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Research Paper

Direct Nose-to-Brain Transfer of Morphine After Nasal Administration to Rats

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Purpose. The aim of this study was to quantify the olfactory transfer of morphine to the brain hemispheres by comparing brain tissue and plasma morphine levels after nasal administration with those after intravenous administration.
Methods. Morphine (1.0 mg/kg body weight) was administered via the right nostril or intravenously as a 15-min constant-rate infusion to male rats. The content of morphine and its metabolite morphine-3-glucuronide in samples of the olfactory bulbs, brain hemispheres, and plasma was assessed using high-performance liquid chromatography, and the areas under the concentration–time curves (AUC) were calculated.
Results. At both 5 and 15 min after administration, brain hemisphere morphine concentrations after nasal administration were similar to those after i.v. administration of the same dose, despite lower plasma concentrations after nasal administration. The brain hemispheres/plasma morphine AUC ratios for the 0–5 min period were thus approximately 3 and 0.1 after nasal and i.v. administration, respectively, demonstrating a statistically significant early distribution advantage of morphine to the brain hemispheres via the nasal route.
Conclusion. Morphine is transferred via olfactory pathways to the brain hemispheres, and drug transfer via this route significantly contributes to the early high brain concentrations after nasal administration to rats.

KEY WORDS: brain uptake; morphine; nasal drug delivery; nose–brain transport; olfactory pathway; rats.

INTRODUCTION

Nasal administration of analgesics has become a promising alternative to oral or parenteral administration because pain relief is rapid and the patients can self-administer and control the dosage as required. The noninvasive nasal route is also preferable for opioid administration to children (1,2).

Most of the dose of a nasally administered drug will be absorbed across the nasal respiratory mucosa, which covers much of the human nasal cavity, into the systemic blood circulation. However, some of the dose may also be swallowed and possibly absorbed orally (1). Part of the dose could also be transferred from the nose to the brain via direct olfactory pathways. This direct nose-to-brain drug transfer has been demonstrated in several animal studies (3), and a few studies have suggested that it also exists in humans (4).

Morphine is a widely used analgesic, and cancer patients are commonly prescribed oral morphine on an as-needed basis against breakthrough pain (5). However, because of extensive first-pass metabolism, the oral bioavailability of morphine is only approximately 20–32% (6,7). Furthermore, the onset of pain relief after oral administration of morphine occurs later than could be desired (20–120 min), with a mean time to maximum plasma concentrations (T_{max}) of 1.1 h; this effect delay is partly caused by limited blood–brain barrier (BBB) permeability (8–13).

Nasal administration of morphine is currently under development, but early studies using a simple nasal morphine solution achieved only 10% bioavailability (14), although improvements were subsequently seen after changes to the formulation. For example, in preliminary clinical studies of morphine–chitosan formulations administered nasally to healthy volunteers and cancer patients, T_{max} was 15 min and the systemic bioavailability was nearly 60% (14,15). Efficacy and safety evaluations of nasal morphine gluconate for breakthrough pain in cancer patients demonstrated a rapid onset of perceptible pain relief (2.4 ± 2.1 min), whereas adverse effects were limited to nasal irritation (16). In addition, when comparing possible analgesics for nasal administration, the risk of severe side effects associated with misuse is lower for morphine than for alternatives such as fentanyl (1,17).

As morphine is a centrally acting analgesic with limited BBB transport, rapid direct nose-to-brain transfer circum-

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ABBREVIATIONS: CSF, cerebrospinal fluid; HPLC, high-performance liquid chromatography; LOB, left olfactory bulb; M3G, morphine-3-glucuronide; PBS, phosphate-buffered saline; ROB, right olfactory bulb.

venting the BBB would be beneficial in pain treatment. We have previously shown that morphine is transferred along the olfactory pathway to the olfactory bulbs and to the longitudinal cerebral fissure in the brain after nasal administration to rodents (18). However, that study did not conclusively demonstrate that morphine was further transferred to the cerebrum after olfactory transfer. Betbeder *et al.* (19) demonstrated increased antinociceptive activity with nasal morphine in mice by coadministering nanoparticles. However, antinociceptive activity was not increased after subcutaneous administration of the same formulation. They suggested that the result was a consequence of increased direct transfer of morphine from the nose to the brain (19). However, quantification of the olfactory transfer of drugs by comparing brain tissue and plasma area under the time-concentration curve (AUC) values after nasal and i.v. administration have only been performed in a few studies (20–22).

