Study of the regulatory mechanisms of glycogen metabolism, its alterations in pathologies and characterisation of new therapeutic targets

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Our group is devoted to the study of the regulatory mechanisms of glycogen metabolism. We have a long tradition in the study of glycogen synthase (GS), the key enzyme in the regulation of glycogen synthesis. To this end we combine our knowledge of biochemistry and metabolism with a wide variety of techniques from molecular biology, cell biology, proteomics, RNA silencing, gene transfer, mutant mouse generation and structural biology, thereby allowing us to address biological issues using a multidisciplinary approach. We have made a series of discoveries that have contributed to revitalizing the field of glycogen metabolism. Our main achievements during 2007 are summarized below.

Neurons have the enzymatic machinery to accumulate glycogen, which is pro-apoptotic for this cell type

Although glycogen is present in most cells, its metabolism has been studied mainly in liver and muscle. Nevertheless, there are some cell types that do not accumulate this polysaccharide, like neurons. The central nervous system is an interesting case regarding glycogen metabolism. In embryonic stages, glycogen appears both in glial and neuronal cells but in adults this polysaccharide is present exclusively in astrocytes. Although the total concentration of glycogen in brain is lower than in muscle or liver, glycogen is a crucial source of energy for neurons. It is accepted that neurons, through neurotransmitters and neuromodulators, stimulate the mobilization of astrocyte glycogen reserves, which are converted into lactate to be taken up and utilized by neurons.

GS is the only enzyme able to synthesise glucose polymers in mammals. We have recently demonstrated that neurons express GS, specifically the muscle isoenzyme of GS (MGS). This is a remarkable finding since, as noted above, these cells do not normally accumulate glycogen. Our results also show that neurons have the capacity to synthesise glycogen when MGS becomes active. Since these cells do not express glycogen phosphorylase (the key enzyme for glycogen degradation) once glycogen is synthesised it cannot be degraded. Interestingly, the glycogen accumulated in neurons in these conditions is poorly branched (Vilchez *et al*, 2007). Furthermore, the deposition of this glycogen is pro-apoptotic. Therefore, when synthesised inside the neurons, glycogen acts as a Trojan horse, triggering mechanisms that lead to neuronal death. The concept that glycogen is harmful for neurons completely changes our vision of the field. A review of the literature indicates that the presence of glycogen in neurons in certain neurological diseases was reported long ago. Moreover, the presence of intracellular bodies composed mainly of glucose polymers has been recognised in many pathologies. The nomenclature used to describe these structures is varied: polyglucosan bodies, corpora amylacea and Lafora bodies among others, although all these structures have in common that they are essentially formed by poorly branched glycogen (Vilchez *et al*, 2007).

New regulatory mechanism of glycogen metabolism: laforin-malin complex blocks glycogen synthesis in neurons by inducing the proteasome-dependent degradation of MGS and PTG

We have shown that although neurons have the enzymatic machinery for synthesising glycogen, it is kept silent by a series of well-coordinated intracellular mechanisms: a) GS is confined in the nucleus; b) GS is fully inactivated by phosphorylation; and c) GS and PTG, a regulatory subunit of protein phosphatase 1 that activates GS by stimulating its dephosphorylation, are degraded by a novel regulatory mechanism involving the proteasome. Failure to keep GS under control,

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which results in glycogen synthesis, damages neurons by triggering apoptotic signalling. In addition, the glycogen that neurons synthesise is abnormal and causes the accumulation of granular deposits that cannot be mobilized. Such deposits, referred to as Lafora bodies, are characteristic of a form of progressive myoclonus epilepsy, Lafora disease (Vilchez *et al*, 2007).

The laforin-malin degradation system regulates PTG stability in hepatic cells

In collaboration with Pascual Sanz (IBV—CSIC) and Santiago Rodríguez de Córdoba (CIB—CSIC), we have also reported that the laforin-malin complex down-regulates PTG-induced glycogen synthesis in hepatic cells. Furthermore, the interaction between laforin and malin is a regulated process that is modulated by the AMP-activated protein kinase (AMPK). These data unravel a novel link between the energy sensor AMPK and glycogen metabolism (Solaz-Fuster *et al*, 2007).

