Simposio Internacional: Mecanismos de los factores neurotróficos en la salud y la enfermedad

***International Symposium**: Mechanisms of neurotrophic factors in health and disease*

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Convergence of BDNF and glucocorticoid signaling mechanisms.

Moses V. Chao

BDNF and glucocorticoids share many biological actions, including the regulation of cell proliferation, survival and differentiation, as well as synaptic plasticity and higher order functions, such as memory, anxiety, depression and obesity. Both molecules trigger distinct signaling pathways involving the BDNF receptor, TrkB, and the glucocorticoid receptor (GR). We have found that glucocorticoids are capable of regulating the activity of Trk receptors. Moreover, distinct new phosphorylation sites in GR at Ser155 and Ser287 are modified in cortical neurons treated with BDNF. Examination of the transcriptional activities of dexamethasone and BDNF through expression profiling analysis of cortical neurons reveals unique sets of genes specifically regulated by each condition. The results indicate that BDNF can influence the transcriptional activity of the glucocorticoids by altering phosphorylation of the GR in central neurons, in particular the two BDNF-dependent phosphorylation sites in rat GR at Ser155 and Ser287. A major target is the hypothalamic peptide, CRH, which controls the secretion of ACTH from the anterior pituitary and glucocorticoids from the adrenal gland. These findings indicate that bi-directional signaling occurs between BDNF and glucocorticoid receptors, which impact upon the activities of the HPA axis and may explain maladaptive actions resulting from changing levels of BDNF

Volver

Endoreduplication and tetraploidy in vertebrate neurons: regulation by neurotrophins

José María Frade

Endoreduplication is characterized by genome replication in the absence of cell division, thus leading to polyploidy. In the vertebrate nervous system, subpopulations of projection neurons including retinal ganglion cells (RGCs) and layer V/VI cortical neurons undergo endoreduplication and become tetraploid during development. This process gives rise to large neurons that are functional and survive in the adult brain. Tetraploidization in neurons requires one round of genome duplication followed by G2/M transition blockade, being both processes regulated by neurotrophins. In this regard, precursors of tetraploid projection neurons express the neurotrophin receptor p75 ($p75^{NTR}$), which is required for NGF-induced cell cycle reentry as these neurons differentiate. In addition, the expression of TrkB is crucial for BDNF-dependent blockade of G2/M transition in neurons that become tetraploid. In differentiating chick RGCs, the interaction of NGF with $p75^{NTR}$ triggers the activation of p38 mitogen-activated protein kinase, which is required for cell cycle reentry after phosphorylation of E2F4, a transcription factor that regulates cell cycle progression. In these neurons, the interaction of BDNF with TrkB results in Wee1-independent

phosphorylation of cdc2 in Tyr15, thus preventing both G2/M transition and the subsequent cell death. The possible participation of both $p75^{NTR}$ and TrkB in pathological tetraploidization leading to neurodegenerative diseases will be discussed.

Volver

Dissecting TrkB Receptor isoforms role in BDNF signaling.

Lino Tessarollo

BDNF is the highest expressed neurotrophic factor in the mammalian brain where it regulates the development and function of a variety of neural circuits. Some of these circuitries exert key roles in learning and memory and specific behaviors. As a consequence, impairments in BDNF signaling has been associated to neurological and psychiatric disorders, obesity, drug addiction and even connected to a possible neural control of tumor development. BDNF functions are transduced by TrkB receptors, which are transmembrane receptors with an extracellular ligand binding domain and an intracellular domain with or without tyrosine kinase function. While most of the data generated so far has been obtained from studying the receptors with kinase activity very little is known about how BDNF functions trough the truncated receptors lacking kinase activity. My laboratory has been studying the in vivo role of TrkB.T1 by gene targeting in mouse. We have found that this TrkB isoform, in addition to regulate the TrkB kinase activity, have other intrinsic functions including BDNF sequestering roles and signaling. For example, we have found an essential signaling role for TrkB.T1 in the heart where it controls cardiomyocyte function. Specific data will be presented.

Volver

Neurotrophins and adult neural stem cells meet at the neurovascular niche

Isabel Fariñas

A characteristic feature of all stem cell populations in long-lived metazoans is their capacity to balance self-renewal with differentiation during lifespan. One approach to the understanding of self-renewal is to analyze the mechanisms that regulate the maintenance of normal stem cells in their natural environment. The adult brain subependymal zone (SEZ) is a very active neurogenic niche in which a relatively quiescent population of radial glia/astrocyte-like GFAP+ neural stem cells (NSC) continually produce new neurons and oligodendrocytes, via a population of rapidly-diving transit-amplifying progenitor cells. Although some intrinsic determinants are known to

regulate stem cell division, the observation that stem cells can respond to excessive cellular demand in pathological situations or after traumatic injury suggests that they have ways to increase their number in response to external signals. Within the specialized microenvironments in which stem cells reside, vascular elements appear to play an important role in the regulation of stem cell self-renewal *vs.* commitment, both under normal and pathological conditions but the signalling pathways involved are still under investigation. We will present our data on the role of vasculature-derived neurotrophin-3 on the regulation of adult NSCs.

