Genetic Diseases of Neurotransmitter Metabolism

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Although there are many (established and putative) neurotransmitters, known diseases due to hereditary defects in the metabolism of these substances are rather few. The established neurotransmitter systems can be divided into amino acidergic [mainly the inhibitory γ-aminobutyrate (GABA) and glycine, and the excitatory aspartate and glutamate], cholinergic (acetylcholine), monoaminergic (mainly adrenaline, dopamine, noradrenaline, and serotonin), and purinergic (adenosine, ADP, AMP, and ATP), while increasing numbers of peptides are considered putative neurotransmitters. Possibly involved in neurotransmission and/or neuromodulation are the N-acetylamino acids and N-acetyl peptides. This chapter deals with hereditary diseases in the metabolism of glycine, GABA, dopamine, and N-acetylaspartate.

NONKETOTIC HYPERGLYCEMIA

Nonketotic hyperglycinemia, probably the most frequent of the known genetic diseases of neurotransmitters, was first reported in 1963 (1). It has to be differentiated from the ketotic hyperglycinemia syndrome occurring in disorders of organic acid metabolism such as methylmalonic and propionic acidemia (2).

Clinical Picture

The majority of patients present with a generalized hypokinesia, hypotonia, and hyporeflexia within a few hours or days after birth. As a rule there is rapid progression to coma and apnea requiring artificial ventilation. Seizures are a prominent feature of this disease, presenting mostly as myoclonic jerks. The electroencephalogram shows early diffuse disturbances with a typical periodicity or burst suppression pattern. However, this pattern is not pathognomonic since it can also occur in newborns with herpes encephalitis or anoxia. After a few weeks hypsarrhythmia
may follow. About half of these patients die rapidly (in a few days or weeks), while in the others death ensues after several years of evolution toward severe spasticity accompanied by extreme psychomotor retardation and intractable convulsions. Different degrees of involvement may be seen in the same family.

Apart from this classical neonatal form, atypical presentations have been reported with a later onset and a more benign clinical picture (3) or with a rapid "degenerative" course (2). Recently, Schiffman et al. (4) discovered a transient hyperglycinemia in two neonates with convulsions; the outcome was favorable. Inheritance is autosomal recessive.

Biochemical Defect

The metabolic marker of this disease is increased glycine in the cerebrospinal fluid (5). There are also increases of glycine in plasma (inconstant) and urine. Hyperglycinemia and hyperglycinuria secondary to organic acid disorders or to treatment with the antiepileptic drug dipropylacetate (6) are not associated with hyperglycinorachia.

The basic defect is in the glycine cleavage system of brain and liver. This system, located in the mitochondria, is the major catabolic pathway of glycine. It is composed of four protein components: P-protein (a pyridoxal phosphate-dependent glycine decarboxylase), H-protein (a lipoic acid-containing protein), T-protein (a tetrahydrofolate-requiring enzyme), and L-protein (a lipoamide dehydrogenase). These components interact to degrade glycine to CO₂ and NH₃. In nonketotic hyperglycinemia, defects have been identified in each of these proteins except the L-protein. Most patients with the classical neonatal form have an abnormality of the P-protein. The extent of the glycine cleavage defect may differ in brain and hepatic mitochondria (3). As the glycine cleavage system exists in placenta, prenatal diagnosis seems feasible on chorionic villi. It is not detectable in fibroblasts and amniocytes.

Treatment

No effective treatment is known. Strychnine blocks glycinerigic inhibition and it has been used with moderate success in a few patients with the milder form of the disease; those with the classical phenotype do not respond (7).

Glycine may have excitatory effects on cortical neurons via facilitation of N-methyl-D-aspartate excitation. N-Methyl-D-aspartate receptor inhibition has therefore been proposed as a possible treatment for nonketotic hyperglycinemia (4).

INBORN ERRORS OF GABA METABOLISM

Three genetic diseases due to a defect in the brain GABA metabolism have been reported: pyridoxine-responsive convulsions, considered to be the consequence of
a GABA synthesis defect (glutamate decarboxylase deficiency) and two defects in the GABA catabolism: GABA transaminase deficiency and succinic semialdehyde dehydrogenase deficiency (Fig. 1).

