of glutamate receptors

Previous experiments suggested that ammonium toxicity could be mediated by the NMDA type of glutamate receptors. To assess this hypothesis MARCAIDA et al.(1992) tested if MK-801, a specific antagonist of the NMDA receptor, is able to prevent ammonium toxicity. The remarkable protection afforded by MK-801 indicates that ammonia toxicity is mediated by the NMDA receptor (MARCAIDA et al., 1992).

The aim of the work of MINANA et al (1995) was to assess whether perinatal hyperammonemia impairs the function of NMDA receptors in rats and whether this impairment affords protection against acute ammonia toxicity and glutamate and NMDA neurotoxicity. Their results indicate that exposure to ammonia during the prenatal and lactation periods results in long-lasting impairment of NMDA receptor function. This would be the reason for the delayed protection afforded by exposure to low ammonia levels against acute ammonia toxicity in animals and against glutamate and NMDA toxicity in neuronal cultures.

Ammonia is a main factor in the pathogenesis of hepatic encephalopathy, acute ammonia toxicity is mediated by activation of NMDA receptors. FELIPO et al. (1998) have tried to identify intracellular events involved in the process of neuronal death. It is known that the rise of Ca2+ is an essential step. Glutamate (Glu) leads to depletion of ATP; some compounds (e.g. carnitine) prevent Glu-induced neuronal death without preventing ATP depletion: additional events are required for neuronal death. Glu induces activation of Na+/K+-ATPase, which could be involved in the toxic process. Inhibitors of protein kinase C, calcineurin or nitric oxide synthase prevent Glu toxicity. FELIPO et al. (1998) results indicate that Glu toxicity can be prevented at different steps or by activating receptors coupled to the transduction pathways interfering with the toxic process. Agents acting on these steps could prevent excitotoxicity in vivo in animals.

During ammonia intoxication, NMDA receptors are excessively stimulated, resulting in a larger influx of Ca^{2+} than usual into neurons. This would elicit a cascade of reactions and eventually lead to neuronal cell death. How does ammonia cause excessive activation of NMDA receptors? It has been shown that NH_4^+ induced depolarization in cultured rat cortical astrocytes (ALLERT et al., 1998). This ammonia-induced depolarization could also take place in neuronal membranes and result in removal of Mg^{2+} that normally blocks the NMDA receptor channel, leading to excessive activation of the NMDA receptor (FELIPO and BUTTERWORTH, 2002).

It was shown (KOSENKO et al., 1999) that MK-801, an antagonist of NMDA receptors prevents ammonia-induced changes in superoxide dismutase, glutathione peroxidase and catalase. Ammonia intoxication also induces a depletion of glutathione and an increase in lipid peroxidation. Both effects, as well as ammonia-induced increase in superoxide formation are prevented by MK-801. These results indicate that ammonia-induced oxidative stress in brain is mediated by excessive activation of NMDA receptors and support the idea that oxidative stress can play a role in the mechanism of ammonia toxicity (KOSENKO et al., 1999).

Ammonia plays a key role in contributing to the astrocytic dysfunction of the HE

Hyperammonemia is a key factor in the pathogenesis of hepatic encephalopathy (HE) as well as other metabolic encephalopathies. Excess ammonia is toxic to the brain resulting in deleterious effects, by both direct and indirect mechanisms, on cerebral metabolism and neurotransmission. Acute HE results in increased brain ammonia (up to 5 mM), astrocytic swelling, and altered glutamatergic function. This high level of brain ammonia is a key factor in the pathogenesis of central nervous system dysfunction in acute and chronic liver failure. The nature and severity of the central nervous system disorder mainly depend upon the degree and acuteness of the onset of hyperammonemia (FELIPO and BUTTERWORTH, 2002).

Because hyperammonemia is thought to contribute to the pathogenesis of hepatic encephalopathy, IZUMI et al.(2005) examined the effects of ammonia on ATP levels, neuronal morphology, and synaptic function in rat hippocampal slices - indicating that ammonia impairs neuronal function via altered metabolism and untimely NMDA receptor activation. Their results suggest that L-carnitine and NMDA receptor antagonists have the potential to preserve neuronal function during hyperammonemia. Ammonia is a neurotoxic substance which accumulates in brain in liver failure and it has been suggested that ammonia plays a key role in contributing to the astrocytic dysfunction characteristic of hepatic encephalopathy. In particular, the effects of ammonia may be responsible for the reduced astrocytic uptake of neuronally-released glutamate and high extracellular glutamate levels consistently seen in experimental models of hepatic encephalopathy. CHAN et al. (2000) found that the reduced capacity of astrocytes to reuptake glutamate following ammonia exposure may result in compromised neuron-astrocyte trafficking of glutamate and could thus contribute to the pathogenesis of the cerebral dysfunction characteristic of hyperammonemic syndromes such as hepatic encephalopathy.

The role of ammonia in the glutamatergic dysfunction demonstrated in HE is supported with a positive correlation between extracellular brain concentrations of glutamate and arterial ammonia concentrations in acute liver failure (ALF) in rats (MICHALAK et al., 1996). In addition, using mild hypothermia as a treatment in rats with ALF, extracellular brain glutamate concentrations were normalized concomitantly with a lowering of brain ammonia (ROSE et al., 2000).

Acute hyperammonemia results in alterations of mitochondrial and cellular energy function resulting from ammonia -induced inhibition of the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase and by activation of the NMDA receptor. Antagonists of this receptor prevent acute ammonia-induced seizures and mortality and prevent acute ammonia-induced changes in mitochondrial calcium homeostasis and cellular energy metabolism. Acute hyperammonemia also results in decreased activities of free radical scavenging enzymes and again, free radical formation due to ammonia exposure is prevented by NMDA receptor (FELIPO and BUTTERWORTH, 2002).

The brain must expend energy to detoxify and to export the ammonia it produces. This is accomplished in the process of producing adenosine diphosphate (ADP) from adenosine triphosphate (ATP) by the enzyme glutamine synthetase, which is responsible for mediating the formation of glutamine from an amino group. Synthesis of glutamine also reduces the total free ammonia level circulating in the blood; therefore, a significant increase in blood glutamine concentration can signal hyperammonemia.

Hyperammonemia leads to calcium-dependent glutamate release from astrocytes

Excess ammonia is toxic to the brain resulting in deleterious effects, by both direct and indirect mechanisms, on cerebral metabolism and neurotransmission. Hepatic encephalopathy (HE) resulting from acute liver failure (ALF) disorder; mainly depend upon the degree and

acuteness of the onset of hyperammonemia. Under normal circumstances, both the liver and the brain generate ammonia in this removal process, contributing substantially to total body ammonia production. The urea cycle is completed in the liver, where urea is generated from free ammonia. Over the past 10 years, there has been an increasing body of evidence demonstrating that ammonia toxicity is involved in alterations of glutamatergic synaptic regulation which is implicated in the pathophysiology of HE in ALF. The total soluble ammonia level in a healthy adult with 5 L of circulating blood is only 150 mcg, in contrast to approximately 1000 mg of urea nitrogen present. Because urea is the end product of ammonia metabolism, the disparity in blood quantities of the substrate and product illustrates the following 2 principles:

- The central nervous system (CNS) is protected from the toxic effects of free ammonia.
- The metabolic conversion system that leads to production of urea is highly efficient.

An individual is unlikely to become hyperammonemic unless the conversion system is impaired in some way. In older individuals, the impairment is more often the consequence of a diseased liver. However, a growing number of reports address adult-onset genetic disorders of the urea cycle in previously healthy individuals.

An acute exposure to ammonia, resulting in cytosolic alkalinization (pH action), leads to Ca-dependent glutamate release from astrocytes. A deregulation of glutamate release from astrocytes by ammonia could contribute to glutamate dysfunction consistently observed in acute HE (ROSE et al., 2005). A rapid increase in ammonia results in an increase in pH_i (intracellular alkanization) in all cell types, including astrocytes. It is commonly known that ammonia (NH4+/NH3) application induces an increase in pH_i in many different cell systems. This alkaline shift is simply due to the rapid permeation of the gaseous NH_3 into the cytosol and the subsequent formation of a new NH4+/NH3 equilibrium according to the Henderson-Hasselbach equation rendering the cytosol alkaline (MARCAGGI and COLES, 2001). It has been also demonstrated that intracellular alkalinization is accompanied with an increase in $(Ca^{2+})_i$ in cultured acinar cells (SPEAKE and ELLIOT, 1998), in enthothelial cells (DANTHULURI et al., 1990), in pituitary cells (SHORTE et al., 1991), and in neurons (MIRONOV and LUX, 1993). Furthermore, ammonia-induced intracellular alkalinization has been demonstrated to increase $(Ca^{2+})_i$ in microglia initiating Ca^{2+} release from thapsigargin-sensitive stores (MINELLI et al., 2000).

Urea production in the liver is controlled by magnesium

Both, chronic and acute liver insuficiencies are associated with increased blood ammonia levels. Hyperammonemia in cirhosis is the result of a diminished capacity of the liver to synthesize urea and to a decrease in glutamine synthetase (KAISER et al., 1988). At elevated concentrations ammonia is toxic to the central nervous system (ADAMS and FOLEY, 1953). The normal value for fasting arterial blood ammonia in humans is less than 50 uM/l (COODER and PLUM, 1987). The brain ammonia to blood ammonia concentration ratio is in the range of 1.5 : 1 to 3 : 1. This ratio is maintained by a complex interaction of blood flow, the pH difference across the blood- brain barrier, and a balance between enzymatic removal and synthesis.

The urea level in blood of animals is closely related to rhythmic intake of dietary protein (EGGUM, 1970) and is strictly reversely proportional to the biological value of the dietary protein (MUNCHOW and BERGNER, 1968). The plasma urea level and its urinary excretion also shows circadian rhythmic changes in man (KANABROCKI et al., 1973). In dairy cattle the meal time - dependent circadian rhythm of blood urea is small (ERBERSDOBLER et al., 1980). Since urea is mostly excrected by the kidneys, the blood urea is increased in renal failure. However, in healthy animals the serum urea level is primarily affected by their feeding regime. The protein intake (IDE et al., 1966) and particularly the ratio of protein to

energy affect the urea levels in blood and milk of cows (PAYNE et al., 1970).

The hepatic urea cycle is the major route for disposal of waste nitrogen generated chiefly from protein and amino acid metabolism. The brain must expend energy to detoxify and to export the ammonia it produces. This is accomplished in the process of producing adenosine diphosphate (ADP) from adenosine triphosphate (ATP) by the enzyme glutamine synthetase, which is responsible for mediating the formation of glutamine from an amino group. Synthesis of glutamine also reduces the total free ammonia level circulating in the blood; therefore, a significant increase in blood glutamine concentration can signal hyperammonemia.

The CNS is most sensitive to the toxic effects of ammonia. Many metabolic derangements occur as a consequence of high ammonia levels, including alteration of the metabolism of important compounds, such as pyruvate, lactate, glycogen, and glucose. High ammonia levels also induce changes in NMDA and gamma-aminobutyric acid (GABA) receptors and causes downregulation in astroglial glutamate transporter molecules (ROTH, 2006).

A high rate and extent of degradation of crude protein causing high concentrations of ammonia – N in rumen results in hyperammonemia, because of diminished capacity of liver to synthetise urea in ornithine cycle. Of prime importance in the control of carbamoyl-phosphate synthese activity in ornithine cycle; is the mitochondrial concentration of N-acetylglutamate, a compound that is indispensable for enzyme activity. In addition to the absolute concentration of mitochondrial N-acetylglutamate, the concentration of liver mitochondrial free Mg^{2+} may be relevant, since binding N-acetylglutamate to carbamoyl-phosphate synthase is dependent on this action (MEIJER, 1985).

The function of activity changes in carbamoyl- phosphate synthetase, via the well-documented alterations in the intramitochondrial concentration on N-acetylglutamate, is to buffer the intrahepatic ammonia concentration rather than to affect urea production per se. At constant concentration of ammonia the rate of urea production is entirely controlled by the activity of carbamoyl- phosphate synthetase (MEIJER et al., 1985). In other words; N-acetylglutamate synthetase is a mitochondrial matrix enzyme which catalyses the synthesis of N-acetylglutamate, which activates carbamoyl-phosphate synthetase (CPS), which initiates the first step of urea synthesis from ammonia. Regulation of CPS activity depends upon the levels of N-acetylglutamate. In cases of homozygous deficiency of CPS, the ability to fix waste nitrogen is completely absent, which results in increasing levels of free ammonia with the attendant effects on the CNS. Overall, activity of the cycle is regulated by the rate of synthesis of N-acetylglutamate, the enzyme activator of CPS (which also is a mitochondrial enzyme) which initiates incorporation of ammonia into the urea cycle.

In addition, the increased glutamate leads to glutamine formation. This depletes glutamate stores which are needed in neural tissue since glutamate is both a neurotransmitter and a precursor for the synthesis of gama- -aminobutyrate (GABA); another neurotransmitter. Therefore, reductions in brain glutamate affect energy production as well as neurotransmission. Additional untoward consequences are the result of elevations in neural glutamine concentration. Glial cell (astrocytes) volume is controlled by intracellular organic osmolyte metabolism. The organic osmolyte is glutamine. As glutamine levels rise in the brain the volume of fluid within glial cells increases resulting in the cerebral edema (KING, 2006).

The apparent effect of pH on the afinity of glutaminase for phosphate was found (SZWEDA and ATKINSON, 1989). The strong response of liver glutaminase to pH and the fact that the reaction can supply metabolites for urea synthesis suggest a possible regulatory role of glutaminase in ureagenesis. It was also found (SZWEDA and ATKINSON, 1990), that the activity of rat liver glutaminase is strongly affected by variation in the Mg2+ concentration within the approximate physiological range of activators. A rise in the Mg2+

concentration stimulates glutaminase by increasing the apparent affinity of the enzyme for its positive modifer phosphate. Since Mg2+ stimulates glutaminase, as does a rise in pH, by increasing the apparent affinity of the enzyme for phosphate, it reduces the inhibitory effect of a decrease in pH and/or phosphate concentration over a physiologically relevant range (SZWEDA and ATKINSON, 1990).

Glutaminase activity in intact mitochondria from rat liver is activated by spermine, as indicated both by increased glutamate production from glutamine and by increased respiration with glutamine as sole substrate. It was found (KOVACEVIC et al., 1995) that spermine was effective in the presence of physiological concentrations of Mg2+. Authors suggest that spermine may be a physiological activator of hepatic glutaminase.

Hypomagnesaemia and neuronal toxicity

In the 1980s; new NMDA receptor and a Mg2+ sensitivity was discovered, it was found in a unique manner

Calcium in cells is tightly regulated and mostly unrelated to necessary dietary calcium (Ca). However, a high content of calcium in the ration increases the Mg requirements of the animal. The lower the Mg level in the animal ration (and in the tissue cells); the more marked is "Ca- effect excitotoxicity". It can be also accentuated; a low temperature raises Mg requirements.

The experiments demonstrated (Van MOSEL, et al, 1990) that younger cows are better able to mobilize Mg from the body reserves than older cows. Also as well known, older especially high milk yielding cows have hepatopathy in connection with high dietary protein intake.

