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Review

Evaluation of intranasal delivery route of drug administration for brain targeting

Franciska Erdő[a,*], Luca Anna Bors[a], Dániel Farkas[a], Ágnes Bajza[a], Sveinbjörn Gizurarson[b]

[a] Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Práter u. 50A, H-1083 Budapest, Hungary
[b] Faculty of Pharmaceutical Sciences, University of Iceland, Hofsvallagata 53, 107 Reykjavík, Iceland

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ABSTRACT

The acute or chronic drug treatments for different neurodegenerative and psychiatric disorders are challenging from several aspects. The low bioavailability and limited brain exposure of oral drugs, the rapid metabolism, elimination, the unwanted side effects and also the high dose to be added mean both inconvenience for the patients and high costs for the patients, their family and the society. The reason of low brain penetration of the compounds is that they have to overcome the blood-brain barrier which protects the brain against xenobiotics. Intranasal drug administration is one of the promising options to bypass blood-brain barrier, to reduce the systemic adverse effects of the drugs and to lower the doses to be administered. Furthermore, the drugs administered using nasal route have usually higher bioavailability, less side effects and result in higher brain exposure at similar dosage than the oral drugs. In this review the focus is on giving an overview on the anatomical and cellular structure of nasal cavity and absorption surface. It presents some possibilities to enhance the drug penetration through the nasal barrier and summarizes some *in vitro*, *ex vivo* and *in vivo* technologies to test the drug delivery across the nasal epithelium into the brain. Finally, the authors give a critical evaluation of the nasal route of administration showing its main advantages and limitations of this delivery route for CNS drug targeting.

1. Introduction

Since the eighties, intranasal drug administration has gained growing interest. The nasal pathway represents a non-invasive administration route of active pharmaceutical ingredients for local, systemic and CNS action. Although, the nasal epithelium appears as a tight barrier, the tightness of the intercellular junctional complex of the nasal mucosa is low due to leaky epithelial tissue (Deli, 2009; Wolburg et al., 2008). In addition, the extensive vascularization of the mucosa, lamina propria and the leaky epithelium provide an optimal absorption surface for the drug delivery (Lungare et al., 2016; Wengst and Reichl, 2010). The direct absorption of the molecules through the trigeminal and olfactory pathways from the nasal cavity provides a direct entrance to the brain and results in beneficial pharmacokinetic/pharmacodynamics (PK/PD) profile for CNS acting drugs. Furthermore, this route of administration is a new, promising alternative pathway to enteral and systemic drug administration for dosing highly potent and efficacious CNS targeted drugs to reach the brain parenchyma by bypassing the main physiological barriers: blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (B-CSF-B). The brain capillary endothelial cells surrounding with pericytes and astrocyte endfeet and also by the basal lamina forms the BBB which limits the brain entrance of xenobiotics. B-

Abbreviations: AD, Alzheimer's disease; ADNP, activity-dependent neuroprotective protein; ALI, air–liquid interface; ANG II, angiotensin II; AUC, area under the curve; BBB, blood-brain barrier; CaCo-2, Caucasian colon adenocarcinoma cell line; CNS, central nervous system; CSF, cerebrospinal fluid; E, exosome; EN, endosome; EPO, erythropoietin; FGF, fibroblast growth factor; FST, forced swimming test; GA, golgi-apparatus; GALP, galanin-like peptide; GLP-1, glucagon-like peptide-1; HD, hungtinton's disease; HIV, immunodeficiency virus; IF γ, interferon γ; IFN-β1b, interferon-β1b; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; IN, intranasal; LCC, liquid-covered culture; L-DOPA, L-3,4-dihydroxyphenylalanine; MCAO, middle cerebral artery occlusion; MCP1, monocyte chemoattractant protein 1; MOG, myelin oligodendrocyte (protein epitope); MSC, mesenchymal stem cells; NGF, nerve growth factor; OEC, olfactory ensheathing cells; ONF, olfactory nerve fibroblast; OSN, olfactory sensory neurons; PACAP, pituitary adenylate cyclase-activating polypeptide; PD, Parkinson's disease; PD, pharmacodynamics; P-gp, P-glycoprotein; PK, pharmacokinetics; RPMI 2650, human nasal epithelial cell line; SC, support cells; SCA-1, spinocerebellar ataxia type 1; scFv, single-chain variable fragment; TEER, Transepithelial electrical resistance; TGF-β1, transforming growth factor β1; TJ, tight junction; TST, tail suspension test; V1, Ophthalmic division of Trigeminal nerve; V2, Maxillary division of Trigeminal nerve; V3, Mandibular division of Trigeminal nerve; VEGF, vascular endothelial growth factor

* Corresponding author.
E-mail address: erdo.franciska@itk.ppke.hu (F. Erdő).

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CSF-B includes the interface of brain and cerebrospinal fluid at the surface of cerebral ventricles and this barrier limits the transport between the liquor (CSF) and the brain parenchyma. At the nasal cavity the molecular diffusion bypass these barriers and the drugs are oriented directly toward the brain.

Active pharmaceutical ingredients absorbed in the nasal mucosa can not only have local and/or systemic effect, but they can also be used to target the brain directly (Gartziandia et al., 2016; Singh et al., 2016). The nasal route of drug administration has several advantages over oral or intravenous administration, which include non-invasiveness, self-administration, shorter time to onset of effect and higher bioavailability due to avoidance of hepatic first-pass metabolism. Moreover, bypassing the BBB may potentially increase central nervous system (CNS) availability of the drug (Dufes et al., 2003).

This article gives an overview of the nose-to-brain route, focusing on the anatomy of nasal cavity and the cellular and molecular mechanisms playing an important role in the nasal drug administration and drug penetration to the brain. After this introductory part various *in vitro*, *ex vivo* and *in vivo* models for investigation of intranasal drug delivery will be presented based on the latest literature of the field. Then some studies which evaluate the intranasal penetration of drugs for different CNS indications will be shown. Finally the methods to improve the nasal drug delivery will be summarized and a critical evaluation of the nasal drug administration route will be given based on the limitations and the advantages of this technique.

2. Anatomy and cellular structure of the human nasal cavity

To study nasal drug absorption and the pathways molecules need to penetrate before reaching the brain, it is crucial to get to know its function and the anatomical and cellular structure of the nasal cavity. The nose is responsible for multiple physiological functions such as olfaction and respiration. It is comprised of two symmetric cavities, divided by the septum which lies along the midsagittal plane (Crowe et al., 2018) (Fig. 1A). The nasal cavity and the oral cavity are separated from each other by the palatine bone. The cavities are lined with a layer of mucosa, and the total area of both nasal cavities is ~150–160 cm^2 (Lochhead and Thorne, 2012; Mygind and Änggård, 1984; Mygind and Dahl, 1998).

These cavities can be further divided into three regions (Fig. 1B). The first is the vestibular region, which is the most anterior and is located immediately at the nostril openings. Its surface area is around 0.6 cm^2 and contains nasal hairs which serve to filter inhaled particles. The primary cell type in this area are squamous epithelial cells, with few if any ciliated cells. Due to the small surface area and the cellular structure, the absorption of drugs is very limited in this region. The respiratory region covers the lateral walls of the nasal cavities, including the three projecting turbinates (lower, middle and upper nasal turbinates). This region has the largest area at ~130 cm^2, and it is also the most vascular region (Watelet and Van Cauwenberge, 1998). There are four principal cell types: goblet, ciliated, non-ciliated columnar and basal cells. Goblet cells secrete mucin to create the mucus layer together with some of the nasal glands, which is turned over at varying rates (Merkus et al., 1998) depending on the environment such as humidity and temperature, but also depending on various circadian factors. Basal cells are the key cells in the nasal cavity being able to develop into all the other types, when needed.

The mucus is able to trap numerous molecules and deliver them to the throat where after they are swallowed into the GI tract. Therefore, drugs must pass through this mucus layer to reach the surface of the epithelium and be absorbed. Higher viscosity of the mucus causes lower clearance rate, but may also ideally allow larger percentages of doses of drugs to penetrate the mucus and reach their intended target. Basal cells are located on the basolateral aspect of the epithelium in contact with the basement membrane. They are able to grow and mature into the cells needed in every region of the nasal cavity. The ciliated and columnar cells possess numerous microvilli (and cilia) which additionally increases the surface area. This large surface area combined with the high degree of vascularity makes the respiratory region a great site for drug absorption to the systemic circulation rather than to the CNS (Arora et al., 2002). This respiratory region is innervated by the maxillary and ophthalmic branches of the trigeminal nerve (V1, V2), which originates in the pons of the brainstem, and presents a possible target nerve for transporting drugs to the CNS in addition to the olfactory pathway (Figs. 2–4) (Crowe et al., 2018).

The nasal septum also contains a small opening or a small depression close to the base of the nasal septum where the vomeronasal-terminalis nerve is accessible *via* the vomeronasal organ (VNO) (Gizurarson, 2012). The VNO is known to be essential in newborns (Leon, 1998), but it is still debated if it is available in adults. If available, active drugs may be absorbed through the VNO and *via* the terminalis nerve directly into the brain (Monti-Bloch et al., 1998) (Fig. 5).

