BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **22**: 213–219 (2001)
DOI: 10.1002/bdd.281

Intranasal Absorption of Sumatriptan and Naratriptan: No Evidence of Local Transfer from the Nasal Cavities to the Brain Arterial Blood in Male Rats

Niels Einer-Jensen[a], Lise Larsen[a], Stephanie Deprez[b], Elaine Starns[c], and Sheila Schwartz[c,*]
[a] *Physiology and Pharmacology, Institute of Medical Biology, University of Southern Denmark, Winsloewparken 21, DK-5000 Odense C, Denmark*
[b] *Cellular and Molecular Toxicology, Safety Assessment Division, GlaxoSmithKline, Park Road, Ware, Hertfordshire, SG12 0DP, UK*
[c] *Drug Metabolism and Pharmacokinetics Division, GlaxoSmithKline, Park Road, Ware, Hertfordshire, SG12 0DP, UK*

ABSTRACT: Nasal administration to rats of small molecules (tritiated water, tyrosine, and propanol) results in a higher concentration in the brain arterial blood than in other arteries. The preferential distribution is based on a counter current transfer, which takes place between nasal vein blood and brain arterial blood in the cavernous sinus-carotid artery complex. This model was used to investigate whether the antimigraine $5HT_{1B/1D}$ receptor agonists sumatriptan and naratriptan may also be transferred by the system. The ratio of 'head':'heart' plasma concentrations obtained from two carotid catheters after intranasal administration was not different from 1.00 for either compound, and thus, there was no experimental evidence of a preferential local transfer of drug from the nose to the carotid artery circulation. However, plasma concentrations increased from the first minute after intranasal dosing suggesting that sumatriptan and naratriptan are absorbed into the general systemic circulation from the nasal cavity in rats in a first-order fashion with no lag time. This is consistent with the clinical onset of efficacy of sumatriptan after an intranasal dose which occurs as early as 15 min post dose. Copyright © 2001 John Wiley & Sons, Ltd.

Key words: sumatriptan; naratriptan; absorption; cavernous sinus-carotid artery complex

Introduction

Direct local transfer of heat and substances between veins and arteries has been observed in a number of organs [1]. In the head, transfer of heat has been suggested to take place between the venous blood in the cavernous sinus and the arterial blood in the carotid artery. A set of parallel small arteries (Rete Mirabile) within the cavernous sinus is thought to be important by increasing the transfer area [2]. We have shown that brain cooling takes place in the intubated rat when the nasal cavities are flushed with oxygen [3]. The rat, like humans, does not have a Rete Mirabile thus, the Rete is not essential for the transfer. In addition to heat, local transfer of substances has been found between the blood vessels in the base of the brain. Transfer of substances (steroid or peptinergic hormones) was found during perfusion of pig or sheep heads with oxygenated blood at 37°C [4,5]. Local transfer of substances of low molecular weight (tritiated water, tyrosine, propanol) to cranial arterial blood was found following intranasal administration in the rat [6]. A similar transfer was found with diazepam, but not with cocaine [7].

*Correspondence to: Drug Metabolism and Pharmacokinetics Division, GlaxoSmithKline, Park Road, Ware, Hertfordshire, SG12 0DP, UK. E-mail: sis38884@glaxowellcome.co.uk

Received 19 March 2000
Accepted 26 September 2001

The antimigraine agents sumatriptan and naratriptan are agonists of the $5HT_{1B/1D}$ receptor subtypes, probably exerting their therapeutic effect by selective vasoconstriction in the carotid arterial circulation [8,9]. Sumatriptan is marketed as an oral, subcutaneous, suppository or intranasal formulation (dependent on country), while naratriptan is available as an oral formulation only. This study was undertaken in an attempt to elucidate the mechanism by which these $5HT_1$ agonists administered by the intranasal route gain access to the site of action in the carotid circulation. A rat model which detects direct local transfer of substances from the nose to the arterial blood was used to determine whether drug concentrations in blood may be increased in the carotid circulation following an intranasal dose.

