

## Intranasal treatment of neurodegenerative diseases and stroke

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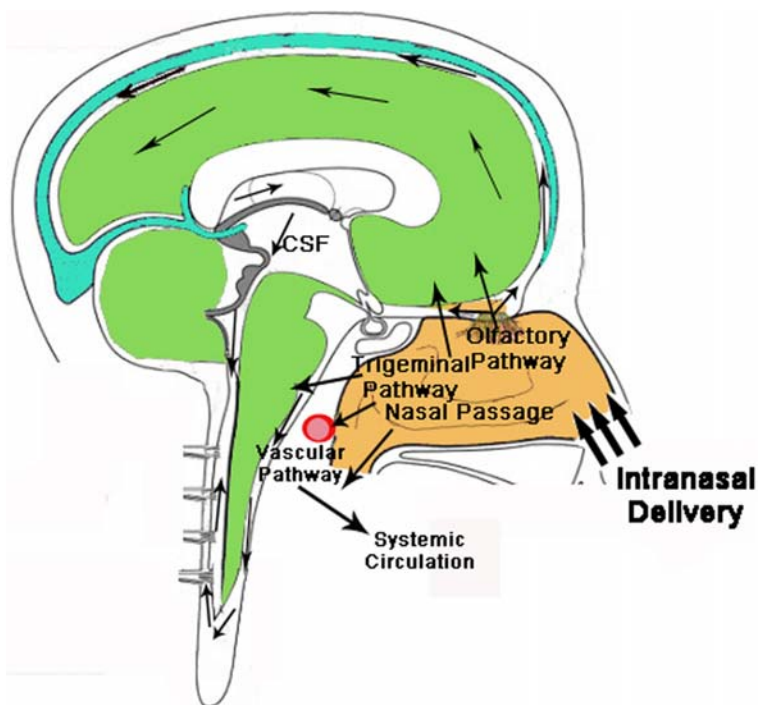
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## 1. ABSTRACT

Although the blood–brain barrier (BBB) restricts access to the central nervous system (CNS) for the use of systemically administered therapies, an alternative approach, the non-invasive method of intranasal delivery, can rapidly target delivery of molecules to the CNS. Intranasal delivery has the distinct advantages of circumventing the BBB while minimizing systemic exposure. This novel approach to treating neurological illnesses will be examined in detail in this review. We will review current understanding of the mechanisms underlying intranasal delivery to the CNS, along with discussion of pathways permitting entry from the nasal cavity into the CNS, particularly those involving the olfactory and trigeminal nerves. Significant preclinical research has been performed to develop and improve our current approaches to intranasal treatments. We will examine the evidence behind the use of intranasal delivery in chronic neurodegenerative conditions such as Alzheimer's Disease and diabetes-mediated cerebral degeneration, as well as in acute conditions such as stroke.

## 2. INTRODUCTION

There are many hurdles preventing interventions from reaching the diseased brain. Systemic delivery of therapeutics to the central nervous system (CNS) is ineffective for most small molecules and nearly all large molecules (1). The main impediment in most cases is the blood–brain barrier (BBB). Necessary for protection against bacterial infections, the BBB prevents most foreign substances, including potential therapeutics, from entering the brain from capillaries. Meanwhile, the BBB permits the diffusion of small hydrophobic molecules, which is often insufficient for pharmacotherapeutic targeting of the diseased brain. As a result, large doses of therapeutics including small molecules or peptides are required to achieve therapeutic levels in the brain, increasing the risk for adverse systemic effects (2). Although therapeutics can be directly targeted to the brain via intracerebroventricular or intraparenchymal delivery, these invasive techniques are not without risk and can necessitate repeated surgical intervention.



**Figure 1.** There are several routes of entry and distribution into the CNS and systemic circulation when molecules or therapeutics are delivered intranasally (50). There is no direct evidence for which particular brain regions are targeted by the olfactory pathway alone or by the trigeminal pathway alone, and delivery in different species of animals differs as well due to multiple differences between species with regards to nasal and brain structures, distribution of the olfactory and trigeminal nerves in the nasal epithelium, etc. Nonetheless, olfactory pathways likely distribute most efficiently to the olfactory bulb and other rostral brain structures such as the anterior olfactory nucleus, and frontal cortex. Trigeminal pathways distribute mainly to the trigeminal ganglion, the olfactory bulb and to more rostral brain structures such as the pons, cerebellum, brain stem and cervical spinal cord (12). Each of these pathways may also contribute to delivery of the therapeutic to the CSF (12, 50). Intranasal drugs which can enter the vasculature from the nasal mucosa will lead to systemic exposure to the drug. All of these pathways can potentially contribute to CNS delivery of intranasally administered drugs.

In humans, the development of intranasal delivery began in the 1960s, with the intranasal administration of oxytocin for induction of labor in pregnancy (3-5). Since that time, intranasal delivery has expanded to other indications, with initial intentions for the systemic delivery of the agent selected (6, 7). It was realized that intranasal systemic delivery was often inconsistent, and even of low yield. As a result of these difficulties, intranasal delivery's use for systemic delivery waned at this time. In 1989, Frey developed a new concept: the use of direct intranasal delivery of therapeutic agents to the brain as a means of bypassing the BBB (8, 9). This direct intranasal delivery involves extracellular delivery along the olfactory and trigeminal nerves connecting the nasal mucosa to the CNS (10-13). This review will concentrate on the use of this non-invasive method to deliver drugs directly and rapidly from the nose to the brain and spinal cord with the goal of treating neurodegenerative disorders and stroke. As the mechanisms behind intranasal delivery have become better understood, new intranasal therapeutics have been investigated in a host of conditions. It is anticipated that some of these therapies will become clinically relevant, but more human clinical trials are required prior to widespread clinical use.

### 3. PATHWAYS AND MECHANISMS OF INTRANASAL DELIVERY

Although the exact mechanisms underlying intranasal drug delivery to the CNS remain incompletely understood, it has been shown that pathways involving the olfactory and trigeminal nerves connecting the nose to the brain and spinal cord are critical. In addition, pathways involving vasculature, cerebrospinal fluid, and even the lymphatic system have been identified as potentially important in the transport of molecules from the nasal cavity into the CNS (10). It is believed that a combination of these pathways is responsible for intranasal delivery as a whole (Figure 1). Depending on the properties of the molecule/peptide being delivered, the formulation being delivered, and the delivery method and device used, certain individual pathways may be dominant.

#### 3.1. Olfactory Nerve Pathways

Within the nose, the dendrites of olfactory receptor neurons extend into the mucous layer of the olfactory epithelium to permit sensation of odors. The axons of these bipolar neurons extend centrally, passing through the subarachnoid space containing CSF in order to

synapse with mitral cells in the olfactory bulbs. From here, neural projections extend diffusely to the olfactory tract, anterior olfactory nucleus, piriform cortex, amygdala, and hypothalamus (14). Chemosensory neurons located anteriorly in the nasal cavity also project to the olfactory bulbs (15, 16). Techniques to demonstrate the role of olfactory tracts have been performed - wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP), a tracer, supplied intranasally is transported within olfactory axons to the olfactory bulbs in the CNS (17). Fluorescent tracers delivered intranasally can be seen along olfactory nerves traversing the cribriform plate (18). However, this intraneuronal transport process within the olfactory tract is slow, and is not involved in the rapid intranasal delivery of drugs to the brain which can be identified within minutes. Although slower than with other methods, it has been shown that olfactory nerve pathways are a major component of intranasal delivery in most situations - drug concentrations in the olfactory bulbs are generally among the highest CNS concentrations observed after intranasal delivery (12, 19-21).

A unique process occurs at the level of the olfactory receptor neurons, in that they regenerate every month due to direct contact with toxins externally - this leads to a "leaky" BBB due to their constant turnover (17, 22). Meanwhile, Schwann cell-like cells termed olfactory ensheathing cells surround the axons of olfactory receptor neurons, creating continuous, fluid-filled perineurial channels which remain open, permitting travel along their length as well (23). Thus, it is possible for intranasally administered therapies to reach the CNS via extracellular or intracellular mechanisms of transport. The extracellular mechanisms may permit transport to occur over only several minutes (11, 17) using bulk flow mechanisms (24, 25) within the olfactory ensheathing cells. Intracellular transport mechanisms involve passive diffusion and receptor-mediated endocytosis in olfactory receptor neurons, leading to slower axonal transport over several hours or even days (26-28). Due to this limited speed, intracellular mechanisms are unlikely to be the predominant mode of transport into the CNS for most molecules/peptides. High speeds of movement of many molecules following intranasal delivery suggests that receptor-mediated transport mechanisms are unlikely to be prevalent and the wide variety of therapeutics possible for delivery to the CNS also argues against any receptor-mediated processes occurring. Even for large molecules such as nerve growth factor (NGF) and insulin-like growth factor-I (IGF-I), intranasal delivery to the brain is non-saturable and therefore, not receptor mediated (12, 29, 30).

### 3.2. Trigeminal Nerve pathways

Another important pathway connecting the nose to the CNS is the trigeminal nerve, which innervates the respiratory and olfactory epithelium of the nasal passages (31, 32). For the trigeminal nerve, branches from the ophthalmic division (V1) innervate the dorsal nasal mucosa and the anterior nose, while branches of the maxillary division (V2) innervate the lateral walls of the nasal mucosa. The trigeminal nerve enters the brain from the respiratory epithelium of the nasal passages at two

locations: (1) into the pons; and (2) through the cribriform plate near the olfactory bulbs, permitting entry into both caudal and rostral brain with intranasal administration.

Small portions of the trigeminal nerve also terminate in the olfactory bulbs (33). Intranasal drug delivery to the brain along trigeminal pathways was first demonstrated by Thorne *et al* (12). Using <sup>125</sup>I-IGF-I, Thorne *et al.* observed high concentrations of radioactivity in the trigeminal nerve branches, trigeminal ganglion, cervical spinal cord, medulla, pons, and olfactory bulbs with significant delivery to other CNS structures as well (12). Intranasal studies with other drugs, including interferon- $\beta$ 1b (IFN- $\beta$ 1b) (19, 34), hypocretin-1 (35, 36), and peptoids (20), also identified high levels of radioactivity in the trigeminal nerve.