The transportation of molecules from the nasal cavity to the parenchyma of the brain occurs along both the olfactory or trigeminal nerves (Figs. 2–4). Once the molecules are delivered to the origins of the nerves in the cerebrum and pons, respectively, they are able to disperse throughout the brain following certain pathways. This transportation process occurs *via* two pathways – intracellular and

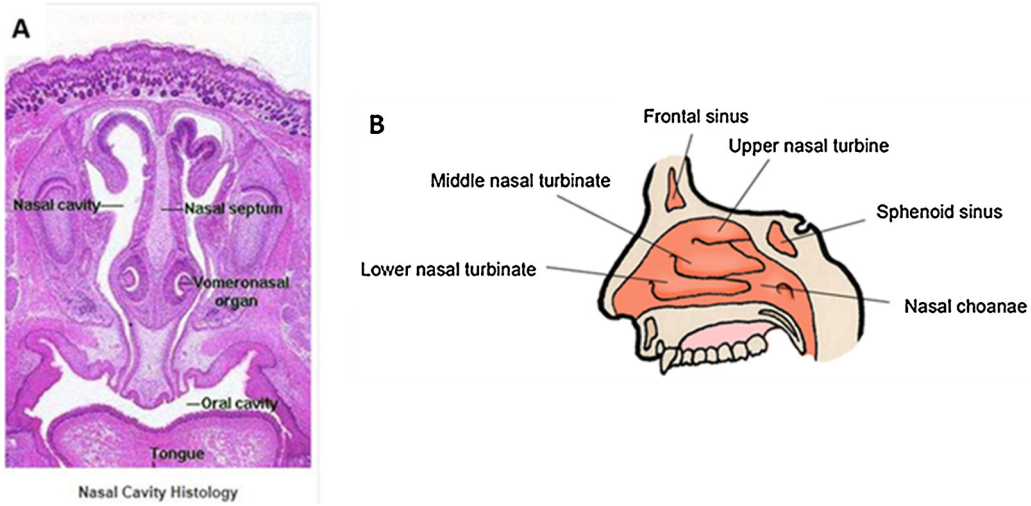

Fig. 1. The anatomy of the human nasal cavity. (A) Histological section of human nasal cavity (Nasal Cavity, 2017, http://www.therespiratorysystem.com/nasal-cavity/), (B) schematic view of the internal structure of human nasal cavity.

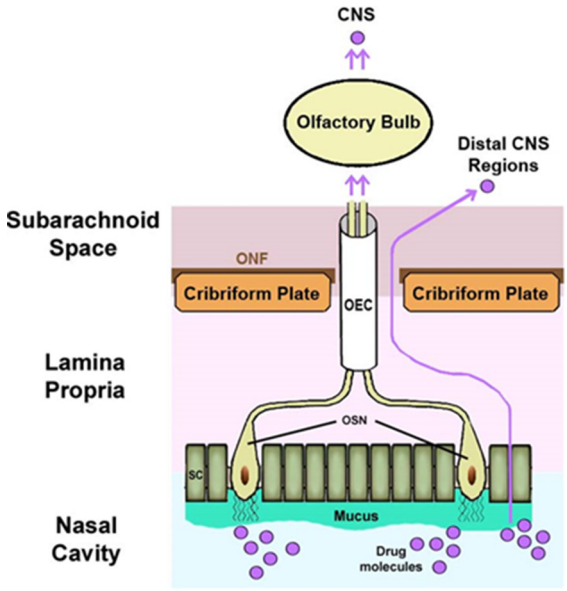

Fig. 2. Intranasal drug transport through the olfactory route to the CNS by intracellular and extracellular pathways. Drug is taken up by OSNs which project to the olfactory bulb. The extracellular route is between the SCs, where the drug passing through the tight junctions (TJs), paracellular cleft, the lamina propria, perineural space, and ultimately to the subarachnoid space where it is transported to distal targets around the CNS. Abbreviations: Support cells (SC), olfactory sensory neurons (OSN), olfactory ensheathing cells (OEC), olfactory nerve fibroblasts (ONF). After Crowe et al. (2018).

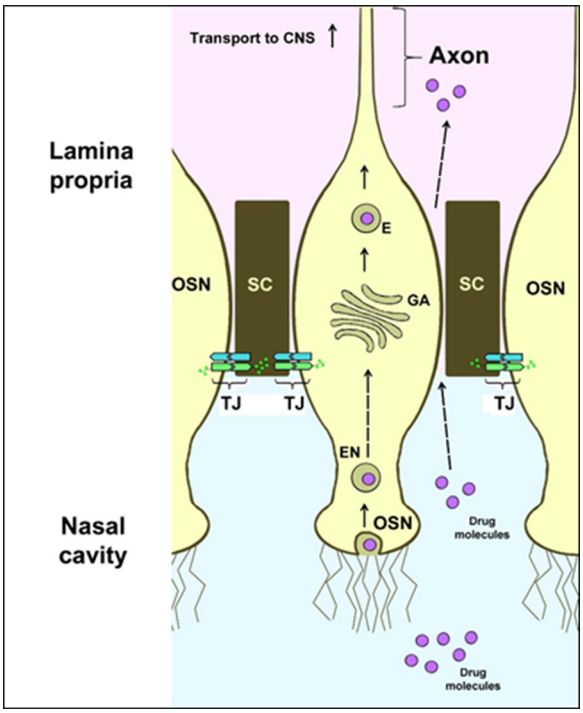

Fig. 3. The initial processes at intracellular and extracellular mechanisms of intranasal drug transport to the CNS. Intracellular shows pinocytosis/endocytosis (1), trafficking of endosome to Golgi apparatus (2), sorting with the Golgi stacks (3), and axonal transport toward olfactory bulb (4). Extracellular pathway shows movement of drug to the paracellular space and translocations through an absent tight junction (TJ) (6), and finally translocation to the lamina propria through the paracellular cleft (7). Abbreviations: olfactory sensory neuron (OSN), supporting cells (SC), endosome (EN), Golgi apparatus (GA), exosome (E) and tight junction (TJ).

extracellular (Fig. 3). The intracellular mechanism starts with internalization of the molecule by an olfactory neuron, trafficking of the endocytic vesicle within the cell to the neuron's projection site, and finally release *via* exocytosis (Fig. 3). The extracellular pathway starts with the drug crossing the nasal epithelium to the lamina propria where the neurons are located and especially in the olfactory region of the nasal cavity, before being transported externally along the length of the neuronal axon by bulk flow processes. The axon leads into the CNS, where the drug is distributed further *via* fluid movement. The penetration of drugs through endothelial cells in the lamina propria or from the subarachnoid CSF into the brain parenchyma suppose the ability of the molecules to cross BBB and blood-CSF barrier (Crowe et al., 2018).

2.1. Olfactory and trigeminal pathways

Intracellular and paracellular pathways of drug transport from nasal cavity to the CNS through olfactory neurons and supporting cells are shown in Fig. 3.

The trigeminal nerve conveys sensory information from the nasal cavity, the oral cavity, the eyelids, and the cornea, to the CNS *via* the ophthalmic division (V1), the maxillary division (V2), or the mandibular division (V3) of the nerve (Clerico et al., 2003; Gray, 1978). Branches from the ophthalmic division of the trigeminal nerve provide innervation to the dorsal nasal mucosa and the anterior portion of the nose, while branches of the maxillary division provide innervation to the lateral walls of the nasal mucosa. The mandibular division of the trigeminal nerve extends to the lower jaw and teeth, with no direct neural inputs to the nasal cavity. The three branches of the trigeminal nerve come together at the trigeminal ganglion and extend centrally to enter the brain at the level of the pons, terminating in the spinal trigeminal nuclei in the brainstem (Dhuria et al., 2010) (Fig. 4).

3. Models for testing direct nose-to-brain delivery

Drug delivery into CNS *via* nasal pathway has been reported in humans and animal models of AD (Jogani et al., 2008), brain tumors (Hashizume et al., 2008), epilepsy (Barakat et al., 2006), pain (Westin et al., 2005) *etc*. The nose-to-brain route *via* olfactory and respiratory epithelium may involve paracellular, transcellular and neuronal transport (Illum, 2003, 2000).

Models of nasal drug delivery can be used for detecting and testing nasal drug absorption and permeation, for PK/PD studies, toxicological and electrophysiological studies, and also for assessment of drug transporter interaction and the nasal barrier.

Models commonly used in nasal drug delivery experiments are *in vitro*, *in vivo* and *ex vivo* models. The different models can be used for various studies. *in vitro* techniques permit permeation and diffusion studies, while *in vivo* models are suitable for characterization of nasal absorption and also for pharmacokinetic profile determination of a drug and finally, ex vivo technique can be performed to study the nasal perfusion (Chhajed et al., 2011). All these models are discussed below.

3.1. In vivo models models

For efficiently studying the nasal delivery systems, adequate *in vivo* models are essential. It is important to study the anatomy of the nasal cavity of the animal before selecting appropriate animal model for an *in vivo* nasal absorption studies. The rat model was the first animal model, presented in the late 1970s, and afterwards, with the development of the nasal absorption studies the mouse, rabbit, dog, sheep and monkey were used as well. Representative reports on different species/strains of experimental animals used for intranasal drug administration studies are presented in Table 1.

Mouse and rat models are very useful for preliminary studies of nose-to-brain drug absorption, while rabbit, dog and sheep models are more frequently used for pharmacokinetic studies.