Method

Experimental

The experimental work was conducted in the laboratories of the University of Southern Denmark. Groups of ten (intravenous dose) or 20 (intranasal dose) mature male Sprague–Dawley rats (450–550 g) were used for the experiment. The animals were anaesthetised with Hypnorm (Fetanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml) intramuscularly at 0.3 ml/kg, and 5–10 min later Dormicum (Midazolam 5 mg/ml) intraperitoneally at 0.4 ml/kg. A catheter was inserted into the trachea to secure the ventilation during the nasal application of the drugs. Two catheters were inserted into the same carotid artery, the tip of one pointing towards the head ('Head', inner diameter 0.58 mm, outer diameter 0.96 mm), the other towards the heart ('Heart', inner diameter 0.40 mm, outer diameter 0.80 mm). The catheters were used to obtain parallel samples. 'Head' samples represent blood modified during the passage from the other carotid through the cavernous sinuses and the circle of Willis, and 'Heart' samples represent 'not-modified' arterial blood. One jugular vein was ligated; an occluding catheter pointing towards the head was inserted into the other. It was intended for the collection of the venous head blood to diminish the influence of recirculation. The blood volume was measured, plasma separated and frozen. Two catheters (inner diameter 0.40 mm, outer diameter 0.80 mm) were inserted 5 mm into the nasal cavities. They were used for infusion of solutions of the substances tested (50 µl into each during a 7 s period, Harvard Instruments infusion pump). A catheter for intravenous infusion of the test substance was inserted into the control animals. The animals were heparinised (0.1 ml, intravenous, 5000 iU/ml, Sygehus Apotekerne, Denmark) before the start of the blood collection.

Sumatriptan was formulated as the hemisulphate salt *in situ* (50 mg/ml) and maintained at approximately pH 5.5. The solution was 'clean', but not sterile. The stock solution was equally distributed to 25 vials, which were kept frozen until the day of experiment. Each rat received 5 mg sumatriptan, either as 50 µl into each nasal cavity during 7 s or 100 µl infused into the femoral artery over 8.5 s. Naratriptan was formulated as a solution in water (approximately 30 mg/ml) fresh each day or every second day. Each rat received approximately 3 mg naratriptan intranasally or 2 mg intravenously, either as 50 µl into each nasal cavity during 7 s or 100 µl infused into the femoral artery over 8.5 s.

Parallel blood samples were obtained every 60 s from the 'Head' and 'Heart' catheters immediately before and for 10 min after the start of the nasal infusion and bleeding from the jugular catheter. Then samples were collected every 120 s for an additional 20 min after which the rats were close to a circulatory collapse. In total, the blood volume removed was several millilitres and may be considered a cardiovascular strain. The blood samples were centrifuged to provide plasma which was frozen at $-20^{\circ}C$. The frozen plasma samples were transferred to the laboratories of Glaxo Wellcome, Ware for assay of sumatriptan or naratriptan.

The plasma concentration ratio (ng/ml) of sumatriptan and naratriptan in the 'Head' samples compared to the 'Heart' was calculated for each set of samples. A ratio greater than unity (>1.00) indicates that the 'Head' blood content of drug is preferentially increased by the local transfer of drug from the nasal vein blood to the carotid artery blood and subsequent passage

to the brain arteries. When the drugs are given intravenously, a ratio of 1.00 is expected since no modification of the 'Head' blood would be taking place.

The animals were sacrificed with an intravenous overdose of barbiturate after the last blood collection. The protocol followed the national rules for use of experimental animals.

Bioanalysis

The plasma samples from rats treated with either naratriptan or sumatriptan were prepared by a solid-phase extraction method (SPE) followed by separation and analysis of the solutions by liquid chromatography coupled to tandem mass spectrometry (LC/MS-MS). Methods used for both analytes are detailed.

Method for naratriptan. Plasma samples were thawed and mixed thoroughly. To maintain concentrations of naratriptan within the calibration range (0.5–250 and 2.00–1000 ng/ml for nasal and intravenous routes, respectively), samples were diluted with control rat plasma if necessary. A 50 mg Varian C18 Microlute solid-phase extraction block was primed with 400 µl of methanol and 400 µl of water and then vacuumed. A 200 µl aliquot of sample together with 25 µl of a 1 µg/ml solution of [$^{2}H_{3}$–^{13}C]-naratriptan internal standard were loaded onto the block and again vacuumed. The block was then washed with 400 µl of water and 400 µl of 40% methanol in water (v/v) before being eluted in 400 µl of 99.8% methanol/0.1% trimethylamine/0.1% formic acid (v/v). The resulting extracts were evaporated to dryness under a stream of heated nitrogen (nominally 40°C) and reconstituted with 100 µl of 90% acetonitrile/9.9% water/0.1% formic acid (v/v).