**Pyridoxine-Responsive Convulsions**

This disorder was first reported in 1954 (8). It is a rare cause of convulsions in early childhood (9).
Clinical Picture

Typical pyridoxine-responsive convulsions have to be differentiated from the more recently identified atypical presentation. The typical form satisfies the following criteria:

Onset of convulsions before or shortly after birth (10)
Rapid response to pyridoxine
Refractoriness to other anticonvulsants
Dependence on a maintenance dose
Absence of pyridoxine deficiency (11)

The disease may start as intrauterine convulsions as early as the fifth month of pregnancy. Some patients have suffered from peripartal asphyxia probably as a consequence of this disorder. The seizures are intermittent at onset but may proceed to status epilepticus. There is a pronounced hyperirritability that can alternate with flaccidity. Abnormal eye movements are often reported (nystagmus, "rolling" eyes, miosis, and/or poor reaction of the pupils to light).

The atypical presentation (12,13) differs from the typical one by:

Later onset of the attacks (up to the age of 14 months)
Prolonged seizure-free intervals without pyridoxine (as long as 5 months)
The need for larger pyridoxine doses in some of these patients
Higher incidence

All types of seizures can be observed; long-lasting seizures and repeated status epilepticus are most common, but brief convulsions (generalized or partial), atonic attacks, and infantile spasms also occur.

Biochemical Defect

Pyridoxine-responsive convulsions are considered to be due to brain GABA deficiency resulting from a genetic defect at the pyridoxal phosphate coenzyme binding site of glutamate decarboxylase, the rate-limiting enzyme in GABA synthesis (15). Yoshida et al. (16) presented evidence but no definitive proof of glutamate decarboxylase deficiency in their patient. Brain GABA has been measured (postmortem) in only one patient (17) and cerebrospinal fluid GABA in another (18); values were low in both patients. No data are available on cerebrospinal fluid homocarnosine concentrations.

Treatment

The disease responds promptly to pyridoxine but is refractory to other antiepileptic medications. The minimum effective daily dose is at least 10 times the minimum daily amount recommended for healthy infants and usually varies between 10 and
100 mg orally. Treatment with isoniazid increases the minimum effective dose. The convulsions cease within a few minutes when pyridoxine is administered parenterally, and within a few hours when it is given orally. In the same patient the effect of a single dose lasts for a consistent period (usually 2–5 days) and is independent of the amount or method of administration. When treatment is interrupted, the seizures return, although there might be exceptions to this rule (delayed "maturation" of enzyme activity?) (18).

In case of (suspected) intrauterine convulsions, treatment of the mother with pyridoxine is effective (around 100 mg/day). In later onset presentation, doses of 100–200 mg may be necessary to control the seizures. Here also the minimum effective maintenance dose has to be determined individually. In the absence of early appropriate treatment, severe psychomotor retardation is the rule and if untreated the disease runs a fatal course, at least in the neonatal form.

In conclusion, a trial of pyridoxine should be performed in all unclear seizure disorders with onset before the age of 15 months.

**Gamma-Aminobutyric Acid Transaminase Deficiency**

Gamma-aminobutyric acid transaminase deficiency was first reported in 1984 in a brother and sister from a Flemish family (19). No other patients seem to have been described since then.

**Clinical Picture**

Both patients showed feeding difficulties from birth, often necessitating gavage feeding. They had a pronounced axial hypotonia and generalized convulsions. A high-pitched cry and hyperreflexia were present during the first 6–8 months. Further evolution was characterized by lethargy and psychomotor retardation (neither patient attained the level of a 4-week-old infant). Corneal reflexes and reaction of the pupils to light remained normal. A remarkable, continued acceleration of length growth was noted from birth to death (Fig. 2). This was explained by increased fasting plasma growth hormone levels (8–39 ng/ml; normal < 5); these could be suppressed by oral glucose. In one of the patients, head circumference showed a rapid increase during the last 6 weeks (from the 50th to the 97th percentile).

**Biochemical Defect**

Using ion-exchange chromatography with fluorescence detection (20), we found very high free GABA concentrations in the cerebrospinal fluid of the index patient (up to 60 times the median control value). Total GABA, homocarnosine (a GABA-histidine dipeptide), and "unidentified" GABA compounds (total GABA minus
homocarnosine and free GABA), as well as β-alanine, were only slightly increased in the plasma and urine. Liver GABA and β-alanine concentrations were normal.