Volver

Regulation of appetite by neurotrophins and beyond

John Lin

Loss of function mutations in the brain derived neurotrophic factor (BDNF) or tyrosine kinase receptor TrkB result in hyperphagia and obesity in human and rodents. Likewise, peripheral or central stimulation of TrkB by its natural ligands BDNF or NT4 reduced body weight and food intake in mice supporting the idea that TrkB activation provides a key anorexigenic signal. In non-human primates, TrkB agonists were anorexigenic when applied centrally, but unexpectedly orexigenic, leading to gain in appetite, body weight, fat deposits and serum leptin levels, when given peripherally. The orexigenic and pro-obesity effects of peripherally administered TrkB agonists appear to be dose dependent, not associated with fluid retention nor with evidence of receptor down regulation. I will discuss recent data that confirm and further extend these findings with monoclonal antibodies that mimic the agonist activity of BDNF or NT4.

Volver

BDNF and the regulation of central feeding circuits

Maribel Rios

Lifestyle changes and increased availability of energy-dense foods have greatly contributed to the current obesity epidemic. However, inheritable biological factors that disrupt the tightly regulated equilibrium between caloric intake and energy expenditure also appear to play a critical part. Mounting evidence obtained from human and rodent studies suggests that perturbed brain-derived neurotrophic factor (BDNF) signaling in

appetite-regulating centers in the brain might be a culprit. BDNF is a multifunctional growth factor that promotes the survival, maturation and function of diverse neuronal cell populations. Mice selectively lacking BDNF in the brain eat excessively, develop dramatic obesity and other aspects of the metabolic syndrome including leptin and insulin resistance, dyslipidemia andhyperglycemia. Data will be presented informing mechanisms underlying the appetite-suppressing effects of BDNF and its receptor TrkB in energy balance and reward centers of the brain impacting feeding behavior. They include recent findings informing molecular mediators of the anorexigenic actions of BDNF in the ventromedial nucleus of the hypothalamus. Expression of BDNF and TrkB in this energy balance-regulating center is robustly regulated by nutritional status and required for the effective control of food intake and body weight.

Volver

Mechanism of rapid antidepressant action

Lisa Monteggia

Recent clinical data has consistently demonstrated that a single low dose of ketamine, an ionotropicglutamatergic n-methyl-d-aspartate receptor (NMDAR) antagonist, produces fast-acting antidepressant responses in patients suffering from major depressive disorder (MDD), although the underlying mechanism is unclear. The clinical data has shown that depressed patients report alleviation of MDD symptoms within two hours of a single low-dose intravenous infusion of ketamine with effects lasting up to two weeks, unlike traditional antidepressants (i.e. serotonin reuptake inhibitors such as Prozac), which take weeks to reach efficacy. This delay is a major drawback to current MDD therapies, leaving a need for faster acting antidepressants particularly for suicide-risk patients. We investigated the ability of ketamine to produce rapid acting, long-lasting antidepressant responses in rodent models. We found that ketamine and other NMDAR antagonists produce fast-acting behavioral antidepressant-like effects in mouse models that depend on rapid synthesis of brain-derived neurotrophic factor (BDNF). We also found that ketamine-mediated NMDAR blockade at rest deactivates eukaryotic elongation factor 2 (eEF2) kinase (also called CaMKIII) resulting in reduced eEF2 phosphorylation and desuppression of BDNF translation. An important aspect of this study is that we were able to demonstrate that inhibitors of eEF2 kinase were sufficient induce fast-acting behavioural antidepressant-like effects. Our findings suggest that protein synthesis regulation by spontaneous neurotransmission may serve as a viable therapeutic target for fast-acting antidepressant development.

Volver

BDNF levels and deregulation of local translation in Down's syndrome

María Luz Montesinos

Local translation plays important roles in synaptic connectivity and plasticity. Among other functions, the Akt-mTOR pathway regulates the rates of local dendritic translation, and has been shown to be hyper or hypoactive in mouse models of Fragile X, Tuberous Sclerosis and Rett's syndrome. Therefore, although these intellectual disabilities have different molecular origins, they converge in deregulation of the same signaling pathway.
Down's syndrome (DS) is the most frequent genetic cause of intellectual disability. We have recently found that, due to increased basal levels of BDNF/pro-BDNF, the Akt-mTOR cascade is hyperactive in the hippocampus of a DS mouse model. As a consequence, the rates of local dendritic translation are augmented. The impact of this phenotype on synaptic plasticity, and the therapeutic potential of rapamycin/rapalogs (which are specific FDA-approved mTOR inhibitors) for improving cognition of DS-affected patients will be discussed.