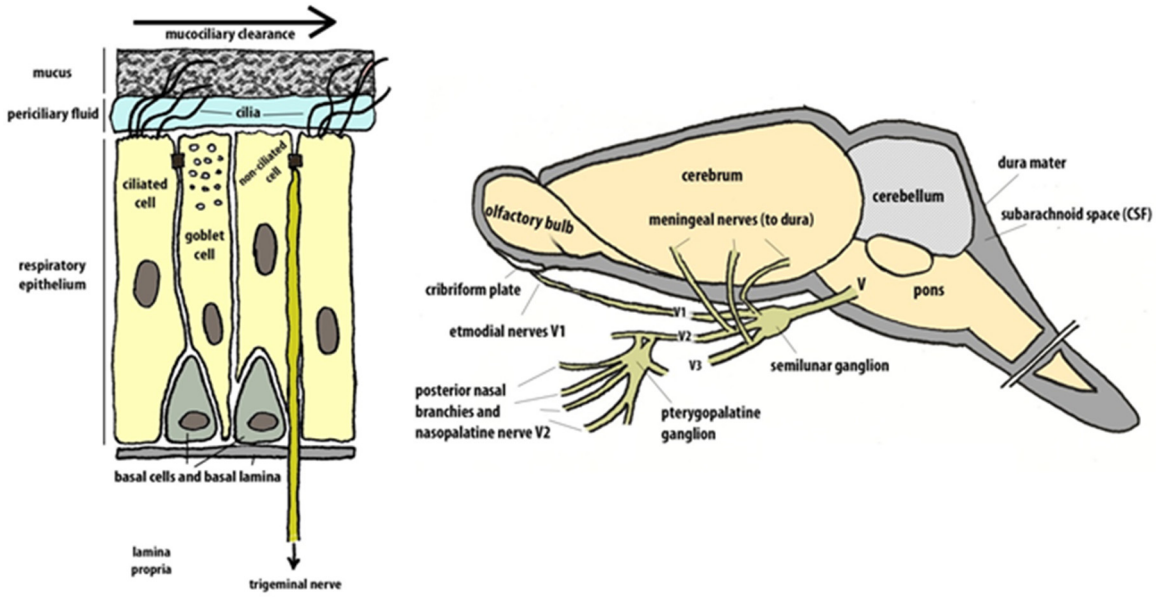

Fig. 4. Trigeminal innervation of the nasal respiratory region in rodents. The respiratory mucosa includes the respiratory epithelium and its underlying lamina propria (panel A). Fibers of the trigeminal nerve, important for conveying chemosensory, nociceptive, touch, and temperature inputs, are found throughout the nasal epithelium where their free nerve endings extend nearly to the epithelial surface, just below tight junctions (TJ). Central projections of the trigeminal nerve are shown in panel B. The cell bodies of the trigeminal nerve fibers are located in the semilunar ganglion; their axons project into the brainstem at the level of the pons. Of the three main trigeminal nerve divisions (V1, the ophthalmic nerve; V2, the maxillary nerve; and V3, the mandibular nerve), only V1 and V2 send branches to the nasal epithelium. Modified from Lochhead and Thorne (2012).

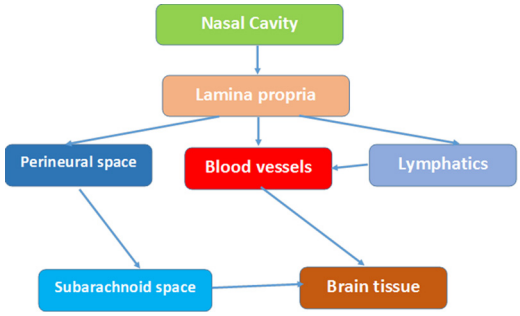

Fig. 5. The key extracellular routes of molecules from the nasal penetration surface to the CNS following intranasal administration. Modified from Crowe, Life Sciences, 2018 (Crowe et al., 2018).
Compounds that may be absorbed by the trigeminal nerve are transported to the ganglion, where after they enter the brain.

However, results of studies obtained from animal models do not always correlate well with those of humans, because of the anatomical and physiological differences of their nasal cavities (Cho et al., 2010). The direct transfer of drugs from the olfactory mucosa to the CNS is usually divided into transfer within the nerve axon, or outside the nerve. Both pathways provide potential for bypassing the blood-brain barrier (BBB).

The solubility and the potency of the drug is usually the limiting factor, since the absorption capacity is limited. It is important to note that direct nose-to-brain absorption avoids preabsorption metabolism, first pass effect, as well as dilution caused by distribution and protein binding. The dosage to be delivered to the olfactory region or to be absorbed neuronally may easily be as low as 0.01–1% of oral dosage. It is also important that the drug is soluble in those few microliters that will be administered intranasally. The clearance inside the nasal cavity is so rapid that if the drug need to be dissolved before being absorbed, the time is usually not enough for the dissolution process, before being absorbed.

The nasal formulation of drugs is usually administered with a

Table 1
Commonly used animals in intranasal drug administration studies.

Animal	Strain	Tools for administration	Volume administered per nostril (μl)	Subject of the study	References
dogs	Labrador Retriever	mucosal atomizer device	2400	analgesic treatment	Micieli et al. (2017)
guinea pigs	Hartley	pipette/syringe	10	induction of rhinitis	Mizutani et al. (2003)
mice	CD-1	micropipette	10	insulin delivery to the brain	Salameh et al. (2015)
	Balb/c	micropipette	5	morphin administration	Westin et al. (2005)
monkeys	Rhesus monkey	modified nasal atomizer	50–200	opioid administration	Saccone et al. (2016)
rabbits	New Zealand	spray	200	insulin absorption	Najafabadi et al. (2004)
	Japanese White	nasal actuator	100	new-generation corticosteroid treatment	Sato et al. (2007)
rats	Wistar	intranasal cannula	40	minimal-stress model for intranasal administration in freely moving rats	Stevens et al. (2009)
	Sprague-Dawley	pipette/syringe	50	morphin administration	Westin et al. (2005)
sheeps	Karaman	cannula	2000	admnistration of different nasal formulations	Karasulu et al. (2008)
	Suffolk	syringe	1000	intranasal inoculation with prion to compare different scrapie strains	Moore et al. (2016)

Table 2
Characteristics of nasal cavity of different species (modified from Gizurarson, 1990).

Animal species	Mean nasal volume (ml)	Nasal length (cm)	Mean nasal epithelial surface (cm^2)	Structure of conchae	Presence of septal window	Reference
dog	20	10	210	branched conchae	no	Craven et al. (2007)
guinea pig	0.9	3.4	27	double scroll	yes	Schreider (1983)
mouse	0.03	0.5	2.8	double scroll	yes	Gross et al. (1982)
monkey	8	5.3	62	single scroll	no	Schreider (1983)
rabbit	6	5.2	61	branched conchae	no	Gizurarson (1990)
rat	0.4	2.3	14	double scroll	yes	Schreider (1983)
Sheep	114	18	327	double scroll	No	Gizurarson (1990) and Proctor and
Human	20	7.5	160	single scroll	no	Anderson (1982)

pipette or using a polyethylene tube attached to a micropipette, inserted approximately 3 mm (in mice) or 5 mm (in rats) into the nostril. In the study of Westin et al. the received volume of the intranasal administration is 5 µl for mice and 50 µl for rats and the drug was administered into the right nostril (right-sided administration), so the left olfactory bulb could serve as a control (Westin et al., 2005). It is important to keep the animals in a supine position in order to increase the chance for the drug to reach the olfactory region or the upper part of the nasal cavity where is direct access to the brain. In humans, the olfactory region covers about 10% of the nasal cavity with limited access. In mice and rats, however, the olfactory region covers about 50% of the nasal cavity. The olfactory region of monkeys is similar to that of humans and lies in the upper part of the nasal cavity. Nasal anatomy of rabbits and dogs are similar, where there are branched complex conchae inside the nasal cavity, although the surface area of dogs is larger and the olfactory region is located primarily on the ethmoidal conchae (Gizurarson, 1990). Anatomical factors affecting nasal absorption are summarized in Table 2.

3.2. *In vitro models*

Whereas *in vivo* studies are most substantial for any nasal drug absorption and permeation tests, mechanistic aspects of nasal absorption and the drug transport can be more clearly studied and controlled by *in vitro* studies. Mainly two cell lines (RPMI 2650 and CaCo-2) are used to assess nasal absorption and permeability. It should be noted that these cellular models provide information on the transport across the cells or paracellular, but concurrent factors such as mucus, mucins, clearance, anatomical and physiological factors involved in keeping the nose functional may also affect the absorption. Additionally, the cellular models contain receiving lumen that does not fully reflect the required transport from the mucosa to the receiving nerves.

3.2.1. *RPMI 2650 a cell culture model of the nasal barrier*

RPMI 2650 is derived from the human nasal epithelial tissue, a human nasal squamous cell carcinoma (a spontaneously formed tumor) of the nasal septum. A cell culture model based on human RPMI 2650 cell line does not form monolayers, only grows to multilayer, thus it is commonly used for nasal metabolism studies and also for toxicity assays (Kürti et al., 2013; Wengst and Reichl, 2010). Although RPMI 2650 cell line is not suitable for drug transport study, its application for drug permeation studies has been reported (Bai et al., 2008). The serially passaged RPMI 2650 cells form confluent cell monolayer under controlled conditions. These conditions include cultivating medium for cells and the additives for the cellular differentiations. Each monolayer creates tight junctions with a transepithelial electrical resistance (TEER) value enabling transportation of drugs.

Werner and coworker studied the transportation of drugs *in vitro* using transwell insert, which is a permeable support device that provide independent access to both sides of a monolayer (Schmidt et al., 1998; Werner and Kissel, 1995). Although the contamination of epithelial cells with pathogens can be a problem, this method is convenient for evaluating the transport and metabolism of peptides (Hoang et al., 2002).

There are two types of culture conditions of these passaged monolayers, the air-liquid interface (ALI) and the liquid-covered culture (LCC) (Dolberg and Reichl, 2016). In LCC model both apical and basolateral sides are filled with cell culture medium. Morphologically is characterized with denuded and flattened ciliated cells with relatively weak mucin expression and TEER value peak on the second day and then decrease rapidly. In ALI model both apical and basolateral sides are filled with cell culture medium, and then apical side is exposed to the air, after which the medium in basolateral side is changed every second days. Morphological appearance is much close to the nasal tissue *in vivo* with number of ciliated cells and stronger mucin gene expression. Maximum TEER value is appeared on day 5, and high TEER value is maintained over 10 days. These differences between LCC and ALI models show that ALI condition compared to LCC could provide more adequate environment for drug transport studies.

