Lake Como School of Advanced Studies - Summer School

Watching at the "D" side:
D-amino acids and their significance in neurobiology

5-9 June 2016
Università degli Studi dell'Insubria

Venue: Villa del Grumello, Via per Cernobbio, 11
22100, Como - ITALY

Site link at : http://dasn.lakecomoschool.org/

Topics:

Sunday, june 5th - Introducing lecture

Lecture Loredano Pollegioni h 14.00-15.30

D-amino acids: description and significance

Every amino acid (except glycine) can occur in two forms because of the possibility of forming two different enantiomers (stereoisomers) around the central carbon atom. This property is known as "chirality", i.e. when a compound has four different functional groups around a central carbon atom it will generate two non super-imposable mirror images, designated as right-handed (D) and left-handed (L). Before the emergence of life, both D- and L-amino acids should have existed on the primitive earth.

Until recently, living organisms were thought to contain mostly L-amino acids, which clearly predominate in Nature since they are the main components of proteins, and D-amino acids have been thought to have relatively minor functions in biological processes. The development of novel analytical methods has revealed that D-amino acids are present in many organisms such as invertebrates and vertebrates including humans, and that some of D-amino acids have important physiological functions. E.g., D-amino acids are found in proteins that are not synthesized by ribosomes - especially as components of certain peptide antibiotics - and in the peptidoglycan cell wall of certain microorganisms. Recent findings show that D-amino acids have previously unappreciated regulatory roles in the bacterial kingdom (D-amino acids govern cell wall remodeling and biofilm disassembly) as well as in mammals (D-serine and D-aspartate are important neuromodulators). Indeed, biochemical specific systems for synthesis and degradation of D-amino acids have also been found and characterized.

Monday, june 6th – D-amino acids metabolism

Lecture Stefano Bruno h 09.00-10.30

Serine racemase: biochemistry, regulation and inhibition

Serine racemase is the enzyme that catalyzes both the synthesis and degradation of D-serine, an obligatory co-agonist of the glutamatergic NMDA receptors. The fine tuning of serine racemase depends on a number of orthosteric and allosteric effectors, including divalent cations, ATP, NADH and glycine. The seminar will focus on the reactivity of pyridoxal phosphate in serine racemase and on the exploration of its conformational space by enzymatic and spectrofluorimetric methods. The discovery of novel inhibitors and the strategies for the identification of activators will be discussed.

Lecture Herman Wolosker h 11.00-12.30

D-Serine synthesis – serine racemase regulation

The seminar will consist of an overview about the mechanisms regulating D-serine production ranging from protein interactors, covalent modifications, subcellular targeting of serine racemase. Furthermore, we will present data supporting the role of the serine shuttle between neurons and astrocytes, whereby astrocytes export L-serine required for the neuronal production of D-serine.

Lecture Silvia Sacchi h 14.00-15.30

D-Serine metabolism: spotlights on D-amino acid oxidase

D-amino acid oxidase (DAAO) has been considered the paradigm of the dehydrogenase-oxidase class of FAD-containing flavoproteins. It catalyzes the oxidative deamination of D-amino acids and is significantly enriched in the mammalian brain, where it has been proposed to play (with serine racemase) an essential role in the catabolism of D-serine. Notably, the cellular concentration of this allosteric activator of the NMDA receptor (NMDAr) depends on the expression of active DAAO. Alterations in the DAAO functionality and/or its cellular levels/localization might contribute to D-serine-mediated signaling dysregulation and the associated NMDAr dysfunctions that occur in several pathological conditions, including neurodegenerative diseases and psychiatric disorders.

Here the main biochemical properties and the knowledge concerning the modulation of DAAO enzymatic activity (by inhibitors, mutations and interacting proteins) will be presented with the final aim to highlight DAAO significance as a therapeutic target.

Lecture Gianluca Molla h 16.00-17.30

Structure/function relationships in D-aspartate oxidase, the key enzyme for the modulation of D-Aspartate in the brain

D-Aspartate plays important roles in the regulation of developmental processes of the nervous system and in the modulation of the glutamatergic neurotransmission through binding, as agonist, to the L-glutamate binding site of NMDA receptors. Pathological decrease of the levels of D-Asp has been correlated to an increased susceptibility to various mental disorders, including schizophrenia.