The aim of this study was to quantify the olfactory transfer of morphine to the brain hemispheres by comparing brain and plasma morphine levels after nasal administration with those after intravenous (i.v.) administration. If the brain concentrations are a result of morphine transfer across the BBB, the level of morphine in the brain should be proportional to the level of morphine in the plasma. If the brain/plasma AUC ratio is higher after nasal administration than after i.v. administration, the surplus can be attributed to olfactory transfer.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (B&K Universal, Stockholm, Sweden) were housed at room temperature with a 12-h light-dark cycle. The rats (n = 24) weighed 236–324 g (average, 273 g) on the day of the experiment. They had free access to a standard pellet diet and tap water and were allowed to adapt for 1 week prior to the experiment. The animal studies were conducted in accordance with the guidelines of the Swedish National Board for Laboratory Animals (CFN) policy LSFS 1988:45 and were approved by the local Ethics Committee for Animal Research (C 223/2).

Chemicals

Morphine hydrochloride trihydrate was purchased from Apoteket AB (Stockholm, Sweden). The morphine metabolite, morphine-3-glucuronide (M3G), was purchased from Lipomed (Arlesheim, Switzerland). Hypnorm® (0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone) was obtained from Janssen Animal Health (Brussels, Belgium) and Dormicum® (midazolam, 5 mg/mL) was obtained from Roche AB (Stockholm, Sweden). Heparin was purchased from Apoteket AB (Stockholm, Sweden). All chemicals and solvents used were of analytical grade.

Experimental Setup

All rats were anesthetized with an intraperitoneal injection of a 1:1:2 mixture of Hypnorm®, Dormicum®, and Milli-Q water (0.27 mL per 100 g body weight) and placed on a heating pad (37°C).

Twelve rats received the study drug through the right nostril. These rats were placed on their backs to keep the formulation in contact with the olfactory mucosa. The morphine dose (1.0 mg morphine/kg body weight) was dissolved in phosphate-buffered saline (PBS, pH 7.4), and 50 µl was administered approximately 5 mm into the nostril using a polyethylene tube (PE 50) attached to a micropipette. This placed the tip of the tube where the nostril ends, at the opening of the nasal cavity.

Twelve further rats received i.v. administration of the same dose, 1.0 mg morphine/kg body weight. Before drug administration, these rats underwent surgery, performed under anesthesia, and two indwelling polyethylene catheters were inserted into the arteria carotis and vena jugularis for the collection of blood and administration of morphine, respectively. The rats were allowed to recover under anesthesia after surgery for 30 min before the start of the experiment. The morphine dose was dissolved in physiological saline and 1.5 mL was administered at 100 µL/min (controlled by a Harvard Apparatus 22 infusion pump) over 15 min, to resemble the morphine plasma profile after nasal administration, with a T_{max} of 15 min (14,18).

Three animals from each group (nasal and i.v. administration) were sacrificed by exposure to gaseous CO_2 5, 15, 60, and 240 min after the start of the experiment. After decapitation, the skull was cut open and tissue samples were collected in the following order: cerebrum, left olfactory bulb (LOB), and right olfactory bulb (ROB). The cerebrum was separated into left and right hemispheres. For the rats in the nasal group, the brain hemispheres were further divided into two equal parts, inferior and superior. As there were no significant differences in morphine concentration between the inferior and superior parts of each brain hemisphere when investigated by Westin *et al.* (18), the average morphine concentrations from the left and right hemispheres were used in this study for comparison with the i.v. data. For the rats in the nasal group, 250 µl blood was collected directly after decapitation with a syringe and a coarse needle from the general blood flowing from the neck part of the rat body. For the rats in the i.v. group, 250 µl blood was collected from the arteria carotis after 5, 15, 30, 60, 180, and 240 min; the volume removed was compensated for by the administration of physiological saline. The catheterization of the i.v. rats enabled blood sampling at all time points until the scheduled sacrifice of the rats, resulting in the collection of 12, 9, 6, and 3 blood samples at the 5-, 15-, 60-, and 240-min time points, respectively. All the blood samples were put in heparinized Eppendorf tubes and centrifuged for 5 min at 7,200 × g. The plasma was then transferred to new tubes and the tissue and plasma samples were frozen at −20°C until analysis.

Analytical Methods

The volumes of the cerebral hemispheres and olfactory bulbs were measured and homogenized with 5- and 10-fold larger volumes, respectively, of 0.1 M perchloric acid. The homogenates were centrifuged for 10 min at 1,000 × g. The supernatant and the plasma samples (100 µl of each) were pretreated using a slight modification of the method by Joel

et al. (23) for solid phase extraction. Morphine and M3G were eluted with 3 mL methanol, and the solution was evaporated under a stream of nitrogen at 45°C. The residue was dissolved in 150 µl of the mobile phase, and 55 µl was injected by a Triathlon auto injector (Spark Holland, Emmen, the Netherlands) onto the high-performance liquid chromatography (HPLC) system.

