Translational Neuroscience

From the bench to the bedside: Gene therapy for Parkinson’s disease, The roles of the habenula and nucleus accumbens in depression, Microglia and neurodegeneration

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GENE THERAPY FOR PARKINSON’S DISEASE

Article: AAV2-GAD gene therapy for advanced Parkinson’s disease: A double-blind, sham-surgery controlled, randomised trial. Lewitt PA, Rezai AR, Leehey MA, Ojemann SG, Flaherty AW, Eskandar EN, Kostyk SK, Thomas K, Sarkar A, Siddiqui MS, Tatter SB, Schwalb JM, Poston KL, Henderson JM, Kurlan RM, Richard IH, Van Meter L, Sapan CV, During MJ, Kaplitt MG, Feigin A.

Lancet Neurol. 2011 Apr;10(4):309-19.

Key points
• Parkinson’s Disease (PD) symptoms are in part due to disinhibition of the subthalamic nuclei (STN). This is felt to be why STN lesions and deep brain stimulation (DBS) act to reduce the severity of PD.
• In this randomized, controlled clinical trial, the authors inject a genetically engineered virus containing the GAD gene into the bilateral STN. Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the production of γ-aminobutyric acid (GABA).
• At 6 months after surgery, patients treated with AAV2-GAD experienced significantly greater reduction in PD motor scores. Furthermore, there were anecdotal reductions in freezing episodes and more reported consistent medication effects.
• AAV2-GAD was shown to be safe.
• This landmark paper is the first successful randomized, controlled trial of gene therapy for a neurological disease. This is a particularly exciting development for future studies.

It is always exciting at Bench to Bedside to see clinical studies based on work initiated in animal models. This is a true representation of translational neurosurgical science and suggests that the key to advancement in the field is with sound science coupled with sound clinical practice. In this past month’s Lancet Neurology, the results of a clinical trial for gene therapy in PD were presented. Also, while some may feel that the results are not as robust as what has been seen in other therapies (such as DBS), this is a landmark article in that it opens the door to further developments of gene therapy in neurosurgery. It shows that gene therapy is safe and has the potential to be effective.

In this double-blind, randomized, sham-surgery controlled trial, the authors examined the delivery of the gene GAD, the enzyme that is critical for the production of the neurotransmitter GABA, to the bilateral STN. The underlying theory as to why GAD delivery would be effective in PD is the loss of GABAergic inputs to the STN, leaving the nucleus uninhibited and leading to many Parkinsonian symptoms. This is a common
theory shared by proponents of subthalamotomy and DBS of the STN to ameliorate some of the Parkinsonian symptoms. So, by inducing production of GABA within the STN, GAD delivery and subsequent expression could potentially serve to intrinsically inhibit the STN (locally) and decrease the abnormal STN excitatory output (by now having some STN neurons release GABA instead of glutamate) without the need to device implant or lesioning. In order to accomplish this, the authors use a genetically engineered virus (AAV2) that essentially inserts the GAD gene into neurons surrounding the area into which it is injected.

In the study, Parkinsonian patients were randomized into two groups: the first group received bilateral injection of AAV2-GAD into their STN, while the second received a sham surgery involving partial thickness burr holes and a simulation of the normal stereotactic procedure. Patients were followed for up to 6 months following the procedure. The authors found that patients who underwent AAV2-GAD injection had a significantly greater improvement (~25%) in the PD symptoms (measured by the UPDRS motor score) at 6 months compared to sham controls. Interestingly, two of the sham control patients had significant improvement following surgery, creating a small but significant sham effect (12.7% improvement). AAV2-GAD treated patients also experienced a reduction in their clinicians’ impression of the global severity of their PD as well as a reduction in their off-medication severity scores. Some patients reported reductions in freezing episodes as well as more consistent medication effects. Importantly, this therapy was safe – only one patient had a serious adverse event that was deemed unrelated to the AAV2-GAD treatment.

In brief, this exciting clinical study, which was based originally on the work done in rodents and primates, provides a new avenue for treatment of chronic neurological disease. Gene therapy, while still in its infancy, could potentially treat a wide variety of neurological diseases currently beyond the scope of neurosurgical practice – including a wide array of neurodegenerative and genetic diseases.

HABENULA NEURONS PROVIDE INSIGHT INTO DEPRESSION

Article: Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. Li B, Piriz J, Mirrione M, Chung C, Proulx CD, Schulz D, Henn F, Malinow R, Li B, Mirrione M, Chung C, Proulx D, Schulz D, Henn F, Malinow R.

Nature. 2011 Feb 24;470(7335):535-9.