In the 1980s great strides have been made toward better undersanding the function of neurotransmitters, in particular because of the application of voltage – and patch- clamp techniques to cultured neurons that express the receptors and because of the development of specific receptor antagonists (COLLINGRIDGE and LESTER, 1989; MAYER and WESTBROOK, 1987; MONAGHAN et al.,1989; STONE and BURTON, 1988). This surge in information has not only resulted in a detailed understanding of the currents that underlie the fast excitatory amino acid- mediated transmission at many central synapses but has unveiled an exciting new receptor type, NMDA receptor, the activity of which is gated in a unique manner both by ligand binding and by voltage.

Aspartate appeared to act at the NMDA receptor as well as at additional sites, since part of the aspartate response showed a Mg2+ sensitivity similar to that of the NMDA response (SEKIGUCHI et al.,1987). These results suggest that cerebellar Purkinje cell dendrites do posses NMDA channels, but their functional role was still unclear. The receptor pharmacology in cerebellum is apparently slightly different from other brain regions. First, APV does not entirely block NMDA responses (CREPEL et al., 1983; SEKIGUCHI et al.,1987). Second, aspartate seems preferentially to activate NMDA- like receptors, some of which mediate effects that are entirely blocked in 1 mM Mg2+ (SEKIGUCHI et al.,1987) and others at which NMDA may act as a competitive antagonist (CREPEL et al., 1983; KIMURA et al., 1985; SEKIGUCHI et al.,1987).

NMDA receptor is crucial to a process known as exitotoxicity, in which excessive release of glutamate leads to over- excitation of neurons producing neuronal injury and death (CHOI, 1988). Because excitotoxicity has been implicated in many disorders (acute neurological insults- e.g. Status epilepticus; chronic neurological disorders- e.g. Huntington´s disease; metabolic disorders- e.g. hyperammonemia), development of NMDA receptor antagonists has become a major area of pharmacological investigation (CHOI,1988; CHOI et al.,1988; CHOI and ROTHMAN, 1990; LIPTON, 1993).

Glutamic acid (Glutamate –"Glu") as the major excitatory neurotransmitter in the mammalian CNS; acts postsynaptically at several receptor types named for their prototypic pharmacological agonist. In excess, glutamate triggers a process called excitotoxicity, causing neuronal damage and eventual cell death, particularly when NMDA receptors are activated. This may be due to:

- High intracellular Ca2+ exceeding storage capacity and damaging mitochondria, leading to release of cytochrome and apoptosis,
- Glu/Ca^{2+}-mediated promotion of transcriptions factors for pro-apoptotic genes, or downregulation of transcription factors for anti-apoptotic genes.

Elevations in extracellular glutamate are not necessary to invoke an excitotoxic mechanism. Excitotoxicity can come into play even with normal levels of glutamate if NMDA receptor activity is increased, e.g., when neurons are injured and thus become depolarized (more positively charged); this condition relieves the normal block of the ion channel by Mg^{2+} and thus abnormally increases NMDA receptor activity (ZEEVALK and NICKLAS, 1992).

Activation of NMDA receptors requires binding of both glutamate and the co-agonist glycine for the efficient opening of the ion channel which is a part of this receptor. In addition, a third requirement is membrane depolarization. A positive change in transmembrane potential will make it more likely that the ion channel in the NMDA receptor will open by expelling the Mg2+ ion that blocks the channel from the outside. In neurons from spinal cord , 10 mM Mg2+ is required to block NMDA responses at potentials negative to 0 mV (MAYER and WESTBROOK, 1985). It may be that the NMDA channel in Purkinje cells has a more extreme sensitivity to Mg2+ than that in other brain regions.

When glutamate and glycine bind and the cell is depolarized to remove Mg^{2+} block, the NMDA receptor channel opens with consequent influx of Ca^{2+} and Na^{+} into the cell, the amount of which can be altered by higher levels of agonists and by substances binding to one of the modulatory sites on the receptor. The two modulatory sites that are most relevant to this review are the Mg^{2+} site within the ion channel and an S-nitrosylation site located toward the N terminus (and hence extracellular region) of the receptor (LIPTON, 2004).

The ion channels coupled to classical NMDA receptors are generally the most permeable to Ca^{2+}. Excessive activation of the NMDA receptor in particular leads to production of damaging free radicals and other enzymatic processes contributing to cell death (LIPTON and ROSENBERG, 1994; LIPTON and NICOTERA, 1998).

Under pathological conditions; depolarization of neurons relieves the normal Mg^{2+} block of NMDA receptor

Under normal conditions of synaptic transmission, the NMDA receptor channel is blocked by Mg^{2+} sitting in the channel and only activated for brief periods of time. Under pathological conditions, however, overactivation of the receptor causes an excessive amount of Ca^{2+} influx into the nerve cell, which then triggers a variety of processes that can lead to necrosis or apoptosis. The latter processes include Ca^{2+} overload of mitochondria, resulting in oxygen free radical formation and activation of caspases, Ca^{2+}-dependent activation of neuronal enzyme nitric oxide synthase (NOS), leading to increased nitric oxide (NO) production and the

formation of toxic peroxynitrite ($ONOO^-$), and stimulation of mitogen-activated protein kinase p38 (MAPK p38), which activates transcription factors that can go into the nucleus and influence neuronal injury and apoptosis (BONFOCO, 1995; DAWSON et al., 1991; DAWSON et al., 1993; LIPTON et al., 1993; TENNETI et al., 1998; YUN et al., 1998; BUDD et al., 2000; OKAMOTO et al., 2002).

Energetically compromised neurons become depolarized (more positively charged) because in the absence of energy they cannot maintain ionic homeostasis; this depolarization relieves the normal Mg^{2+} block of NMDA receptor-coupled channels because the relatively positive charge in the cell repels positively-charged Mg^{2+} from the channel pore. Hence, during periods of ischemia and in many neurodegenerative diseases, excessive stimulation of glutamate receptors is thought to occur. These neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease…, are caused by different mechanisms but may share a final common pathway to neuronal injury due to the overstimulation of glutamate receptors, especially of the NMDA subtype (LIPTON and ROSENBERG, 1994).

Compelling evidence supports contributions of glutamate receptor overactivation ('excitotoxicity') to neurodegeneration in both acute conditions, such as stroke, and chronic neurodegenerative conditions, such as amyotrophic lateral sclerosis. However, anti-excitotoxic therapeutic trials, which have generally targeted highly Ca2+ permeable NMDA-type glutamate channels, have to date failed to demonstrate impressive efficacy (KWAK and WEISS, 2006). Whereas most AMPA type glutamate channels are Ca2+ impermeable, an evolving body of evidence supports the contention that relatively unusual Ca2+ permeable AMPA channels might be crucial contributors to injury in these conditions. These channels are preferentially expressed in discrete neuronal subpopulations, and their numbers appear to be upregulated in amyotrophic lateral sclerosis and stroke. In addition, unlike NMDA channels, Ca2+ permeable AMPA channels are not blocked by Mg2+, but are highly permeable to another potentially harmful endogenous cation, Zn2+. The targeting of these channels might provide efficacious new avenues in the therapy of certain neurological diseases (KWAK and WEISS, 2006).

Mg^{2+} can protect against NMDA- induced neurodegeneration

An important consequence of NMDA receptor activation is the influx of Ca^{2+} into neurons. Excessive NMDA receptor stimulation is thought to be an important factor in neuronal cell damage, mediated by excessive Ca entry into the cell (OLNEY, 1989: McMASTER et al., 1991). Studies have demonstrated that Mg can protect against NMDA-induced neurodegeneration, brain injury, and convulsions in rats (McDONALD et al., 1990: WOLF et al., 1990).

Neuronal free Ca concentrations correlates with the likelihood of irreversible ischaemic cell death (EIMERL and SCHRAMM, 1994: CHOI, 1985), and free intracellular Ca increases may result from Ca entry via the NMDA ion channel and voltage - gated Ca channels, and release from endoplasmic reticulum and other intracellular stores. Mg competes with Ca at voltage- gated Ca channels both intracellularly and on the cell surface membrane (ISERI and FRENCH, 1984). It may thereby impede Ca- dependent presynaptic release of glutamate and prevent neuronal Ca overload via voltage- gated channels during ischaemia. Mg also enhances mitochondrial buffering of raised intracellular free Ca ions (FAVARON and BERNARDI, 1985), and prevents release of intracellular Ca stores from endoplasmic reticulum.

The NMDA receptor channel is additionally blocked by Mg^{2+} and phencyclidine (PCP). Protons suppress NMDA receptor activation, and polyamines, such as spermine, relieve the proton block. Mg is capable of blocking NMDA receptors both intracellularly and

extracellularly (KUPPER et al., 1998).

Another important endogenous allosteric inhibitor of NMDA receptor activation is pH. The frequency of NMDA receptor channel openings is reduced by protons over the physiological pH range, with a midpoint at pH 7.4, such that at pH 6.0 receptor activation is suppressed nearly completely (NOWAK et al., 1984). This suggests that an ionizable histidine or cysteine may play a key role in receptor activation. One or more modulatory sites that bind polyamines, such as spermine and spermidine, also are found on NMDA receptors. Occupancy of one of the polyamine sites relieves tonic proton block and, thus, potentiates NMDA receptor activation in a pH-dependent manner (TRAYNELIS et al., 1995). At higher concentrations, however, polyamines act on an extracellular site to produce a voltage-dependent block of the ion channel and, thus, inhibit receptor activation.

NOTE; The molecular bases for prion diseases are not yet fully understood. Why are some proteins infectious while others are not? Writing in the journal Angewandte Chemie, the researchers (WASNER et al., 2008) report that the molecular structures of the infectious and non-infectious forms are very different. Prions usually consist of β-sheet structures. These are accordion-like folded protein ribbons that can easily aggregate into thread-like structures, known as amyloid fibrils, which are present in the brains of CJD and Alzheimer's sufferers. The research team took on the prion-forming domain of the fungal protein HET-s. At a pH value of 7—under physiological conditions—this domain forms infectious fibrils. In acidic solution, at pH 3, it also forms fibrils, but these are not infectious.

The permeation pathway of NMDA receptors has a property that sets them apart from other conventional ligand-gated receptors. At hyperpolarized membrane potentials more negative than about −70 mV, the concentration of Mg^{2+} in the extracellular fluid of the brain is sufficient to virtually abolish ion flux through NMDA receptor channels even in the presence of the coagonists glutamate and glycine (NOWAK et al., 1984).

Mg^{2+} affect guanine nucleotide binding proteins (G proteins) in several ways

In 1980s as the coupling mechanisms between receptor and ion channel have been defined, it has become clear that the great majority of neurotransmitters in the CNS act throufh coupling proteins to activate or block the action of voltage- dependent channels. The simplest kind of coupling that involves an intermediary protein apparently occurs through a single protein (G protein), as first described in atrial cells of heart (BREITWEISER and SZABO, 1985; PFAFFINGER et al., 1985; YATANI et al., 1987).

Receptor occupation leads to G protein activation by allowing the coupled G protein to exchange its bound GDP for GTP. The binding of GDP results in the dissociation of the G protein´s regulatory subunit (beta gama) from its catalytic subunit (alpha), which is responsible for carrying out most of the G protein´s known intracellular activities. The G protein does not remain permanently activated because of its inherent guanosinetriphosphatase (GTPase) activity. The hydrolysis of GTP to GDP results in the reassociation of the beta gama-and alpha- subunits such that the G protein is now ready to be activated again by ligand occupation of the receptor (GILMAN, 1984; STRYER and BOURNE, 1986). There are several species of G proteins originally named because of their ability to stimulate (Gs) or inhibit (Gi) adenylate cyclase and Go- as a G protein is found in very high concentrations in brain ((GILMAN, 1984; STRYER and BOURNE, 1986).

In the central nervous system (CNS) magnesium (Mg^{2+}) ion has two major functions: the stabilization of synaptic connectivity and widespread enhancement of neurochemical enzymatic functions. The Mg^{2+} has been shown to affect guanine nucleotide binding proteins

(G proteins) in several ways: nanomolar concentrations of Mg^{2+} are required for GPT-ase activity (GILMAN, 1987: HIGASHIJIMA et al., 1987), micromolar concentrations of Mg^{2+} are required for receptor mediated activation of G proteins (GILMAN, 1987: GIERSCHIK et al., 1988), milimolar concentrations of Mg^{2+} increase the affinity of various types of receptors for agonists, an effect thought to result from increased receptor- G- protein coupling (HULUME et al., 1983: BIRNBAUMER et al., 1990), voltage- dependent- Ca^{2+} channel (AUGUSTINE et al., 1987), and NMDA receptor operated ionic channel (CRUNELLI and MAYER, 1984: NOWAK et al., 1984). The inhibitory effect of milimolar concentrations of Mg^{2+} on neurotransmitter release have been already demonstrated by in vivo microdyalisis experiments (OSBORNE et al., 1991: OKADA et al., 1996, 1998).

Astrocytes regulate neuronal Ca2+ levels through the Ca-dependent release of glutamate

The brain has two types of cells; neurons and glia. Neurons contain neurotransmitters, which are chemicals that trigger signals to pass messages. Until recently, neuroscientists believed neurons were the only brain cells transmitting message signals. Glial cells (astrocytes) were thought to serve only as support. Glia, once thought to simply provide structural support for their more important neuronal cousins, have been found, in the past decade, to have a wide variety of important biological functions. One of the most important of these is to foster a proper chemical environment for neuronal function by removing excess glutamate. Why is this important? Because glutamate is a neurotransmitter, i.e., it can bind to receptors on the neuronal membrane and cause it to fire. Thus, glutamate is key to proper neurological functioning. Too much glutamate, however, is a problem, because it could cause neurons to work too hard, fatigue and die a premature death. This phenomenon is called glutamate toxicity.

Astrocytes express receptors for many neurotransmitters, and their activation leads to oscillations in internal Ca^{2+}. These oscillations induce the accumulation of arachidonic acid and the release of the chemical transmitters glutamate, D-serine, and ATP. The Ca^{2+} oscillations in astrocytic endfeet can control cerebral microcirculation through the arachidonic acid metabolites prostaglandin E_2 and epoxyeicosatrienoic acids that induce arteriole dilation, and 20-HETE that induces arteriole constriction. In addition to actions on the vasculature, the release of chemical transmitters from astrocytes regulates neuronal function. Astrocyte-derived glutamate, which preferentially acts on extrasynaptic receptors, can promote neuronal synchrony, enhance neuronal excitability, and modulate synaptic transmission. Astrocyte-derived D-serine, by acting on the glycine-binding site of the NMDA receptor, can modulate synaptic plasticity (HAYDON and CARMIGNOTO, 2006).

Results of a five-year study by Iowa State neuroscientists Vladimir Parpura and Philip Haydon (2000) give evidence supporting a relatively new theory about communications between brain cells. It was found that astrocytes can release glutamate in a Ca-dependent manner and consequently signal to adjacent neurons. Whether this glutamate release pathway is used during physiological signaling or is recruited only under pathophysiological conditions is not well defined. One reason for this lack of understanding was the limited knowledge about the levels of Ca (PARPURA et al., 1994) necessary to stimulate glutamate release from astrocytes and about how they compare with the range of physiological Ca levels in these cells. PARPURA et al. (1994) demonstrated an additional signalling pathway in which glutamate is released from astrocytes and causes an NMDA receptor-mediated increase in neuronal Ca. Thus, astrocytes regulate neuronal Ca levels through the Ca-dependent release of glutamate.