### 3.3. Vascular pathways

The intranasal route of administration has traditionally been utilized to deliver drugs to the systemic circulation through absorption into capillary blood vessels within the nasal mucosa, a highly vascular structure (31, 37). The vasculature in the respiratory region of the nasal mucosa has both continuous and fenestrated endothelia (38, 39), permitting both small and large molecule passage, although delivery from the nasal mucosa to blood is more limited for large hydrophilic molecules. Delivery to the CNS following vasculature absorption is possible, particularly for small, lipophilic drugs, for which passage into the blood stream and crossing of the BBB is easier than with large, hydrophilic peptides and proteins. However, delivery utilizing the systemic circulation results in a degree of drug elimination due to hepatic and renal metabolism. Such vascular transport is also subject to binding of drugs to plasma proteins, plasma protease-degradation, and enhancement of any potential systemic side effects.

Mechanisms involving perivascular channels are likely involved in intranasal drug delivery as well. Perivascular spaces act as a lymphatic system for the brain, where neuron-derived substances are cleared from brain interstitial fluid through entry to perivascular channels associated with cerebral blood vessels. Perivascular transport occurs due to bulk flow mechanisms, rather than simply diffusion (40, 41), with arterial pulsations forming a driving force for perivascular transport, acting as a "perivascular pump" mechanism (42-44). The widespread distribution of intranasally delivered therapeutics observed within the CNS could be the result of perivascular transport mechanisms (12). Several intranasal studies have demonstrated high levels of delivered molecules within the walls of cerebral blood vessels after removal of blood (12, 19, 35), suggesting that intranasally administered drugs may gain access to perivascular spaces.

### 3.4. Pathways involving the cerebrospinal fluid and lymphatics

CSF-containing pathways connecting the subarachnoid space, perineurial spaces surrounding olfactory nerves, as well as the nasal lymphatics are required for CSF drainage; these pathways provide access

for intranasally delivered therapeutics. Tracers injected into the CSF in the cerebral ventricles or subarachnoid spaces drain to the olfactory bulbs, reaching the nasal lymphatic system and cervical lymph nodes (45-49). Drug passage can also occur through nasal passages to the CSF to the brain's interstitial spaces and perivascular spaces. Therapeutics are directly delivered to the CSF following intranasal delivery, without significant entry into blood (50). Overall, it is difficult to experimentally separate contributions of different pathways into the CNS after intranasal administration. In most cases, it can be assumed that multiple pathways are permitting entry to the CNS for intranasal molecules/peptides.

#### 4. DELIVERY AND FORMULATION CONSIDERATIONS IN INTRANASAL DELIVERY

The assessment of distribution of intranasally delivered molecules to different brain regions provides insight into pathways and mechanisms of intranasal delivery. A number of considerations are required when planning intranasal therapy provision in animal models or in human subjects. Whole brain measurements of drug concentration, in general, underestimate the extent of delivery due to the effects of dilution. Drug targeting is of greater value, as this will evaluate the relative distribution of the drug to therapeutic target sites such as specific brain regions and provide comparison to sites not targeted, such as with blood or peripheral tissues. Detection of radiolabelled molecules can also be performed to determine distribution into the CNS as well as systemic tissues (51, 52). Intravenous delivery can be used as a control for evaluating blood-mediated delivery to the CNS; this is particularly useful as a measure of potential absorption through the nasal vasculature occurring through intranasal delivery. Thus, the difference between intranasal and intravenous delivery can be viewed as a measure of direct transport to the brain using intranasal methods (35, 53). In general, when planned properly, intranasal compared to intravenous administration generally yields high brain: blood ratios, demonstrating drug targeting efficiency.

Natural protective barriers within the nasal mucosa contribute to the low efficiency of intranasal delivery, as typically less than 1% of the administered dose reaches the brain (25, 54). Overcoming these barriers has been a concerted research effort in order to improve intranasal delivery efficiency. For example, efflux transport proteins, including p-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP1), are expressed within nasal mucosa (55-57) significantly limiting substrate uptake into the brain (56, 58, 59). Also, drug metabolizing enzymes (60, 61) and tight junction proteins (62) occur in the nasal epithelium, further limiting the efficiency of intranasal delivery to the CNS. Potential methods used in overcoming these barriers involve the improvement of drug solubility, increasing permeability across nasal epithelium, and reduction of vascular passage into systemic circulation.

Improvement of solubility can include the encapsulation of drugs in carriers including cyclodextrins, microemulsions, and nanoparticles. For example, the mixture of galanin-like peptide (GALP) and  $\alpha$ -cyclodextrin

enhances delivery to numerous brain regions by 2-3X (21), possibly via modulation of perivascular transport. Microemulsion and nanoemulsion formulations improve drug solubility and opportunities for direct transport into the CNS. These use an oil-in-water dispersion to increase small molecule uptake for drugs such as clonazepam (63), sumatriptan (64), risperidone (65), and nimodipine (66). Polymeric nanoparticles are comprised of a hydrophobic core of polylactic acid and a hydrophilic shell of methoxy-poly (ethylene glycol); these may also improve solubility and targeting to the CNS. When a drug such as nimodipine is loaded into nanoparticles, a significant increase in CSF delivery (14X) occurred when compared to a simple nimodipine solution (67). However, such dramatic improvements in CSF delivery do not always occur – when chitosan nanoparticles were loaded with estradiol, there was no benefit above that of regular intranasal delivery (68, 69).

Efficient delivery to the CNS following intranasal administration also depends upon membrane permeability. Permeation enhancers such as surfactants, bile salts, lipids, cyclodextrins, polymers, and tight junction modifiers have been attempted. These compounds are often accompanied by nasal toxicity and increased permeation into the nasal vasculature, a problem for therapeutics having systemic side effects (70, 71). However, nasal perfusion studies to evaluate the brain distribution of vasoactive intestinal peptide demonstrated that permeation enhancement with lauroylcarnitine improved brain uptake compared to a formulation that lacked the permeation enhancer (72).

The pH of the formulation used and ionization status of the drug used also impacts the efficiency of intranasal delivery (73). Brain uptake of vasoactive intestinal peptide was greater with a pH of 9 (when the peptide takes a unionized form) when compared to a pH of 4, when the peptide is positively charged (72). Positively charged drugs may form electrostatic interactions with the negatively charged nasal epithelial cells, preventing transport beyond the nasal mucosa. This effect may depend on the drug, as negatively charged drugs can have greater CNS bioavailability after intranasal administration when compared to neutral drugs of similar size (74).

Clearance through mucociliary mechanisms can rapidly eliminate drugs from the nasal epithelium. Mucoadhesive agents, surface-engineered nanoparticles, efflux transporter inhibitors, and vasoconstrictors have been tried to reduce clearance by prolonging the exposure time for the intranasally delivered formulation at the nose. Such use of mucoadhesives and microemulsion formulations can greatly increase drug delivery to the CNS (63-65, 75). Mucoadhesives include acrylic acid derivatives, lectin, and low methylated pectin which form a viscous gel upon contact with nasal epithelium, slowing clearance from the nose (76-79). Nanoparticles containing ligands that bind to specific cell surfaces is another hopeful approach for the reduction of clearance and enhancement of targeted CNS delivery. One example is nanoparticles bound to *Ulex europaeus* agglutinin I (UEA I) which bind to receptors within the olfactory epithelium, while other

nanoparticles expressing wheat germ agglutinin can recognize sugar molecules, binding to receptors expressed throughout the olfactory and respiratory epithelia. Nanoparticles can be used in this way to help target specific pathways in the CNS via intranasal delivery, leading to changes in functional outcomes. Intranasal delivery of wheat germ agglutinin-conjugated nanoparticles carrying vasoactive intestinal peptide enhanced brain uptake and improved spatial memory in an Alzheimer's mouse model (80). Finally, the reduction of clearance from the nasal cavity due to efflux or absorption into nasal vasculature is another additional strategy to increase efficiency of intranasal delivery. Intranasal pre-treatment with an inhibitor (rifampin) of the P-gp efflux transport protein before intranasal administration of a P-gp substrate (verapamil) produced significantly greater brain concentrations due to reduced clearance (59). The use of a vasoconstrictor to prevent vascular uptake could also assist in CNS delivery of intranasal delivered agents. Intranasal administration of hypocretin-1 with a vasoconstrictor, phenylephrine, decreased the blood concentrations of hypocretin-1 (35). All of these techniques need to be considered for the optimal formulation to most efficiently intranasally deliver therapeutic agents to the CNS.

### 5. LIMITATIONS OF INTRANASAL DELIVERY

The use of intranasal delivery has some limitations that need to be considered. First, during long-term intranasal delivery, nasal irritation can develop. Particular drugs may have potentially adverse effects on the olfactory or trigeminal nerves, necessitating preclinical toxicology studies. The reduction of nasal irritation depends upon the specific drug being studied and the cause of nasal irritation. Second, the use of potent therapeutics which are active at very low concentrations (micromolar or lower) is required for drugs delivered via the intranasal route. Third, systemic delivery to some extent will occur with many molecules delivered intranasally, especially those that are small and lipophilic. These limitations must be considered in the design of any interventions to be delivered via the intranasal route.

### 6. APPLICATION OF INTRANASAL DELIVERY TECHNIQUES TO NEURODEGENERATIVE PROCESSES

#### 6.1. Alzheimer's disease and dementia

Alzheimer's disease (AD) is the most common form of dementia affecting our population, thought to be responsible for 70% of dementia (81). Dementia can be defined as the significant loss of intellectual abilities such as memory capacity, severe enough to interfere with social or occupational functioning. The clinical hallmarks of Alzheimer's disease are a progressive impairment in memory, judgment, decision making, orientation to physical surroundings, and language. The clinical diagnosis of probable Alzheimer disease is usually made on the basis of the history obtained, supported by findings on neurologic examination and blood work to exclude metabolic and vitamin deficiencies, for example. However, a definitive diagnosis can be made only at autopsy. Pathological changes in the Alzheimer's disease brain

include cerebral amyloid plaques (82), inflammation and neurofibrillary tangles (83). It is believed by many that these neuropathological changes are responsible for most of the condition (84), although other molecular changes occur and likely play an important pathophysiological role.

In our aging population, it has been estimated that 25 million people throughout the world are impacted by AD (85). It is known that the prevalence of dementia increases exponentially with age, with one third of our population  $\geq 85$  years currently suffering from dementia (86, 87). According to the Alzheimer Society Report, 1.5% of Canada's population suffered from dementia in 2008; it is expected that by the year 2038, that prevalence will rise to 2.8% (1,135,200 cases), bringing the actual incidence to about 103,700 cases per year, or one new diagnosis every 5 minutes in Canada alone. Economic impact of dementia has soared around the world - for Canada, AD cost our society about \$15 billion (Canadian dollars) in 2008, with this expected to increase to \$153 billion by the year 2038. Obviously, this is a disease that needs new approaches to its management before we are engulfed in its high prevalence and associated costs.

