Intranasal insulin – effects on the sense of smell*

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Abstract
Intranasal insulin (IN) administration is a promising way to deliver the peptide to the central nervous system (CNS), bypassing the blood-brain-barrier and gastrointestinal absorption inhibition. IN receptors are localized in the olfactory mucosa and the brain, mainly in the olfactory bulb, hypothalamus, hippocampus, amygdala, cerebral cortex, and cerebellum. The pleiotropic mechanism of insulin action is characterized by its anti-inflammatory properties, antithrombotic, vasodilatory, and antiapoptotic effects. It prevents energy failure and has regenerative properties, affects neuro-regeneration and counteracts insulin resistance. Hence, insulin has been suggested for various pathological states including neurocognitive disorders, obesity, and as a therapeutic option for smell loss.
A sharply increased prevalence of olfactory dysfunction was observed due to the COVID-19 pandemic. The pandemic also emphasized the lack of therapeutic options for smell loss. Intranasal insulin administration has therefore been suggested to serve as potential treatment, influencing the regenerative capacities of the olfactory mucosa.
This narrative review summarizes current knowledge on possible effects of intranasal insulin on the sense of smell.

Key words: COVID-19, insulin, intranasal administration, obesity, olfaction

Introduction
The sense of smell warns us of potential environmental dangers and it is important for social communication. It is significant for the choice of foods, influences the nutritional status, and energy homeostasis. Without a sense of smell, foods and drinks have no flavour [1,2].

The sense of smell decreases gradually with aging, and is compromised in various diseases, e.g., diabetes mellitus (DM), Alzheimer’s (AD) and Parkinson’s Disease [3-5]. A sudden loss of the sense of smell is seen, for example, after head trauma or after viral infections of the upper respiratory tract. In SARS-CoV-2 infection smell impairment occurs so often, that smell tests are used to screen for SARS-CoV-2 infections [6]. The overall prevalence of a decreased olfactory function (resulting from infections, head traumas, sinonasal diseases, etc.) in the general population is approximately 20% [7,8].

Intranasal insulin (IN) receptors in the olfactory mucosa (OM) are present in the olfactory sensory neurons (OSN) [9]. Hence, IN has been listed among the potential approaches for treatment of smell impairment.

IN was discovered 1921 by Best and Banting [10]. Since that time the hormone was of great interest to scientists mainly because of its use in diabetes therapy. As new research emerges, IN is in the spotlight again because of its actions in the central nervous system affecting cognitive functions, influencing neuroprotection and also having an impact on the sense of smell [11]. IN receptors are mainly localized in the mitral cells of the olfactory bulb and various parts of the brain [3,12-14]. They are also widely distributed in capillaries and small vessel walls influencing the regional perfusion [15]. More recent research shows that IN acts not only in neurons but also astrocytes and other types of cells in the brain such as microglia, oligodendrocytes and tanyocytes [16]. Figure 1 shows selected areas of the central nervous system (CNS) where insulin receptors (IR) are found. Importantly, these areas are also significant for processing olfactory information.

First studies regarding IN focused on its action in peripheral tissues (mainly muscle, liver, and adipose tissue) [16]. More recent studies focus on its central action stimulating smell function, ac-
Intranasal insulin and the sense of smell

Intranasal insulin and the sense of smell, even providing neuroprotection in acute ischemic stroke (15,17,18). The subcutaneous (SC) way of IN administration is the traditional and most often used one. Nowadays new approaches to deliver IN are being made due to the various disadvantages of SC use and also new scientific data providing evidence that IN might be a therapeutic option in various pathologic conditions, for example, when administered directly to the CNS (19,20).

One of the more recent methods of IN delivery is the inhalation route. In 2006 the first product for inhalation was approved by the Food and Drug Administration, but later withdrawn from the market by the manufacturer due to low commercial interest. Still, there are other routes of IN administration taken into consideration in clinical trials e.g. oral, oral colon delivery, buccal, intra-peritoneal, transdermal, and nasal administration (19).

