Review

Intranasal administration: a potential solution for cross-BBB delivering neurotrophic factors

Juehua Zhu, Yongjun Jiang, Gelin Xu and Xinfeng Liu

Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu Province, People's Republic of China

Summary. Neurotrophic factors (NTFs) are endogenous polypeptides that regulate the growth, survival, differentiation, and functioning of neurons. The neuroprotective effects of NTFs in experimental animals give strong rationale for developing therapies for neurological disorders. However, when NTFs are applied in clinical trials, great expectation leads to equal disappointment. NTFs are large molecular-weighted and hydrophilic proteins, which limits their access to the central nervous system (CNS) after systemic administration, principally due to poor blood-brain barrier (BBB) permeability and unfavorable pharmacokinetic profiles. Although intracerebral infusion may transport NTFs into the CNS, the invasiveness limits its clinical application. Intranasal administration has been under research for decades and presents promising outcomes in preclinical studies for brain delivering of NTFs. After intranasal delivery, NTFs gain direct and quick access into the CNS at concentrations high enough to elicit their biological effects, bypassing the BBB and minimizing systemic exposure. Due to its invasiveness and convenience, intranasal delivery is feasible for NTFs administration. Although direct evidence of nose-to-brain pathway in human is lacking due to ethical problems, the existence of the nose-to-cerebral spinal fluid pathway has been verified in men. Furthermore, there is abundant indirect evidence for the nose-to-brain pathway as determined by the efficacy of intranasally administered neuroproteins, such as insulin, oxytocin, and vasopressin in clinical trials. Based on the solid preclinical research supporting the efficacy of intranasal NTFs, and the successful clinical application of neuroproteins (not NTFs), it is time to evaluate clinical application of NTFs in treating both acute and chronic CNS diseases.

Key words: Intranasal, Neurotrophic factors, Neuroprotection, Neurodegenerative diseases, Stroke

Introduction

Neurotrophic factors (NTFs) are a large group of endogenous peptides that regulate the growth, survival, differentiation, and functional protein synthesis of neurons via interaction with specific cellular receptors (Semkova and Krieglstein, 1999). The discovery of nerve growth factor (NGF) in the early 1950s presented the start point of the now flourishing area of NTF research. According to their structure and receptor characteristics, NTFs could be classified into more than six families, including neurotrophin family, fibroblast growth factor (FGF) family, neurokine family, transforming growth factor-ß (TGF-ß) family, epidermal growth factor (EGF) family, and insulin-like growth factor (IGF) family (see Table 1 in Thorne and Frey, 2001).

Early preclinical research brought great promise for NTFs in treating neurological disorders. For instance, NGF is potent and selective for forebrain cholinergic neurons (Will and Hefti, 1985) and is considered to be a potential treatment for Alzheimer's diseases (AD) (Ad-Hoc-Working-Group, 1989). Exogenous bFGF successfully reduces the infarct size induced by cerebral ischemia and alleviates the neurological deficit (Ay et al., 1999). Glia cell-line derived neurotrophic factor (GDNF) is indeed a potent survival and differentiation factor for dopaminergic neurons (Lin et al., 1993) and therefore promotes a significant improvement of the cardinal symptoms of parkinsonism (Gash et al., 1996). The observation that ciliary neurotrophic factor (CNTF) effectively blunts the progression of motor neuropathy gives new perspective for the treatment of human

Offprint requests to: Xinfeng Liu, Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, 305 East Zhongshan Road, Nanjing 210002, Jiangsu Province, China. e-mail: xfliu2@yahoo.com.cn

degenerative motor neuron diseases (Sendtner et al., 1992), such as Parkinson's disease (PD). IGF-1 is a neurotrophic factor able to stimulate cell survival in many different cell types, including both the motor and sensory neurons and also the oligodendrocytes (Gluckman et al., 1992; Kooijman et al., 2009). The neuroprotective effects of IGF-1 give rise to the rapid push toward development of IGF-1 as another treatment for ALS and stroke.

Despite high enthusiasm, early attempts to develop treatments with NTFs turned out to be failures in general (Table 1). It is not due to insufficient drug efficacy but due to lack of feasible delivering methods. NTFs cannot enter the brain efficiently after systemic administration (e.g. intravenous, subcutaneous) due to the limitation of the blood-brain barrier (BBB) and the poor

Table 1. Clinical application of neurotrophic factors in CNS diseases.

NTFs	Disease	Number	pathway	Significant improvement	Side effects	Reference
NGF	AD	1	ICV	No	No report	Olson et al., 1992
		3		Slight	Back pain, weight reduction	Eriksdotter Jonhagen et al., 1998
	DPN	250	SC	Yes	Injection site pain	Apfel et al., 1998
		1019		No	Pain syndrome	Apfel et al., 2000
	HIV-associated SN	270	SC	Safe, yes	Injection site pain	McArthur et al., 2000
		200	SC	Safe, yes	Injection site pain	Schifitto et al., 2001
BDNF	ALS	25	IT	Safe	Mild sensory symptoms	Ochs et al., 2000
		13		No	Not report	Beck et al., 2005
		1135	SC	Survival advantage	Injection site reactions	The-BDNF-Study-Group, 1999
	GBS	10 RCT	SC	No	Frequency of SAE 50%	Bensa et al., 2000
	DPN	30 RCT	SC	No	Injection site reactions	Wellmer et al., 2001
bFGF	Acute stroke	66	IV	Safe	Nausea, vomiting, leukocytosis	The-Fibroblast-Safety-Study-Group, 1998
		286		No	Hypotension, leukocytosis, increased mortality rate	Bogousslavsky et al., 2002
CTNF	ALS	57	SC	Safe	febrile reactions, fatigue, cough	ACTS-Phase-I/II-Study-Group, 1995
		730		No	Anorexia, weight loss, and cough	ALS-CNTF-Treatment-Study-Group, 1996
		570, RCT		No	Injection site reactions, cough, asthenia, nausea, anorexia, weight loss, and increased salivation	Miller et al., 1996
		4	IT	No	Pain syndrome (headache, radicular pain)	Penn et al., 1997
GDNF	PD	50	ICV	No	Nausea, anorexia, vomiting, weight loss, paresthesias, hyponatremia	Nutt et al., 2003
		5	IP (Ipu)	Yes for 1 patient	No SAE, 1 requires catheter repositioning	Gill et al., 2003; Patel et al., 2005
		10	IP (Ipu)	Yes	Transient Lhermitte symptoms	Slevin et al., 2005
		36, RCT	IP (Ipu)	Yes	Serious, device-related adverse events	Lang et al., 2006
		10	IP (Ipu)	Yes	Risk of infection	Slevin et al., 2007
IGF-1	ALS	266	SC	Yes	No SAE	Lai et al., 1997
		183	SC	No	No SAE	Borasio et al., 1998
		7	SC	No	No SAE	Frank et al., 2002
		9	IT	Yes	No SAE	Nagano et al., 2005
		330	SC	No	Site reaction, headache, et al. SAE: serious thrombotic events, et al.	Sorenson et al., 2008
TGFß	MS	11	IV	No	Reversible nephrotoxicity	Calabresi et al., 1998

ICV: intracerebroventricular; IT: Intrathecal; SC: subcutaneous; IV: intravenous; IP: intraparenchymal; Ipu: intraputaminal catheter implantation; ALS: amyotrophic lateral sclerosis; GBS: Guillain-Barre Syndrome; PD: Parkinson's disease; DPN: Diabetic polyneuropathy: SN: sensory neuropathy; MS: multiple sclerosis; SAE: severe adverse effect; RCT: randomized clinical trial

pharmacokinetic profiles of NTFs themselves, while intracerebral administration (e.g. intraparenchymal, intraventricular, intrathecal) is not clinically practical for its invasiveness and brings risks of infections to some extent. Intranasal administration, a novel method for brain delivery of therapeutics, is non-invasive, bypasses the BBB, and targets the central nervous system (CNS) directly with minimal systemic exposure. In this sense, intranasal administration might be an alternative for central delivery of NTFs.

The current review is intended to provide evidence for intranasal pathway as a potent choice for clinical NTFs delivery, based on the problems of previous NTFs trails, the transport mechanism, distinguishing features, promising preclinical benefits, and relevant clinical achievements of intranasal delivery.