One of the molecules deficient in the human AD brain is the neurotransmitter acetylcholine. Most of the AD-specific treatments attempted to date have used oral agents targeting the enzyme acetylcholinesterase with the intention of increasing central acetylcholine levels. Treatments such as donepezil (88), galantamine and rivastigmine (89) have shown benefit, but remain considered as symptomatic therapies that fail to modify the course of the disease itself. These medications are also limited due to high hepatic metabolism and gastrointestinal side effects (89). More than 50% of patients treated with oral cholinesterase inhibitors have developed serious adverse drug reactions (ADR) or adverse events leading to discontinuation (90).

Parenteral treatments attempted in AD have included injections of antigenic portions of beta-amyloid ( $A\beta$ ) as a means of directly stimulating an immune response against the protein. Murine studies, however, demonstrated meningoencephalitis, a severe adverse effect, which also occurred in human AD patients during clinical trials (91). Further attempts to minimize such inflammatory responses led to parenteral injections of DNA prime adenovirus containing  $A\beta$  portions, leading to reduced levels of  $A\beta$  plaques without evident inflammation (92, 93). Other different immunogen variants have improved memory acquisition in mouse models of AD (94). Although early findings have been complicated by cases of meningoencephalitis, newer agents such as the humanized anti-  $A\beta$  monoclonal antibody, bapineuzumab, may be useful in reducing cortical fibrillary  $A\beta$  load in human patients (95). Further work is clearly needed before this route of management can be considered viable.

A number of molecules have been examined for efficacy via intranasal delivery in models of AD and human studies for patients with AD. Delivery of Nerve Growth Factor (NGF) has been used in models of AD, based upon

the beneficial effects of NGF in basal forebrain cholinergic neurons. Dysfunctional NGF signaling and/or processing from its precursor proNGF may also directly and causally be related to the aberrant activation of amyloidogenesis leading to neurodegeneration. Thus, NGF can be considered as an anti-amyloidogenic factor, adding to its potential for the treatment of AD. As well, the intranasal delivery of nerve growth factor (NGF) has been associated with reduced tau hyperphosphorylation and A $\beta$  accumulation in a mouse model of AD (96, 97). Furthermore, an engineered mutein of human NGF called hNGF-61, with an identical potency and bioactivity profile as NGF, was able to rescue phenotype changes in transgenic AD mice more effectively when provided intranasally as compared to intraocularly (98); the intranasal route of administration for NGF has been proven superior to intraocular routes in other studies as well (96, 99).

Intranasal vaccination with 25  $\mu$ g A $\beta$  (100, 101) or proteosome-based adjuvant added to glatiramer acetate, a synthetic copolymer used in the treatment of multiple sclerosis, both decreased A $\beta$  plaque volume in AD mouse models (102). As an intervention geared towards reduction of A $\beta$  accumulation, a significant lowering of the A $\beta$  burden in the brains of APP transgenic mice was also accompanied by some improvement in cognitive deficit (100).

Intranasal delivery of NAP (Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln), an octapeptide derived from activity dependent neuroprotective protein (ADNP), protects against A $\beta$ -induced neurotoxicity and significantly lowers levels of A $\beta$  present in brain (103). Following intranasal treatment with NAP at a daily dose of 500 ng for 3 months, mouse brains demonstrated decreased levels of A $\beta$  plaques, tau dephosphorylation, and greater stabilization of microtubules (103). Also called AL-108, the intranasal delivery of NAP has been performed in humans with mild cognitive impairment with initial, promising (but unpublished) results. Intranasal delivery of NAP has also currently entered Phase II clinical trials for patients with schizophrenia, and will soon be examined in clinical studies for AD in the near future as well (104).

The use of anti-acetylcholinesterases through other delivery systems has led to their use via intranasal delivery. The decreased levels of acetylcholine in the brain can be targeted by inhibiting central acetylcholinesterase, with the potential of promoting cholinergic neurotransmission. Again, this approach is largely symptomatic, and does not address the cause of the disease itself (105). Intranasal delivery of the anti-acetylcholinesterase colivelin improved memory impairment and activated the signal transducer and activator of transcription-3 (STAT3) pathway, upregulated acetylcholine (106). Improvement in memory with intranasal colivelin was comparable to intravenous and intraperitoneal injections (106). The intranasal delivery of acetylcholinesterase inhibitors such as tacrine (75), one of the first medications used for treatment of AD, and galantamine (107), has also been attempted to improve

brain targeting with this drug class. Intranasal delivery of NXX-066, a physostigmine analog and an acetylcholinesterase inhibitor (108), has been well tolerated in healthy subjects up to a single dose of 64 mg and multiple daily doses of 60 mg. A randomized, placebo-controlled, double-blind, single-center, inpatient bridging study was performed to determine the maximum tolerated dose of NXX-066 in the target patient population, determined to be 70 mg twice daily, with higher doses leading to nausea and/or vomiting, mild to moderate dizziness, headache, asthenia, and gastric symptoms. Wide variability in plasma levels of NXX-066 was observed for all dose schedules. Interestingly, AD patients tolerated larger daily doses of NXX-066 on a twice daily regimen than healthy normal subjects had tolerated with daily dosing (109). Further studies will determine if NXX-066 may be of therapeutic utility in the AD patient population.

Intranasal insulin delivery is currently under investigation for the treatment of Alzheimer's disease and for the management of cognitive decline related to diabetes. Initially, intranasal insulin was thought to represent a non-invasive alternative to subcutaneous insulin injections used by many diabetic patients. Insulin cannot be given orally due to rapid and thorough gastrointestinal degradation. In order to try to optimize insulin's entry into the systemic circulation, intranasal insulin has required assistance with enzyme inhibitors, mucoadhesives, and absorption enhancers to enhance systemic bioavailability. Nasal irritation from these additives, as well as high and frequent dosing, led to limited clinical success with intranasal insulin for diabetes management and its subsequent abandonment (110). Consequently, use of the intranasal method was initiated for direct insulin delivery to the brain for the treatment of neurodegenerative conditions, including Alzheimer's disease (111). Sometimes referred to as "type 3 diabetes" (112), investigations have demonstrated abnormalities in insulin and insulin receptor signalling in the brains of AD patients and in models of AD (113-120). The use of the intranasal delivery method was associated with improvements in memory and mood in normal subjects using either insulin (121) or an insulin analog (122). Importantly, intranasal insulin failed to impact upon blood insulin or glucose levels in the systemic circulation. In patients with AD, intranasal insulin dose-dependently improves memory after acute treatment (123, 124), and improved attention, memory, and cognitive function after 21 consecutive days of intranasal treatment (124). Intranasal insulin delivery (20-40 units/day) provided in human patients with AD led to improvements in verbal memory (124); these promising results require more detailed and prolonged cognitive studies, as well as further studies to determine optimized dosages. In an AD rat model with concurrent streptozotocin (STZ) injection to induce a model of type 1 diabetes, poor cognitive function, neurofilament phosphorylation, and downregulation of phosphoinositol-3-kinase (PI3K) pathway occurred in exaggerated fashion with diabetes and concurrent AD. It is possible that intranasal insulin treatment could help to reverse such changes and play an important role in AD and its related cognitive impairment (125).

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In addition to insulin, other peptides and proteins administered by the intranasal route can be beneficial in AD. Intranasal delivery of the peptide hormone, oxytocin, has led to significant changes in behavior, such as with increased trust (126, 127), decreased fear and anxiety (128, 129), and improved social behavior and memory (130).

Finally, beyond peptides and molecules, DNA plasmids, mesenchymal stem cells and glioma cells have been delivered intranasally. Intranasally administered plasmid DNA has led to both distribution in the brain and systemic absorption. A  $\beta$ -galactosidase protein encoded by these plasmids was significantly expressed in brain tissues after intranasal delivery, and kinetic studies identified intranasal delivery to be nearly 3000 fold more efficient than intravenous delivery for accessing the brain. Quantitative polymerase chain reaction studies demonstrated a direct route through the olfactory bulb accessed through intranasal delivery of plasmid DNA to the brain (131). Filamentous phages with a myelin oligodendrocyte glycoprotein epitope fused to a main coat protein have also been successfully delivered into the brain intranasally, impacting experimental autoimmune encephalomyelitis (EAE) (132). Stem cells have also been delivered intranasally, becoming apparent in the rodent brain within 1 hour of administration, suggesting that intranasal delivery may facilitate the use of stem cells for treating CNS disorders where neurodegeneration or cell death is prominent (133). Furthermore, intranasal delivery of mesenchymal stem cells in young mice suffering cerebral hypoxic-ischemic encephalopathy has led to attenuation of both grey and white matter loss and also improved function during cylinder rearing testing (134).

### 6.2. Diabetes and the Neurodegenerative Brain

Another syndrome that leads to neurodegenerative impairment is diabetes mellitus (DM). There are two forms of diabetes: type 1 (juvenile) diabetes, characterized by an inability to synthesize insulin; and type 2 diabetes, usually caused by insulin resistance in the periphery (135). It has been estimated that 170 million people world-wide currently have diabetes, and this number is expected to double by the year 2030 (136, 137). Initially investigated to identify a potentially non-invasive method of delivery of insulin to manage systemic hyperglycemia, intranasal insulin was also investigated as a potential prophylactic agent to prevent onset of type 1 diabetes in susceptible children. In mouse models of diabetes, prophylactic administration of insulin reduced incidence of the disease. However, the delivery of intranasal insulin to children born with diabetes-associated autoantibodies did not prevent or delay onset of type 1 diabetes (138).

It has been suggested that insulin may serve as a neuroprotective factor via activity at both insulin and insulin-like growth factor receptors. Murine models of DM share several pathological and behavioral characteristics with murine models of AD, demonstrating impaired learning, attenuation of the insulin signaling pathway, as well as accumulation of pathological markers seen in AD, including tau hyperphosphorylation and A $\beta$  plaque accumulation. Subcutaneous delivery of insulin at low

doses (0.3 U insulin/day) can repair some of these deficits (115).