PARPURA and HAYDON (2000) demonstrated that the astrocytic glutamate release pathway is engaged at physiological levels of internal Ca. Consequently, the Ca-dependent release of glutamate from astrocytes functions within an appropriate range of astrocytic Ca levels to be used as a signaling pathway within the functional nervous system. So the amount of Ca to be within the normal range, indicating that astrocytes are part of the brain's communication network.

The evidence obtained during the last few years has established a new concept of the synaptic physiology, the tripartite synapse, in which astrocytes play an active role by exchanging information with the synaptic elements (ARAQUE et al., 1999; CARMIGNOTO, 2000; AULD and ROBITABILE, 2003; NEWMAN, 2003). This concept is based on the demonstration that astrocytes display a form of excitability based on intracellular Ca^{2+} variations (PASTI et al., 1997; VERKHRATSKY et al., 1998; HAYDON, 2001; NEDERGAARD et al., 2003), respond to synaptically released neurotransmitters (PORTER and McCARTHY, 1996; PASTI et al., 1997; GROSCHE et al., 1999; LATOUR et al., 2001; ARAQUE et al., 2002), and modulate neuronal excitability and synaptic transmission by releasing neuroactive substances through, at least some of them, Ca^{2+}-dependent mechanisms (ARAQUE et al., 1998a, 1998b; KANG et al., 1998; NEWMAN and ZAHS, 1998; ROBITAILLE, 1998; PARRI et al., 2001; BEATTIE et al., 2002; BROCKHAUS and DEITMER, 2002: NEWMAN, 2003; ZHANG et al., 2003; FIACCO and McCARTHY, 2004; LIU et al., 2004).

The ability of astrocytes to release glutamate through a Ca^{2+}-dependent mechanism is well established (BEZZI et al., 1998, 2004; ARAQOE et al., 2000; PARPURA and HAYDON, 2000; PASTI et al., 2001; ZHANG et al., 2004). On the other hand the ability of most neurotransmitters to increase astrocytic Ca^{2+} levels is firmly established (PORTER and McCARTHY, 1997; VERKHRATSKY et al., 1998). Recent reports have shown that astrocytic receptor activation by exogenously applied transmitters may have synergistic effects that increase the Ca^{2+} signal (FATATIS et al., 1994; CORMIER et al., 2001; SUL et al., 2004). Ca^{2+} elevations in astrocytes stimulate the release of glutamate, which acting on presynaptic or postsynaptic receptors modulates synaptic transmission and neuronal excitability (ARAQUE et al., 1998a, 1998b; KANG et al., 1998; PARRI et al., 2001; PASTI et al., 2001; BROCKHAUS and DEITMER, 2002; FIACCO and McCARTHY, 2004; LIU et al., 2004).

Astrocytes, a subtype of glial cells, have numerous characteristics that were previously considered exclusive for neurons. One of these characteristics is a cytosolic Ca2+ oscillation that controls the release of the chemical transmitter glutamate and atrial natriuretic peptide. These chemical messengers appear to be released from astrocytes via Ca^{2+}-dependent exocytosis. Glutamate can be released from astrocytes, and several mechanisms have been proposed. Glutamate has been demonstrated to be an important signaling molecule for neuron-glia communication. Astrocytes express receptors and transporters for glutamate and recently have also been demonstrated to contain the protein machinery necessary to release glutamate by exocytosis through vesicles (BEZZI et al., 2004) and a fusion-related mechanism (ZHANG et al., 2004; KREFT et al., 2004). Overall, astrocytes have many characteristics that were previously considered exclusive for neurons and are therefore actively involved in cell signaling by releasing glutamate. Astrocytic glutamate release is Ca-dependent and can be triggered by any ligand that stimulates an increase in Ca2+, such as bradykinins (PARPURA et al., 1994), prostaglandins (BEZZI et al., 1998). Even a spontaneous $Ca2+_i$ increase leads to glutamate release from astrocytes (PASTI et al., 2001).

Astrocytes as cellular elements involved in the information processing by the nervous system

Astrocytes establish rapid cell-to-cell communication through the release of chemical transmitters. The underlying mechanisms and functional significance of this release was, however, not well understood. BEZZI et al. (2004) identified an astrocytic vesicular compartment that is competent for glutamate exocytosis. After activation of metabotropic glutamate receptors, astrocytic vesicles underwent rapid (milliseconds) Ca^{2+}- and the vesicular SNARE protein (cellubrevin) -dependent exocytic fusion that was accompanied by glutamate release. These data document the existence of a Ca^{2+}-dependent quantal glutamate release activity in glia that was previously considered to be specific to synapses (BEZZI et al., 2004).

Astrocytes in the brain form an intimately associated network with neurons. They respond to neuronal activity and synaptically released glutamate by raising intracellular Ca concentration Ca2+, which could represent the start of back-signalling to neurons. Glutamate has been demonstrated to be an important signaling molecule for neuron-glia communication. Astrocytes express receptors and transporters for glutamate and recently have also been demonstrated to contain the protein machinery necessary to release glutamate by exocytosis through vesicles (BEZZI et al., 2004) and a fusion-related mechanism (ZHANG et al., 2004; . KREFT et al., 2004).

Although cell culture studies have implicated the presence of vesicle proteins in mediating the release of glutamate from astrocytes, definitive proof requires the identification of the glutamate release mechanism and the localization of this mechanism in astrocytes at synaptic locales. To further determine whether vesicular exocytosis mediates Ca-dependent glutamate release from astrocytes, ZHANG et al.(2004), performed whole cell capacitance measurements from individual astrocytes and demonstrate an increase in whole cell capacitance, coincident with glutamate release. Together, these data allow to conclude that astrocytes in situ express vesicle proteins necessary for filling vesicles with the chemical transmitter glutamate and that astrocytes release glutamate through a vesicle- or fusion-related mechanism.

The synaptic control of the astrocytic intracellular Ca^{2+} is crucial in the reciprocal astrocyte-neuron communication. Using electrophysiological and Ca^{2+} imaging techniques in rat hippocampal slices, PEREA and ARAQUE (2005) investigated the astrocytic Ca^{2+} signal modulation induced by synaptic terminals that use glutamate and acetylcholine. Ca^{2+} elevations were evoked by glutamate released from Schaffer collaterals and by acetylcholine, but not glutamate, released by alveus stimulation, indicating that astrocytes discriminate the activity of different synapses belonging to different axon pathways. The Ca^{2+} signal was modulated bidirectionally by simultaneous activation of both pathways, being depressed at high stimulation frequencies and enhanced at low frequencies. The Ca^{2+} modulation was attributable to astrocytic intrinsic properties, occurred at discrete regions of the processes, and controlled the intracellular expansion of the Ca^{2+} signal. In turn, astrocyte Ca^{2+} signal elicited NMDA receptor-mediated currents in pyramidal neurons. Therefore, because astrocytes discriminate and integrate synaptic information, PEREA and ARAQUE (2005) proposed that astrocytes can be considered as cellular elements involved in the information processing by the nervous system.

D-serine coactivates postsynaptic NMDA receptors together with glutamate; mediated by intracellular Ca2+

Other neurotransmitter is an amino acid D-serine. Astrocyte-derived D-serine, by acting on the glycine-binding site of the NMDA receptor, can modulate synaptic plasticity (HAYDON and CARMIGNOTO, 2006). This differs in structure from any known molecule in its class found in mammals and other higher animals. D-serine is what chemists call a right handed amino acid. Normally, amino acids have atoms that extend from the left side of the

molecule. These L-amino acids, as they're called, are the rule in vertebrates, whose biochemistry is set up to deal with these forms. Some primitive organisms, however, notably bacteria, have a mixture of both L-amino acids and their mirror images called D-amino acids. But to find a D-amino acid in humans "is unprecedented" (SNYDER, 2000).

Moreover, unlike dopamine, serotonin or other traditional nerve transmitters, D-serine isn't secreted at nerve cell endings in the brain. Instead, it comes from adjacent cells called astrocytes, which enclose nerve cells in the brain's gray matter like a glove. The current study adds conclusive evidence to the idea that D-serine (released from astrocytes) activates receptors on key nerve cells in the brain. Activating these NMDA receptors has long been linked with learning, memory and higher thought. NMDA receptors are also known culprits in stroke damage in the brain, and have become a focus for anti-stroke research (SNYDER and FERRIS, 2000).

Classic criteria for transmitters were based on the properties of acetylcholine but were markedly revised with the recognition of the catecholamines, serotonin, gama -aminobutyric acid (GABA), and other amino acid transmitters and neuropeptides. Nitric oxide and carbon monoxide are notably atypical, as they are not stored in synaptic vesicles, are not released by exocytosis, and do not act at postsynaptic membrane receptor proteins. D-Serine, recently appreciated as the endogenous ligand for the glycine site of the glutamate NMDA receptor, overturns fundamental axioms of biology as well as those of neuroscience. It is a D-amino acid, and it is synthesized and stored in glia rather than neurons. Released glutamate acts on receptors on the protoplasmic astrocytes closely apposed to the synapse to release D-serine, which coactivates postsynaptic NMDA receptors together with glutamate. D-Serine is formed by serine racemase (enzyme), which directly converts L-serine to D-serine. Inhibitors of this enzyme should reduce NMDA neurotransmission and might be therapeutic in stroke and other conditions associated with glutamate excitotoxicity (SNYDER and FERRIS, 2000).

These researchers also found D-serine and serine racemase concentrated in astrocytes adjacent to NMDA receptors, but less common or nonexistent in other neural tissues. For years, neuroscientists assumed that NMDA receptors could only be stimulated by a single neurotransmitter, an amino acid called glutamate. They now know that two neurotransmitters are needed to stimulate the NMDA receptors. D-serine was recently proposed by Hopkins scientists (Baltimore University) as the second, largely because microscope images of tagged D-serine show it's physically near NMDA receptors in the synapse. Also, knocking D-serine out with enzymes quickly stops NMDA receptors from being active.

COOK et al. (2002) found that divalent cations such as Ca or manganese were necessary for complete serine racemase (SR) enzyme activity, whereas the presence of chelators such as EDTA completely inhibited the enzyme. Moreover, direct binding of Ca to SR was evidenced using $^{45}Ca2+$. Treatment of astrocytes with the Ca ionophore as well as with compounds that augment the intracellular Ca levels such as glutamate or kainate led to an increase in the amount of D-serine present in the extracellular medium. These results suggest that there might be a glutamatergic-mediated regulation of SR activity by intracellular Ca2+ concentration (COOK et al. 2002).

Conclusions about the reasons of the BSE incidence decrease;

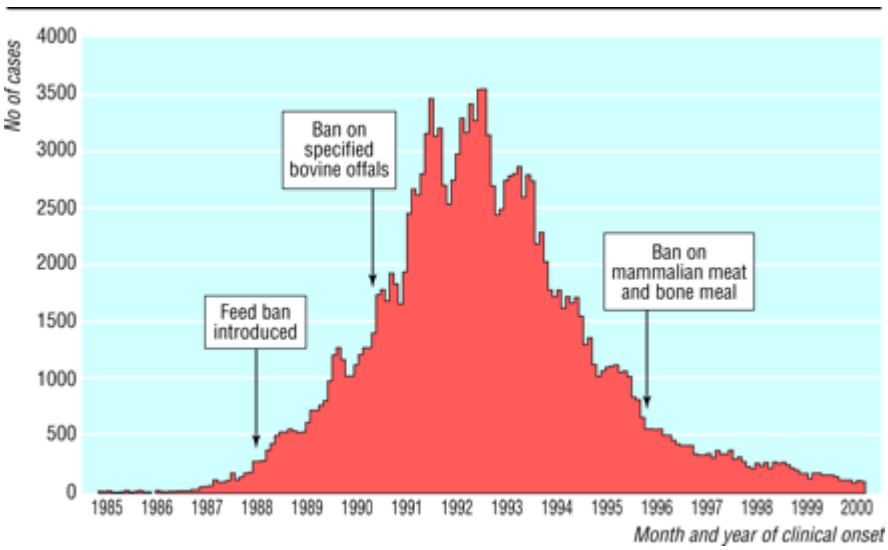
1. Increase of magnesium in Mineral Feed Supplement (by 160%) for dairy cattle at the beginning of 1990s (see later; a decrease of BSE incidence in the UK).

From Czech Republic (to the end of 1980s) there is well known experiment (HLASNY, December 1989); see the „Evaluation of a new mineral feed supplement used in feeding young breeding cattle in the winter season „"(http://www.ncbi.nlm.nih.gov/pubmed/2631375?dopt=Abstract):

- In total, 260 tons of a New Mineral Feed Supplement (NMFS) was used on farms in the period from January to April, 1988.
- The NMFS, containing 8 to 10% calcium, 3 to 4% phosphorus, 6 to 7% magnesium and 8 to 10% sodium, was used in all first-calver barns in the 1987/88 winter feeding season.
- It was administered to about 7300 highly pregnant heifers and first-calvers at rates of 0.15 to 0.2 kg per head and day.

It has been concluded;
- The mortality of newborn calves decreased by 2.9% (from 10.4 to 7.5%) in comparison with the same period of 1987 when (in Mg lower) Former Mineral Supplement (FMS) had been used.
- The NMFS , containing much more Mg (by 180%) and less P (by 46%) than the FMS and other commercially produced mineral supplements (in Europe), it was able to meet all the P requirement, together with a rational management of Mg, in young breeding cattle.
- Apart from a higher effect, especially in the compensation of hypomagnesiaemia and metabolic acidosis, the use of the NMFS in first-calver stocks allowed to reduce the total financial costs of mineral feed supplements by 20%. The basis for the reduction of costs is the saving of phosphates.

However, the official scientific statement about the 5-years incubation period of the BSE is different. It is based on the feed ban of meat and bone meal (1988) and the BSE incidence decrease (after 1993)- it is and was reproduced by this figure (http://www.bmj.com/cgi/content/full/322/7290/841):



Chronology of epidemic of bovine spongiform encephalopathy in United Kingdom, 1986-2000

2. Recommendations about lower protein intake in dairy cows as a „phenomenon“ at the 2001/2002 period; and later steady decrease of BSE incidence, in European states with high producing dairy cows, especially

The research during 1990s resulted to decrease of protein content in dairy rations; see the comparison of the NRC (1989 and 2001);

Dairy cow:600-680 kg body weight							
	Lactation				Early lactation		Dry pregnant
Milk yield (kg/day)	25	35	45	55	25	35	
Degradable protein – „DP" (%):							
NRC,1989	8,8	9,7	10,4	10,4		9,7	-
NRC,2001	9,5	9,7	9,8	9,8	10,5	10,3	9,9
Undegradable protein- „UDP" (%):							
NRC,1989	5,4	5,7	6,0	6,3		7,2	-
NRC,2001	4,6	5,5	6,2	6,9	5,4	5,6	3,2
Crude protein – „CP"- (%):							
NRC,1989	15,0	16,0	17,0	17,5		**19,0**	12,0
NRC,2001	14,1	15,2	16,0	16,7	15,9	**15,9**	13,1

Prion diseases and alternative manganese theory

According to some researchers, the deformed prions would become more prone to binding with manganese (Mn), and it is this combination that makes them dangerous. It is postulated that Mn -bound prions then become rogue prions that have the ability to deform other normal prions in a chain reaction that eventually destroys the brain. This hypothesis contends that TSEs are likely linked to environmental conditions rather than prion-contaminated feed. The researchers discovered that prions require copper (Cu) to develop properly. If Cu is low and exposure to high levels of Mn occurs, the prion may bind to Mn and turn into the fatal form that eventually burns holes in the brain.