Morphine and M3G in the brain tissue and plasma samples were separated using a Nucleosil C18 column (150 × 4.6 mm i.d.; 5 µm particles; Chrompack, Nacka, Sweden). Morphine was detected using an electrochemical detector (Coulochem II, ESA Inc., Chelmsford, MA, USA) with a guard cell (ESA 5020, ESA Inc.; potential set at 600 mV) and two analytical cells (ESA 5011, ESA Inc.; potentials set at 300 and 450 mV, respectively). M3G was detected by fluorescence detection (Jasco 821-FP, Japan; excitation and emission wavelengths 212 and 340 nm, respectively) coupled in series with the electrochemical detector. The mobile phase consisted of 720 mL 0.01 M phosphate buffer (pH 2.1), containing 0.2 mM SDS, 280 mL methanol, and 50 mL tetrahydrofuran, and was delivered at 1 mL/min (ESA 580, ESA Inc.). The peak height was evaluated using CSW32 integrating software (Data Apex Ltd., Prague, Czech Republic) and was compared with a standard curve to quantify morphine and M3G. The standard curve for plasma was linear up to 1,200 ng/mL for morphine and 6,700 ng/mL for M3G. The standard curve for brain tissue was linear up to 1,000 ng/g for morphine and 5,500 ng/g for M3G. Quality control samples in all analyses never deviated by more than 20% from their respective nominal values.

Data Analysis and Statistics

Before subsequent data analysis, the morphine and M3G concentrations in the olfactory bulbs and the brain hemispheres were corrected individually for the contribution from morphine and M3G content in the blood compartment of tissues by using an average blood volume of 0.03 mL/g brain tissue (22,24). The plasma concentrations of morphine were corrected for a blood to plasma ratio of 1.08 (25), and thereafter used in the calculations.

Results are expressed as means ± SD. The AUC values for olfactory bulbs, brain hemispheres, and plasma were calculated using the trapezoidal rule (26) from the mean drug concentrations at 5, 15, 60, and 240 min after administration, because only one set of brain tissue samples per animal per time point could be collected. The variance for the AUC values and AUC ratios were therefore calculated according to Yuan (26) and Bevington and Robinson (27), respectively. All AUC values are presented as values from 0 to t min.

The standard deviation estimates of morphine concentrations in olfactory bulbs and brain hemispheres, respectively, were calculated as the pooled variance of left and right sides and at several time points (28). Similarly, the standard deviation estimates of morphine and M3G concentrations in plasma were pooled from values at several time points. Levene's test was used to determine the groups wherein standards deviation could be regarded as equal. SPSS® was used for these calculations.

The weighted variances for the AUC values and the AUC ratios could be approximated being χ^2-distributed, where the degrees of freedom (ν_{df}) were calculated according to Eqs. (1) and (2) (29):

$$\nu_{AUC} = \frac{\left(s^2_{AUC}\right)^2}{\sum_{i=2}^{n} \frac{\left(\left(\frac{1}{2}(t_i - t_{i-2})\right)^2 s^2_{i-1}\right)^2}{\nu_{i-1}} + \frac{\left(\left(\frac{1}{2}(t_n - t_{n-1})\right)^2 s^2_n\right)^2}{\nu_n}} \quad (1)$$

where s^2_{AUC} is the AUC variance (26), t_i is the time point at sample occasion i (i = 1, 2, …, n), s_i is the corresponding estimated variance of concentration with the degrees of freedom ν_i

$$\nu_{ratio} = \frac{\left(s^2_{ratio}\right)^2}{\frac{\left(\left(\frac{1}{AUC_y}\right)^2 \cdot s^2_{AUC,x}\right)^2}{\nu_x} + \frac{\left(\left(\frac{AUC_x}{AUC_y^2}\right)^2 \cdot s^2_{AUC,y}\right)^2}{\nu_y}} \quad (2)$$

where s^2_{ratio} is the variance of the AUC ratio (27), $s^2_{AUC,x}$ is the estimated variance of the numerator (26) with the degrees of freedom ν_x calculated according to Eq. (1), and $s^2_{AUC,y}$ is the estimated variance of the denominator (26) with the degrees of freedom ν_y calculated according to Eq. (1). With that, ordinary t tests between AUC values and AUC ratios could be used. When multiple comparisons were made, Bonferroni correction for each type of comparison was applied in the t testing (30). A p value of less than 0.05 was considered statistically significant.

The proportion of morphine in the brain hemisphere that was due to olfactory transfer was calculated according the following equation:

$$\text{Olfactory proportion} = \frac{\left(AUC_{observed} - AUC_{expected}\right)}{AUC_{observed}} \times 100 \quad (3)$$

The $AUC_{expected}$ was defined as the AUC expected if there was no direct olfactory contribution to the morphine concentrations in the brain. This was calculated as the fraction of a dose entering the brain after i.v. administration (the brain/plasma AUC ratio) times the nasal plasma AUC. The $AUC_{observed}$ was the AUC after nasal administration.

The nasal morphine raw data was collected from our previous study Westin *et al.* (18).

