

# Pharmacological manipulation of cyclic GMP levels in brain restores learning ability in animal models of hepatic encephalopathy: therapeutic implications

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**Abstract:** Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome present in patients with liver disease that includes impaired intellectual function. To develop therapeutic treatments to restore cognitive function, it is important to understand the molecular mechanisms that impair cognitive function in HE. This review summarizes data showing that: (a) cognitive function and learning are impaired in patients with liver disease and in animal models of chronic liver failure or hyperammonemia; (b) the glutamate–NO–cGMP pathway modulates some forms of learning; and (c) the function of this pathway is impaired in brain in vivo in rats with chronic hyperammonemia or liver failure and from patients who died from HE. Learning ability of hyperammonemic rats was restored by increasing cGMP by: (1) continuous intracerebral administration of zaprinast, an inhibitor of the cGMP-degrading phosphodiesterase; (2) chronic oral administration of sildenafil, an inhibitor of the phosphodiesterase that crosses the blood–brain barrier; and (3) continuous intracerebral administration of cGMP. The data summarized indicate that impairment of learning ability in rats with chronic liver failure or hyperammonemia is due to impairment of the glutamate–NO–cGMP pathway. Moreover, increasing extracellular cGMP by pharmacological means may be a new therapeutic approach to improve cognitive function in patients with HE.

**Keywords:** hepatic encephalopathy, cognitive impairment, cyclic GMP

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## Hepatic encephalopathy

Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome present in patients with chronic or acute liver disease. HE covers a wide range of neuropsychiatric disturbances ranging from minimal changes in personality or altered circadian rhythms (sleep–waking cycle) to alterations in the intellectual function, personality, conscience, and neuromuscular coordination. HE is usually reversible, but in the worse cases can lead to coma and death.

The neurological alterations in HE are the result of a previous failure of liver function. Liver failure leads to impaired detoxification of ammonia and other toxic substances that can reach the brain and alter its function. Many studies have been carried out to identify factors responsible for the neurological alterations in HE. Clinical experience and basic research indicate that ammonia is the main factor responsible for HE. Ammonia is a product of degradation of proteins and other nitrogenated compounds but at high concentrations ammonia is toxic, leading to alteration of cerebral function which can lead to coma and death.

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Hyperammonemia is therefore considered the main factor contributing to the neurological alterations found in HE both in acute and chronic liver disease (Ferenci et al 1984; Lockwood et al 1991; Felipo and Butterworth 2002; Wang and Saab 2003). Classical clinical treatment of HE is mainly directed to reducing ammonia concentration by lowering ammonia production by the intestinal bacteria and by reducing ammonia transport from intestine to the blood flow by acidification of the intestinal lumen. Overt HE is usually elicited by a precipitating factor (high protein ingestion, gastrointestinal constipation, bleeding, diuretics) usually associated with increased ammonia levels. Cognitive, motor (extrapyramidal and cerebellar signs), and sleep alterations (impairment of sleep–wake cycle) are commonly observed in patients with HE and their intensities vary with the grade of HE. Patients with HE show alterations in cognition, consciousness, attention, memory, and learning.

Motor alterations include increase in muscular tone, reduced speed of rapid alternating movement, ataxia, an increase in deep tendon reflexes, abnormal movements such as tremors, and, particularly, asterixis. Also hypomimia, dysarthria, bradykinesia, and hypokinesia could be detected on careful neurological examination.

Alterations in the regulation of biological rhythms such as sleep, appetite, melatonin production, and in sexuality are common in patients with liver disease (Iguchi et al 1982; Steindl et al 1995; Garfinkel and Zisapel 1996; Cordoba et al 1998).

Patients with liver cirrhosis with normal neurological and mental status examination may present minimal forms of HE, showing intellectual function impairment that cannot be detected through general clinical examination but can be unveiled using specific neuropsychological and neurophysiologic examination (Ferenci et al 2002; Amodio et al 2004).

This neurophysiological examination includes the use of EEG as a tool to monitor the severity of HE and the changes due to treatment. Alterations of EEG patterns in cirrhosis are roughly related to the alterations of mental state (Parsons-Smith et al 1957). Both qualitative and semi-quantitative scales for grading of the EEG alterations due to HE have been proposed (eg, Parsons-Smith et al 1957; Amodio, Marchetti, et al 1999). Spectral analysis of EEG tracing to quantify and classify the alterations due to HE is also being used (eg, Van der Rijt et al 1992; Amodio et al 1996, 2001). This technique provides a quantitative estimation of the EEG based on the relative power of frequency bands and the mean dominant frequency. These EEG techniques may detect minimal HE.