Volver

Neuregulin/Erb4 function in cortical interneuron wiring

Beatriz Rico

Schizophrenia is a complex psychiatric illness affecting nearly 1% of the population that is thought to arise as a consequence of abnormal brain development. Structural deficits are subtle in schizophrenia, but increasing evidence suggests that there are significant alterations in inhibitory circuits in patients with this disorder. Recent genetic studies suggest that Neuregulin-1 (Nrg1) and its receptor ErbB4 are susceptibility genes linked to the disorder. Other and we have recently found that ErbB4 function is required for the normal wiring of specific populations of interneurons, most notably Parvalbumin-expressing chandelier and basket cells. In particular, ErbB4 seems to be required presynaptically in Chandelier cells for the formation of a normal complement of GABAergic synapses onto pyramidal cells. Moreover, ErbB4 seems to be required postsynaptically in basket cells to receive a normal complement of excitatory synapses from pyramidal cells. However, whether ErbB4 plays both pre- and postsynaptic roles in each class of Parvalbumin-expressing interneurons remains unknown. We have addressed this question by combining mouse genetics with a set of different experimental approaches. Our results suggest that ErbB4 may contribute differently to the wiring of specific classes of interneurons. In addition, abnormal wiring of Parvalbumin-expressing interneurons in ErbB4 mutants perturbs cortical excitability and leads to specific behavioral deficits.

Volver

Neurotrophic plasticity in the oculomotor system

Ángel M. Pastor

Axotomy, a widely used strategy for the study of target trophic dependencies, produces severe alterations on injured motoneurons like the loss of the afferent connectivity and changes of their firing properties. Based on the discovery that motoneurons innervating the lateral rectus muscle express neurotrophin receptors and that this muscle expresses and releases neurotrophins also in adult stages, we studied whether the morphofunctional changes seen in abducensmotoneurons after nerve injury are in part due to growth factor deprivation and if exogenous retrogradely-delivered neurotrophins were able to reestablish the non-injured phenotype. Thus, selective aplication of BDNF, NT-3 or NGF to the abducens nerve had different effects over the discharge properties and synaptic afferent connectivity of abducensmotoneurons. BDNF and NT-3 exerted complementary roles, promoting the maintenance and/or recuperation of the impinging afferents and therefore the selective restoration of the tonic or phasic discharge signals of the extraocularmotoneurons. NGF, on the other hand, not only promoted the maintenance of synaptic connectivity, but it also enhanced motoneuronal excitability. These experiments are the first *in vivo* demonstration that neurotrophic exogenous supply promotes the recovery of synaptic connectivity and the activity pattern of abducensmotoneurons after axotomy. The existence of distinct types of abducensmotoneurons, with different firing features depending on their tonic *vs.* phasic discharge signals has long been postulated in several groups of vertebrates. Our findings raise the possibility that different pools of abducensmotoneurons express distinct combinations of neurotrophin receptors and this, in turn, promotes the innervation coming from different premotor nuclei and the scaling of the firing properties of abducensmotoneurons.

Volver

Taking pain out of NGF to improve its therapeutic window

AntoninoCattaneo and SimonaCapsoni

The therapeutic potential for Nerve Growth Factor (NGF) in different pathological conditions, including diabetic neuropathic pain and Alzheimer's Disease, is based on a strong rationale, but the development of NGF-based therapies has been hampered by the strong nociceptive activity of NGF itself. Inspired by a mutation in the gene for NGF found in a rare genetic disease (Hereditary Sensory Autonomic Neuropathy type V), that determines a congenital insensitivity to pain, in the absence of mental retardation or of major neurological symptoms, we developed a human NGF mutant (hNGFP61S/R100E, painless NGF) that has identical neurotrophic properties to human NGF, is traceable against endogenous NGF and has a greatly reduced ability to activate nociception.
The properties of this molecule will be described, as well as its ability to prevent and reverse neurodegeneration upon intranasal delivery in different Alzheimer's Disease mouse models (including APPxPS1 and 5xFAD Tg mice). We conclude that "painless

NGF" is a viable therapeutic option to increase NGF activity in the brain, without unwanted safety issues related to pain.