This metabolic pattern could be explained by a decrease of GABA transaminase activity. In liver this activity was 18% of the median control value: 0.07 μmol/mg protein·h (median 0.38, range 0.31–0.69 in 10 controls); and in lymphocytes 2.2%—48 pmol/mg protein·h (median control value 2,154, range 936–4,176, n = 6). In the patient’s healthy sibling it was 24%, in her father 17%, and in her mother 40%. It can be assumed that the same enzymatic defect exists in the brain since GABA transaminases of human brain and of peripheral tissues have the same kinetic and molecular properties (21). β-Alanine seems to be an alternative substrate for GABA transaminase, hence its increase in this disease (22).

GABA transaminase activity is not expressed in fibroblasts (23) and thus prenatal diagnosis based on enzyme analysis of amniotic fluid cells is not feasible; however, activity is present in chorionic villous tissue (24). The pattern of occurrence in this family as well as the enzyme data strongly suggest autosomal recessive inheritance of GABA transaminase deficiency.

Treatment

We found no clear-cut biochemical or clinical response after administration of pharmacological doses of pyridoxine, the precursor of the coenzyme of GABA transaminase, not with picrotoxin, a potent noncompetitive GABA antagonist (25). To be efficient, any treatment should probably be started before birth. Outcome was fatal in both children at ages 1 year and 2 years, 7 months.
Succinic Semialdehyde Dehydrogenase Deficiency

Succinic semialdehyde dehydrogenase deficiency was first reported as γ-hydroxybutyric aciduria in 1981 (26). It has been documented in at least 15 patients (27–29).

Clinical Picture

The clinical picture comprises nonprogressive ataxia, hypotonia, mild to marked psychomotor retardation, and less frequently, hyperactivity, choreoathetosis, autistic features, convulsions, hyporeflexia, nystagmus, and oculomotor apraxia. Clinical symptomatology as well as metabolite accumulation tend to decrease with age, at least in some patients (30).

Biochemical Defect

The hallmark feature is the increase in urine, plasma, and cerebrospinal fluid of γ-hydroxybutyrate formed by the reduction of accumulating succinic semialdehyde (Fig. 1). γ-Hydroxybutyrate can be higher in cerebrospinal fluid than in plasma and is extremely increased in some patients (31). It is a neuropharmacologically active compound. With currently used methods it is not detectable in the plasma of controls, but small amounts are present in urine (32). Cerebrospinal fluid GABA may (30) or may not be increased (27). Cerebrospinal fluid homocarnosine levels are increased. In about half of the patients, an increase in plasma glycine is mentioned. The enzyme defect, succinic semialdehyde dehydrogenase deficiency, has been demonstrated in lymphocytes and lymphoblasts; enzyme activity in intact cells has ranged from 3.7 to 11% of the mean control value (29). Enzyme activity is absent in control human fibroblasts but present in chorionic villous tissue (24).

Treatment

In an attempt to reduce the accumulation of γ-hydroxybutyrate in a 2-year-old Italian girl with this disease, we used the antiepileptic drug γ-vinyl GABA. This substance is known to cause an irreversible inhibition of GABA transaminase, the enzyme preceding succinic semialdehyde dehydrogenase. This treatment (75 mg/kg-day) has now been given for 2.5 years and has been accompanied by a significant and sustained clinical and biochemical improvement; ataxia and hyperactivity have decreased, electroencephalograms have improved, and the levels of cerebrospinal fluid γ-hydroxybutyrate have dropped to about 25% of the pretreatment value (27). This disease runs a much more benign course than GABA transaminase deficiency and no fatal outcome has been mentioned (at the time of writing the oldest reported patient is 11 years). Inheritance is autosomal recessive.
DOPA-RESPONSIVE HEREDITARY PROGRESSIVE DYSTONIA

Segawa et al. (33) first reported a variant of dystonia called “hereditary progressive dystonia with marked diurnal fluctuation.” A remarkable feature of this disease is a complete or pronounced alleviation of the symptoms with small doses of L-dopa (34,35). At least 80 patients are presently known.