RESULTS

Right-sided nasal administration or a 15-min i.v. constant rate infusion of 1.0 mg morphine/kg body weight to rats resulted in similar morphine concentrations at 5 or 15 min in the brain hemispheres (Fig. 1A) despite lower plasma concentrations after nasal administration (Fig. 2A). That is, there were no statistically significant differences in the brain hemisphere morphine $AUC_{0–5\ min}$ or $AUC_{0–15\ min}$ values between the nasal and i.v. groups (Table I). Furthermore, the highest brain morphine concentration was observed 15 min after either nasal or i.v. administration (Fig. 1A). There were no statistically significant differences in morphine AUC values between the right and the left hemispheres after right-sided nasal administration (Table I).

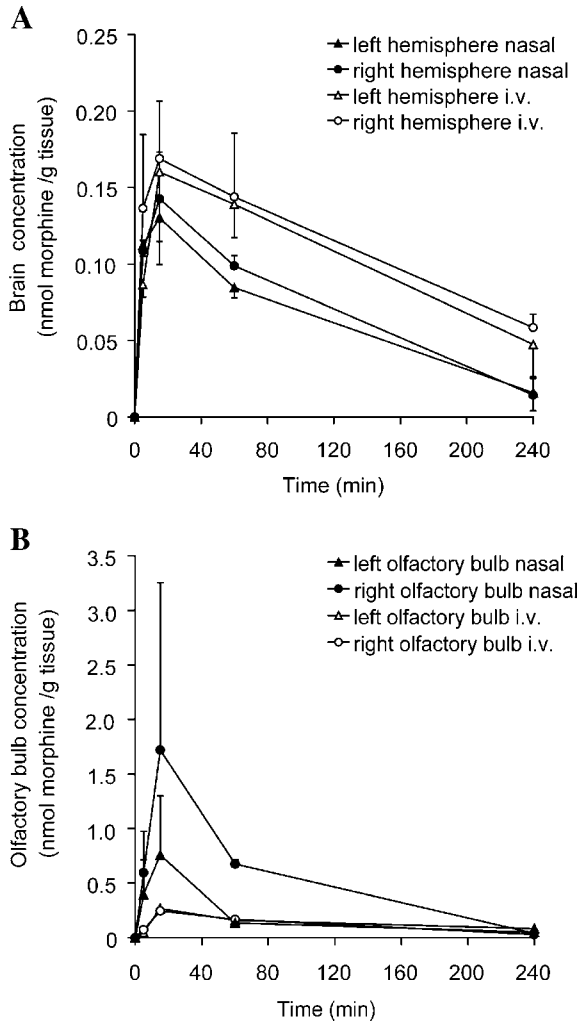

Fig. 1. Morphine concentration–time profiles following right-sided nasal administration or a 15-min i.v. infusion of 1.0 mg morphine/kg body weight to rats. Each point represents the mean of three rats ± SD. (A) Brain hemispheres, (B) olfactory bulbs.

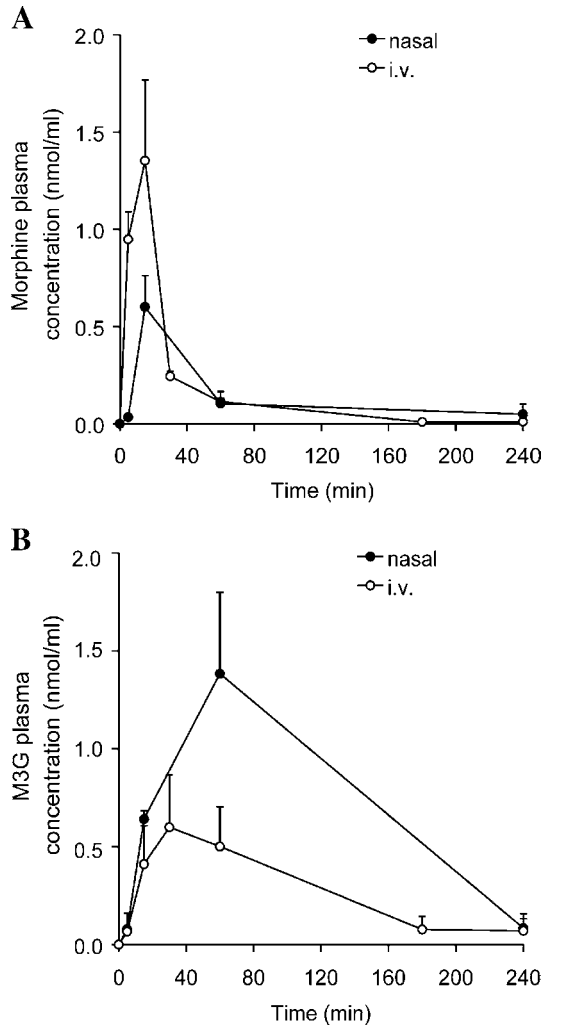

Fig. 2. Plasma concentration–time profiles following right-sided nasal administration or a 15-min i.v. infusion of 1.0 mg morphine/kg body weight to rats. Each point represents the mean ± SD of three rats after nasal administration and of 3–12 rats after i.v. administration. (A) Morphine, (B) morphine-3-glucuronide.