The prevalence of minimal HE ranges from 30% to 84% depending on the kind and number of tests used and the population (etiology and severity of the liver disease) investigated. Cirrhotic patients with minimal HE are “clinically normal” but present cognitive alterations which can be unveiled by a detailed analysis of the patients’ history and by neurological and neuropsychiatric assessment of consciousness and of sensory, cognitive, and motor function.

Even minimal HE has been associated with reduced quality of life and ability to work and to drive (Schomerus et al 1981; Srivastava et al 1994; Groeneweg et al 1998; Marchesini et al 2001; Schomerus and Hamster 2001). Moreover, patients suffering minimal HE have increased probability of suffering later overt HE (Amodio, Del Piccolo, et al 1999; Hartmann et al 2000; Romero-Gomez et al 2001).

## Intellectual function is impaired in HE

Early manifestations of intellectual dysfunction in HE include psychomotor slowing and impaired ability to perform tasks that require sustained attention (McCrea et al 1996; Schomerus and Hamster 1998; Amodio, Del Piccolo, et al 1999). As encephalopathy worsens, impairment in speech and inability to copy simple drawings (eg, a star) appear. In grade II HE, temporal and spatial disorientation and reduced vigilance state or delirium have been observed. Grade IV HE is characterized by the appearance of stupor and coma.

Patients with minimal HE show impaired ability to perform memory tasks, mainly because of deficits in attention and visual perception (Tarter et al 1987; Weissenborn et al 2003). These patients also perform worse than healthy controls in motor function, visual perception, visual orientation, visuo-constructive abilities, and attention (Rehnström et al 1977; Rikkers et al 1978; Gilberstadt et al 1980; Tarter et al 1984). Performance in recognition and free recall tasks is also impaired in these patients (Weissenborn et al 2003).

The psychomotor slowing present in patients with minimal HE is due to cognitive rather than motor deficits, as indicated by the fact that delays in choice reaction times are more strongly impaired than the delays in simple reaction times (Rikkers et al 1978; Schomerus et al 1981).

Sustained attention is also impaired in cirrhotic patients even when memory, language, constructive, or pure motor alterations are absent (McCrea et al 1996; Weissenborn et al 2001, 2003). Patients with minimal HE have a tendency to be easily distracted.

## Animal models of chronic HE show neurological alterations similar to those in patients with HE

Hyperammonemia is considered the main contributor to the pathogenesis of HE, but the mechanisms by which liver failure and hyperammonemia lead to the associated neurological alterations are not well understood.

To try to study these mechanisms a few animal models are being used. The most usual model to study the neurological alterations in HE is the rat with chronic liver failure induced surgically by portacaval anastomosis. In this model a surgical shunt is constructed between the portal and the inferior cava veins. Thus blood does not pass through the liver, and ammonia and other substances are not properly detoxified, can reach the brain, and lead to neurological alterations.

This animal model reproduces some of the neurological alterations found in patients with HE. Rats with portacaval anastomosis show impaired circadian rhythms of locomotor activity and food intake (Zee et al 1991; Steindl et al 1996; Cordoba et al 1997; Lozeva et al 2000, 2002; Lopez et al 2002).

Reduced motor activity is also observed in rats with portacaval anastomosis, which is similar to the motor slowing, hypokinesia and bradykinesia present in patients with HE (Bengtsson et al 1986, 1989; Martin et al 1986; Apelqvist et al 1998, 1999; Lozeva et al 2000). Reduced motor activity has been also observed in rats with bile-duct ligation, another model of HE (Chan et al 2004).

## Learning ability is reduced in animal models of HE

Cognitive function is also altered in animal models of chronic liver disease. When normal rats are moved from the cage where they usually live and are placed in a novel cage, they start to explore it, resulting in increased motor activity. If rats are placed in the same cage during consecutive days, the exploratory behaviour decreases since the animal remembers the novel cage and the interest for it lowers. In rats with portacaval anastomosis this long-term habituation is impaired. This has been interpreted as consequence of a possibly impaired learning–memory capacity in these rats (Apelqvist et al 1999).

A more direct demonstration of impairment of learning ability in rats with portacaval anastomosis has been reported recently by Erceg et al (2005a), who showed that the ability of these rats to learn a conditional discrimination task in a Y maze is lower than that of control rats.