In vivo concentration of this D-amino acid is mainly controlled by the activity of the flavoprotein D-aspartate oxidase (DASPO) which degrades it to oxaloacetate. This important role renders human DASPO an attractive therapeutic target for the design of novel high-efficient antipsychotic drugs aimed to prevent an excessive D-Asp enzymatic degradation.

The deep understanding of the structure-function relationships in this enzyme is central for the thorough comprehension of the D-Asp metabolism and for the developing of novel, extremely efficient drugs by critical computational (in silico screening) approaches.

Tuesday, june 7th – D-amino acids neurobiology

Lecture Herman Wolosker h 09.00-10.30

D-Serine transport mechaninsms

The seminar will consist of an overview on the mechanisms of D-serine release from cells. We will discuss the discrepancies in the literature regarding the different sources of D-serine and the different pathways that may control its release.

Lecture Jean-Pierre Mothet h 11.00-12.30

Co-agonists regulation of NMDAR in the developing and mature brain

The N-Methyl D-Aspartic acid (NMDA) receptors (NMDAR) are key glutamate-gated ionotropic receptors that are central for synaptic plasticity across lifespan. Activation of NMDARs always requires the binding of a co-agonist that has long been thought to be glycine. However, intense research over the last decade has have shown that D-serine is the preferential co-agonist for a large proportion of synaptic NMDARs in many areas of the adult brain. Nowadays, a totally new picture of glutamatergic synapses at work is emerging where both glycine and D-serine are involved in a complex orchestration of NMDAR functions in the CNS following temporal and spatial constraints. During my lecture, I will highlight the particular role of each co-agonist in modulating NMDAR at developing and mature central synapses of different brain regions and show how glia contacting synapses may be engaged in these modulations of synapses and neuronal networks. I will also show that the functions of D-serine and glycine at NMDARs can be pressured by specific neuromodulatory systems.

Lecture Alessandro Usiello h 14.00-15.30

D-aspartate exerts an opposing role upon age-dependent NMDAR-related synaptic plasticity and memory decay

D-Aspartate appears with a peculiar temporal pattern of localization, being abundant during embryonic development and strongly decreasing after birth. This phenomenon is the result of the postnatal onset of D-Aspartate oxidase expression, the only known enzyme that strictly controls the endogenous levels of D-Asp. The pharmacological affinity of D-Aspartate for the glutamate site of NMDARs has raised the intriguing question whether this D-amino acid may have some in vivo influence on responses mediated by this subclass of glutamate receptors. In order to unveil the physiological function of D-Aspartate and of its metabolizing enzyme, genetic and pharmacological approaches have been recently developed. It has now become possible to generate animal models with abnormally elevated levels of D-Aspartate in adulthood based on the targeted deletion of the Ddo gene and on the oral administration of D-Asp. These animal models have thus highlighted that D-Aspartate has a direct neuromodulatory role at NMDARs and influences glutamatergic system homeostasis.

Wednesday, june 8th – D-amino acids and pathologies

Lecture Jean-Marie Billard h 09.00-10.30

D-Amino acids and aging

It is now widely admitted that the encoding of new memories is closely tied to adjustments in the strength of neuronal communication through mechanisms of functional plasticity. In the last decades, D-amino acids including D-serine and D-aspartate were found to tightly control the expression of synaptic plasticity through the regulation of the NMDA subtype of glutamate receptors. This presentation will review changes affecting D-amino acid availability and functional properties in the brain during healthy and pathological aging that contribute to induce memory impairments.