Study of the mechanisms which drive glycogen accumulation in embryonic liver in the absence of glucokinase

Glucokinase (GK, hexokinase type IV) is required for the accumulation of glycogen in adult liver. During development, rat liver expresses hexokinase type I (HKI) and hexokinase type II (HKII) but not GK. It is when pups are weaned that the first solid carbohydrate-rich ingestion triggers an insulin peak, which stimulates the insulin-dependent promoter of GK in liver. Surprisingly, mammalian embryonic livers accumulate glycogen despite the absence of GK expression. We have addressed how mammalian embryonic livers, but not adult livers, manage to accumulate glycogen in the absence of this enzyme. Although HKI or HKII would not normally have the capacity to build up high enough levels of glucose-6-phosphate to activate the liver isoform of GS (LGS), embryonic livers choose to express massive levels of HKI and HKII. In these conditions HKI and HKII can synthesise sufficient amounts of glucose-6-phosphate to activate LGS and consequently hepatic glycogen synthesis. Our results in fasted pregnant mice provide a teleological explanation for the hexokinase reorganisation in embryonic liver. Glycogen plays a key role during embryonic development as it ensures pup survival in the period of time between birth and first receiving their mother's milk. Pups use glycogen deposits as a ready source of energy at the moment of birth and need to build their glycogen reserves during development in such a way that they do not depend on their mother's feeding state. In this scenario, by greatly increasing the expression of the high affinity hexokinases HKI and HKII, embryos not

only ensure their capacity to use glucose in all circumstances, but at the same time they may produce enough glucose-6-phosphate to ensure hepatic glycogen accumulation even if the mother is fasting and blood glucose levels decrease. By this mechanism, embryos safeguard their liver glycogen stores and thereby provide a crucial advantage at the moment of birth (Cifuentes *et al*, 2008).

Study of the effects of sodium tungstate and its possible application in the treatment of diabetes, obesity and neurodegenerative diseases

Tungstate is an oral glucose-lowering and anti-obesity agent discovered by our group. This compound has a low toxicity profile in animals and humans, and has successfully completed Phase I clinical trials and is currently undergoing Phase II clinical trials. Tungstate normalises carbohydrate metabolism in liver, stimulates insulin secretion, and regenerates pancreatic beta-cells in neonatally streptozotocin-treated diabetic rats. Tungstate is also efficient in ZDF rats, a genetic model of type 2 diabetes. Much effort has been devoted in the study of the physiological and metabolic actions of tungstate but little information is available on its molecular targets. To this end, we have focussed on the study on the actions of this compound at the molecular level. Our previous results show that tungstate treatment stimulates glycogen synthesis, which is correlated with a transient activation of ERK1 and ERK2. We now have consistent data showing that this compound activates glycogen synthesis through a non-canonical mechanism involving G-proteins (Gα and G$\beta\gamma$ subunits) and ERK phosphorylation (Zafra *et al*, in preparation).

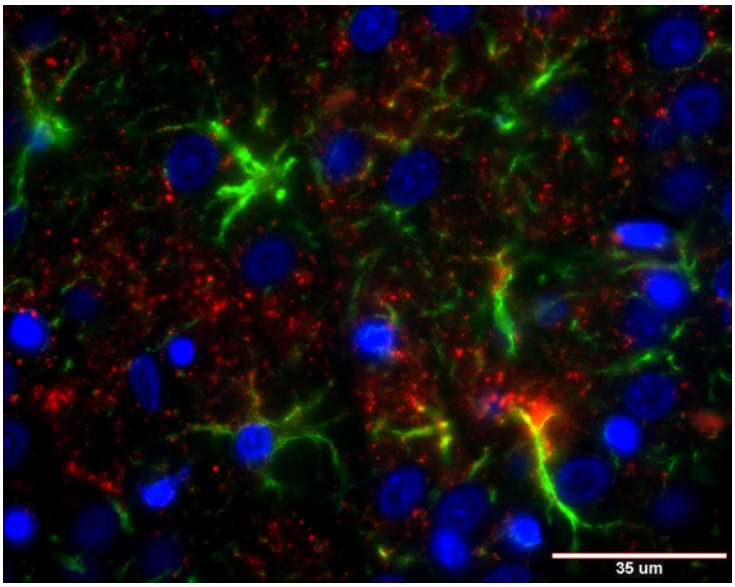

***Figure 1**. Analysis of the distribution of glycogen in rat brain cortex by immunofluorescence. Glycogen (red), astrocytes (green) and nuclei (blue).*

In addition, in collaboration with the group directed by Ramon Gomis (IDIBAPS, Barcelona), we have shown that tungstate also induces phosphorylation and subsequent activation of p38 and PI3K in insulin-secreting cells (Piquer *et al*, 2007). In collaboration with Rafael Salto (University of Granada), we have shown that tungstate increases glucose transport in muscle through a MEF-dependent mechanism (Salto *et al*, in press).

In collaboration with the group directed by Joan Enric Rodríguez Gil from the Veterinary Science Faculty at the Universitat Autònoma de Barcelona, we have shown that tungstate administration improves the sexual and reproductive function in male and female diabetic rats (Ballester *et al*, 2007).

In collaboration with Jesus Avila (CBM-CSIC) and Ramon Gomis, we have performed a study of the effects of tungstate treatment on tau protein, which is involved in Alzheimer's disease. We have demonstrated that tungstate reduces tau phosphorylation in sites that are involved in the aggregation of this protein, which occurs in this disease.