General failure of clinical trials of NTFs

Clinical trials with NTFs in CNS disorders started from 1992 when Olson and his group delivered a total of 6.6 mg of mouse NGF via intracerebroventricular infusion in one patient of Parkinson's disease (PD) (Olson et al., 1992). Since then, many clinical trials have been launched to prove the efficacy of NTFs in treating neurological diseases. Although promise has been shown to treat diseases of peripheral nervous systems, like HIV-associated sensory neuropathy (McArthur et al., 2000; Schifitto et al., 2001) and diabetic polyneuropathy (Apfel et al., 2000), large scale clinical trials of NTFs treating CNS disorders during these two decades have all failed (Table 1). The major obstacle hindering NTFs from eliciting neuroprotection is the difficult availability of NTFs at the target area, resulting from poor BBB permeability, poor pharmacological profiles of NTFs, and the adverse effects accompanied by different delivery pathways (Table 2).

Table 2. Characters of neurotrophic factors and the BBB limitations.

NTFs:
- Large molecular: Mw from 5 to 30 kDa
- Hydrophilic
- Short *in vivo* half-lives
- Potential immunogenicity
- Pleiotropic effects
- Expensive to produce as drugs

Physical barrier of the BBB:
- Physical barrier: tight junction between endothelial cells
- Metabolic barriers: enzymatic degradation

       diverse efflux transport system (e.g. P-glycoprotein)
       protein binding in the circulation
       uptake or sequestration by peripheral tissues

Mechanisms of substances crossing the BBB:
- Transmembrane diffusion: Inversely to the square route of the molecular weight

       High degree of lipid solubility
       Charge, tertiary structure and degree of protein binding
- Saturable transport systems: more efficient but quite specific, e.g. EGF
- Adsorptive endocytosis: specific, e.g. bFGF, BDNF
- Extracellular pathways

Poor blood-brain barrier permeability

The routine NTF central delivery methods can be divided into two categories: systemic pathway which offers potential access to CNS via the vascular system to the CNS blood supply, including intravenous, subcutaneous administration; and central pathway which injects or infuses NTFs directly to the brain parenchyma, ventricles or subarachnoid space. However, due to the existence of the BBB, brain delivery of NTFs especially via the systemic pathway is challenged (Chen et al., 2004; Pardridge, 2005; de Boer and Gaillard, 2007).

The CNS has limited accessibility to the blood compartment due to the existence of the BBB. The BBB shields the brain from toxic substances in the blood, supplies brain tissue with nutrients, and filters harmful compounds from the brain back to the blood stream (Persidsky et al., 2006). Pardridge reported that intact BBB excluded from the brain nearly 100% of large-molecule neurotherapeutics and more than 98% of all small-molecule drugs (Pardridge, 2005). The physical barrier function of BBB is achieved by both the tight junctions between the endothelial cells and the efflux transporters (e.g. P-glycoprotein) in the capillary endothelium. It is estimated that almost half of candidates for CNS disorders are substrates for the P-gp efflux pump (Hanson and Frey, 2007). Transmembrane diffusion allows drugs to be absorbed into the cell membrane, but is only open for low molecule-weight and high lipid-soluble substances (Banks, 2009). Saturable transport systems are demonstrated to deliver epidermal growth factor (EGF) to the CNS (Kastin et al., 1999). However, this specific mechanism is suitable for only a small part of the NTFs. The adsorptive endocytosis mechanism promotes central delivery of drugs via the interaction of a glycoprotein or highly positively charged substance with glycoproteins or highly positively charged regions of the BBB cells (Banks, 2008). Such mechanism is specifically suitable for cationic peptide, like bFGF (Kastin et al., 1999). It might be safe to conclude that, for most of the NTFs which are large molecule and highly hydrophilic proteins, the intact BBB shields them from entering the CNS.

Evidence has been provided that a wide range of neurological conditions, such as AD (Iadecola, 2010), PD (Kortekaas et al., 2005), multiple sclerosis (MS) (Hemmer et al., 2004) and stroke (Gotoh et al., 1985) are associated with perturbations in the normal BBB which contribute to their pathology. In cerebral ischemia, the rupture of BBB in pathological condition is a dynamic and fluctuate process, varying with time and severity of the injury (Chen et al., 2009). BBB permeability to albumin (Mw, 66.4 kDa), which usually indicates BBB opening to proteins, is not apparent until 6 hours after the onset of ischemia, rising to a peak at 3 days after occlusion (Gotoh et al., 1985; Menzies et al., 1993). However, the therapeutic time window for most neuroprotective agents is less than 6 hours. Thus, such neuroprotective agents must cross an 'intact' BBB to

elicit therapeutic effects in stroke if delivered via the systemic pathway (Zhang and Pardridge, 2001). For other CNS degenerative disorders, although there is evidence for the break-down of BBB in pathological situations, none of the clinical trials applied systemic administration turned out to be effective (Table 1). This indicates that the BBB might not be the only obstacle for NTFs brain targeting. For instance, rapid clearance from the blood as a result of short half-life leads to insufficient amounts of the proteins existing in the circulation to reach pharmacologically meaningful concentrations in the target. In addition, systemic administration is frequently accompanied by systemic or local adverse effects which limit the dose applied. Sometimes, although some clinical improvements were achieved, the occurrence of adverse effects outweighed the benefits (Eriksdotter Jonhagen et al., 1998).

Poor pharmacokinetic profiles

For some proteins, it is likely that their unfavorable pharmacokinetic properties are bigger obstacles for brain delivery even than the BBB. A substance with a large volume of distribution, rapid degradation or sequestration by peripheral tissues, and a short half-life in the circulation will simply not be presented to the BBB for possible passage into the CNS (Banks, 2008). In reality, BDNF crosses the BBB by a high-capacity, saturable transport system (Pan et al., 1998). However, BDNF has unfavorable pharmacokinetics, being enzymatically unstable and having a short half-life. This might be one of the reasons why clinical trials of BDNF via subcutaneous pathway failed (The-BDNF-Study-Group, 1999; Bensa et al., 2000; Wellmer et al., 2001). Another example in point is CNTF. Encouraged by the promising pre-clinical result of CNTF in motor neuropathy (Sendtner et al., 1992), human clinical trials with recombinantly produced human CNTF (rHCTNF) were initiated, but produced great disappointment when they failed (ALS-CNTF-Treatment-Study-Group, 1996). Pharmacological research of CNTF indicated that the initial half-time of clearance of CNTF from the blood was 2.9 minutes (Dittrich et al., 1994), which indicated approximately 75% of CNTF was removed from the circulation within 10 minutes following administration. In addition, in contrast to classic chemical drugs, NTFs like the other biological therapeutics, might cause a problem of potential immunogenicity (Thorne and Frey, 2001). In one phase II-III clinical trial of rHCNTF in ALS patients, more than 60% of all patients treated with rHCNTF developed circulating anti-rHCNTF antibodies (ALS-CNTF-Treatment-Study-Group, 1996). The high incidence of anti-NTF antibody means that the protein might be neutralized and inactivated in the circulation, which results in an inability to exert active biological effects in the brain. The development of antibodies was also observed in another human study of GDNF (Slevin et al., 2007).

Adverse effects after NTFs application

Throughout the clinical trials of NTFs, the problem of adverse effects is obvious and troublesome. The mechanism of these adverse effects is uncertain. The unselected effects of NTFs on nontarget receptors might contribute to these undesired responses. Moreover, in central delivery of NTFs, the invasive procedure of injection or infusion causes trauma and pain to the patients. The long-term delivery through the catheter is also accompanied by the risk of infection.

Adverse effects derived from limited selectivity

NTFs are potent polypeptides that interact with specific cellular receptors leading to biological responses. However, it cannot be excluded that specific peptides may themselves exert agonistic or antagonistic effects on nontarget receptors. A lot of known NTFs are expected to produce a number of undesired and adverse effects, given their limited selectivity for target neurons (Hefti, 1997). bFGF, a promising neurotrophic factor in stroke treatment, is reported to be a potent systemic vasodilator (Cuevas et al., 1991). Hence, decreasing arterial blood pressure is one of the major adverse effects of bFGF via systemic administration. Two clinical trials of bFGF performed in North America, Europe and Australia were both terminated as a result of higher incidence of adverse neurological outcomes and mortality (Clark et al., 2000; Bogousslavsky et al., 2002). Other neurotrophic factors, like NGF and BDNF, are shown to induce injection site hyperalgesia after injection. Human application of NGF (Apfel et al., 1998, 2000; McArthur et al., 2000; Schifitto et al., 2001) indicated that injection site hyperalgesia or other pain syndrome was the most frequent adverse effect. One of the studies (McArthur et al., 2000) reported that although it did not frequently prompt study discontinuation, about a third of subjects were potentially unblinded by this stereotypic adverse effect.