Key points
• The lateral habenular nucleus contains excitatory neurons that project to the ventral segmental area (VTA), a midbrain nuclear group involved in the processing of rewards.
• Enhanced excitatory input into the lateral habenula correlates with learned helplessness, an animal model of depression.
• The enhanced excitatory input is because excitatory neurons that project to lateral habenular neurons were more likely to reliably release excitatory neurotransmitter.
• Deep brain stimulation (DBS) of the lateral habenula produces a marked depression of excitatory transmission.
• DBS of the lateral habenula ameliorates depressive behavior in rats as measured in the learned helplessness and forced swim test paradigms.

The neuronal basis of reward and depression is an important area of neuroscience research, with profound implications for treating mood disorders (such as depression) with novel techniques such as deep brain stimulation (DBS). In this paper, the authors study the role of the lateral habenular nucleus in depression using a combination of electrophysiology and behavioral experiments. Using a retrograde tracer (a way of labeling a circuit of neurons by injecting into a target and finding the neurons that project to it), the authors showed that a subset of glutamatergic neurons in the lateral habenula project directly to the VTA, a region of the ventral midbrain involved in reward processing. The authors used the acute learned helplessness (aLH) model in which rats were given inescapable and unpredictable foot shocks as an animal model of depression. A congenital learned helplessness (cLH) strain of rats was also studied which was produced by cross-breeding rats that showed the greatest amount of acute learned helplessness. This means that these animals would be the most genetically predisposed to depression behavior.

The authors compared brain slices from normal rats with slices from rats with acute or congenital learned helplessness. Whole cell patch-clamp recordings (a way of looking at electrical currents within neurons) of lateral habenula neurons were made using brain slices from the three groups of rats to measure miniature excitatory postsynaptic currents (mEPSCs). These mEPSCs represent the responses of these neurons to individual pre-synaptic connections. They noticed that neurons from rats with acute learned helplessness had higher frequency mEPSCs. In other words, there was an association between excitatory neurotransmission in the lateral habenula and depressive behavior. To test whether there was a correlation between the excitatory synaptic activity and helpless behavior, the authors tested animals in a task measuring helpless behavior. They subsequently prepared brain slices from these animals to record from habenular neurons that project to the VTA. They showed that animals with the greatest helpless behavior demonstrated the greatest excitatory synaptic activity onto these VTA-
CREB overexpression in the NAs increases depressive behavior in rats, while disruption of CREB in this region has anti-depressive effects.

In addition to the aforementioned work in the habenula, another recently published study demonstrates another potential anatomic target in the study and therapy for depression. The nucleus accumbens is a deep forebrain nucleus involved in reward encoding and hedonic behavior. Within this nucleus, the transcription factor CREB is known to mediate reward and anhedonia (i.e., inability to experience pleasure) in animal models. In this paper, the authors were interested in studying the role of CREB in the NAs region in mediating stress-related behavior.

The authors first show that in rats subjected to stress, phospho-CREB, the activated isoform of CREB, is elevated within the NAs. Using viral technology, the authors then attempted to overexpress CREB or a mutant inactive form m-CREB in the NAs of awake, behaving animals. The same animals were also implanted with self-stimulation electrodes within the lateral hypothalamus, a model of reward also known as intracranial self-stimulation (ICSS). By measuring the threshold for ICSS, one can measure the effect of viral microinjection on reward (with higher thresholds indicating that ICSS has become less rewarding). The authors found that overexpression of CREB in the NAs led to an increase in ICSS thresholds, indicating that ICSS in the lateral hypothalamus had become less rewarding (anhedonia). In contrast, disruption of CREB by expression of mCREB in the NAs had the effect of increasing reward (decreasing ICSS thresholds).

The authors next extended these studies to another behavioral test known as fear potentiated startle (FPS) in which a previously neutral stimulus (i.e., conditioned stimulus) is paired with footshock. The startle response to the conditioned stimulus is a measure of stress. After the initial learning period, the startle response should decrease as the behavior extinguishes (a phenomenon whereby a learned behavior disappears over time). Overexpression of CREB in the NAs led to increased startle while expression of mCREB led to a decrease in startle responsiveness, suggesting impaired extinction of FPS in the CREB group. The results were the same whether CREB gene transfer occurred before or after the initial training period. Thus, CREB activity in the NAs modulates learned fear behavior as well as anhedonia.

Due to previous studies suggesting that CREB regulates the opioid system, the authors turned to the study of opioids within the NAs to understand a possible relationship between CREB, opioids, and depressive behavior. They hypothesized that elevated dynorphin (an opioid) levels in the NAs should act at kappa opioid receptors (KORs) to decrease dopamine release in the NAs. This in turn should cause anhedonia. The
authors again turned to the ICSS model and showed that microinjection of a KOR agonist into the NAs led to anhedonia. The authors took these results to indicate that the effects of CREB in the nucleus accumbens are mimicked by KOR stimulation in this region.

Overall, the results of this study extend our understanding of the neuronal encoding of stress-related behaviors within the nucleus accumbens and provide further evidence that this region is a logical target candidate for DBS for mood disorders such as post-traumatic stress disorder and depression.