A study of intranasal insulin in streptozotocin (STZ)-induced diabetic mice suggested that noted cognitive decline, brain atrophy and white matter changes can be at least partially related to a relative insulin deficiency (51). Replacement of insulin in the brain using intranasal delivery was compared to subcutaneous delivery of a similar dose in a long term model of murine diabetes. After many months of diabetes, mice had measureable changes in cognitive behavioral testing, as well as neuroimaging demonstrating both cerebral atrophy and white matter deficiencies identified with magnetic resonance imaging and with structural assessment. These changes were also associated with loss of important synaptic proteins and myelin-related proteins. Intranasal insulin delivery over life was associated with partial protection against such pathological changes and improved cognitive outcomes later in life. Meanwhile, delivery of identical doses of subcutaneous insulin was associated with problems with hypoglycemia, less significant penetration into the CNS, and with absence of benefit upon cognitive and structural deficiencies. Such cognitive and pathological changes are compatible with noted cognitive and structural changes in the human diabetic brain after a long duration of either type 1 or 2 diabetes (139-144). Although investigations identified restoration of insulin receptor-mediated pathways involving PI3K and Akt with intranasal insulin, further work will be required to determine the most important contribution of insulin with the diabetic brain. As the diabetes epidemic further evolves, greater understanding of the pathogenesis of these pathway deficiencies may translate to further therapeutic potential. Delivery of intranasal insulin to human subjects with diabetes with the goal of preventing neurodegenerative changes has not yet been attempted, although evidence to date suggests that the method would be safe, without adverse nasal effects and with minimal chance of hypoglycemia (145).

### 7. APPLICATION OF INTRANASAL DELIVERY TO THE TREATMENT OF STROKE

Stroke is the third leading cause of death in the United States and one of the leading causes of severe disability in adults. Tissue plasminogen activator, approved for use in 1996, remains the principal drug indicated for the treatment of acute ischemic stroke (146, 147). However, this treatment has very significant limitations including the short window of time available for effective treatment, the need for rapid imaging and blood pressure control and the poor response of patients with large-artery occlusions. Most problematic is the increased risk of hemorrhage associated with this treatment(147).

Neurotrophic factors, also called neurotrophins and nerve growth factors, are therapeutic proteins which have long been considered as possible treatments for stroke. It has been demonstrated that intracerebroventricular insulin-like growth factor-I (IGF-I) significantly reduces the extent of infarction and global neuronal loss in an adult rat model of transient unilateral

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hypoxic–ischemic injury induced by ligation of the right carotid artery and exposure to 6 % O<sub>2</sub> for 10 min (148, 149). However, intracerebroventricular administration is not practical in humans, and IGF-I does not efficiently cross the blood-brain barrier. Frey first developed the intranasal method for bypassing the blood-brain barrier to deliver neurotrophic factors and other therapeutic proteins to the brain for disease processes such as stroke (10).

Following the demonstration by Thorne *et al* (12) that intranasal IGF-I rapidly bypasses the blood-brain barrier via the olfactory and trigeminal neural pathways to reach the central nervous system (CNS), it was subsequently shown that that intranasal IGF-I reduces infarct volume in the middle cerebral artery occlusion (MCAO) model of stroke (150, 151). Intranasal treatment with doses of 150 µg IGF-I significantly reduced infarct volume by 63% and improved motor, sensory, reflex and vestibulomotor functions. Intranasal treatment with doses of 75 µg IGF-I significantly reduced infarct volumes by 60 % and hemispheric swelling by 45.6 %. Neurologic function, assessed by the postural reflex, flexor response and adhesive tape tests, was also improved by this treatment. To our knowledge, this was the first successful intranasal treatment of stroke in animal models.

Even when intranasal IGF-I treatment was delayed either 2 or 4 hours after the onset of MCAO, infarct volume was reduced by 54 % and 39 %, respectively and improved motor-sensory and somatosensory function was observed when IGF-I was administered 2 hours after the onset of MCAO (152). In addition, treatment with intranasal IGF-I at 2, 4, or 6 hours after MCAO decreased apoptotic cell counts by more than 90% in the hemisphere ipsilateral to the occlusion (152). Effectiveness of intranasal IGF-I treatment of stroke was confirmed by an independent group in another model, as Lin *et al.* reported reduced brain injury by approximately 56 % (based on neuropathologic score) as compared to that in the vehicle-treated neonatal rats up to 1 h after cerebral hypoxic-ischemic injury (153). Moreover, IGF-I treatment improved neurobehavioral performance in juvenile rats subjected to hypoxia-ischemia, where intranasal IGF-I inhibited apoptotic cell death and enhanced proliferation of neuronal and oligodendroglial progenitors after cerebral hypoxia-ischemia (153).

In addition to IGF-I, evidence for effective stroke treatment in animal models using intranasal neurotrophins includes erythropoietin, vascular endothelial growth factor, transforming growth factor-β1, basic fibroblast growth factor, and transforming growth factor-α. Erythropoietin (EPO), when administered intranasally to rats 10 min after MCAO and 1 h after reperfusion, reduced infarct volume, brain swelling and cell damage in the ischemic hemispheres, and improved behavioral function 24 h after stroke (154). IGF-I was reported to be synergistic with EPO as a treatment for reducing apoptosis in a cerebrocortical neuron culture model exposed to NMDA (155). Intranasal administration of EPO plus IGF-I was subsequently shown to reduce infarct volumes in a mouse model of stroke within 24 hours and to improve

neurological function up to 90 days after MCAO. Both EPO and IGF-I were demonstrated to reach the brain within 20 minutes after intranasal administration and to accumulate within the injured areas of the brain. Intranasal delivery of EPO and IGF-I to the brain was more effective than any of intravenous, subcutaneous, or intraperitoneal delivery (12, 156, 157).

Vascular Endothelial Growth Factor (VEGF), which is upregulated in ischemic brain tissue following stroke, is a candidate for prophylactic and therapeutic strategies in ischemic stroke due to its angiogenic, neurotrophic and neuroprotective functions. Nevertheless, attempts to use VEGF therapeutically have been difficult due to the obstruction of the BBB. Intranasal delivery of VEGF to bypass the BBB and to access the brain tissue with minimal tissue affection achieved a significantly higher VEGF tissue concentration when compared to intravenous administration in critical brain regions, including the striatum or the midbrain (158). Intranasal VEGF was shown to reduce infarct volume, improve neurologic behavioral recovery and promote angiogenesis in rats following MCAO (159). Surprisingly, both the reduction in infarct volume and the improvement in behavior were not dependent on dose in the usual fashion but rather were seen only at one dose with both higher and lower doses being ineffective.

Very low doses (4 ng) of transforming growth factor-β1 (TGF-β1), when administered by intracerebroventricular injection or intrahippocampal injection to rats, were found to protect hippocampal neurons against degeneration caused by transient global ischemia (160). Non-invasive intranasal TGF-β1 (1 µg) resulted in significant improvement in neurological function and reduction of infarct volume in mice following MCAO (161). TGF-β1-treated mice had significantly less TUNEL-positive cells in the ipsilateral striatum than that in control groups. The number of BrdU-incorporated cells in the SVZ and striatum was significantly increased in the TGF-β1-treated group. Thus, intranasal TGF-β1 reduces infarct volume, improves functional recovery and enhances neurogenesis in mice after stroke (161).

As noted earlier, intranasal IGF-I treatment reduced infarct volume even when treatment was not begun until 4 h after MCAO (152). However, there were no reports of successful intranasal treatment of stroke after a delay of 24 h until rats were treated with intranasal basic fibroblast growth factor (bFGF) following MCAO (162). It was demonstrated that delayed intranasal bFGF treatment improved functional recovery in adult rats and promoted the proliferation of endogenous neural progenitor cells, even though there was no effect on infarct volume. They also demonstrated that these proliferated cells could differentiate into neurons. From this, it was concluded that the improved behavioral recovery was likely related to the stimulation of neurogenesis by intranasal bFGF (162). This work was based, in part, on the earlier work showing that intranasal bFGF stimulated neurogenesis in the normal adult rat brain (163) and after studies demonstrated that intranasal bFGF was protective when administered



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immediately after a two hour MCAO and induced progenitor cell proliferation (161).

More recently, it was shown that intranasal transforming growth factor- $\alpha$  (TGF $\alpha$ ) effectively improved animals with stroke even when treatment was delayed for 4 weeks after injury (164, 165). Needless to say, demonstrating efficacy after such a considerable delay in treatment is both surprising and promising. TGF $\alpha$  is a mitogen and neurotrophic factor. In a rat MCAO model, intranasal TGF $\alpha$  produced a massive proliferative response in the brain. Near complete (99%) behavioral recovery was observed with this intranasal treatment. The authors concluded that intranasal TGF $\alpha$  is a potential therapeutic strategy for chronic stroke and other neurological damage in humans. Because the stability of therapeutic proteins can sometimes be enhanced by PEGylation, it was determined the effect of intranasally administered PEG-TGF $\alpha$  on cellular proliferation and on animal behavior in a chronic-phase model of stroke (165). Once weekly intranasal treatment was initiated one month after the focal ischemic injury and continued for only 4 weeks. The authors reported that intranasal PEG-TGF- $\alpha$  induces proliferation of neural progenitors and their migration to the damaged part of the brain. This was associated with very significant behavioral improvement in the MCAO model (165).

Not all reports of intranasal neurotrophic factor treatment of stroke have been successful. Cheng *et al.* (2009) recently investigated the effects of intranasal nerve growth factor (NGF), electroacupuncture (EA) or the combination of the two treatments on neurological functional recovery and neural progenitors in the rat MCAO model of stroke (166). Surprisingly while the combination treatment resulted in a significant reduction in infarct volume, increased cell proliferation and survival of neuronal progenitors and improved neurological function, treatment with either intranasal NGF or EA alone produced no significant improvement in any of these parameters. The authors conclude that intranasal NGF and EA may have a synergistic effect in reducing ischemic injury and enhancing recovery of neurological function (166).

In addition to neurotrophins, there is also a report of an intranasally administered small molecule for the treatment of stroke – desferoxamine (DFO). Iron accumulates in the brain during stroke (167). DFO not only binds iron with very high-affinity ( $10^{31}$ ), but also increases hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) which is neuroprotective (168). Hanson *et al.* have reported that intranasal administration targets DFO to the brain approximately 200-fold while reducing systemic exposure (169). Intranasal DFO resulted in significantly higher DFO concentrations in the brain (0.9–18.5  $\mu$ M) at 30 min after intranasal administration than after intravenous administration (0.1– 0.5  $\mu$ M). Further, they have demonstrated that intranasal DFO prevents and treats stroke damage after MCAO in rats. Pretreatment with intranasal DFO (three 6-mg doses) 48 h before MCAO significantly reduced infarct volume by 55 %. Treatment with intranasal DFO (six 6-mg doses) immediately after stroke and

reperfusion significantly decreased infarct volume by 55 %. Intranasal administration of three 6-mg doses of DFO did not cause clinically significant changes in blood pressure or heart rate. Thus intranasal DFO may be a useful treatment for stroke, and a prophylactic for patients at high risk for stroke (169).