Device-related adverse effect

Intraputaminal GDNF infusion was suggested to provide significant improvement in the motor function in PD patients by one study group (Slevin et al., 2005, 2007). However, when a similar treatment protocol was carried out by another group (Lang et al., 2006), the positive effects were not replicated. It suggests that technical differences could influence the outcome. Intracerebral administration of NTFs requires stereotactic surgery for insertion of the catheter into the lateral ventricle or parenchyma (e.g. putamen for PD treatment) and implantation of a pump subcutaneously. In two clinical trials of intraputaminal catheter implantation of GDNF in PD patients, device-related adverse events were reported which required surgical repositioning of catheters or removal of devices due to

the risk of infection (Lang et al., 2006; Slevin et al., 2007).

Intranasal administration

Intranasal administration is based on the hypothesis that since deleterious substances, such as viruses, could move from the nose to the brain via the olfactory neurons (Bodian and Howe, 1940), this pathway could also be applied for brain targeting of beneficial therapeutics. The superiority of intranasal delivery has been verified in CNS delivery of peptides (Thorne and Frey, 2001), chemical drugs (Hashizume et al., 2008), metals (Bondier et al., 2008), virus vector (Broberg et al., 2004), plasmid (Han et al., 2007), bacterial phages (Frenkel and Solomon, 2002) and cells (Jiang et al., 2011b). Even therapeutics which are substrates for the P-glycoprotein efflux transporters are reported to reach the CNS in effective concentration via the nasal pathway (Graff and Pollack, 2003).

The definition and mechanism of nasal pathway

From a broad spectrum, any non-invasive drug delivery route with the nose as the portal could be named intranasal delivery. Intranasal administration as a systemic delivery method for acute pain management has the benefits of rapid drug onset and no first-pass metabolism. Due to the existence of nasal-associated lymphoid tissue, the nasal mucosa has also received attention as a vaccination route for some respiratory diseases. Great success has been achieved in these two areas with marketed intranasal drug formulations (Costantino et al., 2007). This review, however, will focus on the potential of intranasal delivery as a focal administration targeting the CNS, especially for delivering NTFs.

The anatomy of the nose

Nasal cavities are basically divided into three regions, the nasal vestibule, respiratory region and olfactory region. The nasal vestibule is mainly responsible for filtering out the air borne particles and has almost no drug absorption function. The respiratory region, the largest part of the nasal cavity, has the highest degree of vascularity and is mainly responsible for drug absorption into the systemic circulation (Illum, 2004). The olfactory region is known to be the portal for therapeutics to enter from the nose to brain following nasal absorption and is the region most focused on in intranasal administration for brain targeting.

Pathways from nose to brain and/or CSF

After nasal absorption, small lipid soluble molecules which have escaped enzymatic degradation and the normal rapid clearance of the mucociliary clearance system are usually absorbed rapidly across the nasal membrane into the systemic blood (Vyas et al., 2005). Due to the rapid absorption, such molecules do not normally show direct nose-to-brain transport; instead, they present a plasma profile resembling that of an intravenous injection (Illum, 2004).

Less lipophilic or polar molecules with larger molecular weight, such as NTFs, do not rapidly diffuse across the nasal membrane into the systemic circulation and, therefore, have a better chance of reaching the olfactory mucosa and from there being transported across into the CNS. Two possible routes exist by which therapeutics could be transported from the olfactory epithelium to the brain and/or CSF. Extracellular pathway transports drugs into the CNS relying on the direct anatomic connection between the submucosa and the subarachnoid extensions, as well as the perineural space surrounding the olfactory nerves (Vyas et al., 2005). Drugs pass through the tight junctions and the open clefts of the epithelial cells present in the nasal mucosa and are then transported into the subarachnoid space, lymphatic or perivascular spaces, and then diffuse into CSF or brain parenchyma directly (Merkus and van den Berg, 2007; Wu et al., 2008). The pathway of transport appears to be very fast, with drugs appearing in the CSF and brain a few minutes after nasal application (Vyas et al., 2005). In the nasal epithelia, the largest molecular weight drug that was transported extracellularly (albeit at very low amounts) was about 50kDa (Miyamoto et al., 2001). Also, with the addition of permeation enhancers, this molecular limit could be largely expanded with higher central concentration of the therapeutics (Miyamoto et al., 2001; Costantino et al., 2007). The other possible mechanism is via the intercellular axonal transport. The olfactory route nerve pathway would allow the drug to be internalized into the olfactory neurons located in the olfactory epithelium by endocytosis or pinocytosis and then travel along the axon, transverse the cribriform plate and reach the olfactory bulb. It is possible that further transport into the brain can occur by bridging the synapse between the neurons (Merkus and van den Berg, 2007). This axonal route of transport is very slow and it can take up to 24 h before the drug reaches the CNS (Illum, 2004). With the deeper researches of the mechanisms of intranasal delivery, trigeminal nerve, one nerve innervating the olfactory region, is demonstrated to connect the nasal passages with the CNS by helping transporting substances from nose to the CNS (Thorne et al., 2004).

Delivering neurotrophic factors from nose to brain

The intranasal administration system provides a noninvasive and convenient method of drug delivery associated with little pain. The nasal route rapidly targets therapeutics to the CNS with minimizing systemic exposure, no first-pass metabolism, and a decrease in unwanted side effects (Costantino et al., 2007; Hanson and Frey, 2007; Wu et al., 2008, Dhuria et al., 2010). Macromolecular drugs, such as neurotrophic factors

which reach fairly low brain concentrations after routine delivery due to poor BBB permeability and less favorable physio-chemical profiles, are especially suitable for intranasal delivery.

Non-invasive and simple delivery method

An obvious advantage of the intranasal route is its non-invasiveness relative to intravenous injection or intracerebral infusion. The procedure of intranasal administration is as follows (Frey et al., 1997; Jiang et al., 2011a) with slight modifications in different research protocols. Briefly, anesthetized animals are placed in a supine position, and the ventral surface of the head and neck are maintained horizontal using a small role of gauze under the dorsal neck. In the majority of the studies, NTFs are formulated in an aqueous solution such as saline or buffered. The typical delivery volumes are 5-10 μL/nostril in mice, 10-25 μL/nostril in rats and 75-100 μL/nostril in humans (Merkus and van den Berg, 2007). Usually, the contralateral naris (and the mouth in some research (Liu et al., 2001)) is gently occluded during administration of each drop to facilitate snorting of the drops high into the nasal cavity, especially the olfactory epithelium.

As the intranasal procedure is non-invasive and easy, and requires no significant medical training, it could maximize patient convenience, comfort and compliance. Also, due to invasiveness and simplicity, intranasal administration makes dosages repeatable, and therefore is suitable for therapies requiring chronic dosing over a wide length of courses and frequency of therapy (Costantino et al., 2007).

Better brain targeting

The unique character of intranasal delivery lies in delivering therapeutics directly to the brain by circumventing the BBB. To prove the existence of direct drug transport from the nasal cavity instead of via the systemic circulation, the CNS/plasma ratio following intranasal delivery should be significantly higher than that after intravenous administration. In addition, it is preferable to make plasma concentrations similar in both intranasal and intravenous administration, which ensures the rate of passive diffusion from the systemic circulation into the CNS is the same after both delivery pathways (Merkus and van den Berg, 2007). It was demonstrated that (Frey et al., 1997) intranasal delivery of 7.4 nmol ^{125}I-NGF (Mw 26.5 kDa) and intravenous injection of 21 pmol could achieve a similar blood amount of radiolabel. However, the radio accumulation in the brain varied between 0.07 to 2.0 nM, depending on different brain regions after intranasal administration, while it was less than or equal to 0.001 nM after intravenous administration. Another study (Thorne et al., 2004) administered different doses of ^{125}I-IGF-I via intranasal and intravenous administration to reach similar amounts of ^{125}I-IGF-I in the blood and a

comparable distribution in peripheral tissues. The over 100-fold higher concentration of ^{125}I-IGF-I in most CNS areas following intranasal administration was consistent with direct delivery of ^{125}I-IGF-I, bypassing the BBB. When the same dose was delivered, intranasal delivery showed the highest and earliest concentrations in the brain compared with subcutaneous, intravenous, and intraperitoneal injection in delivering ^{125}I-EPO, ^{125}I-IGF-I (Fletcher et al., 2009), TGF-ß (Ma et al., 2007) andvascular endothelial growth factor (VEGF) (Yang et al., 2009).

