Direct Transport of Cocaine from the Nasal Cavity to the Brain Following Intranasal Cocaine Administration in Rats

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Abstract □ Individuals who consume cocaine illegally have long since adopted or explored the nasal route of administration. This study was designed to determine in an animal model whether nasally applied cocaine could be transported directly from the nasal cavity to the central nervous system. Male Sprague–Dawley rats were used in the study. The nasal cavity was isolated to prevent drainage of nasally applied dosing solution to nonnasal regions. Cocaine was then administered, either by intranasal (in) administration or by intravenous (iv) injection. At different times post dose, blood and tissues from different regions of the brain were collected. Cocaine concentrations in plasma and tissue samples were analyzed by HPLC. After iv administration, similar cocaine contents in different brain regions were observed. Following in administration, cocaine content in samples collected within 60 min post dose were found to differ considerably in different brain regions. The highest content was observed in the olfactory bulb, followed by the olfactory tract and then the remaining part of the brain. To allow comparison of brain cocaine content after iv and in administration, brain cocaine contents were normalized by plasma cocaine concentrations. The ratios of the area under the cocaine concentration–time curve (AUC) between the olfactory bulb and plasma at early times following in administration were significantly higher than those obtained after the iv dose (13.4 ± 5.56 vs 6.16 ± 0.94, $p < 0.05$, for AUC ratio up to 2 min post dose; 9.39 ± 1.47 vs 7.34 ± 0.59, $p < 0.05$, for AUC ratio up to 4 min post dose). At 1 min post dose, the olfactory bulb-to-plasma cocaine concentration ratios following in administration was three times those obtained after iv administration. After 1 min, the olfactory bulb-to-plasma concentration ratios following in administration were found to be similar to or smaller than those obtained after iv administration. The tissue-to-plasma concentration ratios in other brain regions following in administration were found to be smaller than those obtained following iv dosing. We conclude that nasally administered cocaine was transported directly from the nasal cavity to the brain but that only a very small fraction of the dose was transported via the direct pathway.

Introduction

Over the past decade, the possibility that intranasal drug administration might be useful has received a great deal of attention. Some relevant research efforts have been reviewed comprehensively.[1] The extensive network of blood capillaries under the nasal mucosa seems to facilitate effective systemic absorption and, because venous blood drains directly into the systemic circulation, this route thereby avoids first-pass metabolism of the drug in the gastrointestinal tract and the liver. For a number of drugs, systemic drug exposure following intranasal administration is comparable to that obtained from intravenous or intramuscular injections.[2−4]

Interestingly, individuals who consume cocaine illicitly have either long ago adopted or have explored this route of administration. It often has been noted that a rise in the physiological and behavior effects of cocaine precedes a rise in the plasma cocaine concentration following intranasal "snorting".[5−7] A clockwise hysteresis loop was consistently observed when behavior or physiological responses were plotted against plasma cocaine concentrations. This indicates that the same plasma cocaine concentration corresponds to a higher response at an earlier time after dosing than that at a later time. Development of acute tolerance can be used to explain such a concentration−response relationship. However, there is no evidence that tolerance would develop rapidly following administration of a single dose of cocaine. To exert CNS activity following nasal drug application, it is typically believed that the drug needs to be absorbed into the systemic circulation via the capillary network under the nasal mucosa and subsequently distributed into target regions in the brain. If this is the only pathway of CNS entry, there will be a time delay in the distribution of cocaine to the brain tissues following nasal administration. A rise in the pharmacological response should therefore occur after a rise in the plasma cocaine concentration. Nevertheless, this outcome is not in agreement with the concentration−response relationship observed following intranasal cocaine snorting.

Evidence exists that substances applied nasally may enter the brain directly from the nasal cavity via the olfactory system.[8−10] If nasally applied cocaine can distribute into the brain directly via this alternative CNS entry pathway, an increase in the brain cocaine concentration could occur prior to a rise in the plasma drug levels following nasal application. This hypothesis would be consistent with the clockwise hysteresis loop observed in the plasma concentration−response relationship following nasal cocaine snorting.