Table I. AUC Values (Mean ± SD) as a Function of a Time for Morphine in Brain Tissue (nmol min/g) and for Morphine and M3G in Plasma (nmol min/mL) Following Right-Sided Nasal Administration to 12 Rats or a 15-min Intravenous Infusion to 12 Rats of 1.0 mg Morphine/kg Body Weight

	Olfactory bulbs		Brain		Plasma	
Time (min)	LOB ± SD	ROB ± SD	LH ± SD	RH ± SD	Morphine ± SD	M3G ± SD
Nasal administration						
0–5	0.99 ± 1.23	1.49 ± 1.23	0.28 ± 0.03	0.27 ± 0.03	0.09* ± 0.02	0.19 ± 0.01
0–15	6.75 ± 4.42	13.1 ± 4.42	1.49 ± 0.12	1.53 ± 0.12	3.26* ± 0.46	3.67 ± 0.12
0–60	26.8 ± 14.0	66.9* ± 14.0	6.32 ± 0.39	6.96 ± 0.39	19.1* ± 2.65	47.7 ± 5.26
0–240	43.4 ± 14.2	131* ± 14.2	15.4* ± 0.93	17.1* ± 0.93	33.0* ± 5.37	175 ± 26.4
Intravenous administration						
0–5	0.12 ± 0.08	0.18 ± 0.08	0.22 ± 0.06	0.34 ± 0.06	2.37 ± 0.22	0.16 ± 0.11
0–15	1.68 ± 0.28	1.76 ± 0.28	1.45 ± 0.20	1.87 ± 0.20	13.9 ± 0.83	2.47 ± 0.41
0–60	11.2 ± 0.83	11.0 ± 0.83	8.18 ± 0.72	8.91 ± 0.72	46.9 ± 2.80	22.3 ± 1.96
0–240	32.9 ± 2.23	28.5 ± 2.23	25.0 ± 2.29	27.1 ± 2.29	58.1 ± 3.06	72.1 ± 10.4

*Significantly different from the corresponding i.v. value, $p < 0.0125$ (Bonferroni corrected).
ROB = Right Olfactory Bulb; LOB = Left Olfactory Bulb; RH = Right Hemisphere; LH = Left Hemisphere.

The plasma morphine concentration–time profiles were comparable after the same morphine dose was administered nasally or by i.v. infusion; the peak concentration occurred at 15 min and declined thereafter as a function of time (Fig. 2a). Furthermore, the plasma AUC values after nasal administration were statistically significantly lower than after i.v. administration, and the bioavailability of morphine was 57%, based on the comparison of plasma morphine $AUC_{0–240\ min}$ after nasal and i.v. administration (Table I).

The brain hemispheres/plasma morphine $AUC_{0–5\ min}$ ratios were approximately 3 and 0.1 after nasal and i.v. administration, respectively, demonstrating an early distribution advantage of morphine to the brain hemispheres via the nasal route. At 240 min, these ratios had evened out to approximately 0.5 for both administration routes (Fig. 3A). The right hemisphere/plasma morphine $AUC_{0–5,\ 0–15,\ 0–60,\ and\ 0–240\ min}$ ratios were 2,200, 348, 91, and 11% higher after nasal administration than after i.v. administration, respectively (Fig. 3A).