Although the quantities of drugs that have been shown to be transported directly from nose to brain is not high, normally less than 0.1% (Illum, 2004), the central concentration may ultimately be increased to some extent by the use of absorption enhancers. But even in their absence, intranasal efficiencies should still be orders of magnitude greater than that after parenteral injection for most proteins. As NTFs are potent therapeutic agents active in the femtomolar to nanomolar range (Thorne and Frey, 2001), the central concentrations obtained by nasal pathway are generally high enough to exert their biological effects. After nasal administration of 7.4 nmol of ^{125}I-NGF (Frey et al., 1997), the lowest brain concentration achieved was 474 pM, which was significantly higher than the 38 pM NGF reported to increase choline acetyltransferase activity in cell culture (Knusel et al., 1990). Intranasal administration of 5 nmol of IGF-I resulted in levels from 0.3 to 3.4 nM in rat brain (Thorne et al., 2004). Given that IGF-I concentrations as low as 10-100 pM can elicit neuroprotective effects (Cheng and Mattson, 1992), delivery of IGF-I to the brain following intranasal administration was expected to be sufficient for achieving pharmacological effects at multiple sites. More recently, one research delivered 70 μg ^{125}I-radiolabeled BDNF (Mw 26.984 kDa), CNTF (Mw 22.706 kDa), NT-4 (Mw 22.428 kDa), or erythropoietin (Mw EPO, 30.4 kDa) to rats (Alcala-Barraza et al., 2010). These NTFs reached the CNS and resulted in 0.1-1.0 nM neurotrophin concentrations within 25 min in brain parenchyma, which were above the concentrations determined by *in vitro* studies for them to elicit neuroprotective effects or sufficient enough to activate the post-survival PI3Kinase/Akt pathway.

Wide distribution in the CNS

Accumulating evidence has demonstrated that, following intranasal administration, the therapeutics are distributed widely in the CNS, with the peak concentrations located in the olfactory bulb, trigeminal nerve, and regions nearby (Thorne et al., 2004; Ma et al., 2007; Lin et al., 2009; Yang et al., 2009). Yang et al. suggested that following intranasal administration of ^{125}I-VEGF, the highest CNS tissue concentration was found in the trigeminal nerve, followed by the optic nerve, olfactory bulb, olfactory tubercles, striatum, medulla, frontal cortex, midbrain, pons, appendix

cerebri, thalamus, hippocampus, and cerebellum. These results are consistent with two routes of rapid entry into the brain: one associated with the peripheral olfactory system connecting the nasal passages with the olfactory bulbs and the rostral brain regions (e.g. anterior olfactory nucleus and frontal cortex), and the other associated with the peripheral trigeminal system connecting the nasal passages with brainstem and spinal cord regions (Thorne et al., 2004). However, this general distribution can be altered by the presence of CNS receptors that may bind to the therapeutic agents and it may also vary between species (Illum, 2004). Evidence has been provided (Ma et al., 2007; Alcala-Barraza et al., 2010) that during the first 30-60min, drug concentrations in the olfactory bulb were among the highest; then the concentration decreased accompanied by a general increase in multiple brain regions at 1-2 hour later. It was suggested that the olfactory bulb might serve as storage for NTFs to distribute from the rostral to caudal and interior part of the brain, and even diffuse into the inner brain regions, like the striatum, thalamus and hippocampus via the interstitial space (Ma et al., 2007). In addition, intranasal administration of NTFs also targets the deep and superficial cervical lymph nodes (Thorne et al., 2004; Yang et al., 2009) which are known to receive lymphatic afferents from the nasal passages.

Relatively rapid CNS delivery

A wide array of evidence has been presented that the uptake of neuropeptides or proteins into the CNS occurs within minutes in both rodents (Thorne et al., 2004) and humans (Born et al., 2002). Following intranasal administration of ^{125}I-NGF, the radiolabel appeared within 20-30 min after intranasal administration in the olfactory bulbs, cerebrum, and brainstem (Frey et al., 1997; Chen et al., 1998). Similarly, IGF-I was demonstrated to bypass the BBB to reach multiple sites in the brain and spinal cord approximately 30 min after the start of intranasal administration (Thorne et al., 2004). Intranasal TGF-ß1 (Ma et al., 2007), EPO and rhIGF-I (Fletcher et al., 2009) were shown to reach the CNS within 20 minutes after intranasal application, and reached the greatest levels at 60 min in most of the brain regions. Given the observation that the vast majority of published intranasal studies demonstrate rapid delivery, with high CNS concentrations and effects observed within 30 minutes of intranasal administration, an extracellular mechanism, instead of intracellular mechanism is considered to contribute more to delivering NTFs from nose to brain (Dhuria et al., 2010). However, since most published experiments last only a few hours, it cannot be ruled out that an intracellular mechanism, which take as long as 24 hours from nose to brain, might take effect at later time (Illum, 2000).

Minimal systemic exposure

Another advantage of intranasal administration is reducing systemic exposure while significantly elevating drug concentrations in the CNS. In research carried out in our laboratory, intranasal administration of TGF-ß1 did not significantly change its concentration in plasma, liver and kidney (Ma et al., 2007). Compared with intranasal delivery, plasma concentration of VEGF delivered via intravenous injection was 43-440% higher (Yang et al., 2009). Higher systemic concentration is always accompanied by the risk of side effects. Delivered via the nasal pathway, such risks could be largely reduced. After intranasal administration of IGF-I (Thorne et al., 2004), the central concentration was higher than the level needed to exert neuroprotective effects and the plasma concentration was approximately 0.5nM at 30 min, with a peak concentration of approximately 5 nM at 6 h. In physiological state, the total IGF-I ranges from 140-175 nM with about 5 % circulating in the free form in rats (Frystyk et al., 1995). This means that after intranasal administration, the plasma concentration was substantially below the normal physiological range of IGF-I, and thereby reduced the inherent risk of causing acute hypoglycemia or other unwanted effects. In systemic administration, NTFs need to experience the potential enzyme degradation and the first-pass metabolism, and then pass the BBB before reaching the CNS. Therefore, at the same dose, the percentage of NTFs reaching the brain after intranasal administration is markedly higher than intravenous administration (Chen et al., 1998), which means a smaller dose is required by intranasal delivery to obtain a similar central effect with respect to the systemic delivery method. Reducing systemic exposure and better brain targeting ensures lower doses, reduces toxicity, decreases economic cost, and leads to better patient compliance.

Therapeutic benefits of intranasal NTFs

Evidence has indicated that intranasal administration delivers NTFs into the CNS intact, and active therapeutic effects of NTFs are exerted. In AD11 anti-NGF mice, a recombinant anti-NGF antibody is secreted by neuronal and glial cells which neutralizes the activity of NGF in the extracellular space, and hence leads to a progressive neurodegenerative phenotype resembling AD. Intranasally delivered NGF was able to fully revert all of the phenotypic markers of neurodegeneration, including the loss of basal forebrain cholinergic neurons, increase of tau hyperphosphorylation in cortex, and intracellular accumulation of Aß in hippocampus (Capsoni et al., 2002). In addition, as AD 11 mice showed a progressive behavioral deficit , intranasal administration of NGF also increased ability of AD11 mice in remembering a familiar object and in associating an object to a particular context (De Rosa et al., 2005).

IGF-I has been shown to protect against cerebral ischemia of rats when injected directly into the lateral ventricles (Guan et al., 1993). Liu provided evidence for the first time of the therapeutic benefits of intranasal

IGF-1 in male SD rats following middle cerebral artery occlusion (Liu et al., 2001). Treatment with 75 or 150 μg IGF-1 significantly reduced the infarct volume by 60-63% and the hemispheric swelling by 45.6% compared with the vehicle treated group, and improved all the neurological deficit tests of motor, sensory, reflex and vestibulomotor function. In addition, intranasal administration of IGF-I to the brain of neonatal rats also turned out to be safe and successful (Lin et al., 2009).