This research line has produced three patents which protect the use of sodium tungstate for the treatment of diabetes, obesity and neurodegenerative diseases. The rights of the first two have been transferred to Bayer.

Publications

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Research Networks and Grants

Ayudas para potenciar y dar soporte a los grupos de investigación
Generalitat Catalunya, 2005-SGR0570: 2005-2009
Project Coordinator: Joan J Guinovart

Efectos del tungstato sobre el síndrome metabólico: Análisis de acciones a nivel plasmático, hepático y muscular. Determinación de dianas terapéuticas
Instituto de Salud Carlos III, ISCIII-PI042402: 2005-2008
Project Coordinator: Joan J Guinovart

Estudio de las alteraciones en la homeostasis iónica e implicación de las proteínas G en el mecanismo de acción del agente antidiabético tungstato de sodio
Ministerio de Educación y Ciencia, SAF 2007-64722: 2007-2008
Project Coordinator: Joan J Guinovart

Investigación de las dianas terapéuticas del agente anti-diabético oral de tungstato de sodio
Ministerio de Educación y Ciencia, SAF2004-06962: 2004-2007
Project Coordinator: Joan J Guinovart

Red de Diabetes y Enfermedades Metabólicas Asociadas
Instituto de Salud Carlos III, Ministerio de Sanidad, RD06/0015/0030: 2007
Project Coordinator: Ramon Gomis de Barbará (network coordinator)

Regulación del metabolismo del glucógeno hepático, muscular y neuronal. Alteraciones en situaciones patológicas
Ministerio de Educación y Ciencia, BFU2005-2253/BMC: 2005-2008
Project Coordinator: Joan J Guinovart

Relación del síndrome diabético con la expresión y localización celular de la fructosa 1,6-Bifosfatasa y la glucógeno sintasa, enzimas claves en la homeostasis de la glucosa
Secretaría de Estado de Cooperación Internacional, Ministerio de Asuntos Exteriores, A/6647/06: 2007-2008
Project Coordinator: Joan J Guinovart

Other Funding Sources

Enfermedad de Lafora: papel de laforina y malina
Fundación La Caixa, BM06-340-1: 2007-2009
Project Coordinator: Joan J Guinovart, Santiago Rodríguez de Córdoba

Mejora de la predicción traslacional de los ensayos de seguridad no clínica al hombre
Neuropharma, Consorcio Melius (CENIT project): 2007-2011
Project Coordinator: Joan J Guinovart

Molecular basis of progressive myoclonus epilepsy of the Lafora type
Fundación La Marató de TV3, 061930: 2007-2009
Project Coordinator: Joan J Guinovart, Santiago Rodríguez de Córdoba, Pascual Sanz Bigorra

Nuevos fármacos y dianas para el tratamiento de la diabetes mellitus
Fundación Marcelino Botín: 2006-2010
Project Coordinator: Joan J Guinovart

Collaborations

Analysis of the toxicity and anti-diabetic potential of GSK3 inhibitors
Neuropharma (Madrid, Spain)

Characterisation of glycogen metabolism in reproductive tissue: analysis of alterations in pathological situations
Joan E Rodríguez-Gil, Universitat Autònoma de Barcelona (Barcelona, Spain)

Characterisation of the anti-diabetic and anti-obesity actions of tungstate
Ramon Gomis, IDIBAPS-Hospital Clínic (Barcelona, Spain)

Determination of the 3D structure of the glycogen synthases
Joan Carles Ferrer, University of Barcelona (Barcelona, Spain) and Ignasi Fita, IRB Barcelona (Barcelona, Spain)

Laser induced forward transfer: a direct writing technique for biosensors preparation
José L Morenza, University of Barcelona (Barcelona, Spain)

Molecular basis of Lafora disease
Santiago Rodríguez de Córdoba, Centro de Investigaciones Biológicas, CSIC (Madrid, Spain) and Pascual Sanz, Instituto de Biomedicina de Valencia, CSIC (Valencia, Spain)

Molecular dissection of the mechanisms of action of the anti-diabetic agent sodium tungstate in skeletal muscle
Rafael Salto, Universidad de Granada (Granada, Spain)

Study of the actions of sodium tungstate on the ionic homeostasis
Miguel A Valverde, Universitat Pompeu Fabra (Barcelona, Spain)

Study of the anti-diabetic actions of tungstate on diabetes induced by immunosupressant treatment
Armando Torres, Hospital Universitario de Canarias (Canary Islands, Spain)

Patents

Método de identificación de compuestos para terapia de enfermedades relacionadas con la acumulación de glucógeno y uso de compuestos para preparar medicamentos contra dichas enfermedades
Patent application number: P200702755
IRB Barcelona (2007)

Awards

Diplôme d'Honneur, Federation of European Biochemical Societies (2007)
Awardee: Joan J Guinovart