RPMI 2650 cells in ALI culture could be used as first screening tool for cytotoxicity and permeability in the preclinical evaluation and comparison of solid formulations for intranasal delivery of drugs (Gonçalves et al., 2016).

3.2.2. *CaCo-2 cell line*

Another common cell culture model is CaCo-2 cell line that has been used for three decades and is suitable to evaluate the nasal absorption of the formulations. The cell line is derived from human colon carcinoma and differentiates to various monolayers slowly. Caco-2 cell culture is the most suitable model to assess the drug absorption and permeability trough the intestinal epithelia (Dolberg and Reichl, 2016; Dyer et al., 2002; Qian et al., 2018; Tan et al., 2018)

Reconstructed human nasal mucosa (the three-dimensional reconstructed human nasal mucosa model of permeation barriers)

Reconstructed three-dimensional model uses isolated human nasal fibroblasts in collagen matrix covered by RPMI 2650 epithelial cells. A collagen matrix containing fibroblasts is used as a growth support for the epithelial cells. The three-dimensional reconstructed nasal mucosa model shows comparable permeation barrier properties and four –to five- times faster paracellular permeation than in the epithelial cell model. Although the disadvantage of this three-dimensional model is the more complex handling of the constructs it is promising model to evaluate passive permeation of substances trough the nasal mucosa (Wengst and Reichl, 2010).

3.3. *Ex vivo models*

In the development of medications for nasal administration, reliable study models are essential. Determination of toxic effects of excipients and transmucosal transport of drugs are usually performed *ex vivo* using nasal mucosa from experimental or slaughtered animals. Frequently utilized *ex vivo* excised animal tissue models are obtained from rats, rabbits, dogs, sheep, monkeys, but from humans as well.

Studies with excised tissues are useful to obtain information on

permeation, metabolism, efflux, and toxicity. Despite of the numerous advantages of the *ex vivo* models for nasal delivery of drugs they have also some limitations. The most important limitation factor is the thickness of nasal epithelial tissues of animal species and the lack of interstitial flow rate underneath the mucosa. To obtain information on permeability it becomes difficult to extrapolate the results to *in vivo* models (Cho et al., 2010).

For nasal drug delivery, it is essential to investigate some factors that may affect the extent and the rate of drug absorption. These factors are metabolic stability, permeation mechanisms, and formulation of the drug.

The well-known *ex vivo* nasal perfusion model for drug permeability is the Ussing chamber. The use of this model is simple so it is very easy to monitor and maintain viability of tissues throughout the study. On the basis of permeability study, it is possible to quantify passive diffusion, active transport, efflux transport as well as identify and characterize (compound)-specific carrier-mediated routes of transport (Li et al., 2004). The efflux pumps, identified in the nasal mucosa, are important to study in these models with and without compounds that may block these pumps (Hosoya et al., 1994). In addition, the Ussing chamber model offers to compare the transport of drugs of the nasal respiratory and olfactory mucosa (Espefält Westin, 2007).

In sum, *ex vivo* models are very popular as drug screening models, especially for drug delivery studies, during the early phase of drug development.

4. Intranasal application of different drugs for CNS indications

Several CNS drugs barely pass through the BBB and even if they can penetrate into the brain efficiently, they cause adverse effects in the periphery (Hanson and Frey, 2008). These are the main reasons why it was important to find an alternative route of administration to deliver CNS drugs directly into the brain. Besides evading the BBB and minimizing peripheral exposure (Born et al., 2002), first-pass metabolism, slow absorption, fast elimination and plasma protein binding (Lindup and Orme, 1981) are additional factors to be considered at administration of CNS acting drugs. Intranasal (IN) administration an alternative option contrary to enteral or intravenous administration. Schiöth et al. (2012) reported that Insulin-like growth factor 1 (IGF-1) had significantly higher CNS exposure when it was administered intranasally compared to IV dosing (Schiöth et al., 2012)

Many CNS-associated diseases have already been treated with IN administration of drugs, like obesity, eating disorders, AD (AD), Parkinson's Disease (PD), Huntington Disease (HD), depression, anxiety, autism, seizure, addiction and stroke and numerous new promising drugs are under development for IN application (Chapman et al., 2013).

4.1. Preclinical data

Many intranasal medications are still in preclinical phase of development. Some of these studies are targeting memory and learning, that show potential treatment for neurodegenerative disorders like AD, PD, epilepsy and other neurotoxic events like oxidative stress and ischemia and other diseases as it can be seen in Table 3.

4.1.1. Learning and memory, neurodegenerative diseases

[Ser(2)]exendin(1–9) is an agonist of glucagon-like peptide-1 (GLP-1) receptor (due to its homology to a conserved domain in the glucagon/GLP-1 family) that used to facilitate learning and has been shown to decreases that IN [Ser(2)]exendin the occurence of kainic acid-induced apoptosis in mouse models after IN administration (During et al., 2003). The radioactive compound can be detected in the lymph nodes and blood and also in the olfactory bulb, that means an efficient uptake through nose-to-brain besides the nose-to-blood-to-brain path.

IN administration of NAP, an eight amino acid peptide (the sequence is NAPVSIPQ) derived from activity-dependent neuroprotective protein (ADNP) has improved memory function in normal and cognitively impaired rats and decreased anxiety in aged mice (Alcalay et al., 2004; Gozes et al., 2000). Gozes et al. had shown with reversed phase-HPLC that (3)H-labeled NAP reaches the brain unchanged after 30 min from administration and by the 60-minute mark it reaches its maximum concentration in the brain cortex (Gozes et al., 2000). In AD models and chronic administration improved spatial learning and memory, increased soluble tau, and decreased neurofibrillary tangles in tauopathy (Shiryaev et al., 2009). In a mouse model of schizophrenia, IN NAP decreased hyperactivity and protected visual memory (Gozes, 2011). Another study also showed that IN NAP reduces oxidative stress in rats subjected to chronic hypoxia (Sharma et al., 2011). However the exact route of NAP was not detailed in these publication only that NAP has reached sufficient amount of concentration in the brain to have its effect.

The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) has shown neuroprotective and neurotrophic properties, beneficial in an AD model, following chronic IN administration (Rat et al., 2011). The radioactive peptide ([^{125}I]PACAP27 was used) has been showed to reach the CNS after 5 min and the maximal concentration (about 0,1% of initial radioactivity) was detected 15 min after administration. However the exact route of the drug (nose-to-brain or nose-to blood-to-brain) was not investigated. For comparison, in another study about 0.11% of the total intravenous dose of PACAP crossed the BBB (Banks et al., 2002).

Recombinant human nerve growth factor (NGF) has been shown to reach the olfactory bulb and most part of the brain in significantly higher concentrations after 30–45 min following IN administration as compared to a matched IV dose (Chen et al., 1998). IN nerve growth factor (NGF) in a NGF-deficient transgenic mouse model of AD showed successful rescue of recognition memory deficits (Capsoni et al., 2002; De Rosa et al., 2005) and a behavioral analysis also showed that it has antidepressant like effects in animals by reduction of the immobility time in forced swimming test (FST) and tail suspension test (TST) (Capsoni et al., 2002). IN NGF also decreased epileptic seizure onset probability and duration in rats (Lei et al., 2017).

IN administration of L-3,4-Dihydroxyphenylalanine (L-DOPA) show that L-DOPA is easily absorbed into the systemic circulation and from there into the CNS. In a study carried out by Chao et al. they pre-treat the animals causing certain lesions to the brain, and following recovery they administer L-DOPA intranasally and carry out tests using bensarazide showing that L-DOPA is able to reach the brain directly, bypassing the blood-brain barrier (Chao et al., 2012).

IN IGF-I was found to be a suppressor of ataxin-1-mediated adverse effects (Vig et al., 2006), the protein that causes spinocerebellar ataxia type 1 (SCA1) (Burright et al., 1995), therefore, this administration may be a therapeutic alternative for this disease. The severity of SCA1 was examined with accelerating rota rod test: the group was treated with saline and as the animals get used to the task they improved their performance while the SCA1 saline treated mice failed to improve. However the IGF1 (60 g) treated animals were almost similar to SCA1 saline treated group, on the third day of the study the animals performed better ($p < 0.05$) than SCA1 saline treated animals (Vig et al., 2006).

There are CNS drugs that not only inhibit the neurodegenerative processes but are also able to prevent it or help in the regeneration. IN Calcitonin gene-related peptide has been shown to improve cerebral blood flow, reduce cortical and endothelial cell death, increase the levels of vascular endothelial growth factor (VEGF) and stimulate angiogenesis and resulted in significantly higher levels in the CSF, cortex, and hippocampus relative to IV administration (Sun et al., 2010).

The IN combination of Erythropoietin (EPO) and IGF-1 significantly reduced infarct volumes in case of middle cerebral artery occlusion (MCAO) and improved neurological function up to 90 days later. A

Table 3
Short summary of some preclinical studies on intranasal drug delivery to the brain with positive outcome.