Neurogenesis, which is critical in brain development, can be stimulated by injury and may have a role in brain repair and is associated with functional recovery (Horner and Gage, 2000). Adult neurogenesis consists of the following processes, including proliferation, survival, migration, and differentiation (Ma et al., 2008a,b). Therefore, the ability to augment one or all injury-induced neurogenesis processes could have therapeutic consequences for neurological disorders. After cerebral ischemic insult, accompanied with the neuroprotection as determined by improved neurological function and reduced infarct volume, intranasal bFGF enhanced BrdU incorporation in subventricular zone (SVZ), striatum (Ma et al., 2008a,b), and subgranular zone (SGZ) of the dentate gyrus (Wang et al., 2008). The new proliferated cells were shown to be of neuronal lineage (BrdU+NeuN+cells) (Wang et al., 2008). Proliferation enhancement was also observed when MCAO rats were treated with TGF-ß1 via the nasal pathway (Ma et al., 2008a,b). Most of these cells were co-labeled with Dcx, which indicated the enhanced migration of progenitor cells from SVZ into the ischemic region. In addition, evidence was provided that an increased number of BrdU+ cells were co-labeled with NeuN, whereas only a few cells were GFAP positive, which suggested that intranasal administration of TGF-ß1 also promoted progenitor differentiation towards a neuronal lineage.

Angiogenesis, the growth of new blood vessels, could be interpreted as a natural defense mechanism helping to restore oxygen and nutrient supply to the ischemic brain tissue (Beck and Plate, 2009). Yang et al. demonstrated that intranasal administration of VEGF elevated the number of cerebral vessels in the ischemic boundary regions which showed vWF positive (Yang et al., 2010). Three-dimensional measurement of FITC-dextran perfused cerebral microvessels also revealed that the number of microvessels in the boundary regions of ischemia was increased. In addition, VEGF treatment significantly increased the number of BrdU+/vWF+ immunoreactive cells.

Clinical application of intranasal NTFs: how far from the clinic?

Partially due to the anatomical differences in species, such as the ratio of olfactory epithelium in nasal mucosa, the CSF volume and its spreading rate between rats and human (Illum, 2004), the ability of therapeutics to access the brain from nose in human is questioned by some papers (Merkus et al., 2003; Merkus and van den Berg, 2007). However, direct and indirect evidence has been presented supporting the existence of a direct pathway from nose to CNS and/or CSF in primates.

Thorne et al. demonstrated in detail that central distribution of a labeled protein after intranasal administration was obvious in non-human primates (Thorne et al., 2008). Intranasal ^{125}I-IFN γ1 in adult cynomolgus monkeys produced measurable, significant concentrations across many different areas of the CNS and its regional lymph nodes with the highest radiolabel level observed in olfactory bulbs and trigeminal nerve. The authors suggested that intranasally applied macromolecules may bypass the BBB and rapidly enter the primate CNS along olfactory- and trigeminal-associated extracellular pathway, as shown in rodents. In human studies, evidence has also been provided for the direct pathway from nose to CSF. Born et al. demonstrated that intranasally administered melanocortin (4-10), vasopressin (Mw 1084.2) and insulin (Mw 5808) achieved direct access to the cerebrospinal fluid within 30 minutes (Born et al., 2002). Meanwhile, concurrent measurement of the concentrations in blood did not reveal a significant increase in MSH/ACTH or insulin and there was no change in plasma glucose concentration after insulin administration.

However, it should be noted that human studies do not normally report absolute magnitude of therapeutics in brain parenchyma, as it is not ethical and practical to take out brain tissue to measure the rate and degree of transport of drugs into the CNS. By far, most of the published studies measured indirectly the pharmacological effects of neuropeptides or proteins in the CNS. Although measuring pharmacological effects rather than drug concentrations in the CNS provides compelling evidence that the intranasal administration of peptides results in positive effects on the CNS, the missing neuroprotective effects after intravenous administration still indicate that the drug does not first pass into the blood stream from the nasal cavity and then cross the BBB (Illum, 2004).

Several clinical trials demonstrated that intranasal administration of insulin helped to improve the declarative memory and attention both in healthy volunteers (Benedict et al., 2004) and AD or mild cognitive impairment patients (Reger et al., 2006, 2008) without changing plasma glucose and insulin levels. Intranasal insulin was suggested to enter the brain either by direct entry through the cribriform plate, along the olfactory nerve and into brain parenchyma, or by entry through specific receptors in BBB and thereby into the brain, or some combinations of the above two (Henkin, 2010). Another peptide holding great promise when delivered intranasally is oxytocin. Human studies indicated that intranasal delivery of oxytocin caused a substantial increase in human trust (Kosfeld et al., 2005), facilitated social behavior (Domes et al., 2007; Ditzen et al., 2009), improved social memory (Savaskan et al.,

2008, Hurlemann et al., 2010), and might be a potential treatment for social phobia (Guastella et al., 2009) and schizophrenia (Feifel et al., 2010) without side effects. Moreover, intranasal administration of arginine vasopressin facilitated aphasias after stroke (Tsikunov and Belokoskova, 2007). Intranasal perillyl alcohol turned out to be a safe and efficient treatment for glioblastoma (da Fonseca et al., 2008a,b).

No drug delivery method is universal for all drugs. When selecting the dosing route, therapeutic considerations are paramount, including the pharmaceutical target (local or systemic, peripheral systems or the CNS), the physiochemical characteristic of the drug, the dosing frequency, the delivery system necessary to deliver the drug safely and efficiently, and the patient population (Costantino et al., 2007). As NTFs are large molecule, hydrophilic proteins, systemic pathways are not sufficient due to poor BBB permeability, unfavorable pharmacokinetic profiles of NTFs themselves, and their pleiotropic effects. Although intracerebral injection brings therapeutics directly into the brain parenchyma, ventricles or CSF, the procedure is invasive and expensive accompanied by the risk of infection when applied for a long period. It should be admitted that nasal delivery method is not a panacea as the quantities of drugs that finally enter the brain is relatively low, and the direct evidence of nose-to-brain pathway in human is yet to be explored. Previous contributions suggest that intranasal administration works best with potent therapeutic agents that are active in the nanomolar range (Dhanda et al., 2005). In addition, it is considered that intranasal administration may be particularly attractive for therapies requiring chronic dosing over a wide length of courses and frequency for a non-orally bioavailable drug. Fortunately, NTFs are such potent neuropeptides and effective in the femtomolar to nanomolar range. Hence intranasal delivery might be an perfectly suitable alternative for central transport of NTFs. Promising therapeutic efficacy of intranasal NTFs in neurological disorders has been demonstrated in preclinical studies, and a great deal of evidence exists for the clinical potential of intranasal neuropeptides and proteins. However, only a few clinical trials of NTFs via nasal pathway have been launched. It seems to be the right time to start the exploration of intranasal NTFs clinical application, and to test the direct pathway from nose to brain in human beings. Isotopic tracing and PET/CT or SPECT imaging might be promising.

References

ACTS-Phase-I/II-Study-Group. (1995). A phase I study of recombinant human ciliary neurotrophic factor (rHCNTF) in patients with amyotrophic lateral sclerosis. The ALS CNTF Treatment Study (ACTS) Phase I-II Study Group. Clin. Neuropharmacol. 18, 515-532.

Ad-Hoc-Working-Group. (1989). Potential use of nerve growth factor to treat Alzheimer's disease. Science 243, 11.

Alcala-Barraza S.R., Lee M.S., Hanson L.R., McDonald A.A., Frey W.H., 2nd and McLoon L.K. (2010). Intranasal delivery of neurotrophic factors BDNF, CNTF, EPO, and NT-4 to the CNS. J. Drug Target 18, 179-190.

ALS-CNTF-Treatment-Study-Group. (1996). A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. ALS CNTF Treatment Study Group. Neurology 46, 1244-1249.

Apfel S.C., Kessler J.A., Adornato B.T., Litchy W.J., Sanders C. and Rask C.A. (1998). Recombinant human nerve growth factor in the treatment of diabetic polyneuropathy. NGF Study Group. Neurology 51, 695-702.

Apfel S.C., Schwartz S., Adornato B.T., Freeman R., Biton V., Rendell M., Vinik A., Giuliani M., Stevens J.C., Barbano R. and Dyck P.J. (2000). Efficacy and safety of recombinant human nerve growth factor in patients with diabetic polyneuropathy: A randomized controlled trial. rhNGF Clinical Investigator Group. JAMA 284, 2215-2221.

Ay H., Ay I., Koroshetz W.J. and Finklestein S.P. (1999). Potential usefulness of basic fibroblast growth factor as a treatment for stroke. Cerebrovasc. Dis. 9, 131-135.

Banks W.A. (2008). Delivery of peptides to the brain: emphasis on therapeutic development. Biopolymers 90, 589-594.

Banks W.A. (2009). Characteristics of compounds that cross the blood-brain barrier. BMC Neurol. 9 (Suppl 1), S3.

Beck H. and Plate K.H. (2009). Angiogenesis after cerebral ischemia. Acta. Neuropathol. 117, 481-496.