In addition to the treatment of ischemic stroke, intranasal delivery of calcitonin gene-related peptide (CGRP) has been provided to rats in a model of subarachnoid hemorrhage; when compared to intravenous delivery, intranasal CGRP delivery led to levels of CGRP 10 times higher in the brain and contributed to amelioration of vasospasm, improved cerebral blood flow and reductions in cell death for cortical neurons and endothelial cells (170). This may be a potential future clinical approach to management of subarachnoid hemorrhage in human patients.

The results of these studies performed in a number of different laboratories over a period of 10 years demonstrates that a variety of therapeutic agents are effective at treating stroke when they are administered by noninvasive intranasal delivery. Toxicology studies are now needed to confirm safety and in animals so that intranasal stroke therapeutics can be tested in clinical trials in both normal adults and patients with stroke.

## 8. THE FUTURE OF INTRANASAL DELIVERY

Important questions remain regarding the safety and efficacy of intranasal therapeutics to treat human neurodegenerative conditions including Alzheimer's disease and stroke. Studies in animal models of Alzheimer's disease, cognitive decline in diabetes, stroke and other CNS disorders have demonstrated efficacy for intranasal therapeutics. Some of the human clinical trials reported to date have also yielded promising results, especially the improved memory, attention and functioning observed in patients in the early stages of AD treated with intranasal insulin. Preclinical success with intranasal therapeutics needs to be translated, following the requisite toxicology testing, to human clinical trials to assess safety and efficacy. All of this will require funding from the NIH, pharmaceutical industry, foundations and private funders.

In addition, there is room for new developments in both therapeutics, formulation and device technology to improve the targeting of intranasal therapeutics not only to the CNS, but if possible to more specific CNS regions and targets. At present, the outlook for intranasal delivery of therapeutics to the CNS is bright because it is non-invasive, rapid, requires no modification of the therapeutic agent and can bypass the BBB to target the CNS while reducing systemic exposure and side effects.

## 9. CONCLUSION

Intranasal delivery is a non-invasive method for targeting therapeutics to the CNS which has yielded beneficial effects in the treatment of neurologic disease in humans and in animal models of disease. One of the major

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advantages of intranasal delivery is the potential of bypassing the BBB to target the CNS while minimizing systemic exposure and lowering the risk of adverse side effects. Intranasal therapeutics can potentially improve the lives of millions of people affected by neurodegenerative diseases and other CNS disorders, but there is still work to be done to translate the preclinical findings to humans and to continue the clinical trials already in progress. Many researchers in different countries around the world are working hard to achieve this goal.

### 10. REFERENCES

1. WM Partridge, WH Oldendorf, P Cancilla, HJ Frank: Blood brain barrier: interface between internal medicine and the brain. *Annals of Internal Medicine* 105, 82-95 (1986)
2. WA Banks: Delivery of peptides to the brain: emphasis on therapeutic development. *Biopolymers* 90, 589-594 (2008)
3. M Suzumura, M Kawamura, S Kikuchi, A Kawada, Y Shibayama, J Otabe: Intranasal oxytocin for the induction and stimulation of labor. *J Jpn Obstet Gynecol Soc* 13, 42-50 (1966)
4. C Jones: Clinical experience with intranasal oxytocin spray. *Med J Aust* 2, 1099-1101 (1966)
5. RT Hoover: Intranasal oxytocin in eighteen hundred patients. A study on its safety as used in a community hospital. *Am J Obstet Gynecol* 110, 788-794 (1971)
6. S Sjostedt: Induction of labour. A comparison of intranasal and transbuccal administration of oxytocin. *Acta Obstet Gynecol Scand Suppl* 7, Suppl 7:1-17 (1969)
7. J Laine: Experience of the use of intranasal, buccal and intravenous oxytocin as methods of inducing labour. *Acta Obstet Gynecol Scand* 49, 149-159 (1970)
8. WHn Frey, inventor Chiron Corporation, assignee. Method of administering neurologic agents to the brain. . US. 1997.
9. Wn Frey, inventor Neurologic Agents for Nasal Administration to the Brain Geneva, Switzerland. 1991 (priority date 5.12.89).
10. SV Dhuria, LR Hanson, WH Frey, 2nd: Intranasal delivery to the central nervous system: mechanisms and experimental considerations. *J Pharm Sci* 99, 1654-1673 (2010)
11. WHn Frey: Bypassing the blood-brain barrier to deliver therapeutic agents to the brain and spinal cord. . *Drug Del Tech* 2, 46-49 (2002)
12. RG Thorne, GJ Pronk, V Padmanabhan, WHn Frey: Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience* 127, 481-496 (2004)
13. DS Dhanda, WH Frey, D Leopold, UB Kompella: Approaches for drug deposition in the human olfactory epithelium. . *Drug Del Tech* 5, 64-72 (2005)
14. LB Buck. The chemical senses. In: *Principles of neural science*. Eds: Kandel ER, Schwartz JH, Jessell TM, eds. pp. 625-652 McGraw- Hill Companies, New York: (2000)
15. SH Fuss, M Omura, P Mombaerts: The Grueneberg ganglion of the mouse projects axons to glomeruli in the olfactory bulb. *Eur J Neurosci* 22, 2649-2654 (2005)
16. DS Koos, SE Fraser: The Grueneberg ganglion projects to the olfactory bulb. *Neuroreport* 16, 1929-1932 (2005)
17. BJ Balin, RD Broadwell, M Salcman, M el-Kalliny: Avenues for entry of peripherally administered protein to the central nervous system in mouse, rat, and squirrel monkey. *J Comp Neurol* 251, 260-280 (1986)
18. B Jansson, E Bjork: Visualization of in vivo olfactory uptake and transfer using fluorescein dextran. *J Drug Target*. 10, 379-386 (2002)
19. RG Thorne, LR Hanson, TM Ross, D Tung, WH Frey, 2nd: Delivery of interferon-beta to the monkey nervous system following intranasal administration. *Neuroscience* 152, 785-797 (2008)
20. TM Ross, RN Zuckermann, C Reinhard, WH Frey, 2nd: Intranasal administration delivers peptoids to the rat central nervous system. *Neurosci Lett* 439, 30-33 (2008)
21. N Nonaka, SA Farr, H Kageyama, S Shioda, WA Banks: Delivery of galanin-like peptide to the brain: targeting with intranasal delivery and cyclodextrins. *J Pharmacol Exp Ther* 325, 513-519 (2008)
22. A Mackay-Sim. Neurogenesis in the adult olfactory neuroepithelium. . In: *Handbook of olfaction and gustation*. Eds: Doty RL, ed. 93-113. Marcel Dekker, Inc. ,New York: (2003)
23. SK Williams, RJ Franklin, SC Barnett: Response of olfactory ensheathing cells to the degeneration and regeneration of the peripheral olfactory system and the involvement of the neuregulins. *J Comp Neurol* 470, 50-62 (2004)
24. RG Thorne, GJ Pronk, V Padmanabhan, WH Frey: Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience* 127, 481-496 (2004)
25. RG Thorne, WH Frey, 2nd: Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. *Clin Pharmacokinet* 40, 907-946 (2001)

## Intranasal treatment of neurodegenerative disease and stroke

26. H Baker, RF Spencer: Transneuronal transport of peroxidase-conjugated wheat germ agglutinin (WGA-HRP) from the olfactory epithelium to the brain of the adult rat. *Exp Brain Res* 63, 461-473 (1986)
27. RD Broadwell, BJ Balin: Endocytic and exocytic pathways of the neuronal secretory process and trans-synaptic transfer of wheat germ agglutinin-horseradish peroxidase in vivo. *J Comp Neurol* 242, 632-650 (1985)
28. K Kristensson, Y Olsson: Uptake of exogenous proteins in mouse olfactory cells. *Acta Neuropathol* 19, 145-154 (1971)
29. XQ Chen, JR Fawcett, YE Rahman, TA Ala, WH Frey II: Delivery of Nerve Growth Factor to the Brain via the Olfactory Pathway. *J Alzheimers.Dis.* 1, 35-44 (1998)
30. HM Zhao, XF Liu, XW Mao, CF Chen: Intranasal delivery of nerve growth factor to protect the central nervous system against acute cerebral infarction. *Chin Med Sci J* 19, 257-261 (2004)
31. DM Clerico, WC To, DC Lanza. Anatomy of the human nasal passages. . In: *Handbook of olfaction and gustation*. Eds: Doty RL, ed. 1-16.Marcel Dekker, Inc. ,New York (2003 )
32. H Gray. Gray's anatomy. , 15th revised (Classic Collectors edition) ed. New York: Bounty Books, 1978
33. ML Schaefer, B Bottger, WL Silver, TE Finger: Trigeminal collaterals in the nasal epithelium and olfactory bulb: a potential route for direct modulation of olfactory information by trigeminal stimuli. *J Comp Neurol* 444, 221-226 (2002)
34. TM Ross, PM Martinez, JC Renner, RG Thorne, LR Hanson, WH Frey: Intranasal administration of interferon beta bypasses the blood-brain barrier to target the central nervous system and cervical lymph nodes: a non-invasive treatment strategy for multiple sclerosis. *Journal of Neuroimmunology* 151, 66-77 (2004)
35. SV Dhuria, LR Hanson, WH Frey, 2nd: Novel vasoconstrictor formulation to enhance intranasal targeting of neuropeptide therapeutics to the central nervous system. *J Pharmacol Exp Ther* 328, 312-320 (2009)
36. LR Hanson, PM Martinez, S Taheri, L Kamsheh, E Mignot, WHI Frey: Intranasal administration of hypocretin 1 (orexin A) bypasses the blood-brain barrier & targets the brain: A new strategy for the treatment of narcolepsy. . *Drug Del Tech* 4, 66-70 (2004 )
37. N Cauna. Blood and nerve supply of the nasal lining. . In: *The nose: Upper airway physiology and the atmospheric environment*. Eds: Proctor DF, Andersen I, eds. 45-69Elsevier Biomedical Press,Amsterdam (1982)
38. G Grevers, U Herrmann: Fenestrated endothelia in vessels of the nasal mucosa. An electron-microscopic study in the rabbit. *Arch Otorhinolaryngol* 244, 55-60 (1987)
39. P Van Diest, MW Kanan: An ultrastructural study of the endonasal microcirculation in the Wistar rat during fetal and early postnatal life. *J Anat* 128, 293-300 (1979)
40. HF Cserr, DN Cooper, PK Suri, CS Patlak: Efflux of radiolabeled polyethylene glycols and albumin from rat brain. *Am J Physiol* 240, F319-328 (1981)
41. DR Groothuis, MW Vavra, KE Schlageter, EW Kang, AC Itskovich, S Hertzler, CV Allen, HL Lipton: Efflux of drugs and solutes from brain: the interactive roles of diffusional transcapillary transport, bulk flow and capillary transporters. *J Cereb Blood Flow Metab* 27, 43-56 (2007)
42. P Hadaczek, Y Yamashita, H Mirek, L Tamas, MC Bohn, C Noble, JW Park, K Bankiewicz: The "perivascular pump" driven by arterial pulsation is a powerful mechanism for the distribution of therapeutic molecules within the brain. *Mol Ther* 14, 69-78 (2006)
43. ML Rennels, OR Blaumanis, PA Grady: Rapid solute transport throughout the brain via paravascular fluid pathways. *Adv Neurol* 52, 431-439 (1990)
44. ML Rennels, TF Gregory, OR Blaumanis, K Fujimoto, PA Grady: Evidence for a 'paravascular' fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space. *Brain Res* 326, 47-63 (1985)
45. MW Bradbury, RJ Westrop: Factors influencing exit of substances from cerebrospinal fluid into deep cervical lymph of the rabbit. *J Physiol* 339, 519-534 (1983)
46. E Hatterer, N Davoust, M Didier-Bazes, C Vuillat, C Malcus, MF Belin, S Nataf: How to drain without lymphatics? Dendritic cells migrate from the cerebrospinal fluid to the B-cell follicles of cervical lymph nodes. *Blood* 107, 806-812 (2006)
47. M Johnston, A Zakharov, C Papaiconomou, G Salmasi, D Armstrong: Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates and other mammalian species. *Cerebrospinal Fluid Res* 1, 2 (2004)
48. S Kida, A Pantazis, RO Weller: CSF drains directly from the subarachnoid space into nasal lymphatics in the rat. Anatomy, histology and immunological significance. *Neuropathol Appl Neurobiol* 19, 480-488 (1993)
49. BA Walter, VA Valera, S Takahashi, T Ushiki: The olfactory route for cerebrospinal fluid drainage into the peripheral lymphatic system. *Neuropathol Appl Neurobiol* 32, 388-396 (2006)