Drug name	Animal and Administration (if mentioned)	Indications	Results	References
NAP peptide	monkey, atomizer mouse	memory problems; hyperactivity; sleep deprivation	Significant difference in potency of IN *vs* i.v orexin-A. Successful memory improvement in NAP-AF64A-treated animals.	Deadwyler et al. (2007) and Gozes et al. (2000)
	mouse	Alzheimer's Disease like pathology; tauopathy, anxiety	Chronic IN NAP reduced anxiety and increased soluble tau and reduces tau hyperphosphorylation.	Alcalay et al. (2004) and Shiryaev et al. (2009)
	mouse	schizophrenia (hyperactivity and loss of visual memory)	Improved cognitive behavior model of schizophrenia	Gozes (2011)
	rat	hypoxia-induced oxidative stress	1) Attenuation in hypoxia induced ROS generation 2) restored antioxidant levels 3) modulates the expression of hypoxia responsive genes during hypobaric hypoxia.	Sharma et al. (2011)
[Ser(2)]exendin(1–9)	rat	Facilitated learning; reduced kainic-acid induced apoptosis (KAIA)	Enhaced spatial learning and attenuation of KAIA in hippocampus.	During et al. (2003)
Pituitary adenylate cyclase-activating peptide	mouse	Alzheimer's Disease (neuroprotection)	Increased sAPPα secretion. Significant reduction of soluble Aβ40 and Aβ42 peptides.	Rat et al. (2011)
Recombinant human Nerve growth factor	mouse	Alzheimer's Disease (memory, depression)	Prevent the reduction of basal forebrain ChAT + neurons. NGF-treated AD11 mice spent more time with the new object exploration. NGF-treated group had higher exploration index than PBS-treatment group.	Capsoni et al. (2002) and De Rosa et al. (2005)
L-DOPA	rat, pipette	Parkinson's Disease	IN L-DOPA group showed fewer ipsilateral, contralateral rotations and forelimb-slips compared to the vehicle group.	Chao et al. (2012)
Insulin-like Growth factor 1	mouse, drops	Spinocerebellar ataxia type 1	Improved performance on rotarod in SCA1 animals.	Vig et al. (2006)
22C4 Single-chain variable fragment antibody	rat	amyloid angiopathy, and plaque pathology, ischemic injury	Reduced mnestic deficit, improved passive avoidance and reduced glutamate overproduction.	Gorbatov et al. (2010) and Romanova et al. (2010)
Erythropoietin + Insulin-like Growth factor 1	mouse, pipette	neuroprotection in Human Immunodeficiency Virus model	Synergic effect: more effective without increasing the dose. Avoid systemic side-effects. Activation of Akt prevents tau phosphorylation.	Kang et al. (2010)
Nerve growth factor	rat, pipette	neurogenesis	Reduced seizure onset and duration. Alleviated neuronal loss.	Lei et al. (2017) and Zhu et al. (2011)
Vascular endothelial growth factor	rat	behavioral recovery, angiogenesis	Improved behavioral recovery, reduced infarct volume in middle-dose IN VEGF-treated animals.	Yang et al. (2009) and Xu et al. (2009)
Transforming growth factor β1	mouse	functional recovery, neurogenesis	Improved behavioral recovery, neurogenesis and decreased infarct volume.	Ma et al. (2008a)
Fibroblast growth factor	rat, drops	neurological functions, neurogenesis	IN bFGF improved behavioral recov. and enhanced proliferation of progenitor cells.	Ma et al. (2008b) and Wang et al. (2008)
MSC	mouse	neurotrophic factors, endogenous cerebral repair	Successfully delievered in CNS and induced recovery. Reversed SAH damage.	Van Velthoven et al. (2010) and Nijboer et al. (2018)
Galanin-like peptide	mouse, cannula attached to a 10-μl syringe	Eating regulation	Successfully delivered into the CNS.	Nonaka et al. (2008)
Leptin	rat	4-aminopyridine- and pentylenetetrazole-induced seizures	Reduced neuronal spiking and AMPA transmission.	Xu et al. (2008)
Interferon-β1b	rat, drops	Sclerosis Multiplex	IN IFN-beta showed high concentration in CNS and less systemic exposure compared to i.v.	Ross et al. (2004)
apolipoprotein B-100	mice	atherosclerosis	Attenuating atherosclerosis and inducing regulatory Tr1 cells that inhibit T effector responses to apoB-100.	Klingenberg et al. (2010)
fusion protein mCTA1–T146	mice	myasthenia gravis	Reinstating tolerance against acetylcholine receptors.	Consonni et al. (2017)
VECTOR + GENE				
Herpes simplex virus type 2 ΔRR + ICP10PK	mouse, rat	kainic acid-induced seizures (KAIS)	prevented KAIS, neuronal loss and inflammation.	Laing et al. (2006)
Filamentous bacteriophage + Myelin oligodendrocyte glycoprotein	mouse	encephalomyelitis	Improved neuronal functions, decreased proinflamatory cytokine level, induced depletion of antibodies against MOG and prevented demyelination.	Rakover et al. (2010)

study has showed that the PI3K/Akt signaling pathway mediates the synergistic effect of EPO + IGF-I and by inhibiting phosphorylation of Akt, the cytokines lose their neuroprotective effect in the cerebrocortical neurons. (Digicaylioglu et al., 2004) EPO + IGF-I increased both Akt phosphorylation and GSK-3 phosphorylation and inhibiting tau hyperphosphorylation (Kang et al., 2010). This study also had confirmed the involvement of the PI3K/Akt/GSK-3 pathway by using a PI3K inhibitor. EPO + IGF-I also prevented HIV/gp120-induced neuronal cell death in cultures and *in vivo* transgenic mouse models of human immunodeficiency virus (HIV) infection (Kang et al., 2010). IN NGF also has enhanced neurogenesis in the striatum and improved functional recovery when administered after a day following MCAO (Zhu et al., 2011) and decreased the neuron loss in the epileptic brain (Lei et al., 2017).

Direct transport of VEGF to the CNS has also been shown after intranasal administration (Yang et al., 2009). Intranasal delivery of recombinant human VEGF also reduced infarct volume, improved behavioral recovery, and enhanced angiogenesis following MCAO (Xu et al., 2009).

The brain distribution of recombinant human transforming growth factor β1 (TGF-β1) was studied in rats and it was found that IN administrated TGF-β1 was successfully delivered to many parts of the CNS within 30 min following IN administration, while there was no increased level of TGF-β1 in the plasma or peripheral organs, suggesting a direct nose-to-brain uptake (Ma et al., 2008a). IN delivery of TGF-β1 also reduced infarct volume, improved functional recovery, and increased neurogenesis following MCAO (Ma et al., 2008a).

Levels of recombinant human basic fibroblast growth factor (FGF) were significantly increased in the olfactory bulb and the striatum of rats following IN administration (Ma et al., 2008b). When basic FGF was delivered intranasally following cerebral ischemia/reperfusion, improved neurological function and reduced infarct volume were observed (Ma et al., 2008b). Rats which received IN basic fibroblast growth factor daily for 6 days starting one day after MCAO also showed enhanced neurogenesis (Wang et al., 2008).

4.1.2. Eating regulation, obesity

Preclinical studies have found promising treatment also for eating disorders. Galanin-like peptide (GALP) is a neuropeptide that has turn out to be a promising treatment for obesity by IN administration as it has been successfully delivered through the olfactory bulb, into the anterior brain, hippocampus, hypothalamus, cerebellum, brain stem, and CSF in mice (Krasnow et al., 2003; Nonaka et al., 2008).

Although the BBB has the ability to transport leptin into the CNS from the blood, this transport is impaired during obesity (Banks et al., 1999), making intranasal delivery of leptin into the CNS a potential strategy to regulate feeding behavior. A pharmacokinetic study of IN administered radioactive leptin showed that more than 80% was delivered in unchanged state into the brain within 30 min, with highest levels to the hypothalamus (Fliedner et al., 2006) while after intravenous ^{125}I-leptin was detected in a little less than 20% brain/serum ratio (Hsuchou et al., 2013). IN administration of leptin has also been shown to delay the onset of pentylenetetrazole-induced generalized convulsive seizures in mice (Xu et al., 2008).

4.1.3. Auto-inflammatory diseases

The anti-inflammatory cytokine interferon-beta-1b (IFN-β1b) was investigated as an intranasally administrated, non-invasive treatment for multiple sclerosis (Ross et al., 2004). At similar blood levels, intravenous IFN-β1b had lower brain concentration compared to IN administration (Ross et al., 2004).

Autoimmune responses to low-density lipoproteins (LDL) are some of the causes of atherosclerosis. Studies have showed that immunization with LDL can induce proatherogenic responses, like they did with intranasal administration of apolipoprotein B-100 (apoB100) fused to the B subunit of cholera toxin (Klingenberg et al., 2010). The treatment induced a protective mucosal immune response in mice model by attenuating atherosclerosis and inducing regulatory Tr1 cells that inhibit T effector responses to apoB-100.

Another study reported the use of experimental autoimmune myasthenia gravis (EAMG) model and intranasal drug administration to find a treatment for myasthenia gravis, an autoimmune disease characterized by muscle weakness and fatigability (Consonni et al., 2017). Consonni et al. had designed a fusion protein, mCTA1–T146, which was able to reinstating tolerance against acetylcholine receptors after several days of intranasal administration.

4.1.4. Antibodies

Antibodies show limited penetration into the brain when delivered peripherally (Banks, 2004). One of the diagnosis of meningitis is the presence of antibodies in the CSF. The IN treatment of 22C4 single-chain variable fragment (scFv) antibodies resulted in a reduction of cerebral amyloid angiopathy and plaque pathology. Additionally, the single chain Fv antibody was detected to bind to amyloid plaques in the brains of these mice. IN delivery of full-length antibodies against glutamate has resulted in anti-amnesic effects in rats subjected to prior injection with an amyloid beta (Aβ) fragment (Aβ25–35) into the nucleus basalis of Meynert. IN administration of the same antibody also improved retention of the conditioned passive avoidance response in rats with ischemic injury of the prefrontal cortex, although neither study examined antibody pharmacokinetics within the brain (Gorbatov et al., 2010; Romanova et al., 2010)

4.1.5. Gene vectors tumors and stem cell therapies

IN administration of gene vectors could be a solution to avoid the blockade of BBB and deliver therapeutic transgenes to brain (Lochhead and Thorne, 2012). For example, growth compromised herpes simplex virus type 2 mutant ΔRR encoding the anti-apoptotic gene ICP10PK has also been successfully delivered to the brain through the IN route to prevented kainic acid-induced seizures, neuronal loss, and inflammation in both mice and rats (Laing et al., 2006).