Rats with biliar obstruction (another model of HE) show impaired learning ability in a working memory test consisting of an object recognition task, ie, show decreased ability to discriminate between novel objects and previously known objects (Garcia-Moreno et al 2005). Working memory is also altered in patients with HE (Elithorn et al 1975).

As mentioned above, hyperammonemia is considered the main factor contributing to the pathogenesis of HE. However, liver failure induces, in addition to hyperammonemia, other alterations (such as decreased muscle mass, altered metabolism of other compounds). To discern the contribution of hyperammonemia to the neurological alterations in HE, we developed an animal model of chronic hyperammonemia without liver failure: rats fed an ammonium-containing diet (Azorin et al 1989). These rats present a level of hyperammonemia similar to that of patients with liver cirrhosis or of rats with portacaval anastomosis, but do not present other alterations associated with liver failure and may be considered therefore as a model of “pure” hyperammonemia.

Learning of a conditional discrimination task in a Y maze is impaired in these rats with chronic hyperammonemia without liver failure (Aguilar et al 2000) as well as in rats with chronic liver failure due to portacaval anastomosis (Erceg et al 2005a).

In patients with liver disease, increase in hyperammonemia worsens (and reduction of hyperammonemia improves) neuropsychiatric and neurological scores in patients with HE.

In summary the above studies show that animal models of HE reproduce some of the neurological alterations found in patients with HE and are therefore adequate to study the molecular mechanisms by which liver failure leads to these neurological alterations. Moreover, the above studies also show that chronic hyperammonemia is responsible for at least some of the cognitive alterations observed in HE.

## Molecular mechanisms modulating learning

The studies summarized above show that cognitive function and learning ability are impaired both in patients with liver disease and HE (either minimal, non clinical HE) and in animal models of hyperammonemia and liver failure.

To try to develop appropriate therapeutic treatments to restore cognitive function it is important to understand the molecular mechanisms that modulate learning ability as well as the mechanisms by which these processes are altered in hyperammonemia and HE. The molecular bases for different

types of learning are not well known. It has been proposed that *N*-methyl-D-aspartic acid (NMDA) receptors play a crucial role in some types of learning. However, the molecular mechanisms by which activation of NMDA receptors modulate learning remain unclear. Activation of NMDA receptors leads to increased calcium in the post-synaptic neuron. Calcium binds to calmodulin and activates neuronal nitric oxide synthase, increasing nitric oxide (NO), which activates soluble guanylate cyclase, increasing guanosine 3',5'-(cyclic)phosphate (cGMP). Part of this cGMP is released to the extracellular space (Figure 1). Several reports suggest that activation of this glutamate–NO–cGMP pathway is involved in some forms of learning (Danysz et al 1995; Chen et al 1997; Meyer et al 1998).

## NMDA receptors modulate some kinds of learning

There is a large amount of evidence that activation of NMDA receptors is involved in some forms of learning. We summarize below some of the data available.

The most frequent experimental approach to analyze the role of NMDA receptors in learning processes is the study of the effects of blocking NMDA receptors with selective antagonists on the ability of experimental animals to learn different kinds of tasks.

This kind of study has shown that both competitive and noncompetitive antagonists of NMDA receptors alter learning and memory processes in a Y-maze (Parada-Turska and Turski 1990; Maurice et al 1994). Administration of NMDA receptor antagonists also alters learning of different kinds of tasks: passive avoidance (Riekkinen et al 1996; Smith et al 1997), active avoidance (Delay 1996; Redolat et al 1998), spacial tasks (Murray and Ridley 1997), classical conditional tasks (Xu 1997), and 14-unit T-maze (Patel et al 1998) and spatial learning in the Morris Water Maze (Morris et al 1986; Packard and Teacher 1997).

In addition to the studies using antagonists, there are genetic studies that support a role of NMDA receptors in certain types of learning (McHugh et al 1996; Tsien et al 1996; Huerta et al 2000; Rampon and Tsien 2000). Mice that do not express the NMDA receptor subunit NR2A have decreased spatial learning ability (Sakimura et al 1995). On the other hand, overexpression of the NR2B subunit in transgenic mice increases NMDA receptor activation and improves learning and memory (Tang et al 1999).

It is therefore clear that NMDA receptors play a role in learning. The subsequent steps by which activation of NMDA receptors mediates learning processes are not so

clear. Some reports suggest that activation of the glutamate–NO–cGMP pathway associated to NMDA receptors is involved in some forms of learning.