## Intranasal treatment of neurodegenerative disease and stroke

50. J Born, T Lange, W Kern, GP McGregor, U Bickel, HL Fehm: Sniffing neuropeptides: a transnasal approach to the human brain. *Nature Neuroscience* 5, 514-516 (2002)
51. GJ Francis, JA Martinez, WQ Liu, K Xu, A Ayer, J Fine, UI Tuor, G Glazner, LR Hanson, WH Frey, 2nd, C Toth: Intranasal insulin prevents cognitive decline, cerebral atrophy and white matter changes in murine type I diabetic encephalopathy. *Brain* 131, 3311-3334 (2008)
52. G Francis, J Martinez, W Liu, T Nguyen, A Ayer, J Fine, D Zochodne, LR Hanson, WH Frey, 2nd, C Toth: Intranasal Insulin Ameliorates Experimental Diabetic Neuropathy. *Diabetes* 58, 934-945 (2009)
53. SV Dhuria, LR Hanson, WH Frey, 2nd: Intranasal drug targeting of hypocretin-1 (orexin-A) to the central nervous system. *J Pharm Sci* 98, 2501-2515 (2009)
54. L Illum: Is nose-to-brain transport of drugs in man a reality? *J Pharm Pharmacol* 56, 3-17 (2004)
55. KK Kandimalla, MD Donovan: Localization and differential activity of P-glycoprotein in the bovine olfactory and nasal respiratory mucosae. *Pharm Res* 22, 1121-1128 (2005)
56. CL Graff, GM Pollack: Functional evidence for P-glycoprotein at the nose-brain barrier. *Pharm Res* 22, 86-93 (2005)
57. MA Wioland, J Fleury-Feith, P Corlieu, F Commo, G Monceaux, J Lacau-St-Guilly, JF Bernaudin: CFTR, MDR1, and MRP1 immunolocalization in normal human nasal respiratory mucosa. *J Histochem Cytochem* 48, 1215-1222 (2000)
58. KK Kandimalla, MD Donovan: Carrier mediated transport of chlorpheniramine and chlorcyclizine across bovine olfactory mucosa: implications on nose-to-brain transport. *J Pharm Sci* 94, 613-624 (2005)
59. CL Graff, GM Pollack: P-Glycoprotein attenuates brain uptake of substrates after nasal instillation. *Pharm Res* 20, 1225-1230 (2003)
60. A Minn, S Leclerc, JM Heydel, AL Minn, C Denizcot, M Cattarelli, P Netter, D Gradinaru: Drug transport into the mammalian brain: the nasal pathway and its specific metabolic barrier. *J Drug Target* 10, 285-296 (2002)
61. MA Sarkar: Drug metabolism in the nasal mucosa. *Pharm Res* 9, 1-9 (1992)
62. F Miragall, D Krause, U de Vries, R Dermietzel: Expression of the tight junction protein ZO-1 in the olfactory system: presence of ZO-1 on olfactory sensory neurons and glial cells. *J Comp Neurol* 341, 433-448 (1994)
63. TK Vyas, AK Babbar, RK Sharma, S Singh, A Misra: Intranasal mucoadhesive microemulsions of clonazepam: preliminary studies on brain targeting. *J Pharm Sci* 95, 570-580 (2006)
64. TK Vyas, AK Babbar, RK Sharma, S Singh, A Misra: Preliminary brain-targeting studies on intranasal mucoadhesive microemulsions of sumatriptan. *AAPS PharmSciTech* 7, E8 (2006)
65. M Kumar, A Misra, AK Babbar, AK Mishra, P Mishra, K Pathak: Intranasal nanoemulsion based brain targeting drug delivery system of risperidone. *Int J Pharm* 358, 285-291 (2008)
66. Q Zhang, X Jiang, W Jiang, W Lu, L Su, Z Shi: Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain. *Int J Pharm* 275, 85-96 (2004)
67. QZ Zhang, LS Zha, Y Zhang, WM Jiang, W Lu, ZQ Shi, XG Jiang, SK Fu: The brain targeting efficiency following nasally applied MPEG-PLA nanoparticles in rats. *J Drug Target* 14, 281-290 (2006)
68. X Wang, N Chi, X Tang: Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting. *Eur J Pharm Biopharm* 70, 735-740 (2008)
69. X Wang, H He, W Leng, X Tang: Evaluation of brain-targeting for the nasal delivery of estradiol by the microdialysis method. *Int J Pharm* 317, 40-46 (2006)
70. SC Chen, K Eiting, K Cui, AK Leonard, D Morris, CY Li, K Farber, AP Sileno, ME Houston, Jr., PH Johnson, SC Quay, HR Costantino: Therapeutic utility of a novel tight junction modulating peptide for enhancing intranasal drug delivery. *J Pharm Sci* 95, 1364-1371 (2006)
71. SS Davis, L Illum: Absorption enhancers for nasal drug delivery. *Clin Pharmacokinet* 42, 1107-1128 (2003)
72. C Dufes, JC Olivier, F Gaillard, A Gaillard, W Couet, JM Muller: Brain delivery of vasoactive intestinal peptide (VIP) following nasal administration to rats. *Int J Pharm* 255, 87-97 (2003)
73. T Sakane, M Akizuki, S Yamashita, H Sezaki, T Nadai: Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the dissociation of the drug. *J Pharm Pharmacol* 46, 378-379 (1994)
74. ST Charlton, J Whetstone, ST Fayinka, KD Read, L Illum, SS Davis: Evaluation of direct transport pathways of glycine receptor antagonists and an angiotensin antagonist from the nasal cavity to the central nervous system in the rat model. *Pharm Res* 25, 1531-1543 (2008)
75. VV Jogani, PJ Shah, P Mishra, AK Mishra, AR Misra: Intranasal mucoadhesive microemulsion of tacrine to improve brain targeting. *Alzheimer Dis Assoc Disord* 22, 116-124 (2008)