In this study, we determined plasma and brain cocaine concentration−time profiles following intravenous and intranasal administration of cocaine in rats in an effort to determine whether cocaine could be transported directly from the nose to the brain following intranasal application.

Materials and Methods

Chemicals and Reagents—Cocaine and tropacocaine were provided by the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse. All other reagents were of HPLC grade or of the highest grade commercially available.

Animal Experimentation—Male Sprague−Dawley rats (250−350 g) were anesthetized with an ip injection of pentobarbital (50 mg/kg) and kept anesthetized throughout the experiment. The anesthetized animals were placed on a warm pad to maintain

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normal body temperature. The nasal cavity was isolated from the respiratory and gastrointestinal tracts using a procedure described by Hussain et al.[11] Briefly, an incision was made in the neck, the trachea was severed, and the upper tracheal segment was tied off with a suture. The lower trachea was cannulated with polyethylene tubing (PE 260; o.d.: 2.8 mm, i.d.: 1.77 mm) to provide a patent airway. Another piece of PE 260 tubing with the ends sealed was inserted from the esophagus to the posterior part of the nasal cavity. The nasopalatine opening was closed with cyanoacrylate glue. This surgical procedure was performed to prevent drainage of nasally applied dosing solution to nonnasal regions.

Following nasal cavity isolation, animals received a cocaine dose over 30 s, either by intranasal administration or by intravenous injection. For intranasal administration, 50 μL of cocaine solution (16.7 mg/kg) was injected via a silastic tubing (o.d.: 1.19 mm, i.d.: 0.64 mm) inserted 2 cm into one of the nares toward the roof of the nasal cavity. For intravenous administration, cocaine dosing solution (5 mg/kg) was injected via an indwelling jugular vein cannula. At 1, 2, 4, 8, 15, 60, and 120 min following the end of the 30-s dosing period, a terminal blood sample was collected from the inferior vena cava. Plasma was separated and immediately stored at −70 °C in tubes containing NaF. The blood collection was generally completed within 15 s. Following the completion of blood collection, the skull of the animals was opened and the brain tissues collected in the following order: the whole brain with the exception of the olfactory tract and olfactory bulb (RB), olfactory tract (OT), and olfactory bulb (OB). The collection of all brain tissues was typically completed within 1 min following the blood collection. The tissue samples were weighed and immediately homogenized in 200 mM NaF solution. An aliquot of the homogenate was stored at −70 °C before analysis. The addition of NaF protected cocaine from undergoing hydrolysis during processing and storage. Four or five rats were used at each collection time. The sample collection process always involved two laboratory personnel: one responsible for dosing and sample collection and the other for tracking the time and sample handling.

Analytical Methods—Plasma and brain cocaine concentrations were determined within 48 h of collection by a published HPLC procedure.[12] Briefly, the plasma (200 μL) and brain homogenates (100−200 μL) were mixed with 50 μL of the internal standard (tropacocaine, 1 μg/mL) and 200 μL of 0.5 M carbonate buffer (pH 9.1). The mixture was extracted with 2 mL of ethyl acetate, and the organic phase was back-extracted with 150 μL of 0.05 M HCl. The final acidic aqueous phase was evaporated with a centrifugal evaporator (Savant, Farmingdale, NY) at room temperature. The residue was reconstituted with the mobile phase (see below) and then injected onto a C_8 column (Nova-Pak, 15 cm × 3.9 mm i.d., 4 μm; Waters, Milford, MA). A mobile phase composed of a mixture of 100 mM pentanesulfonic acid and 50 mM monobasic potassium phosphate (pH 6.0) and acetonitrile in an aqueous-to-organic ratio of 76:24 at a flow rate of 1.3 mL/min was used. The UV absorbance of the effluent was monitored (Waters 486 UV detector) at a wavelength of 235 nm.