## Role of NO, soluble guanylate cyclase, and cGMP in learning

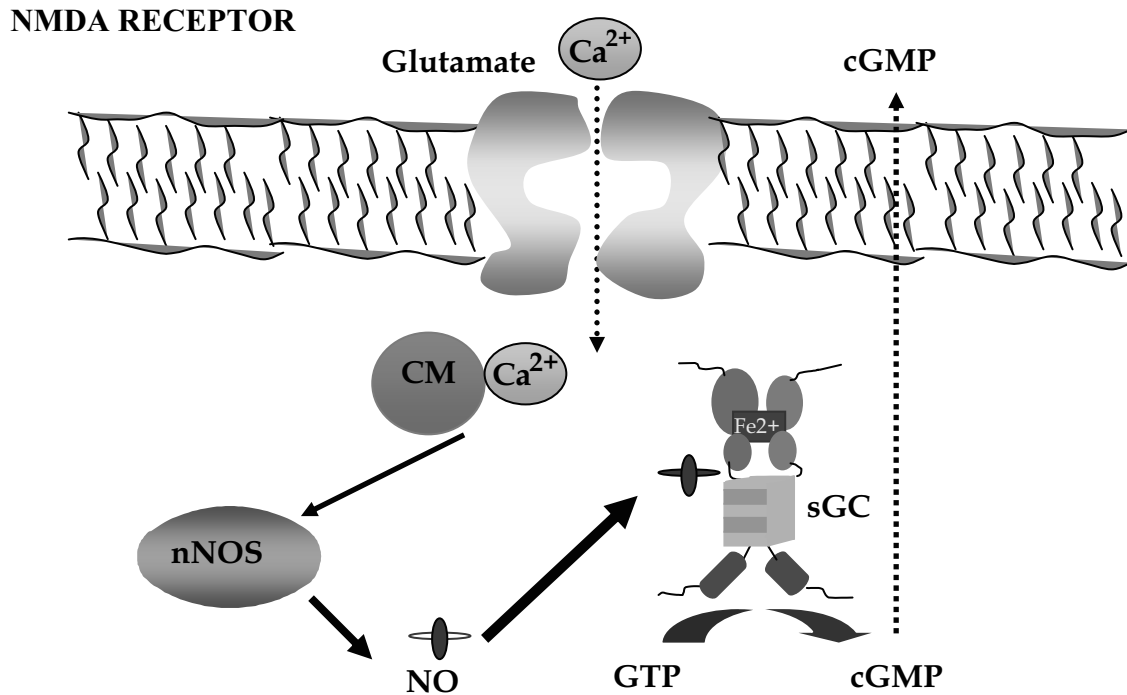
Activation of NMDA receptors leads to activation of NO synthase and to increased formation of NO. NO seems to mediate at least part of the role of NMDA receptors in learning. This is supported by studies showing that inhibition of NO synthase reduces learning of some spatial tasks: 14-unit T-maze (Ingram, Spangler, Kametani, et al 1998; Ingram, Spangler, Meyer, et al 1998) or radial maze (Zou et al 1998). It also impairs memory consolidation in objects recognition tasks (Prickaerts et al 1997) and learning of passive avoidance tasks (Myslivecek 1997).

The role of NO in learning may be mediated by its activation of soluble guanylate cyclase and the increase in cGMP. Some reports indicate that soluble guanylate cyclase and cGMP are important in learning and memory. Bernabeu et al showed that, in rats, passive avoidance learning was associated with a time-dependent, learning-specific increase in cGMP (Bernabeu et al 1996) and in cGMP-dependent protein kinase activity in the hippocampus (Bernabeu et al 1997).

The same group also showed that administration of a membrane permeable analog of cGMP facilitated memory consolidation (Bernabeu et al 1996), while bilateral intrahippocampal administration of an inhibitor of soluble guanylate cyclase caused full amnesia for inhibitory avoidance learning when given immediately after training (Bernabeu et al 1997). These results support a role for soluble guanylate cyclase and cGMP in learning and memory.

Smith et al (2000) tested later whether activation of soluble guanylate cyclase and increased cGMP formation in the brain would improve learning in cognitively impaired animals. They showed that a nitrate ester that activates soluble guanylate cyclase improved learning in scopolamine-pretreated animals in a time- and dose-dependent manner. The authors suggested that stimulation of cerebral soluble guanylate cyclase might be an effective strategy to improve learning and memory performance in individuals in whom cognitive abilities are impaired.