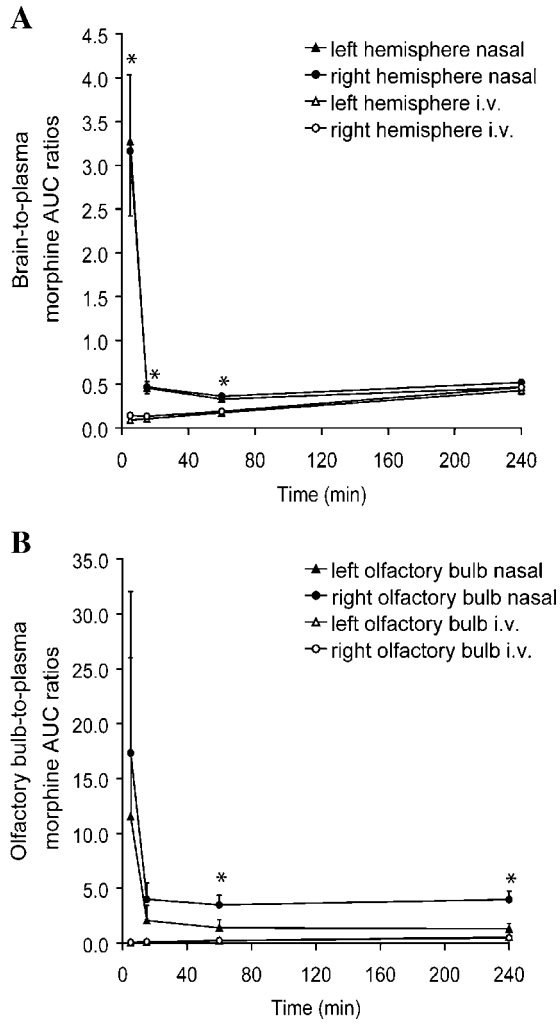

Fig. 3. Brain tissue/plasma morphine AUC ratio as a function of time following right-sided nasal administration to 12 rats or a 15-min i.v. infusion to 12 rats of 1.0 mg morphine/kg body weight. The AUC ratios represent the values from time 0 for all points (0–5, 0–15, 0–60, and 0–240 min) and are presented as means ± SD. (A) Brain hemispheres. *Significant difference between the brain hemisphere/plasma ratios after nasal and i.v. administration, $p < 0.0125$ (Bonferroni corrected). (B) Olfactory bulbs. *Significant difference between the ROB/plasma ratio after nasal and i.v. administration, $p < 0.0125$ (Bonferroni corrected).

The proportions of morphine reaching the right brain hemispheres as a result of direct olfactory transfer, calculated using Eq. (3), were 95, 71, 48, and 10% for the $AUC_{0–5,\ 0–15,\ 0–60,\ and\ 0–240\ min}$ intervals, respectively.

After nasal administration, the ROB morphine $AUC_{0–240\ min}$ was significantly greater than the LOB and brain hemisphere morphine $AUC_{0–240\ min}$ values. As expected, there were no significant differences in morphine $AUC_{0–240\ min}$ values between the ROB, LOB, and brain hemisphere samples after i.v. administration, showing that the olfactory bulbs contained concentrations similar to those in the rest of the brain after systemic distribution. Moreover, the LOB morphine $AUC_{0–240\ min}$ value after nasal administration was not significantly different from the ROB and LOB $AUC_{0–240\ min}$ values after i.v. administration, whereas the ROB morphine $AUC_{0–240\ min}$ value was significantly higher after nasal administration compared to i.v. administration (Table I). The highest morphine concentrations in the olfactory bulbs were observed 15 min after administration for both routes (Fig. 1B). The ROB/plasma morphine $AUC_{0–5,\ 0–15,\ 0–60,\ and\ 0–240\ min}$ ratios were 227, 31, 15, and 8 times higher than the corresponding i.v. ratios (Fig. 3B). Altogether, these results demonstrate that morphine was transferred along the olfactory pathway to the ROB after right-sided nasal administration.

After nasal administration, M3G was detected in the ROB at 15 and 60 min (0.8 ± 0.3 and 1.0 ± 0.4 nmol/g tissue, respectively,) but was not detected elsewhere in the olfactory bulbs or brain hemispheres. After i.v. administration, M3G was detected in neither olfactory bulbs nor brain hemispheres. The M3G concentrations in plasma were higher after nasal administration than after i.v administration (Fig. 2B). The plasma M3G/morphine $AUC_{0–240\ min}$ ratio was 5.3 after nasal administration and statistically significantly higher than the ratio of 1.2 after i.v. administration, which indicates that morphine was more extensively metabolized after nasal administration than after i.v. administration (Table I).

The fraction of the administered dose in the different brain regions after nasal and i.v. administration is presented in Fig. 4. The amounts of morphine present in different brain regions at 15 min were calculated from brain tissue morphine

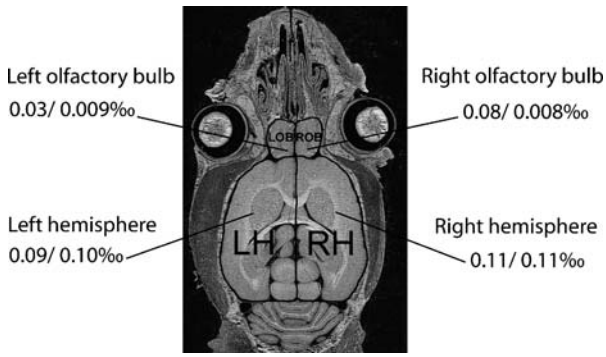

Fig. 4. Fraction of the administered dose (1.0 mg morphine/kg body weight) present in various brain regions 15 min after nasal or i.v. administration (nasal value/i.v. value; $n = 3$). The horizontal brain tissue section is from the autoradiographic study of Westin *et al.* (18).

concentrations (nmol/g tissue) and weights (g). The average weights of an olfactory bulb and a brain hemisphere were 37 and 680 mg, respectively. The amount of morphine for each region was divided by the administered dose to give the fraction of the administered dose. Remarkably, the fractions in each brain hemisphere were similar after nasal and i.v. administration, given the same dose, even though the bioavailability in plasma after nasal administration was only 57%.

DISCUSSION

The results of this study show that morphine is directly transferred from the nose, via olfactory pathways, to the olfactory bulbs and the brain hemispheres. Quantification of the olfactory transfer of morphine to the brain hemispheres indicated that direct olfactory transfer makes a significant early contribution to brain morphine concentrations.