Another successful preclinical study was the investigation of a filamentous bacteriophage, delivered a myelin oligodendrocyte protein epitope (MOG) intranasally as a treatment of murine experimental autoimmune encephalomyelitis (Rakover et al., 2010). Phage MOG treatment improved neuronal function and reduced levels of proinflammatory cytokines such as monocyte chemoattractant protein 1 (MCP1), interferon γ (IF γ), and interleukin-6 (IL-6).

There was also a study to find out, if a brain tumor can be targeted down with telomerase inhibitor GRN163 (Hashizume et al., 2008). The results showed that the drug was delivered successfully to the tumor cells without accumulating either in healthy brain cells or in the body.

Stem cells are potential treatment options for many diseases due to their ability to replace dead cells or deliver trophic factors to damaged areas. IN mesenchymal stem cells (MSC) have been used to treat ischemic brain damage in neonatal mice (Van Velthoven et al., 2010). MSCs stimulate endogenous cerebral repair by up-regulating the repair promoting factors in the ischemic brain (Van Velthoven et al., 2010). Intranasal MSC treatment was also promising in treatment of subarachnoid hemorrhage in a rat model (Nijboer et al., 2018).

4.2. Clinical data

Some drugs, like benzodiazepine derivatives have already been investigated in humans (Table 3). Benzodiazepines are used to terminate seizures and to decrease the neuronal damage that a seizure can cause in the brain. For a successful prevention, the intervention has to be fast and it is also important that the drug should be administrated as soon as possible already in home settings. IN benzodiazepines fulfill these expectations (Gizurarson et al., 1999; Kälviäinen, 2015). Studies have compared different formulations of diazepam like IN midazolam, *versus* IV midazolam (Bistritzer et al., 2000; Mahmoudian and Mohammad,

Table 4
Short summary of some clinical studies on intranasal drug delivery to the brain with positive outcome.

Drug name	Indications	References
Benzodiazepines (midazolam, diazepam)	Epilepsy	Gizurarson et al. (1999), Kälviäinen (2015), Bistritzer et al. (2000), Mahmoudian and Mohammad (2004), Mittal et al. (2006), Thakker and Shanbag (2013), Bhattacharyya et al. (2006), De Haan et al. (2010), Fişgin et al. (2002), Holsti et al. (2010), Pacifici (2014), Wermeling et al. (2006), Agarwal et al. (2013), Henney et al. (2014), Ivaturi et al. (2013) and Streisand and Stanley (1995)
Insulin	memory and mood enhancement, Alzheimer's disease, Mild cognitive impairment	Benedict et al. (2004) and Kern et al. (1999)
		Claxton et al. (2013) and Reger et al. (2006)
	obesity (in men)	Benedict et al. (2011, 2008)
Angiotensin II	high blood pressure (with type 1 receptor block)	Derad et al. (2014)
	increases BBB permeability	Fleegal-Demotta et al. (2009) and Guillot and Audus (1991)
Melanocortin proteins	weightloss (only normal weight people)	Fehm et al. (2001) and Hallschmid et al. (2006)
Oxytocin	social anxiety disorder, autism	Kirsch (2005), Kosfeld et al. (2005), Domes et al. (2007), Heinrichs et al. (2003), Labuschagne et al. (2010) and Guastella et al. (2010)
Orexin-A	Narcolepsy	Baier et al. (2008)

2004; Mittal et al., 2006; Thakker and Shanbag, 2013), and rectal *versus* IN (Bhattacharyya et al., 2006; De Haan et al., 2010; Fişgin et al., 2002; Holsti et al., 2010). In both cases IN administration was faster and more comfortable while in the potency there was no significant difference between the three routes. It should be however mentioned, that the state of the nasal mucous can interfere with IN midazolam absorption. It can help in the absorption into the bloodstream if the blood flow is increased but also could block the absorption from the mucosal surface if the nasal secretion is enhanced (Bistritzer et al., 2000). When normal nasal spray is used, the access to the olfactory region is limited, due to the structure of the nasal cavity (Olafsson and Gizurarson, 2000). In order to improve the access to this region, the subject need to inhale rapidly, similar to when he wants to smell something.

Midazolam has many beneficial properties in IN formulation; it is lipid soluble, can be absorbed fast, has rapid effect and physiological pH and IN formulation has also better bioavailability than IV formulation (Pacifici, 2014; Wermeling et al., 2006). IN diazepam also has high bioavailability compared to rectal 89% and IV 97% formulations (Agarwal et al., 2013; Henney et al., 2014; Ivaturi et al., 2013). Also intranasal sprays and drops are preferred contrary to inconvenient rectal suppositories or invasive injections (better compliance) and can be better applied in emergency situations. Adverse effects in case of nasal administration are nose irritation, bitter taste, and rarely cardiorespiratory depression (Pacifici, 2014; Streisand and Stanley, 1995).

Insulin is one of the most widely studied biologics with regard to its effects on the CNS following intranasal administration. It is mostly known for its role in the regulation of blood glucose level, but also has shown beneficial effects on the CNS to decrease appetite and increase metabolism as well as to improve memory and mood (Benedict et al., 2004; Kern et al., 1999). IN administration of insulin and insulin sensitizers have been used as treatment and as prevention on patients with AD and mild cognition impairment (MCI) (Claxton et al., 2013; Reger et al., 2006). In this way the adverse effects of peripheral insulin, for example the unsafe decrease of blood glucose level can be avoided (Born et al., 2002).

In humans, IN insulin decreased food intake, enhanced postprandial thermogenesis, and decreased postprandial serum insulin, therefore, its potential in helping weight loss proved seems to be evident (Benedict et al., 2011, 2008).

Studying the differences in IN and IV administration of angiotensin II (ANG II) in humans (Derad et al., 1998), it was found that in both cases ANG II levels were increased. However, blood pressure was normalized much faster in IN administration. IN ANG II also reduced plasma norepinephrine and enhanced plasma vasopressin levels compared to the IV administered group. In a later study Derard et al. have also found that intranasal ANG II with ANG II type 1 receptor inhibitor (valsartan) pretreatment can be used to treat high blood pressure (Derad et al., 2014). There is a possibility that these differences are due to IN ANG II binding to brain angiotensin receptors (Culman et al., 2001). However, some studies shown that ANG II has also increase BBB permeability through tight junctional and vesicular mechanisms (Fleegal-Demotta et al., 2009; Guillot and Audus, 1991).

IN Melanocortin(4–10) proteins effect on human body was studied and showed that these proteins reduce focusing of attention (Smolnik et al., 2000) while also decrease body fat in normal weight humans (Fehm et al., 2001). On the other hand in overweight humans the weight regulatory system seems to be resistant to this beneficial effect of melanocortin treatment (Hallschmid et al., 2006). Though MSH-(4–10) barely passes through the BBB, the IN administration has shown to be a viable route to get these peptides in an effective amount into the brain without overdose (Fehm et al., 2000).

Oxytocin is a neuropeptide that has an important role in controlling behavior. The BBB blocks the brain entrance of peripheral oxytocin, therefore IN administration is a plausible solution for oxytocin to reach the CNS (Kang and Park, 2000). IN oxytocin has been shown to increase trust (Kirsch, 2005; Kosfeld et al., 2005) improve empathy (Domes et al., 2007), reduce cortisol and increase anxiolytic effects in stress (Heinrichs et al., 2003), normalize fear in generalized social anxiety disorder (Kirsch, 2005; Labuschagne et al., 2010), and improve emotional recognition in patients with autism spectrum disorders (Guastella et al., 2010), showing that the peptide has been transported into the brain, bypassing the blood-brain barrier.

IN orexin-A has shown to improve the performance and alter the brain metabolic activity in sleep-deprived primate model (Deadwyler et al., 2007) clinical data has also showed that it holds promises to become a treatment for narcolepsy (Baier et al., 2008).

These clinical studies however, were primarily focused on the effect of intranasal drugs, but it should be stated that most of these studies used spray and intranasal puffs as delivery device of the drugs. Therefore, in that case the nose-to-blood- to- brain distribution pathway is also possible contrary to direct nose to brain transport (Table 4).

5. Penetration enhancer techniques at nasal drug delivery route

The penetration of different molecules across the nasal barrier can be improved by various drug formulations, using different devices or by co-administration of *e.g.* transporter modulators which might help in transcellular drug delivery.

5.1. Formulations

5.1.1. Solutions

The physicochemical properties of the drug and its potency are crucial factors when a molecule is formulated in a solution for nasal

delivery. In case of small lipophilic molecules, passive diffusion plays an important role in the nose-to-brain delivery as reported by Kandimalla and Donovan (2005). The size of the molecules to be delivered *via* nose to the brain is also important factor as reported by Pardeshi and Belgamwar (2013).The absorption of dopamine (Mw 153 Da) was compared to that of the NGF (Mw 27 kDa) by Dahlin et al. (2001). They found that brain concentrations were five-fold higher for the small molecule dopamine than the secreted protein NGF when dosed at the same concentration (Warnken et al., 2016). On the other hand, the larger molecules take longer time to get transported from the nasal cavity to the brain. Following intranasal administration, the brain concentrations were higher in the case of small lipophilic drugs, but they often showed larger improvement in the brain compared to other routes of administration. Wang and coworkers studied raltitrexed, a hydrophilic small molecule with a logP of 0.98, for brain levels following intranasal and intravenous administration. They showed a 54–121 fold increase in the area under the curve (AUC) in the brain (depending on the section of the brain) after intranasal administration compared to intravenous route in rats (Wang et al., 2006a). Wang et al. performed similar experiments with methotrexate, which is also a hydrophilic drug. Here, they found that it provided greater than 13 fold higher CSF AUC after nasal administration compared to intravenous administration (Wang et al., 2003).