Yamada et al (1996) also showed that blocking NMDA receptors with dizocilpine or inhibiting NO synthase impaired spatial working memory in mice and suggested that the reduction in NO–cGMP production in the brain may



**Figure 1** The glutamate–NO–cGMP pathway. Activation of ionotropic (mainly NMDA) glutamate receptors leads to increased intracellular calcium ( $\text{Ca}^{2+}$ ) which, after binding to calmodulin (CM), activates neuronal nitric oxide synthase (nNOS) leading to increased production of NO, which in turn activates soluble guanylate cyclase (sGC), resulting in increased formation of cyclic 3'-5'-guanosine monophosphate (cGMP) from guanosine-5'-triphosphate (GTP). Part of the cGMP formed is released to the extracellular space.

be responsible for dizocilpine-induced impairment of learning.

On the other hand, administration of zaprinast, a selective inhibitor of the phosphodiesterase that degrades cGMP, improves early stages of object recognition memory consolidation (Prickaerts et al 1997). Microinjection of 8 Br-cGMP, a membrane permeable analog of cGMP, into the dorsal hippocampus also improves performance in object recognition tasks (Prickaerts et al 2002).

These data indicate that the increase in cGMP produced by guanylate cyclase in response to NO has a crucial role in some types of learning.

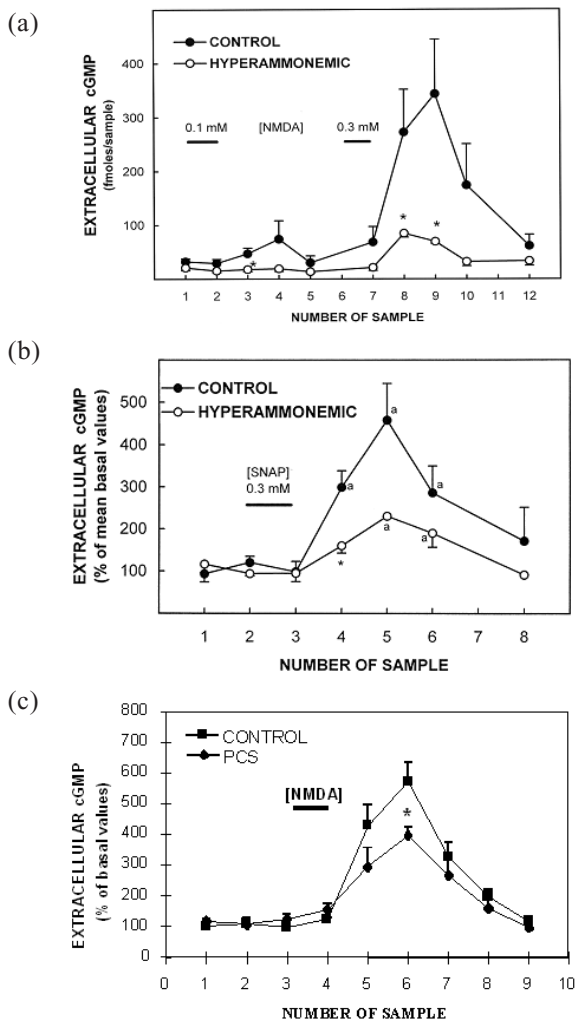
### Chronic hyperammonemia with or without liver failure impairs glutamate–NO–cGMP pathway in rat cerebellum in vivo

Chronic moderate hyperammonemia in rats, similar to that present in patients with liver cirrhosis, impairs the glutamate–NO–cGMP pathway in the cerebellum in vivo, as shown by brain microdialysis in freely moving rats by Hermenegildo et al (1998). Microdialysis probes were inserted in the cerebellum of control or hyperammonemic rats without liver failure. Administration of NMDA through

the microdialysis probe activates the glutamate–NO–cGMP pathway and increases cGMP formation. Part of the cGMP formed is released to the extracellular fluid (Figure 1), and the increase in extracellular cGMP is a good measure of the function of the glutamate–NO–cGMP pathway in the cerebellum in vivo. Hermenegildo et al (1998) showed that the NMDA-induced increase in extracellular cGMP in the cerebellum was significantly lower in hyperammonemic rats than in control rats, indicating that chronic hyperammonemia impairs the glutamate–NO–cGMP pathway in the rat cerebellum in vivo (Figure 2a).

To assess whether the impairment occurs at the level of activation of soluble guanylate cyclase by NO, a NO-generating agent, *S*-nitroso-d,l-penicillamine (SNAP), was administered through the microdialysis probe to activate directly guanylate cyclase. The increase in extracellular cGMP induced by SNAP was also significantly reduced in hyperammonemic rats (Figure 2b). This indicates that chronic moderate hyperammonemia impairs activation of soluble guanylate cyclase by NO in the cerebellum in vivo, resulting in impairment of the glutamate–NO–cGMP pathway.