Data Analysis—The area under the cocaine concentration versus time curve (AUC) was estimated by the trapezoidal rule. The variance for the AUC was estimated by the method of Yuan.[13] The variance (var) for the AUC ratio was approximated using the statistical method described previously,[14]

$$\mathrm{var}\left(\frac{\mathrm{AUC_{in}}}{\mathrm{AUC_{iv}}}\right) \cong \left(\frac{\overline{\mathrm{AUC_{in}}}}{\overline{\mathrm{AUC_{iv}}}}\right)^2 \cdot \left(\frac{\mathrm{var(AUC_{in})}}{(\overline{\mathrm{AUC_{in}}})^2} + \frac{\mathrm{var(AUC_{iv})}}{(\overline{\mathrm{AUC_{iv}}})^2}\right)$$

where $\overline{\mathrm{AUC}}$ denotes the mean AUC value.

A one-way ANOVA followed by a Student−Newman−Keuls test was used to compare the measurements in different brain regions. An unpaired two-sided *t*-test was used to compare the measurements for intravenous and intranasal administration.

Results

Figure 1 illustrates the cocaine concentration versus time profiles in plasma and in different brain regions following intravenous and intranasal administration. Following intravenous administration, plasma and brain cocaine concentrations reached peak levels at 1−2 min after dosing

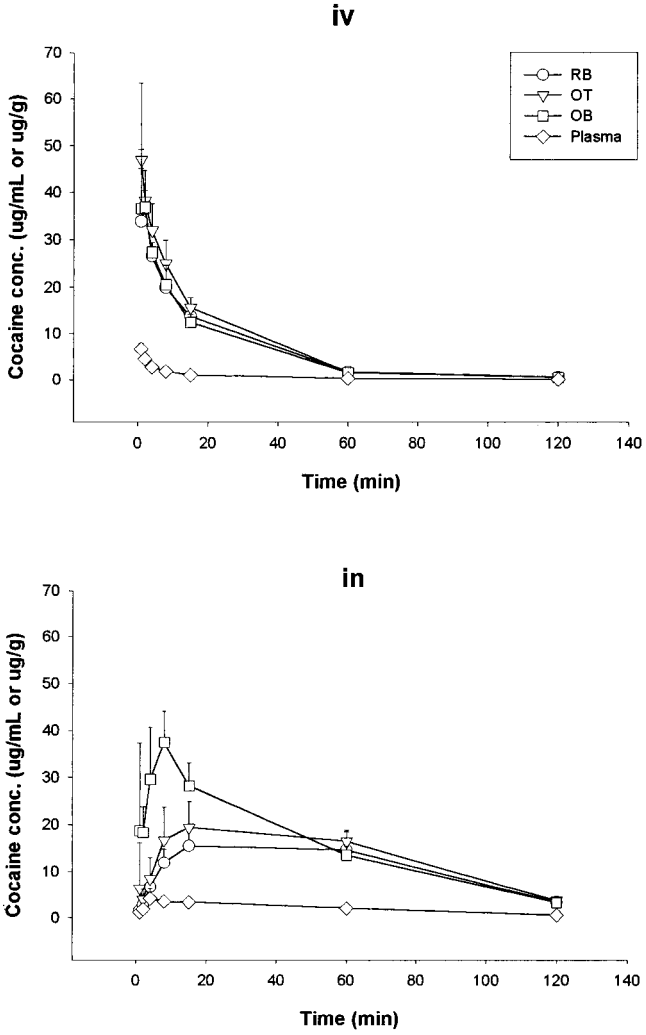

Figure 1—Plasma and brain cocaine concentrations versus time profiles following intravenous (iv) and intranasal (in) administration in rats. Each point represents the average of 4–5 rats and the cross-vertical bars represent one standard deviation of the mean. Key: OT, olfactory tract; OB, olfactory bulb; RB, remaining part of the brain.

and declined exponentially as a function of time. Except for samples collected at 1 min post dose, similar cocaine contents were observed in different brain regions. Following intranasal administration, plasma and brain cocaine concentrations increased gradually to peak levels and started to decline as a function of time. Cocaine contents in samples collected within 60 min post dose were found to differ considerably in different brain regions. The highest content was observed in the olfactory bulb, followed by the olfactory tract and then the remaining part of the brain.