Beck M., Flachenecker P., Magnus T., Giess R., Reiners K., Toyka K.V. and Naumann M. (2005). Autonomic dysfunction in ALS: a preliminary study on the effects of intrathecal BDNF. Amyotroph. Lateral Scler. Other Motor Neuron Disord. 6, 100-103.

Benedict C., Hallschmid M., Hatke A., Schultes B., Fehm H.L., Born J. and Kern W. (2004). Intranasal insulin improves memory in humans. Psychoneuroendocrinology 29, 1326-1334.

Bensa S., Hadden R.D., Hahn A., Hughes R.A. and Willison H.J. (2000). Randomized controlled trial of brain-derived neurotrophic factor in Guillain-Barre syndrome: a pilot study. Eur. J. Neurol. 7, 423-426.

Bodian D. and Howe H.A. (1940). An experimental study of the role of neurons in the dissemination of polymyelitis virus in the nervous system. Brain 63, 135-162.

Bogousslavsky J., Victor S.J., Salinas E.O., Pallay A., Donnan G.A., Fieschi C., Kaste M., Orgogozo J.M., Chamorro A. and Desmet A. (2002). Fiblast (trafermin) in acute stroke: results of the European-Australian phase II/III safety and efficacy trial. Cerebrovasc. Dis. 14, 239-251.

Bondier J.R., Michel G., Propper A. and Badot P.M. (2008). Harmful effects of cadmium on olfactory system in mice. Inhal. Toxicol. 20, 1169-1177.

Borasio G.D., Robberecht W., Leigh P.N., Emile J., Guiloff R.J., Jerusalem F., Silani V., Vos P.E., Wokke J.H. and Dobbins T. (1998). A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-I Study Group. Neurology 51, 583-586.

Born J., Lange T., Kern W., McGregor G.P., Bickel U. and Fehm H.L. (2002). Sniffing neuropeptides: a transnasal approach to the human brain. Nat. Neurosci. 5, 514-516.

Broberg E.K., Peltoniemi J., Nygardas M., Vahlberg T., Roytta M. and Hukkanen V. (2004). Spread and replication of and immune

response to gamma134.5-negative herpes simplex virus type 1 vectors in BALB/c mice. J. Virol. 78, 13139-13152.

Calabresi P.A., Fields N.S., Maloni H.W., Hanham A., Carlino J., Moore J., Levin M.C., Dhib-Jalbut S., Tranquill L.R., Austin H., McFarland H.F. and Racke M.K. (1998). Phase 1 trial of transforming growth factor beta 2 in chronic progressive MS. Neurology 51, 289-292.

Capsoni S., Giannotta S. and Cattaneo A. (2002). Nerve growth factor and galantamine ameliorate early signs of neurodegeneration in anti-nerve growth factor mice. Proc. Natl. Acad. Sci. USA 99, 12432-12437.

Chen B., Friedman B., Cheng Q., Tsai P., Schim E., Kleinfeld D. and Lyden P.D. (2009). Severe blood-brain barrier disruption and surrounding tissue injury. Stroke 40, e666-674.

Chen X.Q., Fawcett J.R., Rahman Y.E., Ala T.A. and Frey I.W. (1998). Delivery of Nerve Growth Factor to the Brain via the Olfactory Pathway. J. Alzheimers Dis. 1, 35-44.

Chen Y., Dalwadi G. and Benson H.A. (2004). Drug delivery across the blood-brain barrier. Curr. Drug Deliv. 1, 361-376.

Cheng B. and Mattson M.P. (1992). IGF-I and IGF-II protect cultured hippocampal and septal neurons against calcium-mediated hypoglycemic damage. J. Neurosci. 12, 1558-1566.

Clark W.M., Schim J.D., Kasner S.E. and Victor S. (2000). Trafermin in acute ischemic stroke. Results of a phase II/III randomized efficacy study (abstract). Neurology 54.

Costantino H.R., Illum L., Brandt G., Johnson P.H. and Quay S.C. (2007). Intranasal delivery: physicochemical and therapeutic aspects. Int. J. Pharm. 337, 1-24.

Cuevas P., Carceller F., Ortega S., Zazo M., Nieto I. and Gimenez-Gallego G. (1991). Hypotensive activity of fibroblast growth factor. Science 254, 1208-1210.

da Fonseca C.O., Linden R., Futuro D., Gattass C.R. and Quirico-Santos T. (2008a). Ras pathway activation in gliomas: a strategic target for intranasal administration of perillyl alcohol. Arch. Immunol. Ther. Exp. (Warsz) 56, 267-276.

da Fonseca C.O., Schwartsmann G., Fischer J., Nagel J., Futuro D., Quirico-Santos T. and Gattass C.R. (2008b). Preliminary results from a phase I/II study of perillyl alcohol intranasal administration in adults with recurrent malignant gliomas. Surg. Neurol. 70, 259-266; discussion 266-257.

de Boer A.G. and Gaillard P.J. (2007). Strategies to improve drug delivery across the blood-brain barrier. Clin. Pharmacokinet. 46, 553-576.

De Rosa R., Garcia A.A., Braschi C., Capsoni S., Maffei L., Berardi N. and Cattaneo A. (2005). Intranasal administration of nerve growth factor (NGF) rescues recognition memory deficits in AD11 anti-NGF transgenic mice. Proc. Natl. Acad. Sci. USA 102, 3811-3816.

Dhanda D.S., Frey I.W., Leopold D. and Kompella U.B. (2005). Approaches for drug deposition in the human olfactory epithelium. Drug Deliv. Technol. 5, 64-72.

Dhuria S.V., Hanson L.R. and Frey W.H. 2nd. (2010). Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J. Pharm. Sci. 99, 1654-1673.

Dittrich F., Thoenen H. and Sendtner M. (1994). Ciliary neurotrophic factor: pharmacokinetics and acute-phase response in rat. Ann. Neurol. 35, 151-163.

Ditzen B., Schaer M., Gabriel B., Bodenmann G., Ehlert U. and Heinrichs M. (2009). Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. Biol. Psychiatry 65, 728-731.

Domes G., Heinrichs M., Glascher J., Buchel C., Braus D.F. and

Herpertz S.C. (2007). Oxytocin attenuates amygdala responses to emotional faces regardless of valence. Biol. Psychiatry 62, 1187-1190.

Eriksdotter Jonhagen M., Nordberg A., Amberla K., Backman L., Ebendal T., Meyerson B., Olson L., Seiger, Shigeta M., Theodorsson E., Viitanen M., Winblad B. and Wahlund L.O. (1998). Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease. Dement. Geriatr. Cogn. Disord. 9, 246-257.

Feifel D., Macdonald K., Nguyen A., Cobb P., Warlan H., Galangue B., Minassian A., Becker O., Cooper J., Perry W., Lefebvre M., Gonzales J. and Hadley A. (2010). Adjunctive intranasal oxytocin reduces symptoms in schizophrenia patients. Biol. Psychiatry 68, 678-680.

Fletcher L., Kohli S., Sprague S.M., Scranton R.A., Lipton S.A., Parra A., Jimenez D.F. and Digicaylioglu M. (2009). Intranasal delivery of erythropoietin plus insulin-like growth factor-I for acute neuroprotection in stroke. Laboratory investigation. J. Neurosurg. 111, 164-170.

Frank J.A., Richert N., Lewis B., Bash C., Howard T., Civil R., Stone R., Eaton J., McFarland H. and Leist T. (2002). A pilot study of recombinant insulin-like growth factor-1 in seven multiple sderosis patients. Mult. Scler. 8, 24-29.

Frenkel D. and Solomon B. (2002). Filamentous phage as vector-mediated antibody delivery to the brain. Proc. Natl. Acad. Sci. USA 99, 5675-5679.

Frey W.H., Liu J., Chen X., Thorne R.G., Fawcett J.R., Ala T.A. and Rahman Y.-E. (1997). Delivery of ^{125}I-NGF to the brain via the olfactory route. Drug Deliv. 4, 87-92.

Frystyk J., Gronbaek H., Skjaerbaek C. and Flyvbjerg A. (1995). Effect of hyperthyroidism on circulating levels of free and total IGF-I and IGFBPs in rats. Am. J. Physiol. 269, E840-845.

Gash D.M., Zhang Z., Ovadia A., Cass W.A., Yi A., Simmerman L., Russell D., Martin D., Lapchak P.A., Collins F., Hoffer B.J. and Gerhardt G.A. (1996). Functional recovery in parkinsonian monkeys treated with GDNF. Nature 380, 252-255.