Chronic liver failure, induced by portacaval anastomosis, also impairs the glutamate–NO–cGMP pathway in the cerebellum in vivo, as shown by brain microdialysis in freely moving rats by Monfort et al (2001) (Figure 2c). NMDA-



**Figure 2** Hyperammonemia impairs the function of the glutamate–NO–cGMP pathway in the cerebellum *in vivo* by reducing NO-induced activation of soluble guanylate cyclase. The effects of NMDA or SNAP on extracellular cGMP in the cerebellum of control, hyperammonemic rats without liver failure (2a and 2b) and with chronic liver failure (2c) were analyzed by *in vivo* microdialysis in freely moving rats. NMDA (0.1 and 0.3 mM) or SNAP (0.3 mM) was administered in the perfusion stream for 20 minutes at the times indicated by the horizontal bars. Perfusion was carried out at 3  $\mu$ L/minute, samples were collected every 20 minutes, and cGMP was determined. Data are presented as a percentage of basal values. Data were analysed using two-way ANOVA.

<sup>a</sup> intragroup comparison values significantly different from baseline ( $p < 0.05$  by Kruskal-Wallis test with post hoc Dunnett's test; \* values significantly different from control ( $p < 0.05$ ).

Figure 2a and 2b from: Hermenegildo C, Montoliu C, Llansola M, et al. 1998. Chronic hyperammonemia impairs glutamate-nitric oxide-cyclic GMP pathway in cerebellar neurons in culture and in the rat *in vivo*. *Eur J Neurosci*, 10:3201–9; Figure 2c from: Monfort P, Corbalán R, Martínez L, et al. 2001. Altered content and modulation of soluble guanylate cyclase in the cerebellum of rats with portacaval anastomosis. *Neuroscience*, 104:1119–25.

induced increase in extracellular cGMP in the cerebellum was significantly lower in rats with portacaval anastomosis than in control rats, indicating that chronic liver failure impairs the function of the glutamate–NO–cGMP pathway in the rat cerebellum *in vivo*.

These results indicate that the function of the glutamate–NO–cGMP is altered in brain *in vivo* in animal models of

chronic liver failure and of chronic hyperammonemia. Moreover, the step of the pathway mainly affected is the activation of soluble guanylate cyclase by NO.

## Modulation of soluble guanylate cyclase by NO is altered in cerebral cortex and cerebellum of patients who died from HE

To assess whether activation of soluble guanylate cyclase by NO is also altered in cirrhotic patients with HE, we measured activation of soluble guanylate cyclase by the NO-generating agent SNAP in homogenates of the frontal cortex or cerebellum from controls and from cirrhotic patients who died from hepatic coma.

The activation of guanylate cyclase by the NO-generating agent SNAP was significantly lower (63%) in the cerebellum from cirrhotic patients than in controls. In contrast to the cerebellum results, activation of guanylate cyclase by NO was higher (about 200% of controls) in the frontal cortex from patients than in controls (Corbalán et al 2002).

The above results show that activation of soluble guanylate cyclase by NO is altered both in the cerebral cortex and cerebellum of patients who died from hepatic coma. However, the effects are opposite in these areas, showing increased activation in the cortex and decreased activation in the cerebellum (Corbalán et al 2002).

The mechanism by which the same pathological situation (liver cirrhosis) can lead to opposite effects in the cerebellum and cerebral cortex is not yet clear. The results obtained suggest that some intrinsic factor in the neurons, different in each cerebral area, may lead to opposite responses to ammonia of the modulation of guanylate cyclase by NO.

Differential modulation of other processes in the cerebellum and cortex has been previously reported. For example, activation of NMDA receptor induces choline release in cortical neurons but not in cerebellar neurons in culture and this release has been related to excitotoxic cell death (Gasull et al 2000). Also, the regulatory subunits of adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinases are differentially expressed in cortical and cerebellar neurons leading to a differential ability of these neurons to transmit cAMP signals to the nucleus (Paolillo et al 1999). Also the role of neuronal NO in the coupling between cerebral blood flow and local neuronal activity is different in these cerebral areas. NO is important in neurovascular coupling in the cerebellum but its role in the neocortex is less important, and it seems that in this area

other neurotransmitters are more relevant in neurovascular coupling (Hayashi et al 2002). It is not surprising therefore that liver cirrhosis may affect differentially the modulation of soluble guanylate cyclase in the cerebellum and cortex.