Table 1 summarizes the brain tissue-to-plasma cocaine AUC ratios as a function of time following intravenous and intranasal administration. Following intravenous administration, the tissue-to-plasma AUC ratios in different brain regions increased consistently toward plateau levels as a function of time. Following intranasal dosing, the RB-to-plasma AUC ratios also increased toward plateau levels as a function of time. The OT-to-plasma AUC ratios decreased initially and then started to increase. The OB-to-plasma AUC ratios were high initially and decreased as a function of time. Up to 2 and 4 min following intranasal administration, the cumulative OB-to-plasma AUC ratios were significantly higher than those obtained after the intravenous dose (13.8 ± 6.29 vs 6.06 ± 1.02, $p < 0.05$, for AUC ratio up to 2 min post dose; 9.62 ± 1.77 vs 7.17 ± 0.67, $p < 0.05$, for AUC ratio up to 4 min post dose). At

Table 1—Brain Tissue-to-Plasma Cocaine AUC Ratios as a Function of Time Following Intravenous and Intranasal Administration

	$AUC_{brain\ tissue}/AUC_{plasma}$					
	iv administratio			in administration		
time, min	OB	OT	RB	OB	OT	RB
0–1	5.52 ± 1.09	7.10 ± 1.42	5.11 ± 0.99	16.1 ± 9.30[c,d]	5.52 ± 4.27	1.27 ± 0.73[a]
0–2	6.06 ± 1.02	7.37 ± 1.31	5.73 ± 0.93	13.8 ± 6.29[a,c,d]	4.23 ± 2.91[a]	1.38 ± 0.54[a]
0–4	7.17 ± 0.67	8.28 ± 0.86[b,c]	6.92 ± 0.62	9.62 ± 1.77[a,c,d]	2.67 ± 0.82[a]	1.52 ± 0.24[a]
0–8	8.30 ± 0.50	9.71 ± 0.69[b,c]	8.03 ± 0.47	9.01 ± 1.40[c,d]	3.05 ± 0.54[a]	2.10 ± 0.30[a]
0–15	9.17 ± 0.54	10.91 ± 0.70[b,c]	9.04 ± 0.56	9.27 ± 0.89[c,d]	4.15 ± 0.51[a]	3.04 ± 0.29[a]
0–60	9.94 ± 0.55	12.11 ± 0.69[b,c]	10.42 ± 0.54	8.15 ± 0.56[a,c,d]	5.93 ± 0.48[a]	4.86 ± 0.34[a]
0–120	9.39 ± 0.49	11.41 ± 0.62[b,c]	9.93 ± 0.49	7.61 ± 0.49[a,c,d]	6.50 ± 0.47[a,c]	5.52 ± 0.51[a]

[a] Significantly different from that after intravenous dosing, $p < 0.05$. [b] Significantly different from OB, $p < 0.05$. [c] Significantly different from RB, $p < 0.05$. [d] Significantly different from OT, $p < 0.05$.

later times, the OB-to-plasma cocaine AUC ratios obtained following intranasal administration were similar to or smaller than those obtained after intravenous dosing. The tissue-to-plasma cocaine AUC ratios in other brain regions following intranasal administration were smaller than those obtained after intravenous dosing.