Clinical Picture

Age at onset of the dystonia is mostly between 1 and 10 years. It starts in the lower extremities mostly with gait difficulties and often remains limited to the extremities with no or minimal axial dystonia. In most patients (not in all!) there is a marked diurnal fluctuation of symptoms characterized by worsening of symptoms and increasing fatigue throughout the day, and marked benefit after sleep. Symptoms noted in a minority of patients are scoliosis, opisthotonus, dysarthria, dysphagia, postural tremor, and/or intermittent eye deviation. There is a great variability in the severity of the disorder. Some patients have been misdiagnosed for as long as 12 years as having cerebral palsy (36). It seems that the age of onset is earlier in girls and that they are generally more severely involved than boys. Available data strongly suggest dominant inheritance with low penetrance in most families.

Biochemical Defect

The precise biochemical defect underlying this disease is still unknown. The dramatic response to L-dopa suggests a dysfunction of dopaminergic systems in the basal ganglia of the brain. No consistent abnormalities of the catecholamines and their metabolites have been found in the cerebrospinal fluid of these patients. De Jong (37) found a biological half-life of dopamine reduced to about 50% of that of the control in three children with this disease after oral loading with deuterated L-tyrosine. This could point to an impaired dopamine storage in the basal ganglia. Recently, cerebrospinal fluid concentrations of homovanillic acid, 5-hydroxyindoleacetic acid, and biopterin were found to be decreased in five patients (38).

Treatment

Low doses of L-dopa (5–30 mg/kg·day) cause a marked improvement with complete or almost complete remission of symptoms, usually within days or weeks. Progressive improvement continues to occur for months in some cases without increase in dosage. On withdrawal of L-dopa there is immediate recurrence of symptoms. Although no long-term adverse effects of L-dopa therapy have been seen in this disease, it should be given at the minimum effective dose.
CANAVAN'S SPONGIFORM LEUKODYSTROPHY:
ASPARTOACYLASE DEFICIENCY

Canavan reported on an early onset cerebral spongy degeneration in 1931 (39). This disorder was further delineated in 1949 by Van Bogaert and Bertrand (40). The basic defect was identified as aspartoacylase deficiency by Matalon et al. (41).

Clinical Picture

Onset is usually in the first 6 months of life. The main features are psychomotor retardation, hypotonia followed by hypertonia, excessive crying, seizures, and blindness. Macrocephaly is common. Progressive deterioration leads to a decorticte condition and death within a few years. Histologic changes involve astrocytic swelling and vacuolation of the myelin. Genetic transmission is autosomal recessive.

Biochemical Defect

The key feature is accumulation of N-acetylaspartic acid in the body fluids, levels in the cerebrospinal fluid being much higher than in the serum (42). The normal function of N-acetylaspartic acid is not well understood. It is abundant in brain, where its concentration is second only to glutamic acid in the free amino acid pool and is higher than that of GABA.

The enzymatic defect was discovered by Hagenfeldt et al. (43), who found aspartoacylase deficiency in fibroblasts from a boy with leukodystrophy. It was the achievement of Matalon et al. (41) to link this defect with Canavan's disease. Since aspartoacylase activity is present in cultured amniotic cells and chorionic villi, it is likely that the assay for this enzyme can be used for prenatal diagnosis (41).

Treatment

It has been proposed that N-acetylaspartic acid serves as a transporter of acetyl groups from mitochondria to the cytosol for lipogenesis. Therefore, in an attempt to supply alternative substrate for lipogenesis in the brain, a ketogenic diet was given to one patient for 5 months. No improvement was seen (43). No other treatment is available.

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DISCUSSION

Dr. Mannaerts: L-Dopa is not only a precursor of dopamine but also of noradrenaline and adrenaline. So the defect in dopa-responsive hereditary progressive dystonia is not necessarily a dysfunction of dopaminergic transmission.

Dr. Jaeken: We have not analyzed this in this disease, but several groups have measured other catecholamine compounds and found normal levels.

Dr. Casaer: In a recent discussion, Dr. Segawa from Tokyo stressed that a more systematic study of catecholamines before, during, and after treatment could perhaps be helpful in understanding some of the underlying mechanisms. From analysis in our observations no clear-cut picture has emerged.