MICROGLIA, NEUROTOXICITY, AND NEURODEGENERATIVE DISEASE

Article: Caspase signalling controls microglia activation and neurotoxicity. Burguillos MA, Deierborg T, Kavanagh E, Persson A, Hajji N, Garcia-Quintanilla A, Cano J, Brundin P, Englund E, Venero JL, Joseph B.

Nature. 2011 Mar 9. [Epub ahead of print].

Key points

- Activated microglia release pro-inflammatory factors that may lead to neurotoxicity in neurodegenerative disorders.
- Caspase-8 and caspase-3/7 are known mediators of apoptotic cell death.
- These caspas activate microglia via a protein kinase C (PKC)-delta-dependent pathway without triggering cell death.
- Inhibition of these caspases interferes with microglial activation and reduces neurotoxicity in vivo and in vitro.
- These caspases are activated in microglia in the ventral midbrain in Parkinson’s disease and the frontal cortex in Alzheimer’s disease.

Microglial activation has been linked to neurotoxicity in several cell and animal models. In this paper, the authors show that intracellular proteins called caspases, known to execute apoptosis (programmed cell death), can act in a pro-inflammatory fashion to activate microglia and lead to microglial-associated neurotoxicity. They begin by showing that lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls, can stimulate caspase-3/7 activity within microglia. Although caspase-3/7 is known to be pro-apoptotic (i.e., it is known to induce programmed cell death), LPS-induced activation of microglia was associated with markers of inflammation but not, interestingly, with microglial cell death.

The authors subsequently selectively knocked down caspase-3 and caspase-7 using small interfering RNAs (siRNAs). SiRNA is a technique that allows researchers to specifically prevent the expression of certain genes of interest (i.e., knockdown). Knockdown of caspase-3 and caspase-7 inhibited the ability of LPS to activate microglial cells, suggesting that these caspases are necessary for microglia activation. When microglia were then co-cultured with dopaminergic neurons, LPS exposure led to microglial activation and subsequent microglial-induced dopaminergic cell death. When the authors used the siRNA technique to again selectively knockdown caspase-3/7, however, dopaminergic cell death was averted.

The authors next turned their attention to determining what upstream mediators are involved in activating caspase-3/7 in LPS-treated microglia. They showed that caspase-8 was required for LPS-induced activation in microglia and that siRNA knockdown of caspase-8 prevented activation of caspase-3/7. The authors then investigated the role of TLR4 receptor (a toll-like receptor; these receptors are critical for pattern recognition within the immune system) in ligation and activation of caspase-8. Selective knockdown of TLR4 was associated with reduced caspase-8 activation and subsequent caspase-3 activation. Thus, the TLR4 receptor acts upstream in LPS-induced microglial activation of caspase-3/7.

Next, the authors turned to downstream mediators of the caspase-3/7 pathway in LPS-activated microglia. It is known that PKC-delta can be cleaved by caspases into an active fragment that subsequently regulates nuclear factor (NF)-kB, a transcription factor involved in the cell’s inflammatory response. LPS treatment of microglia was shown to result in cleavage of PKC-delta into its active form. SiRNA knockdown of caspase-3/7 reduced this effect. These results show that PKC-delta is involved in downstream signaling in LPS-induced microglial activation.

So, why is all of this molecular biology of interest to the clinician? While the above in vitro studies are interesting by themselves, the authors performed further in vivo experiments to elucidate their physiological and clinical relevance. For example, the substantia nigra exhibits a strong inflammatory response upon LPS challenge. The authors injected LPS into the substantia nigra of rats and showed a strong induction of both caspase-8 and caspase-3 not found in the contralateral control substantia nigra. Co-injecting an inhibitor of caspase-3/7 also inhibited this activation. Furthermore, inhibition of PKC-delta also significantly reduced LPS-induced inflammation. The authors then used a MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-lesion mouse model of Parkinson’s disease. Caspase-8 inhibition prevented MPTP-induced microglial activation. Also, inhibition of caspase-8 was shown to reduce MPTP-induced damage to dopaminergic terminals. In their final study, the authors investigated the expression of caspase-3 and caspase-8 in post-mortem Alzheimer and Parkinson’s disease brains and showed that active forms of both caspases were present in the frontal...
lobes and ventral midbrain, respectively, and that they co-localized with microglial cells.

This is an interesting article because it explores a relatively underdeveloped field in neuroscience: the role of inflammation and the immune system in neurological disease. The authors provided convincing evidence that inflammation plays a role in the pathology of two major neurodegenerative diseases (Alzheimer’s disease and Parkinson’s disease). This suggests that future therapies aimed at reducing inflammation, perhaps through the inhibition of caspases or PKC-delta, may slow the progression and ameliorate the symptoms of these challenging diseases.