Figure 2 illustrates the brain-to-plasma cocaine concentration ratios following intravenous and intranasal administration as a function of time. There were no significant differences in these ratios among different brain regions following intravenous administration. The tissue-to-plasma ratios of cocaine in OB following intranasal administration were found to be significantly higher than those in other brain regions before 60 min post dose. At 1 min post dose, the tissue-to-plasma ratios in the olfactory bulb following intranasal administration were found to be three times those obtained after intravenous administration (15.0 ± 4.1 vs 5.5 ± 1.1, $p < 0.05$). After 1 min, the olfactory bulb-to-plasma concentration ratios in samples collected following intranasal administration were found to be similar to or smaller than those obtained after intravenous administration. The tissue-to-plasma concentration ratios in other brain regions following intranasal administration were found to be smaller than those obtained following intravenous administration.

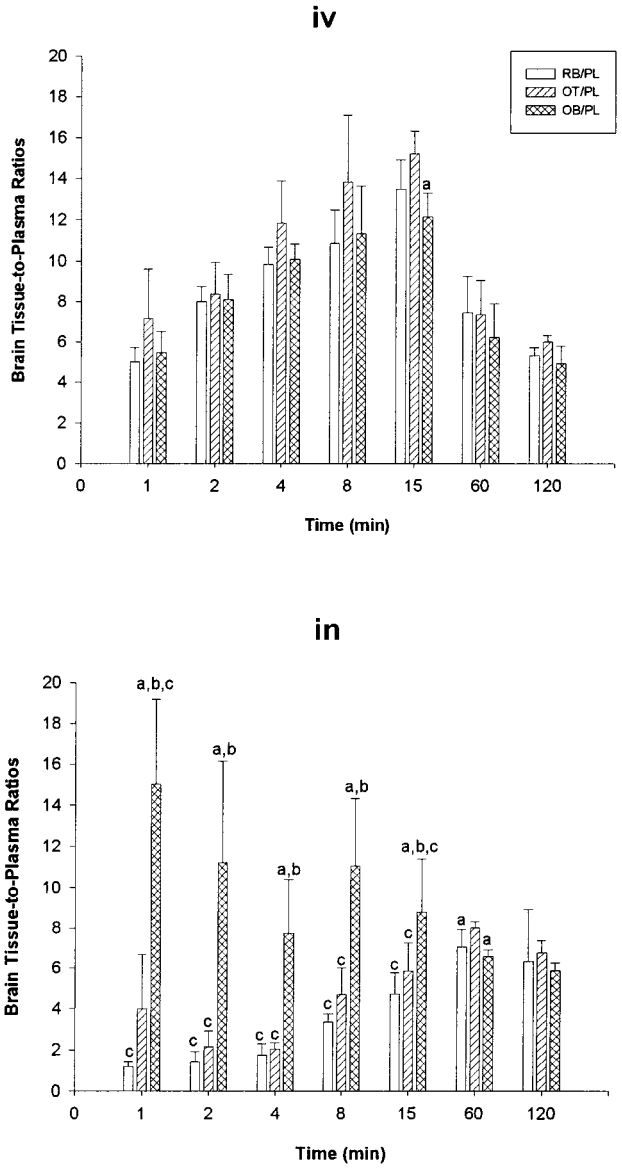

Figure 2—Brain tissue-to-plasma cocaine concentration ratios as a function of time following intravenous (iv) and intranasal (in) administration in rats (mean ± SD, $n = 4–5$ per time point). See legend for Figure 1 for definitions of the abbreviations. a: significantly different from OT/PL cocaine ratio, $p < 0.05$; b: significantly different from RB/PL cocaine ratio, $p < 0.05$; c: significantly different from the corresponding ratios obtained following intravenous administration, $p < 0.05$.