Volver

Modulation of NGF functions through TrkAubiquitination

Juan C. Arévalo

Ubiquitination of receptor tyrosine kinases regulates their endocytosis, sorting and degradation and, therefore, modulates their functions. Different groups have reported that Trkneurotrophin receptors are ubiquitinated in response to neurotrophins. We have previously reported that the E3 ubiquitin ligase Nedd4-2 binding and ubiquitination to TrkA modulates NGF-mediated signaling and survival *in vitro*. However, the role of TrkAubiquitination*in vivo* has not been addressed yet. We have generated a knock-in (KI) mouse expressing a mutant TrkA that does not bind to Nedd4-2 and, therefore, it is not ubiquitinated by this E3 ubiquitin ligase. KI mice present an enhanced sensitivity to thermal and inflammatory stimuli but not to mechanical stimulus compared to wild-type mice. In addition, these mutant mice have behavioral deficits at the CNS level in terms of anxiety and memory. Anatomically, these KI mice have higher numbers of DRG neurons and increase skin innervation than wild-type mice. This data are in agreement with enhanced *in vitro* survival of DRG neurons from KI embryos in limiting amounts of NGF. Furthermore, DRG neurons from KI mice express increased amount of transcripts for CRGP and Substance P, two peptides that a mediators of pain sensation. The mutant TrkA protein shows impaired trafficking and degradation in response to NGF, and an increase in NGF-mediated signaling. All together this data suggest that ubiquitination of TrkA is seminal for NGF-mediated functions not only *in vitro*, but also *in vivo*.

Volver

BDNF is a key modulator of the pathophysiologyof Huntington's disease: a therapeutic approach

Jordi Alberch

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by an expanded CAG repeat in the huntingtin gene. This mutation induces a specific degeneration of striatal and cortical neurons. Although the length of the CAG repeat strongly correlates with the age-at-onset, one prominent feature of HD is the variable age at which the characteristic neurological or psychiatric symptoms appear, suggesting that mutant huntingtin is the trigger, but other factors also influence the disease onset. Between the modifier factors that have been described, the neurotrophin Brain Derived

Neurotrophic Factor (BDNF) has emerged as a potent modulator of the neurodegenerative process in HD.
BDNF is the most potent trophic factor for these neurons regulating survival and differentiation *in vivo* and *in vitro*. Furthermore, BDNF also regulates neuronal dysfunction that can be relevant in early stages of the disease. Several studies have described that mutant huntingtin can alter trophic support for striatal neurons. Thus, BDNF levels are reduced in several transgenic mouse models of HD. This reduction has a functional effect in the pathophysiology of HD, since a down-regulation or up-regulation of BDNF in mice with mutant huntingtin induces a decrease or increase, respectively, in the onset and severity of the symptoms. Therefore, as one pathogenic mechanism leading to dysfunction and loss of striatal neurons in HD might be the reduction in BDNF levels, its administration should improve these alterations. The neuroprotective effect of BDNF has been tested in the excitotoxic and in transgenic mouse models of HD. However, the main challenge is to find the way to administer BDNF chronically and conditionally to the target neurons.
We recently generated a mouse that over-expresses BDNF under the GFAP promoter. When we crossed-mated this mouse with an R6/1, we generated a mouse with mutant huntingtin that only over-expresses BDNF under pathological conditions. These double mutant mice show and improvement in several motor coordination tasks. Furthermore, this over-expression of BDNF also prevented the decrease of presynaptic and postsynaptic markers.
Although the delivery method must be improved, all these results indicate that BDNF is a good candidate to develop new therapeutic strategies for HD due to its positive effects on synaptic plasticity and neuronal survival.

Volver

Neurotrophic factors in Neuroscience: possible applications in neurodegenerative disorders

Beatriz Moreno

Neurotrophin binding to its specific receptors leads to the activation of multiple signalling cascades, culminating in neuroregenerative effects, including neuronal survival and neurite outgrowth. Neurotrophic factors have been used for the treatment of several neurodegenerative diseases. However, their use is limited by their inability to cross the blood-brain barrier, their short half life and their side effects. Small neurotrophin-like molecules may be beneficial in treating a number of neurodegenerative disorders. We will show the capacity of a neurotrophin agonist molecule selected from a library of peptoids to induce differentiation in PC12 cells, promote survival in RN22 cells and activate phosphorylation of different pathways to various extents in both cell lines. In addition this

molecule was able to ameliorate acute experimental autoimmune encephalomyelitis (EAE), a multiple sclerosis (MS) animal model, inhibiting brain inflammation and reducing brain damage. The molecule was also effective in the animal model of glaucoma. These results suggest that small molecules with neurotrophic agonist activity may be beneficial for MS and other neurodegenerative diseases.

Volver