Lecture Joseph T. Coyle h 11.00-12.30

D-Serine and schizophrenia

Schizophrenia is the most devastating serious psychiatric disorder, affecting approximately one percent of the population, and the seventh most costly medical condition to Society because of its life-long disability. The symptomatic onset, typically in the second decade, is heralded by psychosis, a positive symptom; but negative symptoms including anhedonia, asociality and alogia and cognitive impairments correlate best with outcome. The cerebral cortex is reduced in volume at onset of psychosis, and the atrophy progresses over the next decade. Cortical atrophy correlates with the severity of negative symptoms and cognitive impairments but not psychosis. Post-mortem studies reveal reduced dendritic complexity and reduced spine density, resulting in a ~30% reduction in glutamatergic synapses on cortical pyramidal neurons along with a down-regulation of presynaptic markers for the cortical parvalbumin positive (PV+), fast-firing GABAergic neurons. Pharmacologic inhibition of NMDA receptors in normal individuals with ketamine reproduces the positive, negative and cognitive symptoms of schizophrenia. To produce NMDA receptor hypofunction in mice, serine racemase was genetically inactivated (srr-/-), resulting in ~90% reduction in brain D-serine. The adult srr-/- exhibited cortical atrophy, a ~30% reduction in cortical glutamatergic synapses and down regulation of the cortical PV+GABAergic neurons similar to schizophrenia. Hippocampal long-term potentiation (LTP) was impaired in srr-/- but could be restored by treatment with D-serine. Consistent with reduced LTP, srr-/-, memory was found to be impaired in several paradigms including the probe task on the Morris Water Maze, sequential learning and contextual memory, which can be restored by treatment with D-serine. Double-blind, placebo-controlled clinical trials with D-serine, D-cycloserine and sarcosine in chronic schizophrenic patients on stable doses of antipsychotic drugs reduce negative, cognitive and even positive symptoms. A recent sufficiently powered genome-wide association study (GWAS) revealed a number of risk genes for schizophrenia achieving genome-wide significance (5X10-8) that cluster around the NMDA receptor including SRR. Thus, SRR and several genes affecting NMDA receptor function or mediating its post-synaptic effects on spine genesis and PV+GABAergic function play an etiologic role in schizophrenia and represent potential targets for pharmacologic treatment.

Lecture Andrea R. Durrant h 14.00-15.30

N-methyl-D-aspartate receptor (NMDAR) antibodies positive encephalopathies and D-serine

Accumulating data are rapidly leading to the characterization of specific types of autoimmune encephalopathies in which the receptors and proteins critically involved in glutamatergic neurotransmission, e.g., NMDA, AMPA receptors, are antigen targets. Characteristic of these syndromes, antibodies (Abs) cause function deficits of the corresponding neuronal antigen resulting in neuropsychiatric presentations. A proportion of clinically-diagnosed schizophrenia patients may be

seropositive for anti-NMDAR-ABs. The Abs decrease the surface density of NMDAR clusters via antibody-mediated capping and internalization resulting in decreased NMDAR-mediated synaptic currents. The seropositivity prevalence rates seem to be 1 in 10-20 patients, but may differ among patient types. We recently found that: 1) seropositive patients can be identified among chronic schizophrenia patients having illness features that are also characteristic manifestations of anti-NMDAR encephalitis and 2) NMDAR-Abs positive patients respond significantly to treatment with D-serine (DSR) which acts in vivo as NMDAR co-agonist.

Lecture Francesco Errico h 16.00-17.30

D-Aspartate and pathologies

D-Aspartate (D-Asp) is enriched in the embryonic brain and strongly decreases after birth. Temporal reduction of D-Asp levels depends on the postnatal onset of D-aspartate oxidase (DDO) enzyme, which selectively catabolizes this D-amino acid. Pharmacological evidence indicates that D-Asp binds to and activates NMDA receptors (NMDARs). Characterization of genetic (*Ddo* knockout) and pharmacological mouse models with abnormally higher levels of D-Asp has evidenced that increased D-Asp enhances hippocampal NMDAR-dependent synaptic plasticity, dendritic morphology and spatial memory. In line with the hypothesis of NMDAR hypofunction in the pathogenesis of schizophrenia, it has been shown that increased D-Asp levels in preclinical models also improve brain processes relevant to schizophrenia. Recent findings in humans encourage to hypothesize a translational value for this D-amino acid in schizophrenia.

Lecture Jumpei Sasabe h 18.00-19.30

Impaired metabolism of D-amino acids in a motor neuron disease, Amyotrophic Lateral Sclerosis'

Amyotrophic lateral sclerosis (ALS) is an adult-onset motor neuron disease, pathologically characterized by selective motoneuronal loss, neuronal aggregation, and gliosis. No remedy is known for ALS and development of therapeutic target is waited. ALS is recently regarded as a multifactorial disease and, among several etiological hypotheses, vulnerability to hyperexcitation of motoneurons via glutamate receptors is thought to be an important trigger for motoneuronal loss. This seminar reviews the role of D-serine, a coagonist for NMDA-type glutamate receptors, in developing ALS, and discusses if D-serine metabolism can work as a new drug target for ALS.