The results showed no statistically significant differences in the brain hemisphere morphine $AUC_{0-5\ min}$ or $AUC_{0-15\ min}$ values after nasal or i.v. administration of the same dose (1.0 mg morphine/kg body weight), despite statistically significantly lower plasma AUC values after nasal administration. This demonstrates an early distribution advantage for the nasal route. After i.v. administration, morphine concentration in the brain is the result of distribution from the systemic circulation across the BBB to the brain. After nasal administration, concentrations of morphine in the brain could be the result of both distribution across the BBB and transfer via direct olfactory pathways. Thus, higher brain tissue/plasma morphine AUC ratios after nasal administration than after i.v. administration would indicate a preponderance of direct olfactory transfer. The right hemisphere/plasma morphine AUC ratios at 0–5, 0–15, 0–60, and 0–240 min were 2,200, 348, 91, and 11% higher, respectively, after nasal administration than after i.v. administration; this early contribution to brain morphine concentrations from direct olfactory transfer would have been overlooked if the investigation had been confined to later in the process. The proportions of morphine reaching the right hemisphere as a result of olfactory transfer were 95, 71, 48, and 10% for the 0–5, 0–15, 0–60, and 0–240-min intervals, respectively. Hence the impact of olfactory transfer decreased with time. Furthermore, the contribution of olfactory transfer to the brain is easier to separate from that of systemic distribution for drugs such as morphine, which permeate the brain relatively poorly. A lipophilic drug, for example, may well be transferred via the olfactory route (31), but as systemic brain distribution is rapid and extensive, direct olfactory transfer may be disguised and is also of less clinical importance.

Morphine has limited access to the brain from the systemic circulation, with whole brain/blood morphine concentration ratios increasing from <0.1 at 30 min after administration of a single i.v. bolus dose to ~0.4 after 180 min (13). Some of the reasons for the slow transport of morphine across the BBB include its physicochemical properties, the tightness of the BBB cell junctions, and the presence of efflux proteins in the BBB (10,13,32). It is therefore important to use an appropriate pharmacokinetic study design, with brain concentrations determined at several time points and an appropriate i.v. control. In our study, the brain concentrations were determined 5, 15, 60, and 240 min after nasal and i.v. administration, enabling the calculation of AUC values instead of comparison of concentrations at various time points. The parameters of the i.v. administration were adjusted to match those for nasal administration. All rats were anesthetized using the same regime, and the same morphine dose was used for both routes and the infusion rate for the i.v. dose was chosen to give a similar plasma morphine concentration–time profile to that after nasal administration. This approach had the advantage of allowing direct comparisons between brain/plasma AUC ratios after nasal and i.v. administration, while avoiding the potentially erroneous conclusions arising from, say, a comparison of the nasal results with those after an i.v. bolus dose.

Morphine is a substrate for the efflux protein P-glycoprotein (P-gp), which is present at the BBB and there limits morphine's BBB permeability (12,13). P-gp has also been shown to operate at the nose–brain barrier in mice in a study by Graff and Pollack (33); however, the same study showed no difference in brain uptake of morphine 5 min after nasal instillation between P-gp competent and P-gp incompetent mice. As the morphine dose (mg/kg body weight) administered in the present study was higher compared to the dose administered in the study by Graff and Pollack (33), the early olfactory transfer of morphine in this study should not have been attenuated by P-gp. Moreover, a review by Graff and Pollack (34) states that the amount of substrate delivered to the brain tissue after nasal administration was dependent on the presence of P-gp at the nose–brain barrier and that the impact of P-gp on the brain uptake of several nasally administered substrates was similar to that for the substrates administered systemically. This indicates that P-gp substrates present in the systemic circulation after nasal (due to nasal or oral absorption) or i.v. administration and P-gp substrates present at the olfactory mucosa are about equally affected by P-gp at the BBB and nose–brain barrier, respectively. The results on olfactory transfer of morphine in this study are based on comparisons between nasal administration and i.v. administration to control rats, and should therefore be read as net olfactory transfer.

For statistical testing, the ordinary way to handle multiple comparisons would be to apply an ANOVA on the AUC data with appropriate *post-hoc* tests. In this case, the fractal and different degrees of freedom in the groups make it cumbersome or even impossible to apply an ANOVA in a correct way. However, this approach has been performed on data from studies having a similar study design (21,22). Instead, according to some authors (30), some of the *post-hoc* tests are allowed to be used without a preceding ANOVA. We used the Bonferroni correction, which is such a test that is based on pairwise *t* tests and therefore also applicable in cases with fractal and different degrees of freedom in the groups. Notably, Bonferroni correction is developed for uncorrelated observations; and as the observations here are correlated, the test will therefore be conservative.