Discussion

Intranasal drug administration has received considerable recent attention, because it is an attractive noninvasive alternative route that can be used for the systemic delivery of compounds with poor oral bioavailability. Individuals who consume cocaine illicitly have long ago adopted and explored this route of administration. Because of the fear of HIV or other types of infection, there has been a recent shift among drug addicts away from intravenous injection to intranasal use. For inexperienced drug users in particular, the intranasal route has been preferred over intravenous injection. Although some evidence exists that substances may enter the brain directly from the nasal cavity, little attention has been paid to addressing the possibility that nasally applied abused substances could be transported directly from the nasal cavity to the central nervous system (CNS). Using a pharmacokinetic study design, we have obtained results suggesting that, when applied intranasally, a small fraction of the cocaine dose can be transported directly from the nasal cavity to the olfactory bulb. Whether the direct entry of nasally applied substances from the nasal cavity to the brain could result in increased abuse potential of nasally applied neurologically active drugs requires further study.

The olfactory system begins peripherally with the olfactory epithelium that is located on the roof of the nasal cavity. The olfactory receptors are bipolar neurons, one end of which is located in the nasal olfactory epithelium and the other end extending through the holes in the cribriform

plate of the ethmoid bone and ending in the olfactory bulb.[15,16] It has been suggested that there is free communication between the nasal submusocal interstitial space and the olfactory perineuronal space, which appears to be continuous with a subarachnoid extension that surrounds the oflactory nerve as it penetrates the cribriform plate.[17,18] Previous studies in mammals have demonstrated transport of protein tracers such as albumin labeled with Evans blue,[19] horseradish peroxidase,[20−22] and conjugated wheat germ agglutinin−horseradish peroxidase[20−22] from the nasal cavity to the olfactory bulb and its connected structures. Studies examining the transport of metals in the olfactory system have reported the movement of colloidal gold,[23] manganese,[24] and cadmium[25,26] within the olfactory system following intranasal administration. A series of recent studies performed by Sakane and co-workers have demonstrated significant increases of drug concentrations in the cerebrospinal fluid following intranasal administration of a variety of model substances, which have been shown to have low permeability to the CNS following systemic administration.[8,27−30] In contrast, enhanced brain delivery of a number of drugs was not always observed when results of nasal administration were compared with those obtained after intravenous dosing.[2,9,31]

One major limitation of the cited work is that brain and CSF drug concentrations usually were determined at a single time following dosing. Depending on the time of sampling, the brain drug concentration obtained following intravenous administration could have passed the maximal level and already have been in the declining phase, while the brain drug concentration obtained following intranasal administration could have been right around the maximal level. One could then incorrectly conclude that enhanced drug exposure in the brain had been observed following intranasal administration, implying the existence of direct nose−brain drug transport. In this research, a more complete pharmacokinetic examination of drug levels in plasma and different regions of the brain in the rat has been used as a tool to determine the existence of the direct nose−brain drug transport of nasally applied cocaine.

Cocaine is known to be a potent vasoconstricting agent commonly used to constrict nasal blood vessels during rhinologic procedures. This unique property of cocaine could prevent or delay its own systemic absorption following local application. Comparison of the dose−normalized plasma data collected from intravenous and intranasal administration indicates that nasally applied cocaine was rapidly and completely absorbed into the systemic circulation. This suggests that because of its high lipophilicity, movement of cocaine from the nasal mucosa to the blood was not limited by the reduction in the nasal blood flow. The detection of cocaine in the brain following intranasal dosing could have been due to the systemic absorption of the drug via the capillary network under the nasal mucosa and the subsequent distribution from the systemic blood into the target regions in the brain. The brain cocaine content was therefore normalized by the plasma cocaine concentration to allow comparisons of data collected following different routes of administration. The brain-to-plasma cocaine AUC or concentration ratios obtained after intravenous administration should represent the distribution of cocaine from systemic circulation into different brain regions (passing through the blood brain barrier). Because nasally applied cocaine does not appear in the systemic circulation instantaneously, the brain-to-plasma cocaine ratios after intranasal dosing should be similar to or smaller than those obtained after intravenous administration if nasally applied cocaine enters the brain only via the systemic blood. In this study, the cumulative OB-to-plasma cocaine AUC ratios obtained up to 4 min following nasal application was 1.5 to 3 times higher than that obtained after intravenous dosing. At 1 min post dose, the OB-to-plasma cocaine concentration ratio following intranasal administration was 3 times those obtained after intravenous dosing. These results support the existence of an alternative brain entry pathway for cocaine. Furthermore, following intravenous administration, no significant differences in cocaine content were observed among different brain regions whereas cocaine content was found to be region specific (olfactory bulb > olfactory tract > the remaining part of the brain) following intranasal administration. If the substance can be transported directly via the olfactory system, the first anatomical brain region of contact is the olfactory bulb. The olfactory tract connects the olfactory bulb to the remaining part of the brain. The brain cocaine content gradient observed following intranasal dosing but not after intravenous dosing further supports the existence of a direct pathway.