A. Manganese as a diagnostic marker for prion infection

It is almost universally accepted that the normal protein, PrP^c is a metal binding protein. PrP^c was first suggested to be a copper binding protein in the early 1990's and confirmed by David Brown and colleagues in 1997 (BROWN,D.R., 2001). This has been followed by close to a 100 publications that have reafffirmed that PrP^c is a copper binding protein. WONG and his team, University School of Medicine, in Cleveland, Ohio, working with Brown, has also studied metal binding by prion proteins. They found that the brain tissue of patients with CJD contain up to half as much copper and 10 times as much manganese as those with normal brain cells (WONG et al., 2001). They suggest that manganese, which is detectable by MRI, might be used as a diagnostic marker for the progress of the disease.

Recently the team of professor BROWN repeatedly pointed out that elevated Mn was associated with prion infection - in field cases and experimentally infected animals (BSE and scrapie). Although some central nervous system regions showed elevated Mn, other regions did not. The most consistent finding was an elevation of Mn in blood. So sheep infected with scrapie and cows infected with BSE have elevated levels of Mn in their blood before clinical symptoms appear, according to new research. These findings, published in the Journal of Animal Science, (HESKETH et al., 2007) are following (see the abstract);

Prion diseases, or transmissible spongiform encephalopathies, are neurodegenerative diseases that can only be accurately diagnosed by analysis of central nervous system tissue for

the presence of an abnormal isoform of the prion protein known as PrP^{Sc}. Furthermore, these diseases have long incubation periods during which there are no clear symptoms but where the infectious agent could still be present in the tissues. Therefore, the development of diagnostic assays to detect a surrogate marker for the presence of prion disease is essential. Previous studies on mice experimentally infected with scrapie, an ovine spongiform encephalopathy, suggested that changes in the levels of Mn occur in the blood and brain before the onset of symptoms of the disease. To assess whether these findings have relevance to the animal diseases scrapie and bovine spongiform encephalopathy, tissues from bovine spongiform encephalopathy- and scrapie-infected cattle and sheep were analyzed for their metal content and compared with values for noninfected animals. In field cases and experimentally infected animals, elevated Mn was associated with prion infection. Although some central nervous system regions showed elevated Mn, other regions did not. The most consistent finding was an elevation of Mn in blood. This change was present in experimentally infected animals before the onset of symptoms. In scrapie-infected sheep, elevated Mn levels occurred regardless of the genotype of the sheep and were even detected in scrapie-resistant sheep in which no symptoms of disease were detected. These findings suggest that elevated blood Mn could be a potential diagnostic marker for prion infection even in the absence of apparent clinical disease (HESKETH et al., 2007).

So it was repeatedly pointed out that elevated tissue Mn could be a diagnostic marker for prion disease-infection. However, also another interesting findings was that although levels of Mn were elevated, there were differences in the blood levels of selenium (Se) in experimental and field case of BSE in cows. This suggests that the way a cow acquires the disease affects the metabolic processes involved the origin of the inreased Mn in the brains and blood of infected animals remains unknown. The three possibilities are that there is decreased secretion of Mn from the body, release of Mn from other tissues or increased absotption of Mn from the environment.

However, there could be a fourth possibility; increased the Mn absorption and retention can be found in the Mg deficiency. Mn^{2+} is very similar to Mg^{2+} in terms of its chemical properties, including inner and outer shell complexation.

B. Several possible points of interaction between manganese (Mn) and magnesium (Mg); Mg deficiency increases Mn absorption and retention in tissues

In biological systems, only Mn^{2+} is readily capable of replacing Mg^{2+}, and only in a limited set of circumstances. The body can replace Mn with Mg with similar efficiency in Mn-activated proteins (WAPNIR, 1990). Similarly, Mn can occupy Mg allosteric sites in Mg-activated proteins, such as the sarcoplasmic reticulum Ca- ATPase (CHIESI and INESI, 1981). Mn^{2+} effectively binds ATP and allows hydrolysis of the energy molecule by most ATPases. Mn^{2+} can also replace Mg^{2+} as the activating ion for a number of Mg^{2+}-dependent enzymes, although some enzyme activity is usually lost. Sometimes such enzyme metal preferences vary among closely related species: for example is that the reverse transcriptase enzyme of lentiviruses like HIV, SIV and FIV is typically dependent on Mg^{2+}, whereas the analogous enzyme for other retroviruses prefers Mn^{2+} (COWAN, 1995).

Investigated the relationship between Mg deficiency and Mn metabolism, YASUI et al (1995) found that Mn content in central nervous system tissues and visceral organs was highest in rats fed a low Ca-Mg diet. SANCHEZ- MORITO et al. (1999) found that feeding rats a diet deficient in Mg decreased urinary and fecal Mn excretion. They also observed greater Mn retention in skeletal muscle, heart and kidney in Mg-deficient rats as compared to control. They reported that the lack of response by the liver to increased Mn absorption may

have led to the redistribution of this ion to other tissues. So Mg- deficiency increased Mn absorbed, and this favored the deposition of Mn in all tissues studied; except the liver and trabecular bone. Their findings differ from those of KIMURA et al. (1996), who found that an Mg deficit generally led to depletion of tissue Mn. This discrepancy may be related with the shorter duration of the experiments run by KIMURA et al. (14 days), since, under experimental conditions of SANCHEZ- MORITO et al; increased Mn concentrations were first detected after 35 days of feeding with the Mg-deficient diet. SANCHEZ- MORITO et al.(1999) concluded that under experimental conditions, Mg deficiency increased Mn absorption, which was reflected as increased Mn deposits in all soft and hard tissues studied except the sternum and liver. Their finding that Mn did not accumulate in the liver (the key organ in Mn metabolism) is probably related with the formation of Fe deposits in the liver as a result of Mg.

Mn uptake and toxicity in Saccharomyces cerevisiae are strongly influenced by intracellular Mg, possibly through the Mg-dependent regulation of divalent-cation transport activity (BLACKWELL et al., 1997). The Mg content of Saccharomyces cerevisiae was found to vary by up to fivefold at differing stages of batch growth and during growth in the presence of differing Mg concentrations. Excess Mg was primarily sequestered in vacuoles. Mn^{2+}-uptake experiments revealed that Mg-enriched cells had a markedly reduced capacity for Mn^{2+} accumulation. Researchers concluded that Mn^{2+} uptake and toxicity in S. cerevisiae are strongly influenced by intracellular Mg, possibly through Mg-dependent regulation of divalent-cation transport activity.

In humans, the link between hypomagnesemia in the heart and the increased risk of IHD as well as coronary vasospasm, cardiac dysrhythmia and altered cellular respiration has been well-documented (ALTURA and ALTURA,1985; RASMUSSEN, 1993; SEELIG,1989). If high dietary Mn displaces Mg from the heart, then people suffering from depressed Mg status could be at greater risk for Mn toxicity. In conclusion, these data suggest that Mn exacerbates Mg deficiency and is responsible for a decrease in heart muscle Mg concentrations. This reduction of Mg concentrations in the heart may therefore be a contributing factor in the deaths observed in pigs fed high Mn. These data suggest high dietary Mn may exacerbate Mg deficiency in heart muscle and thus may be a complicating factor in the deaths observed in Mg-deficient pigs.

Other cause- example about Mn deposits in tissues is liver disease. People with chronic liver disease have neurological pathology and behavioral signs of Mn neurotoxicity, probably because elimination of Mn in bile is impaired (BUTTERWORTH et al., 1995; HAUSER et al., 1994; SPAHR et al., 1996). This impairment results in higher circulating concentrations of Mn, which then has access to the brain via transferrin. HAUSER and coworkers (1994) reported whole blood Mn concentrations of 18.8 to 45 μg/L in three patients with chronic liver disease, as compared to a normal range of 4.2 to 14.3 μg/L. SPAHR and coworkers (1996) reported blood Mn concentrations of 124.7 nmol/L (6.85 μg/L) in control subjects versus 331.4 nmol/L (18.2 μg/L) in patients with cirrhosis. High concentrations of circulating Mn as a result of total parenteral nutrition have also been associated with Mn toxicity (KEEN et al., 1999). DAVIS and GREGOR (1992) reported that women who ingested 15 mg/day of supplemental Mn had serum Mn concentrations that increased gradually throughout the 125-day study; significant differences were reported after 25 days of supplementation.

On the other hand Mn-supplemented diets increased Mg excretion through the urine in rats (GAILLARD et al., 1996), SCHEUHAMMER and CHERIAN (1983) reported that Mn decreases Mg concentrations in both heart and bone by an undefined mechanism. Interactions between Mg and Mn have also been reported in the digestive tract. The addition of high concentrations of Mg to the water or food reduced the absorption in rats of Mn given orally (Van BARNEVELD and Van Den HAMER, 1984).

In addition, chronic Mg deficiency decreases selenium (Se) absorption and retention and erythrocyte concentrations of this mineral, and increases Se in plasma, kidney and heart (JIMENEZ et al., 1997).

C. Toxicity about "ingested manganese" is low compared with the "inhaled manganese"

In ruminants, Mn absorption is about l to 4 %, regardless of dietary Mn concentration. The Mn in typical animal feeds can range from l0 ug/g in corn to l50 ug/g in ryegrass and red clover, with the concentration being highly dependent on soil conditions and fertilizer practice. Although Mn can produce toxic effects, it is often considered to be among the least toxic of the essential trace elements to birds and mammals. For example, chicks, calves, pigs, and sheep have been reported to tolerate diets up to 3.000; 1.000; 500; and 200 ug Mn/g, respectively (HURLEY and KEEN,l987). Biliary Mn excretion is the principal means of Mn homeostasis. Analyzed Mn contents of various silages and forages, including alfalfa, were lower (HIDIROGLOU, l979) than concentrations indicated in tables of feed composition and were below the requirement(40 ppm) of dairy cattle (NRC,l989). In domestic animals the major reported biochemical lesion associated with dietary Mn toxicosis is an induction of Fe deficiency which is thought to be result of an inhibitory effect of Mn on iron absorption (HO et al. l984).

The Mn toxicity may result in multiple neurologic problems and is a well-recognized health hazard for people who inhale Mn dust. Exposure to Mn dusts and fumes should not exceed the ceiling value of 5 mg/m3 for even short periods because of its toxicity level. A form of Parkinson Disease-type neurodegeneration called "manganism" has been linked to Mn exposure since the early 19th Century (KEEN et al., 1999). Unlike ingested Mn, inhaled Mn is transported directly to the brain before it can be metabolized in the liver (DAVIS, 1999). The symptoms of Mn toxicity generally appear slowly over a period of months to years. In its worst form, Mn toxicity can result in a permanent neurological disorder with symptoms similar to those of Parkinson´s disease, including tremors, difficulty walking, and facial muscle spasms.

II. Shizophrenia as a hypofunction of NMDA receptors; two effects of cannabiods on the NMDA hypofunction

NMDA receptor hypofunction might be important in schizophrenia

The NMDA receptor hypofunction (NRH) hypothesis was initially proposed in 1980, by researchers who had found significantly low levels of the neurotransmitter glutamate (Glu) in cerebrospinal fluid. It wasn't until the later 1980's, however, when the results of several studies showed the ability of psychotomimetic agents phencyclidine (PCP) to block NMDA receptors, that the potential association of NRH to schizophrenia was realized. The idea of a glutamatergic abnormality in schizophrenia was first proposed by Kim, Kornhauber, and colleagues in 1980 (KIM et al., 1980) based on their findings of low cerebrospinal fluid (CSF) glutamate levels in patients with schizophrenia.

In a symposium entitled, "Not Just Dopamine Any More: Emerging Glutamatergic Therapies for Schizophrenia," (2006) professor Joseph Coyle from Harvard Medical School,

Cambridge, Massachusetts, and Editor of the Archives of General Psychiatry, described molecular mechanisms that had recently been identified as being of interest in schizophrenia

It was proposed that the NMDA receptors on the GABAergic interneurons of brain areas affected by schizophrenia are hypofunctional. The results are disinhibition that impairs cortical-hippocampal processing and disruption of the excitatory output sufficient to trigger subcortical dopamine release that leads to psychosis. "Enhancing glycine modulatory site occupancy by agonists is a plausible treatment for schizophrenia, especially the negative and cognitive symptoms," Dr. Coyle maintained. "This strategy … may be more powerful than just the symptomatic treatment that we have been using. By enhancing NMDA receptor function in conjunction with rehabilitation, we may be able not only to deal with symptoms but perhaps to reverse the cognitive and social deficits that are the most disabling aspects of this disorder" (BEGANY, 2006).

Recently, evidence is accumulating that the exclusive dopamine hypothesis of schizophrenia has to be abandoned. Instead, a more integrative approach combines different neurotransmitter systems, in which glutamatergic, GABAergic and dopaminergic pathways interact. This paradigm shift coincides with the recognition that, while typical and modern atypical antipsychotic drugs have efficiently controlled the dramatic psychotic symptoms of schizophrenia, their impact on negative and cognitive symptoms is negligible. Indeed, cognitive decline is now believed to represent the core of schizophrenic morbidity and in this context, impairment of glutamate and more specifically NMDA function is of major importance. Given that astrocytes are important in controlling glutamate homeostasis, it is necessary to assign a significant role to glial-neuronal interactions in the pathophysiology of schizophrenia. Indeed, recent data from several animal and human studies corroborate this notion (KONDZIELLA et al., 2007).

The NMDA receptor antagonist psychotomimetic agents phencyclidine (PCP) has been shown to induce the positive, negative and cognitive symptoms of schizophrenia in healthy patients and cause a resurgence of symptoms in stable patients. These observations led to the NMDA receptor hypofunction hypothesis as an alternative theory for the underlying cause of schizophrenia. According to this hypothesis, any agent that can potentiate NMDA receptor currents has the potential to ameliorate the symptoms of schizophrenia. To date, NMDA receptor currents can be modulated by either direct action on modulatory sites on the NMDA receptor (i.e., the glycine co-agonist binding site) or indirectly by activation of G-protein coupled receptors (GPCRs) known to potentiate NMDA receptor function (i.e., mGluR5). This review will discuss the NMDA receptor hypofunction hypothesis, the NMDA receptor as an emerging target for the development of novel antipsychotic agents and progress towards in vivo target validation with GlyT1 inhibitors and mGluR5 positive allosteric modulators. Other potential targets for modulating NMDA receptor currents (polyamine sites, muscarinic receptors, etc...) will also be addressed briefly (LINDSLEY et al., 2006).