Intranasal delivery
The intranasal route of insulin administration was first proposed by Frey, who started a series of proof of concept studies in animals, showing that intranasal IN administration allows the peptide to enter the brain without peripheral absorption (11). Studies in humans confirmed that after intranasal administration, IN enters the brain without causing elevation of its peripheral concentration (11,21). The intranasal surface area for IN delivery is about 180 cm². The peptide is physiologically secreted into the nasal mucus membrane, where IN-like growth factor (IGF) and IGF receptor 3 are present (22). Table 1 shows factors influencing intranasally administered IN absorption and possible consequences resulting from changes in the physiological status (12,22–24).

The main advantages of intranasal IN delivery relate to the bypassing of gastrointestinal absorption inhibition and the blood-brain barrier (BBB). This enables blood glucose regulation and activation of IN feedback mechanisms directly in the central nervous system (22,25,26).

In his review, Benedict et al. showed that intranasal IN delivery can be a promising alternative for the intravenous route of administration, leading to better patient outcomes and avoidance of peripheral side effects connected with that kind of drug administration, especially hypoglycaemia resulting from high doses which would be needed to achieve proper IN concentrations in the CNS (27).

Although the intranasal application route is thought to be a safe way of IN administration, various authors state that progress has to be made to overcome adverse drug reactions related to this administration type, such as nasal irritation (nasal burning, pain, epistaxis) or respiratory symptoms (cough, sinus pain/irritation, coryza) (11,28,29). A new approach to overcome this problem is the use of fast dissolving films enabling accurate dosing, rapid IN release, and better application properties (30).

Some studies showed that immediate intranasal administration of IN enhances blood pressure. This effect vanishes with prolonged intranasal administration, which allows the use of intranasal IN in longer (8 weeks) therapies without a significant increase of blood pressure (31). Importantly, the elevation of blood pressure as a result of intranasal administration of IN does not exceed normal ranges (32).

Intranasal IN – potential therapeutic use
Several reviews indicate that intranasal IN may be beneficial to patients undergoing acute ischemic stroke (15,33). In this context, intranasal IN has specific neuroprotective qualities including a pleiotropic mechanism of action and a rapid administration route enabling selective cerebral delivery (33).
In 2021 a narrative review highlighted the potentially beneficial role of intranasal IN administration for prevention of delayed neurocognitive recovery and postoperative neurocognitive disorder often reported after hospitalization and connected with anaesthesia, predominantly in cardiac surgery (51).

Brunner et al. found that intranasal IN administration improves the delayed odour-cued reactivation of spatial memory in young men. During the study participants were exposed to eight food and non-food odors. The effect was independent of odour type, and the intranasal IN administration caused no adverse reactions. The authors hypothesized that the use of odours may help maximize the memory-enhancing properties of intranasal IN in cognitively impaired humans (53).

In 2004 Hallenschmid et al. demonstrated that 8-weeks-long intranasal IN administration (4 x 40 IU/day) to healthy volunteers leads to weight loss and adiposity reduction in men but not in women, suggesting a sex dependency of IN catabolic effect. The investigation gave a promising approach to the therapeutic use of intranasal IN in obesity treatment (51). Later Hallenschmid et al. demonstrated in a study with 30 healthy women that postprandially administered intranasal IN enhances the satiating effect of meals and reduces palatable snack intake. Taking into consideration that women are less susceptible to the anorexic effect of IN, the authors hypothesized that the effect could be similar in obese patients, representing general brain IN resistance (54).