The delivery of macromolecules in solutions has also been studied both in animal models (plasmids, IGF-I and NGF) and in humans (arginine vasopressin, insulin, oxytocin and melanocortin melanocyte-stimulating hormone/adrenocorticotropin4–10 (Warnken et al., 2016).

Aqueous based solution formulations are shown to be effective drug delivery systems for water soluble small molecules and many peptides and proteins as it was studied both in preclinical and human studies.

5.1.2. Mucoadhesive agents

Mucoadhesive and viscosity increasing agents have been used to increase drug residence time in the nasal cavity to allow better absorption (Touitou and Illum, 2013). By increasing the viscosity of the formulation, with polymers such as hypromellose or polyvinyl alcohol, it is possible to decrease mucociliary clearance (Nakamura et al., 1996; Pennington et al., 1988). Mucoadhesive agents, such as pectin and chitosan studied by Charlton et al, were effective at extending residence times at the olfactory epithelium. It has also been shown that mucoadhesive and viscosity increasing agents increase bioavailability from nasal formulations designed for systemic delivery (Chaturvedi et al., 2011). To determine how the addition of a mucoadhesive agent can influence the absorption of drugs into the brain, Khan et al. (2009) compared brain concentrations of buspirone after intravenous administration as a solution without chitosan or cyclodextrins and intranasally as a solution with 1% chitosan and 5% hydroxypropyl β-cyclodextrin. They found that the AUC in the brain was 2.5-times higher for buspirone in the mucoadhesive formulation than in the intravenous solution, and two-times as high as buspirone solution when delivered intranasally. The cyclodextrins may have also played a role in the increasing brain concentration by enhancing the permeability of the drug through the tight junctions of the nasal epithelium (Khan et al., 2009).

To test the possible toxicity of natural and synthetic mucoadhesive ingredients in nasal formulations OECD guidelines 420 were followed in some studies (Mangilal and Rao Patnaik, 2014; Singh et al., 2011).

5.1.3. Nanoparticles (nanosuspensions, nanoformulations)

A popular formulation method for many routes of administration is the formation of nanosuspensions of drugs encapsulated in polymeric carriers. These carriers may provide favorable characteristics to the drug like enhanced absorption, mucoadhesion and increased stability. Bhavna et al. (2014) developed a nanosuspension formulation of donepezil, a cholinesterase inhibitor, for enhancing brain exposure to treat AD. In another paper, the authors tested chitosan nanoparticles loaded with bromocriptine (Md et al., 2014) and found that bromocriptine-loaded nanoparticles given intranasally produced two-fold greater brain AUCs than that were in case of intravenous administration of the nanoparticles. Other studies also reported nanoformulation-induced enhanced drug delivery to the brain (wheat germ agglutinin (Gao et al., 2007), olanzapine (Seju et al., 2011), lorazepam (Sharma et al., 2014), rivastigmin (Fazil et al., 2012; Mistry et al., 2015).

Pegylation (Kamiya et al., 2018) and dendrimers are also possible nanotechnological solutions to improve CNS drug delivery (Lu et al., 2014) by nasal drug administration. Pegylation may increase the retentivity in the nasal cavity according to Kamiya et al. (2018).

For a comprehensive review about the preparation and testing of nanocarriers for nose to brain delivery see the recent article of Sonvico et al. (2018). In the same article there are some marketed drugs listed for nasal administration and CNS targeting (nicotine, Nicotrol® NS, Pfizer, New York City, NY, USA, pain management: fentanyl, Intstanyl®, Takeda, Japan and Pecfent/Lazanda®, Archimedes Pharma Ltd., Reading, UK; butorphanol tartrate spray, Mylan Inc., Canonsburg, PA, USA, the treatment of migraine: zolmitriptan, Zomig®, AstraZeneca, Cambridge, UK; sumatriptan, Imigran, GSK, Brentford, UK and Onzetra™ Xsail™, Avanir Pharmaceuticals, Aliso Viejo, CA, USA).

5.1.4. Lipid based systems (microemulsions, lipid based nanoparticles)

Many groups have used lipid components like microemulsions to increase delivery of drugs to the brain. Microemulsions can increase the concentration of hydrophobic drugs to be delivered, as well as increase the permeability across the membranes (Jadhav et al., 2006). Jogani et al. (2008) developed a microemulsion formulation of tacrine for delivery to the brain. Risperidone has also been formulated as solid lipid nanoparticles for nose-to-brain delivery (Patel et al., 2011). Solid lipid nanoparticles have recently received high attention in delivery therapeutics using direct nose-to-brain drug delivery as it was reported in several articles (Dalpiaz et al., 2014; Montenegro et al., 2011; Pardeshi et al., 2013; Patel et al., 2011)

5.1.5. Co-administration with vasoconstrictors for improved delivery

The olfactory region receives its blood supply from the small branches off the ophthalmic artery, while the respiratory region receives its blood supply from a large arterial branch from the maxillary artery. Therefore, the respiratory region is highly innervated with blood vessels, making it an ideal target for systemic drug absorption (Dhuria et al., 2010). The olfactory region is often a target area for nose-to-brain delivery, since this has fewer blood vessels contributing to plasma concentrations, while providing access to the olfactory nerve pathways. Dhuria et al. (2010) studied the effect phenylephrine, a vasoconstrictor drug used for nasal decongestion, to increase the brain to plasma AUC ratio. They tested brain concentrations after nasal administration of neuropeptides, hypocretin-1 or dipeptide L-Tyr-D Arg. The use of the vasoconstrictor significantly decreased the amount of drug absorbed into the systemic circulation, it also significantly increased the amount delivered to the olfactory bulb.

5.1.6. Permeability enhancers

The nasal epithelium can be a rate-limiting barrier for transport of drugs directly to the brain. In targeting drug delivery to the system circulation, many agents have been used to increase the permeation of drugs across the epithelium (Arora et al., 2002; Behl et al., 1998; Drejer et al., 1992; Gordon et al., 1985; Karasulu et al., 2008; Warnken et al., 2016). Agents used to increase the permeability across a membrane are referred to as permeation enhancers. Permeation enhancers have also been used to overcome this barrier for targeting delivery to the CNS. Since the nasal epithelial layer is comprised of tight junctions, permeation enhancers which open tight junctions may be useful in improving drug delivery to the brain. Some studies have used borneol (Lu et al., 2012), chitosan and cyclodextrins (Khan et al., 2009; Wang et al., 2006b) to help improve direct nose-to-brain drug transport.

5.2. Devices to help nasal delivery

The deposition from various nasal devices is typically measured as the amount or percent deposited in defined segments of the nasal cavity. One of the oldest nasal delivery systems is nasal drops (Kublik and Vidgren, 1998). When administered correctly, nasal drops may spread over a larger area than nasal sprays, but are often cleared faster than nasal sprays as well (Hardy et al., 1985). Charlton and coworkers reported that nasal drops possess higher deposition in the olfactory region compared to nasal sprays if administered to the patient in a supine position, and when formulated with mucoadhesive agents it is able to reduce the time in which the formulation is cleared from the area. The longest mean residence time in the olfactory region achieved in the study was about 14 min, compared to 1.3 min for control solution without any mucoadhesive agents (Charlton et al., 2007). An important limitation of nasal drops, and nasal sprays for that matter, is that their efficacy can be affected by patient administration technique. Nasal drops require complex maneuvers by patients to achieve correct head positioning for proper administration (Kublik and Vidgren, 1998).

In order to overcome the disadvantages associated with conventional nasal delivery systems with regards to targeting the olfactory region, novel delivery devices have been developed. Some studies focused on nose to brain delivery in humans utilized the Vianase™ a delivery system designed *e.g.* to delivery insulin intranasally (Craft et al., 2012). Vianase™ is an electronic atomizer device developed by Kurve Technology® which consists of a nebulizer attached to a vortex chamber. Nebulized medication particles move in a vortex in the vortex chamber and continue to exhibit this flow when leaving the device (Giroux, 2007). This promotes deposition to the olfactory region to maximize transport to the brain (Craft et al., 2012). Another device is Opt-Powder by Optinose® is a bi-directional delivery device which uses the patient's own exhalation force to emit the dose from the device (Djupesland and Skretting, 2012). Dr Djupesland studied that access to the olfactory region from behind (from the pharynx) and by using a bidirectional flow, the patient blows himself into one nostril, and since he blows himself he closes the soft palate and the airflow will turn around and flow back to the anterior of the other nostril. This will allow access to the olfactory region. Impel Neuropharma has developed a device to delivery either powder or liquids through an insufflation method similarly to that used by Optinose®, however, instead of using the patient's own exhalation force the device uses pressurized gas to emit the dose (Warnken et al., 2016). SipNose has developed a drinking actuated nasal device which similarly, enables delivery of small particle aerosols without deposition in the lower airways (Shahaf and Hadash, 2016).

For testing the delivery of nasally administered drugs different imaging techniques are used. Gamma camera image information (logarithmic “hot iron” intensity scale) from the nasal cavity can be superimposed on the corresponding sagittal MRI section. The images should be taken from the same subject and present deposition 2 min after delivery using *e.g.* (a) a traditional liquid spray, (b) the breath-powered Bi-Directional™ powder device, and (c) the breath-powered Bi-Directional™ liquid spray device (Djupesland, 2013; Djupesland and Skretting, 2012).