Accumulating evidence from both genetic and clinico-pharmacological studies suggests that D-serine, an endogenous coagonist to the NMDA subtype glutamate receptor, may be implicated in schizophrenia (SZ). Although an association of genes for D-serine degradation, such as D-amino acid oxidase and G72, has been reported, a role for D-serine in SZ has been unclear. In the study (FUJI et al. (2006) identify and characterize protein interacting with C-kinase (PICK1) as a protein interactor of the D-serine synthesizing enzyme, serine racemase (SR). The binding of endogenous PICK1 and SR requires the PDZ domain of PICK1. The gene coding for PICK1 is located at chromosome 22q13, a region frequently linked to SZ. In a case–control association study using well-characterized Japanese subjects, FUJI et al. (2006) observe an association of the PICK1 gene with SZ, which is more prominent in disorganized SZ. Their findings implicating PICK1 as a susceptibility gene for schizophrenia are consistent with a role for D-serine in the disease.

Schizophrenia is characterized by disturbances in sensorimotor gating and attentional processes, which can be measured by prepulse inhibition (PPI) and latent inhibition (LI), respectively. Research has implicated dysfunction of neurotransmission at the NMDA-type glutamate receptor in this disorder. LIPINA et al (2005) examined whether compounds that enhance NMDA receptor (NMDAR) activity via glycine B site, D-serine and ALX 5407 (glycine transporter type 1 inhibitor), alter PPI and LI in the presence or absence of an NMDAR antagonist, MK-801. Authors concluded; D-Serine and ALX 5407 display similar effects to clozapine in PPI and LI mouse models, suggesting potential neuroleptic action. Moreover, the finding that agonists of NMDARs and clozapine can restore disrupted LI and disrupt persistent LI may point to a unique ability of the NMDA system to regulate negative and positive symptoms of schizophrenia (LIPINA et al., 2005).

The hypothesis that NMDA receptor hypofunction might be important in schizophrenia was not given much credence until investigators performed experiments in which healthy volunteers received subanesthetic infusions of the dissociative anesthetic ketamine, a known NMDA receptor antagonist. The infusions reproduced the positive and negative symptoms of schizophrenia, including paranoia, thought disorder, loose associations, illusions, emotional withdrawal, and psychomotor retardation.

NMDA receptor antagonists may play a role in schizophrenia

Evidence from histological and pharmacological challenge studies indicates that NMDA receptor hypofunction may play an important role in the pathophysiology of schizophrenia. It has long been known that treatment with NMDA receptor antagonists produces psychosis and cognitive deficits that are reminiscent of the clinical picture of schizophrenia (JAVITT and ZUKIN, 1991; KRYSTAL et al., 1994). Antagonists of the NMDA receptor have provided an important mechanism for evaluating the pathophysiology of neuropsychiatric disorders, such as Alzheimer's disease, anxiety, depression, and schizophrenia. For example, administration of the NMDA antagonist ketamine to healthy subjects have led to further understanding of the pathophysiology of schizophrenia (ADLER et al., 1999).

NMDA receptor antagonists are induce a state of called "dissociative anesthesia", which is marked by catalepsy, amnesia, and analgesia (PENDER, 1971). Ketamine and other NMDA receptor antagonists are most frequently used in conjunction with diazepam as anesthesia in cosmetic or reconstructive plastic surgery (ERSEK, 2004), and in the treatment of burn victims (CEBER and SALIHOGLU, 2006). Ketamine is a favored anesthetic for emergency patients with unknown medical history because it depresses breathing less than other anesthetics (HESHMATI et al., 2003). The NMDA receptor antagonist dextromethorphan is one of the most commonly used cough supresants in the world (EQUINOZZI and ROBUSCHI, 2006). NMDA receptor antagonists sometimes induce "psychomimetic" side effects, symptoms resembling psychosis. Such side effects caused by NMDA receptor inhibitors include hallucinations, paranoid delusions, confusion, difficulty concentrating, agitation, alterations in mood, nightmares (MUIR and LEES, 1995), catatonia (AARTS and TYMIANSKI, 2003), ataxia (KIM et al., 2002), anaesthesia (KRISTENSEN et al., 1992) and learning and memory deficits (ROCKSTROH et al., 1996). Because of these psychotomimetic effects, NMDA receptor antagonists, especially phencyclidine, ketamine, and dextromethorphan, are used as recreational drugs. At subanesthetic doses, these drugs have mild stimulant effects, and at higher doses, begin induce dissociation and hallucinations (LIM, 2003). Several drugs have been found that lessen the risk of neurotoxicity from NMDA receptor antagonists, such as anticholinergics , diazepam, barbiturates (OLNEY et al., 1991).

Antagonists of the NMDA subtype of glutamate receptor are of considerable interest for various neurotherapeutic purposes, including preventing neuronal degeneration in stroke and CNS trauma, suppressing neuropathic pain and preventing the development of tolerance to opiate analgesics. Unfortunately, NMDA antagonists can cause potentially serious side

effects, including acute neurodegenerative changes in corticolimbic regions of the adult rat brain and psychotic reactions in adult humans. Recreational use or investigator administration of a single low dose of an NMDA receptor antagonist such as phencyclidine (PCP) or ketamine and the powerful NMDA antagonist, MK-801 - produces "schizophrenialike" symptoms in healthy individuals and profoundly exacerbates preexisting symptoms in patients with schizophrenia (see following works);

a/ It was found (JAVITT and ZUKIN, 1991) that PCP-induced psychotomimetic effects are associated with submicromolar serum concentrations of PCP. At these concentrations PCP interacts selectively with a specific binding site (PCP receptor) that is associated with the NMDA-type excitatory amino acid receptor. They concluded that endogenous dysfunction of NMDA receptor-mediated neurotransmission might contribute to the pathogenesis of schizophrenia. The relative implications of the PCP and amphetamine models of schizophrenia are discussed in relationship to the diagnosis and etiology of schizophrenia (JAVITT and ZUKIN, 1991).

b/ Ketamine, a phencyclidine hydrochloride derivative, is a dissociative anesthetic and a noncompetitive antagonist of the NMDA subtype of excitatory amino acid receptor. KRYSTAL et al (1994) found that ketamine - NMDA antagonist produce a broad range of symptoms, behaviors, and cognitive deficits that resemble aspects of endogenous psychoses, particularly schizophrenia and dissociative states (KRYSTAL et al., 1994).

c/. FARBER et al. (1995) examined the sensitivity of rats at various ages to the neurotoxic effects of the powerful NMDA antagonist, MK-801. Vulnerability was found to be age dependent, having onset at approximately puberty (45 days of age) and becoming maximal in early adulthood. This age-dependency profile (onset of susceptibility in late adolescence) in the rat is similar to that for ketamine-induced psychosis or schizophrenia in humans. Susceptibility to the psychotomimetic effects of the ketamine is minimal or absent in children and becomes maximal in early adulthood. These findings suggest that NMDA receptor hypofunction, the mechanism underlying the neurotoxic and psychotomimetic actions of NMDA antagonists, may also play a role in schizophrenia (FARBER et al., 1995).

d/ Phencyclidine (PCP) and ketamine, induce schizophrenia-like symptoms in healthy individuals and worsen some symptoms in schizophrenia (HIRAYASU et al., 2001; HAZLETT et al., 1999).

e/ In addition, ethanol (alcohol) is also an antagonist of the NMDA glutamate receptor. The capacity of ethanol to block N-methyl-D-aspartate (NMDA) glutamate receptors is among its most potent actions in the brain (KRYSTAL et al., 2003). As a result, ethanol and more selective NMDA receptor antagonists share many physiologic, behavioral, and reward-related effects (CREWS et al., 1996; GRANT and LOVINGER, 1995). Ethanol is an antagonist of the NMDA glutamate receptor, and alterations in NMDA receptor function are thought to be involved in ethanol abuse and dependence (PETRAKIS et al., 2004)..

Cannabinoids are known to inhibit calcium channels- glutamate release in schizophrenia

There can be another example about the NMDA antagonism and hypoglutamatergic condition; cannabinoids are known to inhibit Ca^{2+} channels- glutamate release in schizophrenia. Cannabinoids are a group of terpenophenolic compounds present in Cannabis sativa L. Natural cannabinoids are only known to occur naturally in the cannabis plant. The chemical tetrahydrocannabinol (THC) found in marijuana is a cannabinoid, though different from the endogenous cannabinoids naturally produced in the bodies of animals. The broader definition of cannabinoids refer to a group of substances that are structurally related to THC or that bind to cannabinoid receptors. THC is the primary psychoactive component of the plant ,medically, it appears to ease moderate pain and to be neuroprotective.

Endocannabinoids serve as intercellular 'lipid messengers', signaling molecules that are released from one cell and activate the cannabinoid receptors present on other nearby cells. So cannabinoids are naturally occurring compounds in vertebrates, and are known to play an important role in intercellular signaling. In 1992, the first such compound was identified as arachidonoyl ethanolamide and named anandamide. It has a pharmacology similar to THC, although its chemical structure is different.

THC mediates the majority of its activities through stimulation of cannabinoid receptors (CB). Two cannabinoid receptors, CB1 and CB2, were discovered only the last ten years. CB1 exists primarily in the central nervous system, while CB2 is found primarily in the peripheral nervous system. Also, endogenous anandamide binds to both the central (CB1) and peripheral (CB2) cannabinoid receptors, and is found in nearly all tissues in a wide range of animals, it is about as potent as THC. Activation of CB1 receptor inhibits neurotransmitter release in many brain regions. CB1 receptors are essentially absent in the medulla oblongata, the part of the brain stem that is responsible for respiratory and cardiovascular functions. Thus, there is not a risk of respiratory or cardiovascular failure as there is with many other drugs. CB1 receptors appear to be responsible for the euphoric and anticonvulsive effects of cannabis.

Activation of CB1 receptor (CB1R) inhibits neurotransmitter release in many brain regions but the mechanism of action is debated (ALGER, 2002). In expression systems and cell bodies, CB1R couples to activation of K^+ channels or inhibition of neuronal Ca^{2+} channels, or both (HOWLETT et al., 2002). Either of these mechanisms can reduce Ca^{2+} influx at nerve terminals and thereby inhibit transmitter release. Activation of K^+ channels may change the presynaptic action potential (AP) and thus indirectly modulate Ca^{2+} channel activity (DIANA and MARTY, 2003). Alternatively, CB1R activation may directly inhibit presynaptic Ca^{2+} channels coupled to exocytosis. Investigations of CB1R action in nerve terminals have relied on measurements of cytosolic Ca^{2+} concentration with indicator dyes (KREITZER and REGEHR, 2001) and occlusion experiments using specific Ca^{2+} channel blockers (SULLIVAN, 1999; ROBBE et al., 2001; WILSON and NICOLL, 2001); however, direct recordings of CB1R-dependent modulation of presynaptic Ca^{2+} currents or APs are still missing. There is evidence that cannabinoids can regulate glutamate release, oxidant free radicals and Ca influxes (TWITCHELL et al., 1997; HAMPSON et al., 1998; KREITZER and REGEHR, 2001; HOWLETT et al., 2002), which, in excess, can cause neuronal death. Cannabinoids can tonically regulate NMDA glutamate receptor activity in vitro and support the in vivo observation that CB_1 regulates NMDA-induced and ischaemic excitotoxicity (NAGAYAMA et al., 1999; PARMENTIER-BATTEUR et al., 2002).

Communication between the cells requires the release of a glutamate neurotransmitter, triggered by Ca currents passing through a specific Ca channel. Cannabinoids are known to inhibit Ca channels. If we shut down the channel, we shut down the release of glutamate, and profoundly alter the cell's ability to signal.

The mechanism of endocannabinoid synaptic transmission is understood by the following events: an excitatory transmission of the neurotransmitter glutamate causes an influx of Ca ions into the post-synaptic neuron. Through a mechanism not yet fully understood, the presence of Ca post-synaptically induces the production of endocannabinoids in the post synaptic neuron. In standard neurotransmission, the pre-synaptic neuron releases neurotransmitter into the synaptic cleft which binds to cognate receptors expressed on the post-synaptic neuron. Upon binding, the neuron depolarizes. This depolarization facilitates in the influx of Ca into the neuron; this increase in Ca activates an enzyme called [[transacylase] which catalyzes the first step of endocannabinoid biosynthesis. Release of endocannabinoids may occur in response to elevations in intracellular Ca^{2+}, for example during prolonged depolarizations (ALGER, 2002) or during flash photolysis of caged Ca^{2+} (BRENOWITZ and REGEHR, 2003). Endocannabinoid release during mGluR1 activation by mGluRs selective

agonist (S)-3,5-dihydroxyphenylglycine (DHPG)), however, may also be Ca^{2+} independent in the cerebellum (MAEJIMA et al., 2001) and hippocampus (CHEVALEYERE and CASTILLO, 2003).

KUSHMERICK et al. (2004) investigated the mechanisms by which activation of group I metabotropic glutamate receptors (mGluRs) and CB1 cannabinoid receptors (CB1Rs) leads to inhibition of synaptic currents at the calyx of Held synapse in the medial nucleus of the trapezoid body (MNTB) of the rat auditory brainstem. Their data suggest that activation of postsynaptic mGluRs triggers the Ca^{2+}-dependent release of endocannabinoids that activate CB1 receptors on the calyx terminal, which leads to a reduction of presynaptic Ca^{2+} current and glutamate release. This observation may suggest a very close coupling between the site of internal Ca^{2+} release (or Ca^{2+} influx) triggered by group I mGluR activation and a Ca^{2+}-dependent step in endocannabinoid synthesis and release (KUSHMERICK et al., 2004).

Cannabinoids may protect neurons against neurodegenerative process

There is accumulating evidence in vitro and in vivo to support the hypothesis that the cannabinoid system can limit the neurodegenerative possesses that drive progressive disease, and may provide a new avenue for disease control (JACKSON et al., 2005). One important aspect of cannabiod (CBD)-based treatment of neurodegenerative process can be related to the NMDA receptor antagonism properties of CBD (GRUNDY et al., 2001).

The French researchers (DIRIKOC et al., 2007) recently noted that cannabinoid (CBD) may be a promising agent for the treatment of prion diseases, reported that the non-psychoactive cannabis constituent CBD inhibited the accumulation of prion proteins in both mouse and sheep prion-infected cells, whereas other cannabinoids were either weak or not effective. Moreover, after infection with mouse scrapie, a prion disease, CBD limited accumulation of the prion protein in the brain and significantly increased the survival time of infected mice. CBD inhibited the nerve damaging effects of prions in a concentration-dependent manner. They concluded; results suggest that CBD may protect neurons against the multiple molecular and cellular factors involved in the different steps of the neurodegenerative process, which takes place during prion infection. When combined with its ability to target the brain and its lack of toxic side effects, CBD may represent a promising new anti-prion drug (DIRIKOC et al., 2007).