Gill S.S., Patel N.K., Hotton G.R., O'Sullivan K., McCarter R., Bunnage M., Brooks D.J., Svendsen C.N. and Heywood P. (2003). Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. Nat. Med. 9, 589-595.

Gluckman P., Klempt N., Guan J., Mallard C., Sirimanne E., Dragunow M., Klempt M., Singh K., Williams C. and Nikolics K. (1992). A role for IGF-1 in the rescue of CNS neurons following hypoxic-ischemic injury. Biochem. Biophys. Res. Commun. 182, 593-599.

Gotoh O., Asano T., Koide T. and Takakura K. (1985). Ischemic brain edema following occlusion of the middle cerebral artery in the rat. I: The time courses of the brain water, sodium and potassium contents and blood-brain barrier permeability to ^{125}I-albumin. Stroke 16, 101-109.

Graff C.L. and Pollack G.M. (2003). P-Glycoprotein attenuates brain uptake of substrates after nasal instillation. Pharm. Res. 20, 1225-1230.

Guan J., Williams C., Gunning M., Mallard C. and Gluckman P. (1993). The effects of IGF-1 treatment after hypoxic-ischemic brain injury in adult rats. J. Cereb. Blood Flow Metab. 13, 609-616.

Guastella A.J., Howard A.L., Dadds M.R., Mitchell P. and Carson D.S. (2009). A randomized controlled trial of intranasal oxytocin as an adjunct to exposure therapy for social anxiety disorder. Psychoneuroendocrinology 34, 917-923.

Han I.K., Kim M.Y., Byun H.M., Hwang T.S., Kim J.M., Hwang K.W.,

Park T.G., Jung W.W., Chun T., Jeong G.J. and Oh Y.K. (2007). Enhanced brain targeting efficiency of intranasally administered plasmid DNA: an alternative route for brain gene therapy. J. Mol. Med. (Berl) 85, 75-83.

Hanson L.R. and Frey W.H. 2nd. (2007). Strategies for intranasal delivery of therapeutics for the prevention and treatment of neuroAIDS. J. Neuroimmune Pharmacol. 2, 81-86.

Hashizume R., Ozawa T., Gryaznov S.M., Bollen A.W., Lamborn K.R., Frey W.H. 2nd and Deen D.F. (2008). New therapeutic approach for brain tumors: Intranasal delivery of telomerase inhibitor GRN163. Neuro. Oncol. 10, 112-120.

Hefti F. (1997). Pharmacology of neurotrophic factors. Annu. Rev. Pharmacol. Toxicol. 37, 239-267.

Hemmer B., Cepok S., Zhou D. and Sommer N. (2004). Multiple sclerosis -- a coordinated immune attack across the blood brain barrier. Curr. Neurovasc. Res. 1, 141-150.

Henkin R.I. (2010). Intranasal insulin: from nose to brain. Nutrition 26, 624-633.

Horner P.J. and Gage F.H. (2000). Regenerating the damaged central nervous system. Nature 407, 963-970.

Hurlemann R., Patin A., Onur O.A., Cohen M.X., Baumgartner T., Metzler S., Dziobek I., Gallinat J., Wagner M., Maier W. and Kendrick K.M. (2010). Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. J. Neurosci. 30, 4999-5007.

Iadecola C. (2010). The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. Acta. Neuropathol. 120, 287-296.

Illum L. (2000). Transport of drugs from the nasal cavity to the central nervous system. Eur. J. Pharm. Sci. 11, 1-18.

Illum L. (2004). Is nose-to-brain transport of drugs in man a reality? J. Pharm. Pharmacol. 56, 3-17.

Jiang Y., Wei N., Lu T., Zhu J., Xu G. and Liu X. (2011a). Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats. Neuroscience 172, 398-405.

Jiang Y., Zhu J., Xu G. and Liu X. (2011b). Intranasal delivery of stem cells to the brain. Expert Opin. Drug Deliv. 8, 623-632.

Kastin A.J., Pan W., Maness L.M. and Banks W.A. (1999). Peptides crossing the blood-brain barrier: some unusual observations. Brain Res. 848, 96-100.

Knusel B., Michel P.P., Schwaber J.S. and Hefti F. (1990). Selective and nonselective stimulation of central cholinergic and dopaminergic development in vitro by nerve growth factor, basic fibroblast growth factor, epidermal growth factor, insulin and the insulin-like growth factors I and II. J. Neurosci. 10, 558-570.

Kooijman R., Sarre S., Michotte Y. and De Keyser J. (2009). Insulin-like growth factor I: a potential neuroprotective compound for the treatment of acute ischemic stroke? Stroke 40, e83-88.

Kortekaas R., Leenders K.L., van Oostrom J.C., Vaalburg W., Bart J., Willemsen A.T. and Hendrikse N.H. (2005). Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. Ann. Neurol. 57, 176-179.

Kosfeld M., Heinrichs M., Zak P.J., Fischbacher U. and Fehr E. (2005). Oxytocin increases trust in humans. Nature 435, 673-676.

Lai E.C., Felice K.J., Festoff B.W., Gawel M.J., Gelinas D.F., Kratz R., Murphy M.F., Natter H.M., Norris F.H. and Rudnicki S.A. (1997). Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. The North America ALS/IGF-I Study Group. Neurology 49, 1621-1630.

Lang A.E., Gill S., Patel N.K., Lozano A., Nutt J.G., Penn R., Brooks D.J., Hotton G., Moro E., Heywood P., Brodsky M.A., Burchiel K., Kelly P., Dalvi A., Scott B., Stacy M., Turner D., Wooten V.G., Elias W.J., Laws E.R., Dhawan V., Stoessl A.J., Matcham J., Coffey R.J. and Traub M. (2006). Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. Ann. Neurol. 59, 459-466.

Lin L.F., Doherty D.H., Lile J.D., Bektesh S. and Collins F. (1993). GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260, 1130-1132.

Lin S., Fan L.W., Rhodes P.G. and Cai Z. (2009). Intranasal administration of IGF-1 attenuates hypoxic-ischemic brain injury in neonatal rats. Exp. Neurol. 217, 361-370.

Liu X.F., Fawcett J.R., Thorne R.G., DeFor T.A. and Frey W.H. 2nd. (2001). Intranasal administration of insulin-like growth factor-I bypasses the blood-brain barrier and protects against focal cerebral ischemic damage. J. Neurol. Sci. 187, 91-97.

Ma Y.P., Ma M.M., Ge S., Guo R.B., Zhang H.J., Frey W.H. 2nd, Xu G.L. and Liu X.F. (2007). Intranasally delivered TGF-beta1 enters brain and regulates gene expressions of its receptors in rats. Brain Res. Bull. 74, 271-277.

Ma M., Ma Y., Yi X., Guo R., Zhu W., Fan X., Xu G., Frey W.H. 2nd and Liu X. (2008a). 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.

Ma Y.P., Ma M.M., Cheng S.M., Ma H.H., Yi X.M., Xu G.L. and Liu X.F. (2008b). Intranasal bFGF-induced progenitor cell proliferation and neuroprotection after transient focal cerebral ischemia. Neurosci. Lett. 437, 93-97.

McArthur J.C., Yiannoutsos C., Simpson D.M., Adornato B.T., Singer E.J., Hollander H., Marra C., Rubin M., Cohen B.A., Tucker T., Navia B.A., Schifitto G., Katzenstein D., Rask C., Zaborski L., Smith M.E., Shriver S., Millar L., Clifford D.B. and Karalnik I.J. (2000). A phase II trial of nerve growth factor for sensory neuropathy associated with HIV infection. AIDS Clinical Trials Group Team 291. Neurology 54, 1080-1088.

Menzies S.A., Betz A.L. and Hoff J.T. (1993). Contributions of ions and albumin to the formation and resolution of ischemic brain edema. J. Neurosurg. 78, 257-266.

Merkus F.W. and van den Berg M.P. (2007). Can nasal drug delivery bypass the blood-brain barrier?: questioning the direct transport theory. Drugs R. D. 8, 133-144.

Merkus P., Guchelaar H.J., Bosch D.A. and Merkus F.W. (2003). Direct access of drugs to the human brain after intranasal drug administration? Neurology 60, 1669-1671.

Miller R.G., Petajan J.H., Bryan W.W., Armon C., Barohn R.J., Goodpasture J.C., Hoagland R.J., Parry G.J., Ross M.A. and Stromatt S.C. (1996). A placebo-controlled trial of recombinant human ciliary neurotrophic (rhCNTF) factor in amyotrophic lateral sclerosis. rhCNTF ALS Study Group. Ann. Neurol. 39, 256-260.