Dr. Dhondt: I think you definitely have to add biotinidase deficiency to that list, because it is a real inborn error of the metabolism of neurotransmitters and probably is one of the best models for the dopamine and serotonin defect.

Dr. Endres: Concerning the treatment of undiagnosed seizures with pyridoxine I would like to propose that pyridoxal phosphate levels should be determined in cerebrospinal fluid (CSF) as well as in serum before giving pyridoxine. We have developed a very sensitive method and we sometimes find a very low CSF level in the presence of a normal serum level of pyridoxal phosphate.

Dr. Saudubray: I have a general comment and a question. I guess we can make a distinction between the disorders affecting the synthesis of neurotransmitters and the disorders affecting
the catabolism of the neurotransmitters. Of course, as already suggested by Dr. Dhont, it is important to include in the list the disorders affecting the synthesis of biopterin, dihydropteridine reductase deficiency, and even PKU. It is important because in the clinical presentation of PKU offspring, one hypothesis for explaining mechanisms of congenital malformations is the abnormal synthesis of neurotransmitters. Along this line neurotransmitters could be considered as substances necessary for brain development, and we could expect to find congenital malformations as frequent findings in this first category. By contrast, in the second category, including the disorders of catabolism, I feel a little disappointed when faced with the very large variety and the nonspecificity of the clinical symptoms due to these disorders. But there are absolutely no congenital malformations suggesting that consequences of these disorders start after birth.

My question is about the physiological significance of acetyl amino acids. You presented Canavan's disease with acetylaspartaturia, which is a model of accumulation of acetyl amino acids. More generally, do you feel that this is a new field of investigation for inborn errors?

Dr. Jaeken: The significance of these acetyl amino acids and acetyl peptides is not understood. I think we can detect defects in the metabolism of these compounds by doing strong acid hydrolysis of the CSF. For example, we recently found a child with a large increase of alanine in the CSF after hydrolysis; in this child an accumulation of acetyl alanine has to be excluded.

Dr. Van Hoof: Dr. Jaeken makes an excellent point by insisting on cerebrospinal fluid analysis for the detection of abnormal metabolites. Did you note the accumulation of GABA in patients on valproate therapy? As you know, this drug inhibits ω-hydroxyacyl-CoA oxidation (1). 4-Hydroxybutyrate is thus expected to accumulate and this would reduce GABA transamination.

Dr. Jaeken: No, we have never observed this. But it could perhaps be below the detection level.

Dr. Saudubray: I have a comment on γ-hydroxybutyrate aciduria. This compound has been used and may still be used as an anesthetic drug. I remember some babies born from mothers who had been anesthetized with this compound. These babies were completely floppy. My other comment is on the "transience" of nonketotic hyperglycinemia. I totally disagree with Goodman's last paper (2). It is very unwise to treat nonketotic hyperglycinemia. I have personally observed three patients with the so-called "transient" nonketotic hyperglycinemia, and these patients are now mentally retarded after 4 years of life.

Dr. Jaeken: I agree with you. It remains uncertain whether the convulsions are the consequence of the increased CSF glycine in "transient" neonatal hyperglycinemia.

Dr. Schaub: This definition of "transient" is based on what? On the clinical picture? Or is it a biochemical definition?

Dr. Saudubray: The early clinical presentation is identical to the classical type of nonketotic hyperglycinemia with severe hypotonia, myoclonic jerks, and "bursts suppression" on the EEG. In CSF and sometimes in the blood you find high levels of glycine. In contrast to the usual course of the severe form of nonketotic hyperglycinemia, these patients get an unexpected improvement and all the symptoms can resolve. Glycine in CSF decreases slightly and can reach normal levels. But the long-term outcome is poor and all three patients I have observed have developmental delay.

Dr. Schaub: I think there may be a slight improvement with strychnine therapy as in the case of Gitzelmann and Baerlocher. Can Dr. Baerlocher comment on this case. What is the long-term outcome?

Dr. Baerlocher: The child you mention has a severe form of nonketotic hyperglycinemia. It
survived spontaneously for the first 6 months and has since been treated successfully over 10 years with strychnine by Professor Gitzelmann. He has treated two more children (twins) with strychnine; both also showed some improvement but deteriorated rapidly after discontinuation of strychnine and died thereafter (3,4).