In line with these conclusions, a study published in 2020 examined sensitivity to a food and a non-food odorant in the hungry and sated state in 75 young healthy (26 normal weight, 25 overweight, and 24 obese) participants. A negative indirect effect of BMI on odour sensitivity for chocolate mediated by IN was observed (50). Corroborating Hallschmid’s hypothesis (54), Rodriguez-Raecke et al. provided evidence that in healthy male subjects gustatory sensitivity was boosted by single-dose intranasal IN (40 IU) application. This may also impact food intake, as the detection of the sweet taste

### Table 1. Factors influencing absorption and bioavailability of intranasally administered insulin.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Change in physiological status</th>
<th>Consequence</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow</td>
<td>Vasodilatation</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Mucus</td>
<td>↑ Viscosity</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>pH between 5-6,5</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>↑ mucus production</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>↑ ciliary beat frequency</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Mucociliary membrane</td>
<td>↓ permeability</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Enzymes (cytochrome P-450 enzymes, proteases, peptidases)</td>
<td>Possible drug degradation</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Macrophages and other immune competent cells</td>
<td>↑ presence</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Microbes</td>
<td>↑ presence</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Xenobiotics</td>
<td>↑ presence</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

The pleiotropic mechanism of IN action characterized by its anti-inflammatory properties, antithrombotic and vasodilatory effects, antiapoptotic effect, prevention of energy failure and regenerative properties may be used not only in stroke treatment, but also in neurocognitive impairment therapies, obesity treatment and in acute smell loss management. Molecular mechanisms behind the listed effects are presented in Table 2 (15,34-45).

High density of IN receptors can be found in the hippocampus which is mainly involved in memory organization and cognition (12). Positron emission tomography images provided evidence that elevation of IN concentration in the CNS leads to memory improvement in various pathological conditions such as Alzheimer’s disease (17,47,48), Parkinson’s disease (4), Down Syndrome (32), and cognition impairment linked to obesity and DM type 2 (49,50).

In 2021 a narrative review highlighted the potentially beneficial role of intranasal IN administration for prevention of delayed neurocognitive recovery and postoperative neurocognitive disorder often reported after hospitalization and connected with anaesthesia, predominantly in cardiac surgery (51).

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### Table 2. Molecular mechanisms behind pleiotropic effects of intranasally administered insulin.

<table>
<thead>
<tr>
<th>Anti-inflammatory properties</th>
<th>Antithrombotic and vasodilatory effects</th>
<th>Antiapoptotic effect</th>
<th>Prevention of energy failure</th>
<th>Regenerative Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9</td>
<td>LTF</td>
<td>TP1-3K/Akt signalling pathway</td>
<td>Cerebral glucose metabolism ↑ neuronal norepinephrine uptake</td>
<td>↑ neurite outgrowth</td>
</tr>
<tr>
<td>VEGF</td>
<td>LPAI-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF</td>
<td>LRos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-450 enzymes, proteases, peptidases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages and other immune competent cells</td>
<td>↑ presence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbes</td>
<td>↑ presence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xenobiotics</td>
<td>↑ presence</td>
<td></td>
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</tbody>
</table>

MMP-9: matrix metalloproteinase 9; VEGF: vascular endothelial growth factor; TF: tissue factor; PAI-1: plasminogen activator inhibitor-1; ROS: reactive oxygen species; eNOS: endothelial nitric oxide synthase; PI-3K/Akt: phosphoinositide 3-kinases; GSK-3β: glycogen synthase kinase 3 beta.
was mostly enhanced and the bitter taste less enhanced. The aforementioned studies suggest that intranasal IN application can be a potential therapeutic option in obesity treatment counteracting IN resistance and contributing to the restoration of chemosensory perception in overweight patients. Another potential target for intranasal IN use is the treatment of acute smell loss, where the smell-restoring effect may result from enhanced regeneration and maturation of OSN after direct IN application to the olfactory epithelium.

### Intranasal IN and normosmia

In 2011 Ketterer et al. aimed to determine whether IN is involved in the regulation of olfactory function. Odour thresholds were measured in 14 healthy subjects eight of which underwent a hyperinsulinenic–euglycemic clamp. In the intervention group, a decrease in odour threshold was observed during euglycemic hyperinsulinenia. In the control group, no such effect was observed after 2h of fasting, indicating that IN regulates the satiation process by reduction of smelling capacity, which is a regulator of food intake. However, the methodology of this study was invasive and therefore may have led to some biases caused by elevated cortisol levels and altered brain function. Since then, several studies were performed to determine the influence of IN on the sense of smell and the possibilities of intranasal IN application, at the same time addressing the caveats of the first study on the effects of intranasal IN administration on olfactory performance.