Because nasally applied cocaine was extensively and rapidly absorbed into the systemic circulation, the amount available for the direct nose−brain pathway is small. Therefore, the OB-to-plasma cocaine AUC ratios after intranasal administration were higher than those after intravenous administration only at early times after dosing. The distribution of this small amount of cocaine into other brain regions is counteracted by their large masses, thus leading to the insignificant increase in cocaine content in other brain regions. Consequently, the tissue-to-plasma AUC ratios in other brain regions obtained after intranasal administration were not higher than those after intravenous dosing. Similar to the tissue-to-plasma AUC ratios in different brain regions after intravenous dosing, the RB-to-plasma AUC ratios after intranasal administration increased as a function of time. The OT-to-plasma AUC ratios after intranasal dosing were high initially, decreased as a function of time, and subsequently increased as a function of time. The OB-to-plasma AUC ratios after intranasal dosing were high initially and decreased as a function of time. These results suggest that directly transported cocaine also was distributed into the OT regions but, because the amount entered via this pathway was not substantial, the OT-to-plasma AUC ratios after intranasal dosing were not higher than those after intravenous dosing. Similar results were obtained when the data were analyzed in nonaccumulated fashion (brain tissue-to-plasma cocaine concentration ratios).

Mechanisms responsible for the direct nose−brain transport of nasally applied substances are not clear. One suggested anatomical pathway for the direct transport of foreign compounds from the nasal cavity to the brain theorizes that foreign compounds enter into the olfactory sensory neurons by endocytosis or by binding to surface receptors and subsequently undergoing adsorptive endocytosis. The compounds could thereafter be transported within the olfactory sensory neurons by the axoplasmic flow, but whether they could be transported beyond the olfactory system is a question that remains to be answered. Neuronal transport is generally believed to be a slow process and seems to be consistent with the results from studies on heavy metals[23−26] and protein tracers.[19−22] However, this pathway is not likely to explain the observed increase in OB-to-plasma cocaine AUC or concentration ratios within 1−2 min following nasal application.

Another plausible explanation is that foreign substances can diffuse into the nasal submucosa and subsequently travel into the olfactory perineuronal channels because no morphological barrier between the loose submucosal connective tissue and the perineuronal space has been identified.[17,18] Large molecular weight markers injected into the lateral ventricles can be observed to flow down from the

central nervous system and reach the nasal submucosal extracellular space via the open pathway in the perineuronal space.[18] However, an intriguing question to us is the following: How could foreign compounds be transported from the nasal cavity to the brain through this open channel against the direction of flow of the cerebral spinal fluid? More studies are needed to define the exact mechanism(s) responsible for the direct transport of cocaine to the olfactory bulb.

We conclude that nasally administered cocaine can be transported directly from the nasal cavity to the olfactory bulb. Due to rapid and extensive systemic absorption of nasally applied cocaine, only a small fraction was transported to the CNS via the direct pathway. Further studies are necessary to investigate the pharmacological/toxicological consequences of direct nose−brain transport of nasally applied cocaine and other abuse substances.

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