Dr. Jaeken: Regarding the transient type, the CSF glycine values were up to 463 μmol/liter, and it is conceivable that there could be a maturation defect in the glycine cleavage system.

Dr. Endres: I want to pursue the question of dopamine therapy. L-dopa is mainly decarboxylated in the periphery to dopamine, which cannot enter the brain. Since we require dopa to enter the brain, where its active metabolite forms, we administer at the same time a peripheral decarboxylase inhibitor (e.g., carbidopa). We do this in the severe forms of tetrahydrobiopterin deficiency. I saw on your Table 5 that you use 30 mg/kg-day of l-dopa. In comparison to our children this is a very high dose. As there are some long-term side effects such as nightmares and others, I would like to ask: What is the disadvantage of adding carbidopa to the medication of your patients?

Dr. Mannaerts: If you do not add a peripheral dopa decarboxylase inhibitor, most of the administered dopa will be decarboxylated to dopamine by a first-pass effect in the liver. Dopamine cannot penetrate the brain and can cause a number of serious side effects.

Dr. Casae: In our present study protocol, we use L-dopa together with a peripheral decarboxylase inhibitor. As to side effects, we have never seen any gastrointestinal side effects. We monitor sleep and wake behavior very carefully and pay detailed attention during the clinical examination to the possible development of dyskinesia or other "release" symptoms. In contrast to the children with juvenile Parkinson's disease, we have had few or no problems with finding an optimal dose in the children with Segawa syndrome.

Dr. Saudubray: About pyridoxine-responsive disorders, do you keep a difference between the so-called "pyridoxine dependency" and the pyridoxine-responsive convulsions? I remember an old paper with this distinction. Is it true?

Dr. Jaeken: No, it is not relevant. We have several patients with low-CSF GABA who do not respond to pyridoxine and I am sure that there are glutamate decarboxylase-deficient patients who are not responsive to pyridoxine as well, although this has not been yet reported. It is not easy to prove this because glutamate decarboxylase has many isoenzymes and there are different isoenzymes in brain and other tissues. This means that brain tissue is necessary for final diagnosis.

Dr. De Meirleir: In the last Münich meeting there was a presentation of a case of deficiency of L-aromatic decarboxylase, which is an enzyme responsible for the synthesis of serotonin and L-dopa. This is also a new neurotransmitter disease. How can we diagnose this easily?

Dr. Jaeken: This is an interesting disorder recently discovered by K. Hyland and P. Clayton in twins with severe hypotonia and convulsions. They showed decreased CSF and plasma serotonin and increased L-dopa, 5-hydroxytryptophan, and 3-methoxytyrosine, an L-dopa metabolite. A deficiency of aromatic L-amino acid decarboxylase was found, providing evidence that the decarboxylation of L-dopa and 5-hydroxytryptophan is catalyzed by a single enzyme in the human. Treatment with a monoamine oxidase inhibitor clearly improved the symptomatology.

Dr. De Meirleir: Would it be sufficient to measure serotonin levels in CSF or plasma?

Dr. Jaeken: I don't think so; you have to measure dopa itself.

Dr. Schaub: My question is: Can you use urine instead of plasma or cerebrospinal fluid for the diagnosis of deficiencies?

Dr. Jaeken: It depends on the disorder; for example, in GABA transaminase deficiency.
GABA is not increased in the urine and only slightly in the plasma. Therefore, CSF analysis is necessary for diagnosis.

Dr. Mannaerts: It might be possible to follow neurotransmitter release or turnover in the brain by following the urinary excretion of some specific metabolites.

Dr. Endres: I think in measuring urinary metabolites the problem is that we don't know whether they are coming from the brain or from the liver. So only in cases where we have a distinct disease (e.g., tetrahydrobiopterin deficiency) does it make sense to measure, for example, 5-hydroxyindole acetic acid and homovanillie acid in the urine. But, of course, it would be better to measure dopamine and serotonin in the CSF. However, it is clearly easier to obtain urine than CSF.

Dr. D'Hondt: In your patients with 5-dopa responsive dystonia, have you checked prolactin levels?

Dr. Jaeken: These levels were normal.

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