In a double-blind, placebo-controlled, balanced within-subject study with 17 normal-weight normosmic participants (7 women), Brünner et al. demonstrated that after a single dose of intranasally administered IN (40 IU) the sensitivity for n-butanol decreased significantly, whereas olfactory discrimination ability did not change. After intranasal IN administration neither serum IN nor serum cortisol concentrations were altered. However, a small but significant drop in plasma glucose levels was observed, but it was not related to the effects of intranasal IN on olfactory sensitivity.

While these studies in healthy subjects suggested that intranasal IN decreases olfactory sensitivity, results concerning olfactory sensitivity in healthy volunteers according to the gender specificity (similarly to those observed for gustatory sensitivity) are ambiguous. Rodriguez-Raecke et al. demonstrated in a double-blind, placebo-controlled, balanced within-subject study with 30 normal-weight normosmic participants (14 females), that the olfactory sensitivity for n-butanol was lower after intranasal IN administration in women but not in men, as compared to the placebo group. The study was also the first one extending the measures of olfactory sensitivity by utilizing food-related peanut odour, that revealed no significant effects of IN administration.

### Intranasal IN and olfactory disorders

In 2015 Schöpf et al. performed a study among patients with olfactory deficits. Ten anosmic patients received 40 IU of intranasal IN. The study generated different observations than previous ones, performed among healthy volunteers. Two patients presented an immediate increase in odour sensitivity. Odour intensity ratings also increased in a significant manner. Patients with high body mass index (BMI) scores better identified odours immediately after intranasal IN application. Although the observations were the first of their kind, and provided a positive input for further investigations, the study had limitations in terms of the small sample size, lack of randomization and relatively narrow BMI range.

In 2018 Rezaeian performed a double-blind randomized controlled clinical trial among 38 hyposmic patients aiming to evaluate the effect of intranasal IN (40 IU) administration on olfactory recovery. After 4 months follow up there was a significant improvement in the n-butanol threshold test in the intervention group, compared to the placebo group. No adverse drug reactions of intranasal IN were observed during the study. The authors therefore suggested intranasal IN administration for the therapeutic management of olfactory dysfunctions. The study also suffered from a relatively small sample size and the lack of reference to BMI values. The latter being an important omission as obesity has been shown to reduce the sensitivity to odours via IN resistance.

Thanarajah et al. performed an investigation to determine the role of peripheral IN sensitivity, reactive blood IN changes and intranasal IN application on olfactory performance in 36 male normal weight and overweight participants. Three different IN doses (40 IU, 100 IU, 160 IU) or corresponding placebo volumes were administered during the test period to each participant. Olfactory threshold and odour discrimination tests were performed and correlated with the homeostasis model assessment of IN resistance. The investigation revealed enhanced odour perception with a dose-dependent improvement of olfactory thresholds after intranasal IN administration, giving more evidence on favourable effects of intranasal IN use in the treatment of olfactory dysfunction. Blood IN and intranasal IN intervention dose influenced olfactory threshold. Higher systemic IN levels correlated negatively with olfactory performance, while higher intranasal IN doses correlated positively with odour sensitivity. The study also showed that it is important to control the fasting state, blood IN levels and peripheral IN sensitivity during intranasal IN testing. Notably, those variables except for blood IN levels were not taken into consideration in previously mentioned studies.

Figure 2 summarizes differences in intranasally administered IN action between healthy individuals and patients with olfactory loss.
**Issues related to studies on insulin effects in olfactory dysfunction**

The relatively short intervention time and lack of long-term observations of intranasal IN administration consequences on smell deterioration are among factors limiting the conclusions based on the existing empirical evidence. An additional limitation relates to the relatively short follow-up period which in most studies did not take longer than a year, limiting our knowledge on possible long-term and dose related adverse drug reactions after intranasal IN administration. Most studies were performed using between 20 and 160 IU of insulin during a relatively short period of time (11).