## Intranasal treatment of neurodegenerative disease and stroke

76. ST Charlton, SS Davis, L Illum: Evaluation of effect of ephedrine on the transport of drugs from the nasal cavity to the systemic circulation and the central nervous system. *J Drug Target* 15, 370-377 (2007)
77. NS Barakat, SA Omar, AA Ahmed: Carbamazepine uptake into rat brain following intra-olfactory transport. *J Pharm Pharmacol* 58, 63-72 (2006)
78. Z Cai, SX Hou, ZX Yang, XR Song, QH Chen, YB Li, BB Zhao: [Brain targeting of gastrodin nasal in situ gel]. *Sichuan Da Xue Xue Bao Yi Xue Ban* 39, 438-440 (2008)
79. Y Zhao, P Yue, T Tao, QH Chen: Drug brain distribution following intranasal administration of Huperzine A in situ gel in rats. *Acta Pharmacol Sin* 28, 273-278 (2007)
80. X Gao, B Wu, Q Zhang, J Chen, J Zhu, W Zhang, Z Rong, H Chen, X Jiang: Brain delivery of vasoactive intestinal peptide enhanced with the nanoparticles conjugated with wheat germ agglutinin following intranasal administration. *J Control Release* 121, 156-167 (2007)
81. BL Plassman, KM Langa, GG Fisher, SG Heeringa, DR Weir, MB Ofstedal, JR Burke, MD Hurd, GG Potter, WL Rodgers, DC Steffens, RJ Willis, RB Wallace: Prevalence of dementia in the United States: the aging, demographics, and memory study. *Neuroepidemiology* 29, 125-132 (2007)
82. J Hardy, DJ Selkoe: The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353-356 (2002)
83. TC Dickson, HL Saunders, JC Vickers: Relationship between apolipoprotein E and the amyloid deposits and dystrophic neurites of Alzheimer's disease. *Neuropathol Appl Neurobiol* 23, 483-491 (1997)
84. GL Wenk: Neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry* 64 Suppl 9, 7-10 (2003)
85. R Brookmeyer, E Johnson, K Ziegler-Graham, HM Arrighi: Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement* 3, 186-191 (2007)
86. A Ott, MM Breteler, F van Harskamp, JJ Claus, TJ van der Cammen, DE Grobbee, A Hofman: Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam study. *BMJ* 310, 970-973 (1995)
87. RJ Caselli, TG Beach, R Yaari, EM Reiman: Alzheimer's disease a century later. *J Clin Psychiatry* 67, 1784-1800 (2006)
88. S Gauthier, OL Lopez, G Waldemar, RW Jones, J Cummings, R Zhang, R Schindler, E Schwam: Effects of donepezil on activities of daily living: integrated analysis of patient data from studies in mild, moderate and severe Alzheimer's disease. *Int Psychogeriatr* 22, 973-983 (2010)
89. B Borah, P Sacco, V Zarotsky: Predictors of adherence among Alzheimer's disease patients receiving oral therapy. *Curr Med Res Opin* 26, 1957-1965
90. A Pariente, DJ Sanctussy, G Miremont-Salame, N Moore, F Haramburu, A Fourier-Reglat: Factors associated with serious adverse reactions to cholinesterase inhibitors: a study of spontaneous reporting. *CNS Drugs* 24, 55-63 (2010)
91. Y Okura, Y Matsumoto: DNA vaccine therapy for Alzheimer's disease: present status and future direction. *Rejuvenation Res* 11, 301-308 (2008)
92. HD Kim, K Tahara, JA Maxwell, R Lalonde, T Fukuiwa, K Fujihashi, KR Van Kampen, FK Kong, DC Tang, K Fukuchi: Nasal inoculation of an adenovirus vector encoding 11 tandem repeats of Abeta1-6 upregulates IL-10 expression and reduces amyloid load in a Mo/Hu APPswe PS1dE9 mouse model of Alzheimer's disease. *J Gene Med* 9, 88-98 (2007)
93. HD Kim, JJ Jin, JA Maxwell, K Fukuchi: Enhancing Th2 immune responses against amyloid protein by a DNA prime-adenovirus boost regimen for Alzheimer's disease. *Immunol Lett* 112, 30-38 (2007)
94. M Maier, TJ Seabrook, ND Lazo, L Jiang, P Das, C Janus, CA Lemere: Short amyloid-beta (Abeta) immunogens reduce cerebral Abeta load and learning deficits in an Alzheimer's disease mouse model in the absence of an Abeta-specific cellular immune response. *J Neurosci* 26, 4717-4728 (2006)
95. JO Rinne, DJ Brooks, MN Rossor, NC Fox, R Bullock, WE Klunk, CA Mathis, K Blennow, J Barakos, AA Okello, S Rodriguez Martinez de Liano, E Liu, M Koller, KM Gregg, D Schenk, R Black, M Grundman: 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol* 9, 363-372 (2010)
96. R De Rosa, AA Garcia, C Braschi, S Capsoni, L Maffei, N Berardi, A Cattaneo: Intranasal administration of nerve growth factor (NGF) rescues recognition memory deficits in AD11 anti-NGF transgenic mice. *Proc Natl Acad Sci U S A* 102, 3811-3816 (2005)
97. S Capsoni, S Giannotta, A Cattaneo: Nerve growth factor and galantamine ameliorate early signs of neurodegeneration in anti-nerve growth factor mice. *Proc Natl Acad Sci U S A* 99, 12432-12437 (2002)
98. S Covaceuszach, S Capsoni, G Ugolini, F Spirito, D Vignone, A Cattaneo: Development of a non invasive NGF-based therapy for Alzheimer's disease. *Curr Alzheimer Res* 6, 158-170 (2009)
99. S Capsoni, S Covaceuszach, G Ugolini, F Spirito, D Vignone, B Stefanini, G Amato, A Cattaneo: Delivery of NGF to the brain: intranasal versus ocular administration in anti-NGF transgenic mice. *J Alzheimers Dis* 16, 371-388 (2009)
100. CA Lemere, R Maron, DJ Selkoe, HL Weiner: Nasal vaccination with beta-amyloid peptide for the treatment of Alzheimer's disease. *DNA Cell Biol* 20, 705-711 (2001)

## Intranasal treatment of neurodegenerative disease and stroke

101. HL Weiner, CA Lemere, R Maron, ET Spooner, TJ Grenfell, C Mori, S Issazadeh, WW Hancock, DJ Selkoe: Nasal administration of amyloid-beta peptide decreases cerebral amyloid burden in a mouse model of Alzheimer's disease. *Ann Neurol* 48, 567-579 (2000)
102. D Frenkel, R Maron, DS Burt, HL Weiner: Nasal vaccination with a proteasome-based adjuvant and glatiramer acetate clears beta-amyloid in a mouse model of Alzheimer disease. *J Clin Invest* 115, 2423-2433 (2005)
103. Y Matsuoka, AJ Gray, C Hirata-Fukae, SS Minami, EG Waterhouse, MP Mattson, FM LaFerla, I Gozes, PS Aisen: Intranasal NAP administration reduces accumulation of amyloid peptide and tau hyperphosphorylation in a transgenic mouse model of Alzheimer's disease at early pathological stage. *J Mol Neurosci* 31, 165-170 (2007)
104. I Gozes, I Divinski: NAP, a neuroprotective drug candidate in clinical trials, stimulates microtubule assembly in the living cell. *Curr Alzheimer Res* 4, 507-509 (2007)
105. CG Ballard, NH Greig, AL Guillozet-Bongaarts, A Enz, S Darvesh: Cholinesterases: roles in the brain during health and disease. *Curr Alzheimer Res* 2, 307-318 (2005)
106. M Yamada, T Chiba, J Sasabe, K Terashita, S Aiso, M Matsuoka: Nasal Colivelin treatment ameliorates memory impairment related to Alzheimer's disease. *Neuropsychopharmacology* 33, 2020-2032 (2008)
107. HR Costantino, AK Leonard, G Brandt, PH Johnson, SC Quay: Intranasal administration of acetylcholinesterase inhibitors. *BMC Neurosci* 9 Suppl 3, S6 (2008)
108. M Dahlin, E Bjork: Nasal administration of a physostigmine analogue (NXX-066) for Alzheimer's disease to rats. *Int J Pharm* 212, 267-274 (2001)
109. JJ Sramek, J Hourani, SS Jhee, NR Cutler: NXX-066 in patients with Alzheimer's disease: a bridging study. *Life Sci* 64, 1215-1221 (1999)
110. DR Owens, B Zinman, G Bolli: Alternative routes of insulin delivery. *Diabetic Medicine* 20, 886-898 (2003)
111. WHI Frey, inventor Method for administering insulin to the brain. . US Patent: Chiron Corporation. 2001.
112. E Steen, BM Terry, EJ Rivera, JL Cannon, TR Neely, R Tavares, XJ Xu, JR Wands, SM de la Monte: Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? *J Alzheimers Dis* 7, 63-80 (2005)
113. S Craft, GS Watson: Insulin and neurodegenerative disease: shared and specific mechanisms. *Lancet Neurol* 3, 169-178 (2004)
114. L Frolich, D Blum-Degen, P Riederer, S Hoyer: A disturbance in the neuronal insulin receptor signal transduction in sporadic Alzheimer's disease. *Annals of the New York Academy of Sciences* 893, 290-293 (1999)
115. CG Jolival, CA Lee, KK Beiswenger, JL Smith, M Orlov, MA Torrance, E Masliah: Defective insulin signaling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: parallels with Alzheimer's disease and correction by insulin. *J Neurosci Res* 86, 3265-3274 (2008)
116. ES Korf, LR White, P Scheltens, LJ Launer: Brain aging in very old men with type 2 diabetes: the Honolulu-Asia Aging Study. *Diabetes Care* 29, 2268-2274 (2006)
117. JA Luchsinger, MX Tang, S Shea, R Mayeux: Hyperinsulinemia and risk of Alzheimer disease. *Neurology* 63, 1187-1192 (2004)
118. TJ Nelson, MK Sun, J Hongpaisan, DL Alkon: Insulin, PKC signaling pathways and synaptic remodeling during memory storage and neuronal repair. *Eur J Pharmacol* 585, 76-87 (2008)
119. H Pilcher: Alzheimer's disease could be "type 3 diabetes". *Lancet Neurol* 5, 388-389 (2006)
120. L Zhao, B Teter, T Morihara, GP Lim, SS Ambegaokar, OJ Ubeda, SA Frautschy, GM Cole: Insulin-degrading enzyme as a downstream target of insulin receptor signaling cascade: implications for Alzheimer's disease intervention. *Journal of Neuroscience* 24, 11120-11126 (2004)
121. C Benedict, M Hallschmid, A Hatke, B Schultes, HL Fehm, J Born, W Kern: Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* 29, 1326-1334 (2004)
122. C Benedict, M Hallschmid, K Schmitz, B Schultes, F Ratter, HL Fehm, J Born, W Kern: Intranasal insulin improves memory in humans: superiority of insulin aspart. *Neuropsychopharmacology* 32, 239-243 (2007)
123. MA Reger, GS Watson, WH Frey, LD Baker, B Cholerton, ML Keeling, DA Belongia, MA Fishel, SR Plymate, GD Schellenberg, MM Cherrier, S Craft: Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. *Neurobiol.Aging* 27, 451-458 (2006)
124. MA Reger, GS Watson, PS Green, CW Wilkinson, LD Baker, B Cholerton, MA Fishel, SR Plymate, JC Breitner, W DeGroot, P Mehta, S Craft: Intranasal insulin improves cognition and modulates beta-amyloid in early AD. *Neurology* 70, 440-448 (2008)
125. Y Deng, B Li, Y Liu, K Iqbal, I Grundke-Iqbal, CX Gong: Dysregulation of insulin signaling, glucose transporters, O-GlcNAcylation, and phosphorylation of tau and neurofilaments in the brain: Implication for Alzheimer's disease. *Am J Pathol* 175, 2089-2098 (2009)
126. T Baumgartner, M Heinrichs, A Vonlanthen, U Fischbacher, E Fehr: Oxytocin shapes the neural circuitry