Miyamoto M., Natsume H., Satoh I., Ohtake K., Yamaguchi M., Kobayashi D., Sugibayashi K. and Morimoto Y. (2001). Effect of poly-L-arginine on the nasal absorption of FITC-dextran of different molecular weights and recombinant human granulocyte colony-stimulating factor (rhG-CSF) in rats. Int. J. Pharm. 226, 127-138.

Nagano I., Shiote M., Murakami T., Kamada H., Hamakawa Y., Matsubara E., Yokoyama M., Moritaz K., Shoji M. and Abe K. (2005). Beneficial effects of intrathecal IGF-1 administration in patients with amyotrophic lateral sclerosis. Neurol. Res. 27, 768-772.

Nutt J.G., Burchiel K.J., Comella C.L., Jankovic J., Lang A.E., Laws E.R.

Jr., Lozano A.M., Penn R.D., Simpson R.K. Jr., Stacy M. and Wooten G.F. (2003). Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. Neurology 60, 69-73.

Ochs G., Penn R.D., York M., Giess R., Beck M., Tonn J., Haigh J., Malta E., Traub M., Sendtner M. and Toyka K.V. (2000). A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Other Motor Neuron Disord. 1, 201-206.

Olson L., Nordberg A., von Holst H., Backman L., Ebendal T., Alafuzoff I., Amberla K., Hartvig P., Herlitz A., Lilja A., Lundqvist H., Langstrom B., Meyerson B., Persson A., Viitanen M., Windblad B. and Seiger A. (1992). Nerve growth factor affects 11C-nicotine binding, blood flow, EEG, and verbal episodic memory in an Alzheimer patient (case report). J. Neural Transm. Park. Dis. Dement. Sect. 4, 79-95.

Pan W., Banks W.A., Fasold M.B., Bluth J. and Kastin A.J. (1998). Transport of brain-derived neurotrophic factor across the blood-brain barrier. Neuropharmacology 37, 1553-1561.

Pardridge W.M. (2005). The blood-brain barrier: bottleneck in brain drug development. NeuroRx 2, 3-14.

Patel N.K., Bunnage M., Plaha P., Svendsen C.N., Heywood P. and Gill S.S. (2005). Intraputamenal infusion of glial cell line-derived neurotrophic factor in PD: a two-year outcome study. Ann. Neurol. 57, 298-302.

Penn R.D., Kroin J.S., York M.M. and Cedarbaum J.M. (1997). Intrathecal ciliary neurotrophic factor delivery for treatment of amyotrophic lateral sclerosis (phase I trial). Neurosurgery 40, 94-99; discussion 99-100.

Persidsky Y., Ramirez S.H., Haorah J. and Kanmogne G.D. (2006). Blood-brain barrier: structural components and function under physiologic and pathologic conditions. J. Neuroimmune Pharmacol. 1, 223-236.

Reger M.A., Watson G.S., Frey W.H. 2nd, Baker L.D., Cholerton B., Keeling M.L., Belongia D.A., Fishel M.A., Plymate S.R., Schellenberg G.D., Cherrier M.M. and Craft S. (2006). Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. Neurobiol. Aging 27, 451-458.

Reger M.A., Watson G.S., Green P.S., Wilkinson C.W., Baker L.D., Cholerton B., Fishel M.A., Plymate S.R., Breitner J.C., DeGroodt W., Mehta P. and Craft S. (2008). Intranasal insulin improves cognition and modulates beta-amyloid in early AD. Neurology 70, 440-448.

Savaskan E., Ehrhardt R., Schulz A., Walter M. and Schachinger H. (2008). Post-learning intranasal oxytocin modulates human memory for facial identity. Psychoneuroendocrinology 33, 368-374.

Schifitto G., Yiannoutsos C., Simpson D.M., Adornato B.T., Singer E.J., Hollander H., Marra C.M., Rubin M., Cohen B.A., Tucker T., Koralnik I.J., Katzenstein D., Haidich B., Smith M.E., Shriver S., Millar L., Clifford D.B. and McArthur J.C. (2001). Long-term treatment with recombinant nerve growth factor for HIV-associated sensory neuropathy. Neurology 57, 1313-1316.

Semkova I. and Krieglstein J. (1999). Neuroprotection mediated via neurotrophic factors and induction of neurotrophic factors. Brain Res. Brain Res. Rev. 30, 176-188.

Sendtner M., Schmalbruch H., Stockli K.A., Carroll P., Kreutzberg G.W. and Thoenen H. (1992). Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. Nature 358, 502-504.

Slevin J.T., Gerhardt G.A., Smith C.D., Gash D.M., Kryscio R. and Young B. (2005). Improvement of bilateral motor functions in patients with Parkinson disease through the unilateral intraputaminal infusion of glial cell line-derived neurotrophic factor. J. Neurosurg. 102, 216-222.

Slevin J.T., Gash D.M., Smith C.D., Gerhardt G.A., Kryscio R., Chebrolu H., Walton A., Wagner R. and Young A.B. (2007). Unilateral intraputamenal glial cell line-derived neurotrophic factor in patients with Parkinson disease: response to 1 year of treatment and 1 year of withdrawal. J. Neurosurg. 106, 614-620.

Sorenson E.J., Windbank A.J., Mandrekar J.N., Bamlet W.R., Appel S.H., Armon C., Barkhaus P.E., Bosch P., Boylan K., David W.S., Feldman E., Glass J., Gutmann L., Katz J., King W., Luciano C.A., McCluskey L.F., Nash S., Newman D.S., Pascuzzi R.M., Pioro E., Sams L.J., Scelsa S., Simpson E.P., Subramony S.H., Tiryaki E. and Thornton C.A. (2008). Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. Neurology 71, 1770-1775.

The-BDNF-Study-Group. (1999). A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III). Neurology 52, 1427-1433.

The-Fibroblast-Safety-Study-Group. (1998). Clinical safety trial of intravenous basic fibroblast growth factor (bFGF, FIBLAST) in acute stroke (abstract). Stroke 29, 287.

Thorne R.G. and Frey W.H. 2nd. (2001). Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. Clin. Pharmacokinet. 40, 907-946.

Thorne R.G., Pronk G.J., Padmanabhan V. and Frey W.H. 2nd. (2004). 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.

Thorne R.G., Hanson L.R., Ross T.M., Tung D. and Frey W.H. 2nd. (2008). Delivery of interferon-beta to the monkey nervous system following intranasal administration. Neuroscience 152, 785-797.

Tsikunov S.G. and Belokoskova S.G. (2007). Psychophysiological analysis of the influence of vasopressin on speech in patients with post-stroke aphasias. Span J. Psychol. 10, 178-188.

Vyas T.K., Shahiwala A., Marathe S. and Misra A. (2005). Intranasal drug delivery for brain targeting. Curr. Drug Deliv. 2, 165-175.

Wang Z.L., Cheng S.M., Ma M.M., Ma Y.P., Yang J.P., Xu G.L. and Liu X.F. (2008). Intranasally delivered bFGF enhances neurogenesis in adult rats following cerebral ischemia. Neurosci. Lett. 446, 30-35.

Wellmer A., Misra V.P., Sharief M.K., Kopelman P.G. and Anand P. (2001). A double-blind placebo-controlled clinical trial of recombinant human brain-derived neurotrophic factor (rhBDNF) in diabetic polyneuropathy. J. Peripher. Nerv. Syst. 6, 204-210.

Will B. and Hefti F. (1985). Behavioural and neurochemical effects of chronic intraventricular injections of nerve growth factor in adult rats with fimbria lesions. Behav. Brain Res. 17, 17-24.

Wu H., Hu K. and Jiang X. (2008). From nose to brain: understanding transport capacity and transport rate of drugs. Expert Opin. Drug Deliv. 5, 1159-1168.

Yang J.P., Liu H.J., Cheng S.M., Wang Z.L., Cheng X., Yu H.X. and Liu X.F. (2009). Direct transport of VEGF from the nasal cavity to brain. Neurosci. Lett. 449, 108-111.

Yang J.P., Liu H.J. and Liu X.F. (2010). VEGF promotes angiogenesis and functional recovery in stroke rats. J. Invest. Surg. 23, 149-155.

Zhang Y. and Pardridge W.M. (2001). Neuroprotection in transient focal brain ischemia after delayed intravenous administration of brain-derived neurotrophic factor conjugated to a blood-brain barrier drug targeting system (editorial comment). Stroke 32, 1378-1384.