Moreover, cannabinoids are neuroprotectant in a wide variety of in vitro and in vivo models of neuronal injury including neurodegenerative disorders (LASTRES-BECKER et al., 2005). These effects have been ascribed, among others, to antioxidant properties, NMDA antagonism, decrease in glutamate release, and blockade of microglia migration and activation (MECHOLAM et al., 2002). Like memantine, cannabinoids are also capable of increasing brain-derived neurotrophic factor to confer protection against excitotoxicity (KHASPEKOV et al., 2004).

In non-neuronal cells, the induction of nerve growth factor is also facilitated by cannabinoids, acting through the PI3K/PKB pathway (SANCHES et al., 2003), and activation of the CB_1 receptor by the endocannabinoid, 2-arachidonoyl glycerol, can also couple to an axonal growth response, whereas CB_1 receptor antagonists inhibit axonal growth (WILLIAMS et al., 2003). Thus, dampening excessive glutamatergic transmission and excitotoxicity, coupled with neurotrophic actions, may represent interesting actions of cannabinoids that could be exploited for the treatment of Alzheimer´s disease (AD).

There are alternative mechanisms that are pivotal to cannabinoid-mediated protection include inhibition of $[Ca^{2+}]_i$ by reducing Ca release from ryanodine-sensitive stores (ZHUANG et al., 2005), inhibition of protein kinase A and reduced nitric oxide generation (KIM et al., 2006).

Manipulation of the cannabinoid system has several consequences that mirror those observed with memantine. Thus, the protective effects of some cannabinoids are related to the direct regulation of the NMDA receptor, since the non-psychotropic cannabinoid, HU-211, acts as a stereoselective inhibitor of the NMDA receptor and protects rat forebrain cultures (NADLER et al., 1993) and cortical neuronal cultures (ESHHAR et al., 1993) from NMDA-induced neurotoxicity.

Cannabinoids have schizophreniform and neuroprotective effect on NMDA hypofunction

The goal of this "cannabis-review" it was to show that cannabis use can be a cause of schizophrenia; characterize two effects on NMDA hypofunction, related to schizophrenia-associated neurodegenerative impairment;

Cannabinoids activity about NMDA glutamate receptor hypofunction; as a schizophreniform effect;

In expression systems and cell bodies, CB1 receptor couples to activation of K^+ channels or inhibition of neuronal Ca^{2+} channels, or both. Either of these mechanisms can reduce Ca^{2+} influx at nerve terminals and thereby inhibit transmitter release. Activation of K^+ channels may change the presynaptic action potential and thus indirectly modulate Ca^{2+} channel activity. Communication between the cells requires the release of a glutamate neurotransmitter, triggered by Ca currents passing through a specific Ca^{2+} channel. Cannabinoids are known to inhibit Ca^{2+} channels. If we shut down the channel, we shut down the release of glutamate, and profoundly alter the cell's ability to signal.

Cannabinoids can regulate NMDA glutamate receptor by reducing intracellular Ca^{2+} release; as a neuroprotective effect;

There is evidence that cannabinoids can regulate glutamate release, oxidant free radicals and Ca influxes, which, in excess, can cause neuronal death. Cannabinoids can tonically regulate NMDA glutamate receptor activity in vitro and support the in vivo observation that CB_1 regulates NMDA-induced and ischaemic excitotoxicity. Exogenously administered cannabinoids are neuroprotective in several different cellular and animal models. Cannabinoids produce neuroprotection by reducing intracellular Ca^{2+} release. Emerging evidence indicates that cannabinoids may play a role in slowing the progression of certain neurodegenerative diseases.

Calcium deficiency may be a prediposing or causative factor in schizophrenia

Recently, evidence is accumulating that the exclusive dopamine hypothesis of schizophrenia has to be abandoned because significant additional evidence has accumulated supporting a role for NMDA hypofunction in the pathophysiology of schizophrenia. However, treatment studies with NMDA modulators, such as glycine, D-serine, and glycine transport inhibitors (GTIs), have yielded encouraging findings, although results remain controversial. Why? Because- perhaps, NMDA receptors might have a lower affinity for glycine, explaining why administration of exogenous glycine-agonists results in a favorable clinical response in schizophrenia. Additionally, one could imagine that these receptors might be less sensitive to glutamate, and, perhaps, more sensitive to Mg2+ block. So NMDA receptors may differ in their sensitivity to voltage-dependent Mg2+ block, agonists, and antagonists as a function of their subunit composition.

Thus, functional diversity of NMDA receptors may be expected from the assembly of different subunit combinations, and there is very important "Ca2+-dependent manner" which permits activation of NMDA receptors … So dietary Ca- deficiency can be important about

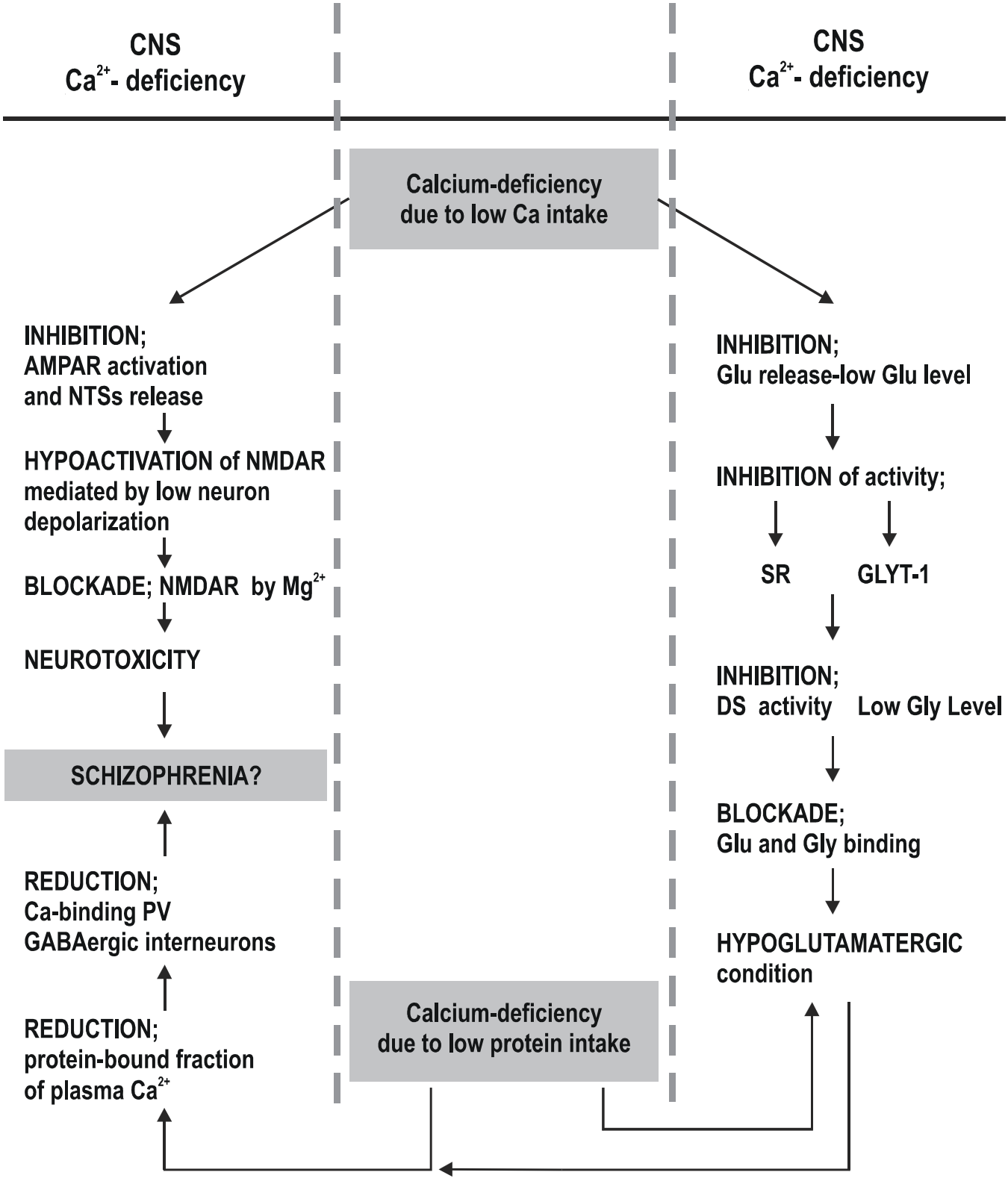
"NMDA hypofunction" in schizophrenia… Calcium in cells is tightly regulated and mostly unrelated to necessary dietary Ca. However, a low content of Ca in the ration decreases the Mg requirements of the animal. The lower the Ca level in the animal- human ration (and in the tissue cells); the more marked is "NMDA receptors blocking effect of Mg2+ "; both intracellularly and extracellularly. Maintenance of the body Ca stores depends mainly on dietary Ca intake, and on absorption of Ca from the gastrointestinal (GI) tract. For example, the majority of Americans do not get enough Ca in their diet- nearly 75 percent of women and 50 percent of men according to the United States Department of Agriculture (USDA). And only 14 percent of teen girls and 35 percent of teen boys are meeting the recommended dietary allowance. This deficit is a crippling statistic considering how critical Ca is to the body's infrastructure. But now with the innovation of a double-Ca fluid milk, every cookie dunk, spoonful of cereal and breakfast smoothie can provide twice the nutrient that can help reduce the risk of osteoporosis, keep teeth strong, battle high blood pressure and may even aid weight loss as part of a reduced calorie diet (Medical News Today, 2004). In addition, diets moderate in protein (in the approximate range of 1.0 to 1.5 g protein/kg) are associated with normal Ca metabolism At low protein intakes, intestinal Ca absorption is reduced, resulting in increases in serum PTH; as induced secondary hyperparathyroidism (KERSTETTER et al., 1998; 2003).

Hypoproteinemia is associated with a decrease in total Ca, hypoproteinemia can reduce the protein-bound fraction of plasma Ca. Calcium is an important component of a healthy diet. Calcium supplements are used to prevent and to treat Ca deficiencies. There are conflicting recommendations about when to take Ca- supplements. However, most experts agree that no more than 500 mg should be taken at a time because the percent of Ca absorbed decreases as the amount of Ca in the supplement increases. It is recommended to spread doses throughout the day, with the last dose near bedtime. Recommended daily Ca intake varies from 1000 to 1500 mg, depending upon the stage of life. Calcium plays a vital role in the physiology and biochemistry of organisms and of the cell, particularly in signal transduction pathways. The amount of total Ca varies with the level of serum albumin, a protein to which Ca is bound. The biologic effect of Ca is determined by the amount of ionized Ca, rather than the total Ca.

The skeleton acts as a major mineral storage site for the element and releases Ca2+ ions into the bloodstream under controlled conditions. In mammals, levels of intracellular Ca are regulated by transport proteins that remove it from the cell. Ca2+ entering the cell plasma causes the specific action of the cell, whatever this action is: secretory cells release vesicles with their secretion, muscle cells contract, synapses release synaptic vesicles and go into processes of synaptic plasticity, etc. Ca2+ ions are one of the most widespread second messengers used in signal transduction. They make their entrance into the cytoplasm either from outside the cell through the cell membrane via Ca channels (such as Ca- binding proteins), or from some internal Ca storages.

So Ca metabolism or Ca homeostasis is the mechanism by which the body maintains adequate Ca levels. However, derangements of this mechanism can lead to "Ca- deficiency", which can have important consequences in health of "schizophrenic individuals". This concept is based on the demonstration that "NMDA receptor hypofunction" can be based on Ca-deficiency, potentiated by nutritional hypoproteinemia (Fig.2):

Fig.2 Calcium deficiency may be a predisposing or causative factor in schizophrenia



CNS (Central nervous system) • NMDAR (N-Methyl-D-Aspartate receptor) • AMPAR (Amino-3-hydroxy -5-methylisoxazole-4-propionic acid receptor) • NTSs (Neurotransmitters) • Glu (Glutamate) • Gly (Glycine) GLYT-1 (Glycine transporter type 1) • DS (D-serine) • SR (Serine racemase enzyme) • PV (Parvalbumin)

Questions about the Czech alternative „BSE ammonia-magnesium theory“:

The World Veterinary Congress is one of the premier events of the Veterinary Profession worldwide and it was expected to draw 2,000+ International attendees to Vancouver. An interview room for Media was available at the Congress. Media contacted presenters during the Congress to arrange for individual interviews. There is one example about journalist´s 15 questions, concerning the Czech alternative „BSE ammonia- magnesium theory“:

1.) Have you personally dealt with any mad cow disease cases in your country, or in other countries in Europe?

Response;
Yes, I described in details (about nutrition and biochemical interpretation); one case of BSE in Czech Republic. There I found the confirmation about my „ammonia- magnesium theory“. However, there I was as the „BSE disident“ and as a private veterinarian; because in Czech Republic only the infectious „scientific“ BSE theory is accepted (by government and media). I described this example in the „Journal of Czech Veterinary Surgeons“, only there I had/have a „great support“.
HLÁSNÝ.J.: Long-term dietary protein surpluss in dairy ration and the BSE- II. part. Journal of Czech Veterinary Surgeons; Brno, 2004(8); 11-13

3.) Given the situations that occurred in England and Germany and subsequent news reports, are there any citizens who are still fearful of mad cow disease?
Response;
About Czech Republic there is still the „BSE-fear“, see the situation about the BSE testing;
First occurrence of BSE was reported in the Czech Republic during the year 2001 (two cases, June and August). Since this time acomprehensive national surveillance programme has been adjusted and applied. There are three laboratories available to perform BSE examinations in accordance with provisions of the EC Regulation No. 999/2001 of the European Parliament and of the Council of 22 May 2001. In accordance with provisions issued in this programme, the following categories of cattle are subject to compulsory BSE testing (from June 2001 to date):
-all bovines older than 30 months slaughtered regularly,
- all cattle older than 24 months emergency slaughtered,
- all animals older than 20 months clinically suspected of BSE,
- fallen stock older that 24 months selectedonrandombasis.

And concerning citizens who are still fearful of mad cow diseaese? In Czech Republic is still a low „beef consumption“ (an example about the „beef fear“; see the low price of „beef liver“ compared to „pig liver“ …).

2.) How did you get involved in studying this phenomenon?

4.) ***Tell me about this Mg-deficient alternative you mention numerous times in your comments. How did you come to this conclusion when it appears nobody else had even mentioned it, at least not in the United States or Canada?***

Responses;

At first; in the library of West Virginia University I found (in „older“ Australian Veterinary Journal);

In cases of protracted ryegrass staggers of sheep and cattle, MASON (1968) described cerebellar lesions involving Purkinje cell axons. These lesions consist of eosinophilic homogenous swellings in the cerebellar granular layer , generally located in groups rather than randomly distributed and with a tendency to be more numerous adjacent to the Purkinje cell layer. In general axons with torpedoes were myelinated at least over part of their traceable length, often on both sides of the swelling. Total encasement with myelin was often demonstrable about small swellings, whereas larger ones sometimes had only vestiges remaining. In one early developing lesion axonal rupture had occurred with with retraction. The myelin sheat, hovewer, remained intact both about the bulb and the empty retraction space. The myelin sheats would appear to remain relatively unaffected about degenerating axons. Vacuoles were observed in some torpedoes. It is believed that torpedo development as a reaction of Purkinje cells to simple atrophy, to be caused by disturbances of neurone metabolism. **It appears that the longer the disturbing syndromes has been present, the greater the likelihood of finding these axonal changes, in protracted ryegrass staggers in sheep and cattle.** Therefore the lesions described are not regarded as pathognomonic of protracted ryegrass staggers but probably arise from a number of factors, which may include disturbed neuronal metabolism, neuronal exhaustion and repeated anoxic insults (MASON, 1968).