In this study, the AUC values were regarded as normally distributed because the brain tissue and plasma drug concentrations could be regarded as normally distributed (26), and there is a linear relation between the concentrations and the AUC values. In general, the ratio of two normally distributed variables, as in the case of the AUC ratios, is not normally

distributed. However, in-house simulations have shown (results not shown) that the distributions of the ratios are, with good approximation, normal in most cases. In cases where the distributions differ more from the normal, the simulations showed the same significance as the *t* tests showed.

In our previous study we concluded that the olfactory transfer of morphine was rapid and that the drug reached the olfactory bulbs and the longitudinal cerebral fissure via this route, but the techniques used in that paper showed no significant further transfer to the cerebrum (18). However, the 5- and 15-min autoradiograms revealed morphine-derived radioactivity in a fissure in the brain close to both olfactory bulbs. The morphine present in this fissure after nasal administration could have spread to both olfactory bulbs, which would explain why they contained high levels of morphine after 5 and 15 min. This rapid transfer of morphine to the longitudinal cerebral fissure, together with the current results of elevated morphine brain concentrations due to olfactory transfer, suggests that some of the morphine transferred via the olfactory route might have been distributed via the local cerebrospinal fluid (CSF) to the brain, which would explain why right-sided nasal administration resulted in early distance-independent elevation of the brain concentrations. The 60-min autoradiogram in our previous study (18) suggested that morphine diffused only within the ROB. Furthermore, 60 min after nasal administration, morphine concentrations were high only in the ROB and not in the LOB. However, no further right-sided diffusion to the right hemisphere was seen. This phenomenon could have been the result of the large right hemisphere masking elevated morphine concentrations due to olfactory transfer. Taken together, this demonstrates the possibility of two olfactory transfer mechanisms for morphine: one rapid method, where morphine is spread via the CSF to the brain, and one slower method, where morphine diffuses at least within the olfactory bulb tissue.

Rats only metabolize morphine into the inactive M3G (35). Metabolism of morphine was more extensive in our study after nasal than after i.v. administration. For example, the plasma M3G/morphine $AUC_{0-240\ min}$ ratio was significantly higher after nasal administration than after i.v. administration. Routes of administration that avoided first-pass metabolism, for example, i.v. administration, resulted in lower metabolite production than oral administration in a study by Faura *et al.* (36). This suggests that, in our study, part of the nasally administered drug was absorbed orally, and then underwent first-pass metabolism. This is supported by our previous study, in which a majority of the nasally administered morphine solution still remained in the nasal cavity after 15 min, but after 60 min had a considerable amount drained to the esophagus (18).

Metabolism of morphine in the brain is low (37), and no M3G was detected in the brain hemispheres after nasal or i.v. administration in this study. However, M3G was detected in the ROB 15 and 60 min after nasal administration, but not after i.v. administration. This indicates that morphine was metabolized to M3G in the nasal cavity and was then transferred along the olfactory pathway to the olfactory bulbs. The rat nasal mucosa contains UDP-glucuronosyl transferases, which metabolize morphine (38) to the same extent as in the liver. The M3G metabolized nasally was probably also transported to the systemic circulation which, along with oral absorption, would help to explain the high M3G plasma concentrations after nasal administration.

The fraction of the dose present in various brain regions or CSF has been discussed previously with respect to olfactory transfer (34,39–41). It has been argued that the fraction transferred via the olfactory pathway to the olfactory bulbs, brain, and CSF is often very small and insignificant with respect to exerting pharmacological effects. However, these fractions have to be compared with corresponding fractions present after i.v. administration, and the size of the brain region has also to be taken into account. For example, only a small amount of morphine enters the brain relative to the administered dose (42). Indeed, in this study, the fractions present in the brain regions were in the order of thousandths of the administered dose, but they were equal after nasal and i.v. administration, even though the driving force from the plasma was significantly lower after nasal administration.

The rapid transport of morphine to the brain hemispheres after nasal administration could be beneficial for the treatment of breakthrough pain compared with the currently used oral administration. However, conclusions concerning the impact of the olfactory transfer of morphine in humans cannot be drawn from the results of rat studies because humans and rats have different nasal morphology, with the olfactory area covering approximately 3% of the total nasal area in humans and 50% in rats (43).

Interestingly, the potential for olfactory transfer may increase with the development of new nasal delivery devices and more effective formulations, improving the distribution to, and the absorption across, the olfactory mucosa (44). In fact, a remarkably rapid onset of perceptible pain relief (2.4 ± 2.1 min) after nasal administration of morphine to cancer patients has already been observed (16). Furthermore, Illum *et al.* (14) recorded higher sedation scores among the volunteers in their clinical trial at the earliest time point after nasal administration than after i.v. infusion over 30 min. These findings are in good agreement with the results of the present study, which demonstrated that morphine is transferred via olfactory pathways to the brain hemispheres, and significantly contributes to the early high brain concentrations after nasal administration to rats. Studies clarifying the significance of olfactory transfer of morphine should therefore be included in future development of nasal administration of morphine.

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