Keeping in mind antiapoptotic effects of insulin action the assumption of potential cancer inducing effects after long-term therapies, may be raised, as the cancer inducing effect of chronic hyperinsulinemia was shown for patients presenting with insulin resistance (11). To our knowledge no studies on the potential cancer inducing effect of intranasally administered IN were performed to date. In general intranasal insulin administration was found to be safe and well tolerated. After intranasal administration, the peptide was found to inhibit the mitochondrial apoptotic pathway, mainly in mature OSN (62). Therefore it seems not to be very likely that olfactory dysfunction treatment with intranasally administered insulin may induce cancer, although studies with long-term observations are needed to confirm this assumption.

A novel approach to the use of intranasal IN could potentially be related to hyposmia and anosmia caused by the COVID-19 virus. The prevalence of olfactory dysfunction during COVID-19 infection with the delta variant is approximately 40%, varying between different populations and virus strains (63). Although most patients recover from smell loss within 1-3 weeks, some remain hyposmic or anosmic for months or years (64). It is hypothesized that in those patients larger areas of sensory epithelium are affected, possibly with a larger number of lost OSN (6,65,66). This group of patients would potentially benefit the most from intranasal IN delivery because IN receptors are also located in sustentacular cells. As stated before IN promotes OM regeneration through the enhancement of newly generated OSN maturation and potentiation of their electrical activity (9,56). It is not certain whether globose basal cells express insulin receptors. A study performed by Lacroix et al. confirmed IR in horizontal cells but failed to identify them in globose basal cells due to an aspecific signal during Western blot analysis (57). According to Leung at al. and Schwob at al. horizontal basal cells are mainly responsible for the regeneration of the olfactory epithelium after severe injury (67,68). In line with this finding stands a study performed by Kikuta et al., demonstrating that higher concentrations of insulin in nasal mucus correlated with preservation of larger amounts of OSN, and indicating a potential protective role of the peptide against olfactory epithelial damage (69). On the other hand it has to be kept in mind that globose basal cells also play an important role in the regeneration of olfactory epithelium. Further studies are needed to determine whether IR are present in those cells, because only the activation of horizontal and globose basal cells will allow a complete regeneration of strongly damaged olfactory epithelium.

A recent study aimed to formulate fast dissolving intranasal IN films for the management of COVID-19 associated anosmia. The investigation contained two phases. In the first step films with different composition of hydroxypropyl methyl cellulose and poly vinyl alcohol were prepared and investigated for in vitro characterizations. In the second step a clinical evaluation was performed in 49 participants with post-COVID olfactory loss. The...
study was conducted in a single-blinded randomized parallel design. The intervention group received 100 IU IN in the form of a fast-dissolving film twice weekly for four weeks. The placebo group received IN-free films. Thirty minutes after the intervention a significant improvement in olfactory sensitivity and odour discrimination scores in the intervention group was detected (59). These results suggest that intranasal IN administration is a promising approach for the treatment of COVID-19-related olfactory loss.

As previously explained insulin may be extracellularly transferred by OSN to the olfactory bulb, which presents high amounts of insulin receptors (69). Taking into consideration that lower sensory input may lead to atrophy of the OB, intranasal insulin also presents a promising approach to treat the shrunken olfactory bulb in a dual mechanism. First, it protects the olfactory epithelium from injury related OSN loss, what may further preserve a sufficient sensory input. In the second mechanism intranasally administered insulin might support the formation of stable neural circuits by inhibition of OB neuron apoptosis (69,70). Studies on this topic are needed. Another field for further investigations are the potential changes of insulin transport to the brain after intranasal application in patients with olfactory dysfunction. Insulin is mainly transported by the extra-neuronal pathway and, as shown in rats, possibly also through the trigeminal nerves (11,71–73). There are problems with studies execution and results interpretation for example due to inaccurate dosing (13,70).

Authorship contribution
All authors contributed to the text of this review, reviewed and approved the final text.

Conflict of interest
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