Thursday, june 9th – D-amino acids: investigation techniques

Lecture Joseph T. Coyle h 09.00-10.30

D-Serine in models of substance abuse, anxiety and stroke

NMDA receptor activity has been implicated In substance abuse, consistent with learning models and the synaptic plastic changes that occur in the nucleus accumbens. We have used the srr-/- and GlyT1-/+ mice, which exhibit increased NMDA receptor function, to explore the role of the NMDA receptor in substance abuse, using the cocaine conditioned place preference (CPP) model. Compared to wild-type (WT) mice, GlyT-/+ displayed hastened extinction of CPP and robust cocaine-induced reinstatement whereas srr-/- mice appeared to immediately "forget" the learned preference because did not exhibit cocaine-induced re-instatement and also displayed attenuated locomoter sensitization. Treatment of srr-/- with D-serine caused them to avoid the cocaine-paired side of the chamber during extinction. Using intracranial self-stimulation in which an electrode is placed in medial forebrain bundle that permits the mouse to electrically stimulate it, we found no difference between WT and srr-/- in the stimulation threshold. However, WT mice reduced their stimulation frequency with escalating doses of cocaine whereas SR-/- mice showed no response to cocaine up to near toxic doses. Thus, srr-/- exhibit anhedonia.

Considerable evidence indicates that both hippocampal and the amygdala NMDA receptors are critically involved in conditioned fear. Mice are placed in a chamber with an electrical grid floor and given a shock ten seconds after hearing a tone. Subsequently, their "fear" response is quantified by the duration of freezing when placed in the chamber (contextual) or after the tone (cue). Srr-/- mice exhibit impairments in both cue (amygdala) and context (hippocampus) induced freezing, which is reversed by D-serine treatment. Acquisition of trace fear conditioning is associated with induction of SR in both the hippocampus and the amygdala, indicating that SR is dynamically involved in learning and memory.

Pharmacologic studies have long implicated NMDA receptor function in the pathophysiology of stroke. We have used srr-/- mice with hypofunctional NMDA receptors to substantiate this inference. Cortical cultures from srr-/- mice markedly diminished nitric oxide (NO) formation (~50%) and decreased sensitivity to oxygen deprivation. Consistent with this, srr-/- brains exhibited >70% reduction of steady-state protein S-nitrosylation. Finally, srr-/- mice were significantly less vulnerable to stroke induced by middle cerebral artery ligation than WT mice.

Lecture Jonathan V. Sweedler h 11.00-12.30

Techniques to measure D-amino acids in the brain

In the nervous system of all animals studied to date, including mammals, D-amino acids are present. While both D-serine and D-aspartate are well known examples, other D-amino acids including D-alanine and D-glutamate are found in specific brain and endocrine regions. Because neurochemistry is well conserved across metazoan life, we find these molecules in a variety of animals. A range of measurement tools are described that can characterize these D-amino acids. These tools include capillary electrophoresis and capillary liquid chromatography separations, and they can characterize the chemical content of small brain regions down to individual neurons. Using capillary electrophoresis with laser induced fluorescence and mass spectrometry detection, we have determined which neurons synthesize D-Asp, and have measured the formation, transport and release of D-Asp in a stimulation dependent manner. Newer data on the presence of a range of D-amino acids in neurons and endocrine structures are highlighted, including their activity-dependent release.

Lecture Annabella Di Giorgio h 14.00-15.30

D-Aspartate as a regulator of brain activity

Neuroimaging-based phenotyping has unique potential to characterize gene effects in brain and to identify genetic mechanisms of disease and of normal human neurobiologic variation. This presentation will introduce the fundamentals of Imaging Genetics, and it will highlight the effectiveness of this strategy to elucidate biological pathways and mechanisms by which individual differences in brain function emerge and potentially bias behavior and risk for psychiatric illness. In particular, it will outline how such strategy was instrumental to unravel the effect of genetic variation in the DDO gene on complex in vivo prefrontal phenotypes in humans.