### **Animal models of chronic hyperammonemia and of liver failure reproduce faithfully alterations in modulation of guanylate cyclase by NO found in brain of cirrhotic patients**

To study the molecular mechanisms responsible for the alterations of modulation of guanylate cyclase by NO in the cerebral cortex and cerebellum of patients with liver cirrhosis and to test possible therapeutic treatments to reverse this alteration, it is important to have animal models that faithfully reproduce the effects found in human patients. We assessed whether animal models of hyperammonemia with or without liver failure reproduce the alterations in the modulation of guanylate cyclase by NO.

Rats with chronic liver failure due to portacaval anastomosis faithfully reproduce the increased activation of soluble guanylate cyclase by NO in the cerebral cortex and the reduced activation in the cerebellum (Monfort et al 2001; Corbalán et al 2002).

Rats with bile duct ligation plus hyperammonemia also reproduce the alterations in the modulation of soluble guanylate cyclase both in cerebral cortex and in the cerebellum (Rodrigo et al 2005). The reduced activation in the cerebellum is also reproduced in rats with chronic hyperammonemia without liver failure (Rodrigo et al 2005).

These results show that animal models reproduce faithfully the alterations in the modulation of guanylate cyclase by NO found in brain of patients who died from HE due to liver cirrhosis. Other forms of HE such as Reye's syndrome or Wilson's disease are much less frequent and have been less studied, and it is not yet possible to compare the neurobiological alterations in these situations with those of cirrhotic patients or to assess the validity of the animal models to study these disorders.

These animal models are therefore adequate to:

- 1) study the molecular mechanisms by which liver failure alters the function of the glutamate–NO–cGMP pathway;
- 2) assess the functional consequences of this alteration and its contribution to the neurological alterations in HE, for example in learning ability;

- 3) test possible therapeutic treatments to normalize the function of the glutamate–NO–cGMP pathway and to normalize the altered neurological functions, for example learning ability.

### **Pharmacological manipulation of extracellular cGMP concentration in brain restores learning ability in rats with chronic liver failure or chronic hyperammonemia**

The data summarized above show that:

- a) cognitive function and learning ability are impaired in patients with liver disease and in animal models of chronic liver failure or of chronic hyperammonemia;
- b) the glutamate–NO–cGMP pathway modulates some forms of learning;
- c) the function of the glutamate–NO–cGMP pathway is impaired in brain in vivo in animal models of chronic hyperammonemia and of chronic liver failure and also in autopsied brain from patients who died from HE

We hypothesized that:

- a) the alterations in the function of the glutamate–NO–cGMP pathway and the decrease in extracellular cGMP in the brain in hyperammonemia and liver disease may be responsible for the impairment in learning ability and intellectual function, and that
- b) pharmacological modulation of extracellular cGMP concentration may restore learning ability in hyperammonemia and HE.

To assess this possibility we tried to reverse the impairment in learning ability of hyperammonemic rats by increasing cGMP. We increased extracellular cGMP by using three different treatments:

- 1) continuous intracerebral administration of zaprinast, an inhibitor of the phosphodiesterase that degrades cGMP;
- 2) chronic oral administration of sildenafil, an inhibitor of the phosphodiesterase that crosses the blood–brain barrier, and
- 3) continuous intracerebral administration of cGMP.

### **Continuous intracerebral zaprinast increases extracellular cGMP and restores learning ability in hyperammonemic rats**

We tested whether increasing cerebral cGMP by inhibiting its degradation is able to restore learning ability in

hyperammonemic rats. We administered intracerebrally zaprinast, an inhibitor of the phosphodiesterase that degrades cGMP, to control or hyperammonemic rats, continuously for 28 days, by using osmotic minipumps. Extracellular cGMP was significantly reduced in hyperammonemic rats and treatment with zaprinast increased extracellular cGMP to the same level present in control rats.

We carried out tests of conditional discrimination learning with control and hyperammonemic rats treated or not with zaprinast. Learning ability was significantly reduced in hyperammonemic rats. Continuous intracerebral administration of zaprinast to hyperammonemic rats completely restored the learning ability of these rats (Erceg et al 2005b). This indicates that increasing cGMP by inhibiting its degradation restores learning ability in hyperammonemic rats.

The changes in extracellular cGMP are parallel to changes in learning ability, supporting a role for cGMP levels in learning ability.