At second; in Research Institute of Rapotin I found;

The **report from MOORBY et al.(2000) described a nutritional experiment** in dairy cows, as a different cause of BSE. During the last six weeks of the dry period they were offered one of three grass silage (first cut perennial ryegrass)- based diets, offered ad libitum. After calving, all the animals received the same lactation diet consisting of ad libitum access to ryegrass silage. After the 21 weeks of lactation, **six from the 47 animals developed clinical signs of BSE**, which was later confirmed by histopathological examination. In addition the BSE developed after 27 weeks of ryegrass feeding- **without meat and bone meal (MBM) in the experimental diet**; unfortunately, without the Mg status of cows testing.

Therefore, the feeding of MBM to cattle per se may not be the main cause of BSE. When this article about „ryegrass toxicity“ on the CNS of cows was published ; **it was prompted to air my views in studies of CNS neurotoxities by nutritional causes** (see the literature sources obtained in West Virginia University and from „Magnesium Research“- from my friend Dr.Steeidl) . I reviewed about 200 papers on the CNS changes associated with BSE, and detected a possibility that these mechanisms have a strong influence on CNS, especially in ruminants, and that the BSE has its roots in a more common nutritional problem (HLÁSNÝ, 2001; 2002).

In addition see; List of scientific papers supplied to Spongiform Encephalopathy Advisory Committee (SEAC); 29th November - 28th February 2001 (http://www.seac.gov.uk/papers/ref_280201.htm), There was presented the paper by J Moorby, M Dhanoa and A Austin, 'Aspects of the incubation of dairy cows during the incubation of bovine spongiform encephalopathy', published in the Veterinary Record, 2000, volume 147, pages 409-412;

Ad 2/

Response;

During 1975- 1990 period in Pisek (the study about the magnesium and potassium antagonism in the field conditions);

Veterinary surgeon and animal nutrition specialist in district of Pisek. During this period, there I evaluated about 40 biochemical examinations of dairy (mostly) herds (from 15 to 20 animal) per year (samples of blood and urine were personally taken; including the metabolic animal profile interpretations - in the connection with their dietary details). **Biochemocal laboratory** in „Veterinary Centre of Pisek“, **Feed laboratory** in „Farmers Corporation of Pisek“; in both I was as a head about the laboratory results interpretation. I published these results to the end of 1980s;

HLÁSNÝ,J.: Content of **macrominerals**, nitrates, and soluble sugars in feedstuffs. Vet.Med.(Prague), 1989; 34: 567-576

HLÁSNÝ,J.:Providement for an optimum supply of sodium and **magnesium** to the feed rations of dairy cows and high pregnant heifers. Biol.Chem. Vet. (Prague), 1989a; 25: 157-169

HLÁSNÝ,J.: Evaluation of a new **mineral supplement** in young cattle feeding during winter season. Vet. Med.(Prague), 1989b; 34: 717-725

HLÁSNÝ,J.: Evaluation of some relationships **between macrominerals and nitrogen** compounds in forages. Agrochémia (Bratislava), 1990(1); 30: 28-32

HLÁSNÝ,J.: Influence of nutrition on acid base balance and serum **magnesium** in dairy cows.Biopharm (Prague), 1991;1: 51-60

During my stay in West Virginia (1991) I found in Evansdale Library that;

The only **common feature of all the cases of BSE investigated** in the UK; was the use of commercially produced **compound feed containing meat and bone meal (MBM)** (**Wilesmith**, Wells, Cranwell & Ryan 1988 Veterinary Record, 123, 638-644). This conclusion was further supported by the fact that the incidence **of BSE in dairy herds was much greater than in beef suckler herds,** closely matching the use of compound feeds in these two types of herd.

However, I had more of experineces (1975-1990) about MBM feeding in beef cattle („no-standard“ MBM for feed mills) - it was impossible fed- about the extraordinary smell. When I read the statement from the BSE Inquiry (http://www.bseinquiry.gov.uk/files/ws/s047.pdf) it was supported about my “ not infectious BSE idea”, and later I also wrote in my “BSE comments”;

Where is a central role of British infectious proteins? (from meat and bone meal- MBM) in BSE- when there is not any evidence about this?

For example see **Statement of Ben GILL (April, 1998) ; former chairman of the Livestock and Wool Committee of the National Farmers' Union (NFU)** , he says:

"I have been involved in the emerging story of BSE since the NFU first learnt of the existence of a nine cattle in July 1987. I was vice chairman and chairman of the Livestock and Wool Committee of the NFU (1986-1991). Feed compounds used for feeding cattle, farmers may buy compound feed from feed producers. The actual ingredients used will vary from time to time and from producer to producer. These are commercial decisions taken by the feed producers. For example, protein could amongst others be generated by soya bean meal, or from processed meat and bone meal (MBM). At the time that feed producers switched from soya bean meal to meat and bone meal, there would have been no restrictions on them doing so. **A farmer buying compound feed would not know what ingredients had been used to provide protein. He would not know if the protein source in the compound was soya bean meal or meat and bone meal(MBM)**. The arguments for a declaration of ingredients are well rehearsed in an NFU paper prepared in March 1983. **The absence of ingredient listing meant that farmers buying compound feedstffs would not know whether or not the feed included meat and bone meal(MBM), and if so, whether it was bovine or ovine meat and bone meal(MBM)?** The alternative to purchasing compound feeds is for a farmer to purchase the individual ingredients (referred to as ???straights???) and to prepare feed

compounds himself "

My conclusion;

In the second half of the 1970s and beyond, UK farmers were being encouraged to increase their milk production. However, where unpalatable "MBM was fed in cows- when a farmer buying compound feed would **not know what ingredients had been used to provide protein?** Meat products (MBM) are valuable only for simple- stomached animals. So, MBM is eaten readily only by pigs (100- 200 g) and poultry- hens (5- 10 g/ animal/ day). This product is not readily acceptable for ruminants **(not acceptable- impossible in wildlife) because of the extraordinary smell**. Therefore; if MBM is fed in domestic ruminants, it **must be introduced into their diets gradually and continuously**. So, a farmer buying compound feed would know what ingredients had been used to provide protein !!! **In high milk producing cows, especially ; is not possible fed MBM one week (day) and does not next week (or day)!!!**

And what is about; to prepare feed compounds himself? This is ???impossible??? in a small farm, especially; because about ???the extraordinary smell??? and a high risk about the low feed intake in other cattle- so, the profitability is very low.

During 1990s in Veterinary University (Brno), and in Research Institute in Rapotin (the study about the magnesium and calcium antagonism);

HLÁSNÝ,J.: News about the pig-stress (PSE) prevency. Výzkum v chovu skotu (Rapotín), 1999 (1),41: 22- 30

HLÁSNÝ,J.: The clinical signs of cow´s paresis puerperalis and the therapeutic effect of calcium. Výzkum v chovu skotu (Rapotín), 2000a (4), 42: 16- 25

Ad 4/ About „Mg- experiencies“

Response;

During 1980s I collaborated with Czech neurologist associate professor Ladislav Steidl (Palacky University, Olomouc). We had a lecture (I preseted in French) in Geneve at „3rd European Congress on Magnesium“ (March, 1990).

HLASNY,J.- STEIDL,L.: Mechanism of magnesium deficiency in feedstuffs and in nutrients. Magnesium Research (London- Paris), 1990, 3: 48

Dr. Steidl, he was a member of the editorial board of „Magnesium Research“ from 1975 to 2001 (http://www.jle.com/fr/revues/bio_rech/mrh/sommaire.md). Later (2001), he called attention about my alternative BSE theory (see in references of; http://www.zdravcentra.cz/cps/rde/xbcr/zcsk/56.pdf).

I received my PhD degree (in Czech Republic as „CSc.“) after „the revolution in 1989“- in March 23, 1991 (University of Veterinary Medicine and Pharmacy in Brno) – concerning my dissertation work „Causes of magnesium deficiency in ruminants“. In Geneve I obtained some connections with „Mg- researchers“ and after few months later I received the invitation (December 1990) from West Virginia University as a „Visiting Research Fellow“.

There I collaborated (April 1- December 31, 1991) with an international team led-headed by professor R.L.Reid (http://www.caf.wvu.edu/avs/faculty/lewis.html), I participated about the experiment concerning mineral metabolism in ruminants. It has been as a cooperative grant between West Virginia University and the USDA-ARS-NAA Appalachian Soil and Water Conservation Research Laboratory, Beckley, WV (CRIS No.l932-23330 - 00l- 00D) on a project entitled "**Improved Forage and Sheep Production Efficiency".**

There the metabolism of 24 ewes has been tested at three different pastures during the five weeks period. Ewes were fed a good quality grass legume hay (Crude Protein- 11%, K- 1.9% of dry matter) after lambing in late February, and in mid April six groups of six ewes (3 with twin lambs, and 3 dry) were allocated to three pastures (Matua, Tall fescue, Kentucky

bluegrass with red clover). Pastures were fertilized with 90 kg N/ ha (as N-nitrate) in late March.

Pasture samples were taken for mineral analysis (N, P, K, Ca, Mg, S, Mn, Fe, Cu, B, Al, Na), and also samples of the blood (Ca, P, Na, K, Mg,Cl, Fe,Zn,Cu, glucose, urea, cholesterol, total protein, creatinine, albumin, globulin, bilirubin, triglycerides, ALP, LDH, SGOT, SGPT, GGT, amylase), and urine samples (Ca, P, Na, K, Mg) were taken.

After five days of young pasture in lactating ewes feeding (Crude Protein; 20-30%, K; 2.4-4.0% of dry matter):

- Mg blood values significantly decreased (from 1.7 to 1.1 mg/ dl)
- with previously significantly increased blood urea (from 20 to 45-50 mg/dl)
- with following significant AP increaee (from 100 to 200-340 IU/l, and with a slightly increase of AST (from 140 to 160- 340 IU/l)).

Blood and urine concentrations of Mg fell further to the end of experiment (matua pasture, especially), and did not recover to the same extent as it was at the begining of pasture feeding. In addition, later (June – July, 1991)- **more of ewes clinical neurological signs showed.** This experiment shows, that **spring pasture feeding, it is an example of the nutrition changes (especially in subclinical hypomagnesemia- and uremia)**, after the winter feeding, when N and K levels in „winter feeeds“ are low, compared with their level in young pasture.

The obtained results (the 390 nutritive values of forages and the 4872 biochemical values measured in ewes blood and urine) obtained in this experiment, were only "partially" published (J. M.COX-GANSER, J.R.PUOLI, J. HLASNY, R.L.REID: Mineral status of ewes and quality of different grass and legume pastures for spring grazing. J. Anim. Sci./Suppl./,70, 1992: 182), however, to this date, without biochemistry results evaluation and interpretation. These results I obtained from professor Reid **(from our working group only I was veterinarian)** with the intention – to support my habilitation in Czechoslovakia. However, the political situation (by communist still involved) in 1992/94 at Veterinary university in Brno (see; I was first Visiting Research Fellow from our Veterinary university in the USA; after „1989 revolution“)- former „science committee of university“ refused my habilitation…

However, we found experimental results with the significant N;K;Mg;P- forage changes , and the hypomagnesemia involved in ruminants - with later clinical neurological signs in ewes. So, I interested **to more intensively study the „world literature“ sources about nutritional connections concerning nervous BSE diseases in ruminants**. Also, during 1990s , I studied the work of Czech Mg- scientist professor of biochemistry PhDr., MUDr. et MVDr.h.c. Jan BECKA (killed in Mauthausen; January 1942). Concerning his work; I presented as the lecture; Professor Jan Becka and his still useful magnesium research from thirties. In: Book of Abstracts of 31st International Congress on the History of Veterinary Medicine (Brno), 2000; 44 (http://wahvm.vet.uu.nl/specific/activities/congresses/brno.html). The experimental works of professor Becka (with the rabbits, especially) are unfortunately , to date almost unknown., especially concerning shorter- longer **Mg- deficiency action in connection with the acid-base status… (in addition; professor Becka is the „first man or scientist“ about the history (from 1918) of Veterinary University in Brno).**

In conclusion; literature sources about my „BSE- web- study“ I obtained from the „**Evansdale Library“**. (http://www.libraries.wvu.edu/evansdale/) (http://www.libraries.wvu.edu/evansdale/images/map.gif), and later (1992- 2001) from my friend Dr. Ladislav Steidl (all editions of „Magnesium Research“).

5.) What is the best way to circumvent this issue? I know you talked about the feed ban and removing the ruminant-to-ruminant diet essentially removed the catalyst for the disease. But is there a way to absolutely prevent the disease with increased mineral intake or a certain

diet? Can a livestock owner dramatically increase the amount of magnesium in their diet as an insurance to prevent mad cow disease?
Responses;
Ad a/ What is the best way to circumvent this issue?
There is a simple response; keep the nutritional values in dairy ration **according to the ; National Research Council's;** Nutrient Requirements of Dairy Cattle, 7th Revised Edition (January 2001)- so keep the **cow nutrition on the „physiological level".**
This can be documented (according to ammonia- magnesium theory) why the BSE incidence (in the Europe) decreased after 2001 to „zero levels" (Switzerland, Neetherlands, France…)- see the following text;

The previous NRC edition was released in 1989. One of the main differences is the size of the later publication. The 2001 edition has 224 more pages than the 1989 publication. **There are changes about the lowering of protein requirements; in early lactation especially.**

During early lactation (0-70 days postpartum) milk production increases rapidly, peaking at 4 to 6 weeks after calving. Protein content is critical **during early lactation; rations may** need to contain 19% of more crude protein (ENSMINGER et al., 1990). For example, the same high protein level is recommended in turkeys- in animals with highest protein requirements from animals (NRC,1994). In growing young turkeys (age; 11 to 14 weeks) there is the recommendation (http://www.nap.edu/books/0309048923/html/) 19 percent of protein of diet (90% dry matter). Almost the same situation is in „monogastric" young rapidly growing pigs allowed ad libitum diet of 90% dry matter (NRC, 1998)- average weight in range 15 kg (20.9% of crude protein) and 35 kg (18% of CP) (http://darwin.nap.edu/books/0309059933/html).

Almost the same high protein recommendations (18.8 % dry matter) are from McCULLOUGH (1994) to dairy rations of high producing „supercows"- **during the „all" lactation.** However, according to the **NRC (1989) this high protein level is recommended only during first three weeks (0-21 days postpartum)** after calving. So, above mentioned recommendation were **„overdosed" in dairy practice**. Recent research , **during 1990s resulted to decrease of protein** content in dairy cows – compared NRC (1989) and NRC (2001)- (http://www.nap.edu/catalog/9825.html).