5.3. Transporter interactions

CNS diseases remain difficult to treat due to poor brain penetration of therapeutic agents. The primary limitation of uptake of these agents is the blood-brain barrier (BBB). The BBB consists of polarized endothelial cells connected by tight junctions that limit paracellular permeability (Graff and Pollack, 2004). Various methods have been evaluated to improve BBB penetration and thereby increase brain uptake. These methods have included increasing substrate lipophilicity (to increase passive permeability), increasing carrier mediated transport across the BBB by conjugation with a substrate of an endogenous uptake transporter (Polt et al., 1994), and decreasing efflux through transport inhibition or chemical modification of the substrate. In addition, nasal delivery has been explored as a means to deliver substrates to the CNS (Graff and Pollack, 2005). Previous work has shown that efflux transporters attenuate brain uptake of substrates after nasal administration, and that this attenuation can be overcome by nasal administration of appropriate transporter inhibitors (Graff et al., 2005; Graff and Pollack, 2003). These observations seem to suggest that uptake and efflux transport systems are operative at this site.

Targeted nano-drug delivery systems conjugated with specific ligands to target selective cell-surface receptors or transporters could enhance the efficacy of drug delivery and therapy. Transporters are expressed differentially on the cell-surface of different cell types, and also specific transporters are expressed at higher than normal levels in selective cell types under pathological conditions (Kou et al., 2018). Targeted nano-drug delivery systems conjugated with specific ligands could enhance the efficacy of drug delivery. Most transporters have a site-specific expression, which provide ideal targets for drug delivery to increase uptake at specific site or enhance permeation across biological barriers such as the blood–brain barrier. Transporters usually have broad substrate selectivity whereas the ligands for the receptors are much more specific. These differences could actually offer certain advantages in selecting cell-surface transporters for nano-drug delivery systems as it provides multiple choices in terms of ligands for modification of the surface of the nanoparticles to target the transporters (Kou et al., 2017). Lack of immunogenicity of such ligands is also an advantage.

Hada et al. (2017) demonstrated that imatinib, a P-glycoprotein (P-gp) substrate is rapidly transported into the brain *via* the olfactory region after nasal administration. If imatinib is given IV then it rapidly penetrates and removed from the brain. Following intravenous administration, the brain/plasma ratio for imatinib was calculated to be 2% and remained at this ratio for 30 min. The brain/plasma ratio following intranasal administration, however, was found to be 5.3% and remained at this ratio for up to 90 min. If imatinib was administered intranasally together with either pantoprazole or elacridar, compounds known to be P-gp and breast cancer resistance protein (BCRP) substrates, the amount of imatinib was significantly increased in the brain, (especially following concurrent administration of elacridar), showing that it is possible to increase both the amount of imatinib inside the brain as well as the duration staying of the drug inside the brain, preventing it from being removed by the P-gp, following intranasal administration. The increased brain concentration of imatinib (0.33 μg/g tissue) achieved by intranasal administration, compared with an IV injection, is likely to provide a model for developing a wide range of CNS active molecules that were previously removed from consideration as drug candidates due to their lack of CNS access.

6. Advantages and limitations of intranasal drug administration

BBB is the delicate network of blood vessels having tightly packed endothelial cells which separates the brain from circulatory system. It protects brain from entry of unwanted or harmful substances such as various chemicals and toxins. Hydrophilic substances, charged molecules, proteins and peptides are unable to cross this barrier, whereas lipophilic drugs such as antidepressants, anxiolytics and many hormones can more easily cross the endothelial cells (Bates, 2014; Khan et al., 2017). Patients suffering from neurological disorders required chronic dosing, leading to side effects in non- targeted organs. It is considered that majority of drugs which are useful to treat the neurological disorders have lost their potential due to the BBB, resulting in limited treatment options for the patients suffering from neurodegenerative diseases and brain cancer (Pardridge, 2005). The non-invasive transport of drugs to brain is highly needed for neurological disorders and brain tumors requiring chronic therapy. Olfactory and trigeminal pathways are reliable alternative to achieve desire therapeutic effects at

Table 5
Advantages and limitations of intranasal drug administration. Modified from Lochhead and Thorne (2012).

Advantages	Limitations
Non-invasive	Limited for potent drugs
Low risks of infections	Small volumes (25–200 μL in humans)
Easy self-administration	Active mucociliary clearance
Relatively large absorption area (160 cm^2 in humans; 13.4 cm^2 in rats)	Short retention time
Large olfactory epithelium area (especially in rodents) (12.5 cm^2 in humans; 6.75 cm^2 in rats)	Enzymatic degradation by nasal cytochrome P450/peptidases/proteases (pseudo first pass effect)
Rapid absorption	Low permeability for hydrophilic drugs
Nasal submucosa is abundant in vascular and limphatic vessels	Absorption enhancers needed
No hepatic first pass metabolism of the drugs	Low nasal epithelial pH
Direct drug delivery to the brain bypassing the blood-brain barrier	Interindividual variability
	Low CNS delivery for proteins
	Nasal secretion has an influence on the absorption

lower doses for treating chronic diseases while minimizing the side effects. Transmucosal delivery of drugs through olfactory or trigeminal pathways to brain bypassing the BBB is referred as the direct IN drug transportation to brain. This is the only route through which brain is in connection with the outside environment (Mistry et al., 2009).

Besides the advantages, the limitations of nose-to-brain delivery have also been identified, and include a relatively small volume for administration of the drugs, limited surface area of the olfactory epithelium, short retention time for drug absorption and influence a nasal secretion on drug delivery (Wu et al., 2008).

The main points of advantages and shortcomings of intranasal drug delivery are summarized in Table 5.

7. Discussion and conclusion

There is a long list of drugs that have been developed for various CNS disorders, that have been discarded because they did not pass through the blood-brain barrier. Majority of research and development projects have, therefore, been focusing on the development of new molecules that are able to pass through BBB. Little focus has been on the delivery systems and searching for routes to bypass the BBB. Recently another factor has made this field, “drug delivery to the brain” more complex, is the discovery of efflux mechanisms in the brain and other membranes. Studies have shown that there are many molecules that may have access into the brain, such as imatinib, but are removed from the brain almost immediately. This leaves us with the question: How do we bypass the blood-brain barrier and keep the drug inside brain? In this review the focus is on one delivery route, that has been shown to bypass the BBB and allows a direct access to the brain. The so-called nose-to-brain pathway. It requires that the molecules get absorbed by the nerves inside the nasal cavity, either the olfactory nerves or even the trigeminal nerve.

There is a growing demand for new and more potent CNS drugs and with increasing knowledge in the pathways and functions inside the brain, the need for new therapeutics increases as well. One field where there has been an increased demand is within neurodegenerative disorders. Here, the pathogenesis of these diseases are not enough clarified, but also that the drug molecules are not able to reach the target tissue in the brain at an appropriate concentration level. By using intranasal administration a proper drug concentration in the brain parenchyma may be reached. This show the importance of finding routes that are able to bypass the blood-brain barrier. Numerous research groups have been looking at the absorption of the drugs from the nasal cavity and how they are transported *via* intercellular (extracellular) or transcellular by endocytosis into the olfactory sensory neurons. After the neuronal uptake, the molecules move away along the axons to the synapse where they are exocytosed onto the olfactory bulb (Fig. 3) and transported further into the brain *via* various neuronal pathways.

There is also another possibility, since the olfactory mucosa is the only site where the central nervous system is in a direct contact with a mucosal surface. Here, a compound may be absorbed to the lamina propria trough the paracellular space of the olfactory mucosa. From the lamina propria, is transported through the perineural space to the subarachnoid space from where it can reach directly the brain tissue (Fig. 2).

Besides the direct nose-to-brain pathways, there are other routes for the drugs to penetrate the brain. Namely from the respiratory route they can be transported partially to the circulation and reach the brain by the “nose-to-blood-to-brain” pathway.

Today, one of the factors that need to be respected is the role of drug transporters or efflux transporters as described in Chapter 5.3. New drugs must be studied with respect to their ability to be removed by these efflux transporters or if it possible to use specific inhibitors that may improve the therapeutic efficacy and pharmacokinetic profile inside the brain of CNS targeted drugs following intranasal or intraolfactory administration. There are also possibilities to use pharmaceutical and technological approaches to improve the CNS access of the therapeutics, such asformulation factors, additives, nanoformulations, co-administration with vasoconstrictors, lipophilicity and permeability enhancement techniques *etc*. (see Chapter 5.1).

Taken together, drug research and development in the field of intranasal or intraolfactory administration to the brain is a rapidly growing area. Several potential drugs have been described that require nose-to-brain transport since they do not pass the blood-brain barrier such as oxytocin, IGF-1, insulin, glutathione, and many more. In order to reach the CNS, all these compounds will benefit from intranasal application, compared with the classical delivery routes. As our understanding of the brain, the pathways inside the brain and the pathophysiology of CNS diseases increases, there will be more demands for new therapeutic targets in the brain, where more specific delivery systems will be required. The olfactory route, trigeminal route (and vomeronasal route) may provide direct access to certain regions of the brain, that will otherwise not be reachable. There is still need for optimization of this route(s) as well as full understanding of dosing and safety following nasal drug administration focusing on reaching the neurons for a direct CNS targeted therapy.

Author contribution statement

FE (anatomy, cellular structure, penetration enhancement, critical evaluation) LB (CNS drug for intranasal delivery, figures, references), DF (anatomy, cellular structure, figures), ÁB (methods for studying intranasal drug delivery), SG (critical reading, discussion, evaluation, comments).

Declaration of interest

None.

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