### **Oral sildenafil normalizes function of glutamate–NO–cGMP pathway and extracellular cGMP and restores learning ability in rats with chronic liver failure or with hyperammonemia without liver failure**

As shown above, zaprinast is effective in restoring learning ability in hyperammonemic rats, but it does not cross the blood–brain barrier and is therefore not suitable for clinical treatment of patients.

We therefore studied whether oral administration of sildenafil, an inhibitor of the phosphodiesterase that degrades cGMP and crosses the blood–brain barrier, restores learning ability in rats with chronic liver failure or with chronic hyperammonemia without liver failure.

Chronic oral treatment with sildenafil normalizes the function of the glutamate–NO–cGMP pathway and extracellular cGMP in rats with portacaval anastomosis and also restored the ability of rats with portacaval anastomosis to learn the Y maze conditional discrimination task (Erceg et al 2005a).

Hyperammonemia is one of the main factors contributing to the neurological alterations in HE. To assess the role of hyperammonemia in the alterations in learning ability, extracellular cGMP, and function of the glutamate–NO–cGMP pathway observed in rats with portacaval anastomosis, we carried out experiments similar to those reported

above using rats with chronic moderate hyperammonemia without liver failure.

Chronic hyperammonemia significantly reduced the ability of rats to learn the conditional discrimination task. Treatment with sildenafil normalized the function of the glutamate–NO–cGMP pathway and extracellular cGMP in hyperammonemic rats without liver failure and restored their ability to learn the conditional discrimination task (Erceg et al 2005a).

### **Continuous intracerebral administration of cGMP restores learning ability in hyperammonemic rats**

To further confirm that changes in extracellular cGMP are responsible for the changes in learning ability, we tested whether increasing only extracellular cGMP without affecting intracellular cGMP is also able to restore learning ability in hyperammonemic rats. To do this we administered cGMP intracerebrally to control or hyperammonemic rats, continuously for 28 days, by using osmotic minipumps. cGMP is not able to cross the cellular membrane and therefore increases cGMP only in the extracellular fluid. We carried out tests of conditional discrimination learning with control and hyperammonemic rats treated or not with cGMP. Continuous intracerebral administration of cGMP to hyperammonemic rats completely restored the learning ability of hyperammonemic rats.

The results summarized above clearly point out a role for extracellular cGMP concentration in the ability of rats to learn the Y maze task. Changes in extracellular cGMP are parallel to changes in learning ability, supporting a correlation between cGMP levels and learning ability.

The possible mechanisms by which extracellular cGMP would modulate learning are not yet clear. Only a few reports suggest some physiological role for extracellular cGMP as a neuroprotector against excitotoxicity (Montoliu et al 1999) and as a modulator of sodium uptake in kidney (Sasaki et al 2004). However, the mechanisms involved remain unclear.

### **Conclusion**

In conclusion, the above data indicate that the impairment of learning ability in hyperammonemic rats with or without chronic liver failure is due to impairment of the glutamate–NO–cGMP pathway. As the function of this pathway is also altered in the brain of patients with liver cirrhosis, this alteration should also contribute to the cognitive impairment in these patients.



Moreover, increasing extracellular cGMP by pharmacological means may be a new therapeutic approach to improve learning and memory performance in individuals in whom cognitive abilities are impaired for different reasons, for example, in patients with evident HE and also in patients with minimal (subclinical) HE who present reduced performance in psychometric tests.

The modulation of extracellular cGMP can be achieved by different means: modulation of its synthesis by soluble or particulate guanylate cyclase or of its degradation by phosphodiesterases. There are already pharmacological tools to apply these treatments to patients. Several companies are selling inhibitors of phosphodiesterases that may increase extracellular cGMP and are also investigating new molecules that may modulate cGMP levels. The inhibitors of cGMP-degrading phosphodiesterase (mainly of phosphodiesterase 5) used in the animal studies described above are currently being used for the clinical treatment of erectile dysfunction and of pulmonary hypertension. In fact, sildenafil is the active component of Viagra®.

However, in the case of cirrhotic patients, caution must be taken considering the possible deleterious increase in the existing vasodilatation in liver disease by sildenafil (Tzathas et al 2002). It has also been reported that in patients with ascites and cirrhosis, inhibition of phosphodiesterase 5 leads to increased plasma levels of the renin–angiotensin–aldosterone system (Thiesson et al 2005). Therefore additional studies seem to be required to find some safe procedure to increase extracellular cGMP in the brain without inducing deleterious effects in peripheral tissues. Pharmacological manipulation of cGMP in the brain by safe procedures may be a useful treatment to restore cognitive and intellectual functions in patients with overt or minimal HE.

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