## Intranasal treatment of neurodegenerative disease and stroke

- of trust and trust adaptation in humans. *Neuron* 58, 639-650 (2008)
127. M Kosfeld, M Heinrichs, PJ Zak, U Fischbacher, E Fehr: Oxytocin increases trust in humans. *Nature* 435, 673-676 (2005)
128. P Kirsch, C Esslinger, Q Chen, D Mier, S Lis, S Siddhanti, H Gruppe, VS Mattay, B Gallhofer, A Meyer-Lindenberg: Oxytocin modulates neural circuitry for social cognition and fear in humans. *J Neurosci* 25, 11489-11493 (2005)
129. KJ Parker, CL Buckmaster, AF Schatzberg, DM Lyons: Intranasal oxytocin administration attenuates the ACTH stress response in monkeys. *Psychoneuroendocrinology* 30, 924-929 (2005)
130. U Rimmele, K Hediger, M Heinrichs, P Klaver: Oxytocin makes a face in memory familiar. *J Neurosci* 29, 38-42 (2009)
131. IK Han, MY Kim, HM Byun, TS Hwang, JM Kim, KW Hwang, TG Park, WW Jung, T Chun, GJ Jeong, YK Oh: Enhanced brain targeting efficiency of intranasally administered plasmid DNA: an alternative route for brain gene therapy. *J Mol Med* 85, 75-83 (2007)
132. IS Rakover, N Zabavnik, R Kopel, M Paz-Rozner, B Solomon: Antigen-specific therapy of EAE via intranasal delivery of filamentous phage displaying a myelin immunodominant epitope. *J Neuroimmunol* 225, 68-76 (2010)
133. L Danielyan, R Schafer, A von Ameln-Mayerhofer, M Buadze, J Geisler, T Klopfer, U Burkhardt, B Proksch, S Verleysdonk, M Ayturan, GH Buniatian, CH Gleiter, WH Frey, 2nd: Intranasal delivery of cells to the brain. *Eur J Cell Biol* 88, 315-324 (2009)
134. CT van Velthoven, A Kavelaars, F van Bel, CJ Heijnen: Nasal administration of stem cells: a promising novel route to treat neonatal ischemic brain damage. *Pediatr Res* 68, 419-422 (2010)
135. AL Rosenbloom: Obesity, Insulin Resistance, beta-Cell Autoimmunity, and the Changing Clinical Epidemiology of Childhood Diabetes. *Diabetes Care* 26, 2954-2956 (2003)
136. S Wild, G Roglic, A Green, R Sicree, H King: Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27, 1047-1053 (2004)
137. LL Lipscombe, JE Hux: Trends in diabetes prevalence, incidence, and mortality in Ontario, Canada 1995-2005: a population-based study. *Lancet*. 369, 750-756 (2007)
138. K Nanto-Salonen, A Kupila, S Simell, H Siljander, T Salonsaari, A Hekkala, S Korhonen, R Erkkola, JI Sipila, L Haavisto, M Siltala, J Tuominen, J Hakalax, H Hyoty, J Ilonen, R Veijola, T Simell, M Knip, O Simell: Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. *Lancet* 372, 1746-1755 (2008)
139. GJ Biessels, FE De Leeuw, J Lindeboom, F Barkhof, P Scheltens: Increased cortical atrophy in patients with Alzheimer's disease and type 2 diabetes mellitus. *J Neurol Neurosurg Psychiatry* 77, 304-307 (2006)
140. T den Heijer, SE Vermeer, EJ van Dijk, ND Prins, PJ Koudstaal, A Hofman, MM Breteler: Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia* 46, 1604-1610 (2003)
141. BM Lobnig, O Kromeke, C Optenhostert-Porst, OT Wolf: Hippocampal volume and cognitive performance in long-standing Type 1 diabetic patients without macrovascular complications. *Diabet Med* 23, 32-39 (2006)
142. SM Manschot, AM Brands, GJ van der, RP Kessels, A Algra, LJ Kappelle, GJ Biessels: Brain magnetic resonance imaging correlates of impaired cognition in patients with type 2 diabetes. *Diabetes* 55, 1106-1113 (2006)
143. T Matsuzaki, K Sasaki, Y Tanizaki, J Hata, K Fujimi, Y Matsui, A Sekita, SO Suzuki, S Kanba, Y Kiyohara, T Iwaki: Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama study. *Neurology* 75, 764-770
144. GJ Biessels, IJ Deary, CM Ryan: Cognition and diabetes: a lifespan perspective. *Lancet Neurol* 7, 184-190 (2008)
145. A Kupila, J Sipila, P Keskinen, T Simell, M Knip, K Pulkki, O Simell: Intranasally administered insulin intended for prevention of type 1 diabetes--a safety study in healthy adults. *Diabetes Metab Res Rev* 19, 415-420 (2003)
146. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. *N Engl J Med* 333, 1581-1587 (1995)
147. JL Frey: Recombinant tissue plasminogen activator (rtPA) for stroke. The perspective at 8 years. *Neurologist* 11, 123-133 (2005)
148. PD Gluckman, GR Ambler: Therapeutic use of insulin-like growth factor I: lessons from in vivo animal studies. *Acta Paediatr Suppl* 383, 134-136 (1992)
149. J Guan, C Williams, M Gunning, C Mallard, P Gluckman: The effects of IGF-1 treatment after hypoxic-ischemic brain injury in adult rats. *J Cereb Blood Flow Metab* 13, 609-616 (1993)
150. XF Liu, JR Fawcett, RG Thorne, TA DeFor, WH Frey, 2nd: Intranasal administration of insulin-like growth factor-I bypasses the blood-brain barrier and protects

## Intranasal treatment of neurodegenerative disease and stroke

- against focal cerebral ischemic damage. *J Neurol Sci* 187, 91-97 (2001)
151. XF Liu, JR Fawcett, RG Thorne, WH Frey: Non-invasive intranasal insulin-like growth factor-I reduces infarct volume and improves neurologic function in rats following middle cerebral artery occlusion. *Neuroscience Letters* 308, 91-94 (2001)
152. XF Liu, JR Fawcett, LR Hanson, WH Frey: The window of opportunity for treatment of focal cerebral ischemic damage with noninvasive intranasal insulin-like growth factor-I in rats. *J.Stroke Cerebrovasc.Dis.* 13, 16-23 (2004)
153. S Lin, LW Fan, PG Rhodes, Z Cai: Intranasal administration of IGF-1 attenuates hypoxic-ischemic brain injury in neonatal rats. *Exp Neurol* 217, 361-370 (2009)
154. YP Yu, QQ Xu, Q Zhang, WP Zhang, LH Zhang, EQ Wei: Intranasal recombinant human erythropoietin protects rats against focal cerebral ischemia. *Neurosci Lett* 387, 5-10 (2005)
155. M Digicaylioglu, G Garden, S Timberlake, L Fletcher, SA Lipton: Acute neuroprotective synergy of erythropoietin and insulin-like growth factor I. *Proc Natl Acad Sci U S A* 101, 9855-9860 (2004)
156. L Fletcher, S Kohli, SM Sprague, RA Scranton, SA Lipton, A Parra, DF Jimenez, M Digicaylioglu: Intranasal delivery of erythropoietin plus insulin-like growth factor-I for acute neuroprotection in stroke. Laboratory investigation. *J Neurosurg* 111, 164-170 (2009)
157. SR Alcalá-Barraza, MS Lee, LR Hanson, AA McDonald, WH Frey, LK McLoon: Intranasal delivery of neurotrophic factors BDNF, CNTF, EPO, and NT-4 to the CNS. *J Drug Target*, (2009)
158. JP Yang, HJ Liu, SM Cheng, ZL Wang, X Cheng, HX Yu, XF Liu: Direct transport of VEGF from the nasal cavity to brain. *Neurosci Lett* 449, 108-111 (2009)
159. JP Yang, HJ Liu, ZL Wang, SM Cheng, X Cheng, GL Xu, XF Liu: The dose-effectiveness of intranasal VEGF in treatment of experimental stroke. *Neurosci Lett* 461, 212-216 (2009)
160. P Henrich-Noack, JH Prehn, J Krieglstein: TGF-beta 1 protects hippocampal neurons against degeneration caused by transient global ischemia. Dose-response relationship and potential neuroprotective mechanisms. *Stroke* 27, 1609-1614; discussion 1615 (1996)
161. M Ma, Y Ma, X Yi, R Guo, W Zhu, X Fan, G Xu, WH Frey, 2nd, X Liu: Intranasal delivery of transforming growth factor-beta1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventricular zone. *BMC Neurosci* 9, 117 (2008)
162. ZL Wang, SM Cheng, MM Ma, YP Ma, JP Yang, GL Xu, XF Liu: Intranasally delivered bFGF enhances neurogenesis in adult rats following cerebral ischemia. *Neurosci Lett* 446, 30-35 (2008)
163. K Jin, L Xie, J Childs, Y Sun, XO Mao, A Logvinova, DA Greenberg: Cerebral Neurogenesis Is Induced by Intranasal Administration of Growth Factors. *Ann Neurol* 53, 405-409 (2003)
164. M Guerra-Crespo, D Gleason, A Sistos, T Toosky, I Solaroglu, JH Zhang, PJ Bryant, JH Fallon: Transforming growth factor-alpha induces neurogenesis and behavioral improvement in a chronic stroke model. *Neuroscience* 160, 470-483 (2009)
165. M Guerra-Crespo, A Sistos, D Gleason, JH Fallon: Intranasal administration of PEGylated transforming growth factor-alpha improves behavioral deficits in a chronic stroke model. *J Stroke Cerebrovasc Dis* 19, 3-9
166. S Cheng, M Ma, Y Ma, Z Wang, G Xu, X Liu: Combination therapy with intranasal NGF and electroacupuncture enhanced cell proliferation and survival in rats after stroke. *Neurol Res* 31, 753-758 (2009)
167. C Justicia, P Ramos-Cabrer, M Hoehn: MRI detection of secondary damage after stroke: chronic iron accumulation in the thalamus of the rat brain. *Stroke* 39, 1541-1547 (2008)
168. YX Li, SJ Ding, L Xiao, W Guo, Q Zhan: Desferoxamine preconditioning protects against cerebral ischemia in rats by inducing expressions of hypoxia inducible factor 1 alpha and erythropoietin. *Neurosci Bull* 24, 89-95 (2008)
169. LR Hanson, A Roeytenberg, PM Martinez, VG Coppes, DC Sweet, RJ Rao, DL Marti, JD Hoekman, RB Matthews, WH Frey, 2nd, SS Panter: Intranasal deferoxamine provides increased brain exposure and significant protection in rat ischemic stroke. *J Pharmacol Exp Ther* 330, 679-686 (2009)
170. BL Sun, FP Shen, QJ Wu, SM Chi, MF Yang, H Yuan, FM Xie, YB Zhang, J Chen, F Zhang: Intranasal delivery of calcitonin gene-related peptide reduces cerebral vasospasm in rats. *Front Biosci (Elite Ed)* 2, 1502-1513 (2010)

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