Dairy cow:600-680 kg body weight							
	Lactation				Early lactation		Dry pregnant
Milk yield (kg/day)	25	35	45	55	25	35	
Degradable protein – „DP" (%):							
NRC,1989	8,8	9,7	10,4	10,4		9,7	-
NRC,2001	9,5	9,7	9,8	9,8	10,5	10,3	9,9
Undegradable protein- „UDP" (%):							
NRC,1989	5,4	5,7	6,0	6,3		7,2	-
NRC,2001	4,6	5,5	6,2	6,9	5,4	5,6	3,2
Crude protein – „CP"- (%):							
NRC,1989	**15,0**	**16,0**	**17,0**	**17,5**		**19,0**	**12,0**
NRC,2001	**14,1**	**15,2**	**16,0**	**16,7**	**15,9**	**15,9**	**13,1**

Ad b/ Can a livestock owner dramatically increase the amount of magnesium in their diet as an insurance to prevent mad cow disease?

There is the same response; keep the nutritional values in dairy ration **according to the ; National Research Council's;** Nutrient Requirements of Dairy Cattle, 7th Revised Edition (January 2001)- so keep the **cow nutrition on the „physiological level". The magnesium (Mg) content in dairy ration is about 0,2 percent of Mg in dry matter. This optimal level can be „overdosed" to 0.3 percent of Mg in dry matter – see the situation about the young grass-forage (first cut - ryegrass especially) feeding…**

6.) Your theory would explain why animals as young as 5 and 3 years old have contracted mad cow disease, but how did they contract it? I

Response;

Older cows are more susceptible to grass tetany (Mg- deficiecy) than those with their first or second calves, because of lowered Mg stores; decreased absorption efficiency, and reduced ability to resorb adequate amounts of Mg from the bone. However, in BSE there is necessary „long-term Mg-deficiency" (chronic subclinical hypomagnesemia). It is not possible in the US because there is „hot weather", however in England there are ideal condititions…

See the copy of my article(Rapotin; December, 2002);

Are common the clinical signs of BSE and magnesium deficiency in cows?

Responses;

a/ The clinical signs of BSE:

Most cases of BSE in Great Britain have occurred in dairy cows between 3 and 6 years of age with the initial clinical sigs : nervous, kicking, locomotor difficulty, loss of condition, loss of weight, reduced milk yield, abnormal behaviour, nervous of entrances, temperament change, falling, aprehension, aggression, dificulty rising, tremors, hyperaesthesia, and recumbency. Other clinical signs analysed for the 17,154 cases; ataxia, kicking in parlour, excessive licking, head pressing or rubbing, abnormal ear position, teeth grinding, abnormal head carriage, head shyness… There were variations in the frequency with which some signs were recorded in animals observed at different times during the epidemic (WILESMITH et al., 1992).

b/ The causes and clinical signs of hypomagnesemia

Forages causing staggers in livestock include perennial ryegrass must be considered in the differential diagnosis of hypomagnesemia. Expression of clinical signs in „ryegrass staggers „ is most directly correlated with the cerebrospinal fluid (CSF) Mg levels (SMITH, 1996). Several factors adversely influence Mg metabolism in cattle and may „trigger" grass tetany.: among them drastic fluctuations in spring temperatures, prolonged cloudy weather, organic acid content of plants, hormonal status of the animal, level of higher fatty acids in plants, energy intake of the animal, and additional stress – such as a dog chasing animals, parasites, or a cold rain. Grass tetany is most likely to occur on pasture plants grown on soils that are low in available Mg and high in available potassium. When the ratio K/(Ca+Mg)in the feed ration is higher than 2.2, it is likely to induce hypomagnesemia (KEMP and t´HART, 1957). Therefore, if calcium is low as well as Mg, the hazard of tetany is even greater. However, in the UK there is well known that rainfall can affect the mineral composition of pasture herbage. Calcium, for example tends to accumulate in plants during periods of drought but to be present in smaller concentration when the soil moisture is high. There the exact cause of hypomagnesaemic tetany in ruminants is unknown, although a dietary deficiency of Mg may be a contributory factor. In addition, in britain book „Animal Nutrition", there are no

informations about the potassium content in forages (McDONALD et al., 1988). Also, according to recently well known book (SMITH, 1996), there is only little informations about cow hypomagnesemia in the UK. There, from more than 40 references, only two are about Mg- research in the UK (MOODIE,1965: COLLINS,1980), signalize however, that there hypomagnesemia- hypocalcemia, can would be a problem.

A deficiency of Mg may cause grass tetany (grass staggers) in cattle; lactating ewes and dairy goats are also susceptible**. It is highly fatal, affecting only ruminant species**. Hypomagnesemia is usually accompanied by hypocalcemia. The highest incidence is in high-producing cows in the third to fifth lactation, within 60 days of calving that are pastured on cool season grasses. Generally, occurs within the first 2 weeks after animals are turned out on new pasture growth, either in spring or fall. Also, the disease is almost likely to strike beef cows during early lactation, especially those with high levels of milk production. Dry cows and bulls are seldom affected. **Older cows are more susceptible to grass tetany than those with their first or second calves, because of lowered Mg stores; decreased absorption efficiency, and reduced ability to resorb adequate amounts of Mg from the bone.**

The initial signs include nervousness, attentive ears, markedly erect ears, ear twitching , hyperestesia, and decreased milk yield are early clinical signs. Cows are alert and hyperexcitable, and they may charge. In more severe cases affected animals may avoid the rest of the herd, walk with a stiff gait, lose their appetite, and urinate frequently. They are nervous, have staring eyes, keep their head and ears in an erect position; twitching of muscles (usually of head and neck), head held high, accelerated respiration, higher temperature, grinding of the teeth, and abundant salivation. Also, they stagger: have a twitching skin, especially on the face, ears, and flanks: and lie down and get up frequently. Animals may be irritable and behave aggressively : they may even charge or fight persons in the immediate area. After a time, extreme excitement and violent convulsions may develop. Animals lie flat on their sides, the fore legs pedal periodically, saliva flows freely, breathing is labored, and the heart pounds rapidly. Violent episodes of opisthotonus and clonic convulsions can be precipitated by any stimuli, and these alternate with periods of tetanic muscle spasms. Nystagmus, exaggerated mastication, and a snapping eyelid retraction occurs. If treatment is not given at this stage, animals usually die during or after a convulsion (SMITH, 1996).

The various symptoms of animals suffering from grass tetany indicate that the nervous system controlling both voluntary and unvoluntary muscles is affected. The Mg concentration must be maintened for the normal production and decomposition of acetylcholine. Low Mg: calcium ratio potentiate acetylcholine release, and alterations of the ratio in the extracellular fluid may contribute to tetany. If uncontrolled, the tetany spasms culminate in cardiorespiratory failure (ENSMINGER et al., 1990; SMITH, 1996). **However, according to professor Bečka findings, not only Ca – Mg disturbances, but also acidemia (surpluss of H ions) cause the inhibition of the sympathetic, and the parasympathetic nervous system action prevails (HLÁSNÝ, 2000; HLÁSNÝ, 2000a; HLÁSNÝ, 1999).**

Because the tetany can develop within a day or two of animals being turned out to graze, the condition has been referred to as the acute form. In the chronic form of the disease plasma magnesium levels fall over a period of time to low concentrations. This type is not uncommon in suckler herds. Clinical signs of the disease are often brought on by „stress“ factors such as cold, wet and windy weather**. Chronic grass tetany is generally slow to develop and muscular affection may be limited to twitching, a clumsy walk or exaggerated motions , but convulsions may occur if animals are driven or handled roughly (McDONALD et al., 1988).**

Why hypomagnesemia is not observed in ruminants on warm season grasses?

There is the explanation; these **grasses are low in crude protein and potassium, and**

higher in magnesium content; grown under a low NPK-fertilizers application (hot weather- water stress is obvious). The main advantages of the grasses are their summer growth habit, providing when temperate (cool) grasses (perennial ryegrass, orchardgrass…) have become semi- dormant , and their ability to grow to use soil moisture efficiently. By the same token, they share the disadvantage of all tropical (warm) or C_4 grasses in that their nutritive quality for livestock is lower than that of temperate (C_3) species. This appears to be related to higher fiber and lower crude protein, and potassium concentrations in the warm season grasses (REID and JUNG, 1982).

It should be noted that grazing cattle preferentially select leaf material and protein consumption would therefore be expected to be markedly higher than indicated by whole plant analysis. It is well established that tropical grasses contain relatively high concentration of fiber and low levels of protein (PAYNE, 1966; BUTTERWORTH, 1967). The fundamental differences in leaf structure (Kranz anatomy) and metabolism of C_4 grasses result in marked differences in composition and nutritional quality of tropical and temperate forages (NORTON, 1982). Environmental conditions exert a strong effect on composition of C_4 grasses result in slower rates of degradation of fiber components in rumen (AKIN, 1986), and lower digestibility by ruminants (MINSON, 1981).

Under tropical or subtropical conditions, pastures based on C_4 grasses are generally considered to provide no more than a maintenance level of nutrition for grazing animals. The effects of temperature on cell wall development were examined by FORD et al. (1979); with increasing temperature , leaf neutral detergent fiber (NDF) concentrations in temperate species perennial ryegrass(Lolium perenne) increased, while in tropical species (Panicum maximum) NDF levels decreased. The decrease was due to a decline in cellulose concentration, while hemicellulose and lignin contents increased. There are clear differences in the concentration of minerals; lower levels of Ca and P, and higher concentrations of Mg, Cu in tropical than in temperate grasses (NORTON, 1982); and K concentrations quite low (mean 1.23% for 378 samples) with a high positive correlation with crude protein(REID and JUNG, 1988)in tropical grasses. The same high positive correlation between K and crude protein were found in temperate forages with highest coefficients in ryegrass forage- Lolium multiflorum (HLÁSNÝ, 1990). Tropical grasses appear to contain higher concentrations of Mg (0.36%) than temperate grasses (0.18%)- according to Norton (1982). However, REID and JUNG (1988) found a mean concentration (in 414 samples) only of 0.16 percent Mg in warm season grasses of Northeast of the USA. It is interesting to note that grass tetany at this area has not been observed in animals on warm season grasses. In balance trials with cattle and sheep fed a range of switchgrass (Panicum virgatum) and big bluestem hays in West Virginia , VONA et al (1984) found that both animal species remained in positive Mg balance at all stages of maturity of the hays.

7.) Is it possible the disease could be transmitted in some other way such as saliva to saliva or one animal directly breathing in the breath of another animal? I ask this question because you mention chronic wasting disease but with little elaboration.

Ad a/ Your question; Is it possible the disease could be transmitted in some other way such as saliva to saliva or one animal directly breathing in the breath of another animal?
Response;
It is impossible.

*Ad b/ Your question**; about the CWD…***
Response; I have no personal experieces about the CWD. However, my theory about the „neurodegeneration in ruminants“ - this shows the possibility about it- described in the

chappter; Prions are a symptom of the (metabolic) „chronic wasting disease“ and do not cause the disease (as a part of my eco-detective study)- see my web www.bse-expert.cz

8.) How does Alzheimer's disease fit into this?
Response;
I am veterinarian; I have no experieces about the AD. However, my theory about the „neurodegeneration“ - this shows the possibility about it- described in the chapter;
Hyperfunction (Alzheimer's disease and Parkinson disease) and hypofunction (schizophrenia) of glutamatergic neurons. See also „Alzheimers Caused by Meat?- high protein intake?; (http://www.vegsource.com/articles/bse_editorial.htm) and other realationships;
(http://www.goveg.com/alzheimers_madcow.asp)

9.) In terms of economics, what has this cost in Europe, in England, in the Czech Republic?

Response;
Unfortunately, I have not „precise informations“. However, Dr.Murphy (October, 2006) says http://www.accessexcellence.org/WN/NM/madcow96.php; **What about the political impact of the BSE epidemic?**

The global political impact has been incredible. We're now reading that this might be the ultimate crisis for Prime Minister Major's Tory government, and could lead to its downfall. There are also huge economic costs. **Some estimates go as high as $50 billion, with 300,000 jobs at risk**. At the recent **Turin (Italy) meeting of the leaders of the European Community**, representatives of European countries even brought up questions concerning the pace of the unification of Europe. Even though it has now been decided that quite a bit of the cost of eliminating BSE from British cattle will be shared among other European governments, I don t think we ve heard the last of these tensions at the highest levels of governments.

10.) Why haven't more people in the industry embraced your theory or hypothesis and I'm talking about people in Canada and the United States. We don't always get accurate news reports out of Europe so we are sometimes naiive to what is going on there, but are quite familiar with this situation in North America.

12.) How does the feed industry feel about your findings? Are they willing to embrace what could ultimately reduce or eradicate this disease?

Response;
Unfortunately, in Czech Republic and in the all EU is the same situation, so only „BSE infectious theory is accepted. There is the similar situation as described by American Thinker (2008);
„In Britain, much of the alarmism about **Mad Cow disease was never justified scientifically**. It was pure, **math-model-driven science fiction**, just like Global Warming. But it was pushed very vigorously by the British science establishment, which has never confessed to its errors, and is therefore likely to make the same ones again. In politicized science**, public hysteria actually builds careers; in real science, it tends to ruin careers**. Years after the Brits realized that Mad Cow was a false alarm, the French admitted that *Oui, Messieurs, we had ze Mad Cow, naturally, but we are not hysterique, comprenez vous? Besides, cow brains are a great delicacy, and one only lives once. Vive la France!* Right

across the Channel in Britain, farmers were required by law to destroy and bury hundreds of thousands of sheep and cows. **It was an economic disaster, and all because of wildly alarmist science.** Britain is even more vulnerable to politicized science than we are, because medicine is controlled by the Left. That is a huge chunk of all science in the age of biomedicine. But the *British Medical Journal* and even the **venerable *Lancet* are no longer reliable sources“**
(http://www.americanthinker.com/2007/11/global_warming_as_pathological.html).

11.) If a pregnant cow is in the beginning stages of mad cow disease, is it passed to the offspring and an incubation period ensues when the animal is born?

Response;
Not only about my „BSE nutritional theory“- but also according to the „BSE infectious theory“- it is not possible.

13.) Was your work done under the auspices of the Czech government or did you work in private laboratories?

Response; my all „BSE work“ this were my private professional hobby activities, without any financial support from Czech government. For example my trip to Vancouver – it was „financially“ supported only by the „Chamber of Czech Veterinary Surgeons“ and only „morally supported“ by the Research Institute for Cattle Breeding in Rapotin.

14.) Are there any products in nature, that cows can eat, that may have increased levels of magnesium, such as alfalfa, clover, grasses, or grains?

Response;
Leguminose such as alfalfa, clover… are very good Mg- sources from forages. The Mg content in grains is low… From the mineral supplements, there the **best and cheap Mg-source is Mg-oxide.**

15.) Is the deficiency of the magnesium the cause of the disease, or is it a trigger just like blood meal is believed to be?

Response;
Yes, **Mg-deficiency in the connection with the dietary „protein surpluss**“ is the cause of the BSE in ruminants. Because only in ruminants- the Mg is predominantly absorbed in the rumen; where can be higher ammonia concentration- that depressed Mg absorption. And about blood meal- there is the connection concerning the highest protein content in this feed- from the „all feed proteins“ used in animals.