Solutions were analysed using a Hewlett-Packard Model 1090 liquid chromatograph (Palo Alto, USA) coupled to a Quattro II tandem quadrupole mass spectrometer (Micromass, Manchester, UK) fitted with a Z-spray electrospray ionisation source (ESI). Separations were achieved on a 50 × 2.1 mm i.d. ODS3 Inertsil column (Capital HPLC Ltd., Broxburn, Scotland) packed with C18 stationary phase and operated at 20°C. The mobile phase consisted of 0.1% (v/v) formic acid in water (solvent A) and acetonitrile/0.1% (v/v) formic acid in water 95:5 (solvent B). The proportion of solvent B was increased linearly from 0 to 100% in 1.5 min, maintained at 100% for 1.5 min, and then decreased to 0% in 0.1 min. After each injection the column was allowed to re-equilibrate with 0% solvent B for 1.9 min. A flow rate of 0.8 ml/min was used for all analyses. The injected volume was 10 µl. Retention time of naratriptan was 1.90 min with a complete cycle time of 5 min.

The column eluent was split 1:10, directing ~80 µl/min to a pneumatically assisted electrospray interface operating with a capillary voltage of 3.5 kV. Source block and desolvation temperatures were 150 and 250°C, respectively. The mass spectrometer was operated in the positive ion mode. Nitrogen was used as nebulisation and drying gas, at flow rates of 20 and 400 l/h, respectively. The collision gas used was argon at a pressure of 1.8×10^{-3} mbar with a collision energy of 20 eV. Selected reaction monitoring (SRM) experiments were performed using a dwell time of 500 ms per transition (335.5 > 97.5 and 339.5 > 101.5 for naratriptan and internal standard, respectively). Each chromatographic run was integrated according to the ratio of naratriptan peak area to internal standard peak area. Quantification was performed by interpolation using calibration curve range 0.5–250 and 2–1000 ng/ml for nasal and intravenous routes, respectively.

Method for sumatriptan. Plasma samples were thawed and mixed thoroughly. To maintain concentrations of sumatriptan within the calibration range 0.5–500 ng/ml, samples were diluted with control rat plasma if necessary. A 50 mg Varian C18 Microlute solid phase extraction block was primed with 400 µl of methanol and 400 µl of water and then vacuumed. A 100 µl portion of sample together with 100 µl of a 300 ng/ml solution of $^{2}H_{3}$ sumatriptan internal standard were loaded onto the block and vacuumed. The block was then washed with 400 µl of water and 400 µl of 80% methanol before being eluted in 400 µl of 99.8% methanol/0.1% trimethylamine/0.1% formic acid (v/v). The resulting extracts were evaporated to dryness under a stream of heated nitrogen (nominally

40°C) and reconstituted with 50 µl of 90% acetonitrile/9.9% water/0.1% formic acid (v/v).

The HPLC system comprised an autosampler LC PAL (CTC Analytics), 1100 pump, gradient unit and degasser all from Hewlett-Packard. Detection was by means of an API 365 triple quadrupole mass spectrometer (PE-Sciex, Ontario, Canada). Separation was achieved on a 50 × 2.1 mm i.d. ODS3 Inertsil column (Capital HPLC Ltd., Broxburn, Scotland) packed with C18 stationary phase and operated at ambient temperature. The mobile phase had a flow rate of 0.8 ml/min and consisted of 0.1% (v/v) formic acid in water (solvent A) and acetonitrile/0.1% (v/v) formic acid in water 95:5 (solvent B). The proportion of solvent B was increased linearly from 0 to 100% in 2 min, and back to 100% A by 2.1 min and then retained at 100% A for a further 1.9 min. The injected volume was 20 µl and the retention time of sumatriptan was 1.75 min with a complete cycle time of 4 min. The flow from the column was split 1:10 with ~160 µl/min directed to a TurboIonspray interface at 450°C. Analytes were ionised by positive ion electrospray and detected by tandem mass spectrometry using SRM. The transitions 296.0 > 58.0 for sumatriptan and 299.5 > 61.0 for sumatriptan internal standard were monitored with dwell times of 250 ms per transition. Each chromatographic run was integrated according to the ratio of sumatriptan peak area to internal standard peak area. Quantification was performed by interpolation using a calibration curve range 0.5–500 ng/ml.

Results and Discussion

After nasal application, the 'head' and 'heart' arterial concentrations of sumatriptan and naratriptan showed a steady increase in plasma concentrations immediately after dosing and for the duration of the 30 min experimental period (Figures 1 and 2). The ratio of 'head':'heart' plasma concentrations obtained from the two carotid catheters after intranasal administration was not different from 1.00 for either compound (Figures 3 and 4) indicating that there was no preferential local transfer of drug from the nose to the carotid artery circulation. As expected, the

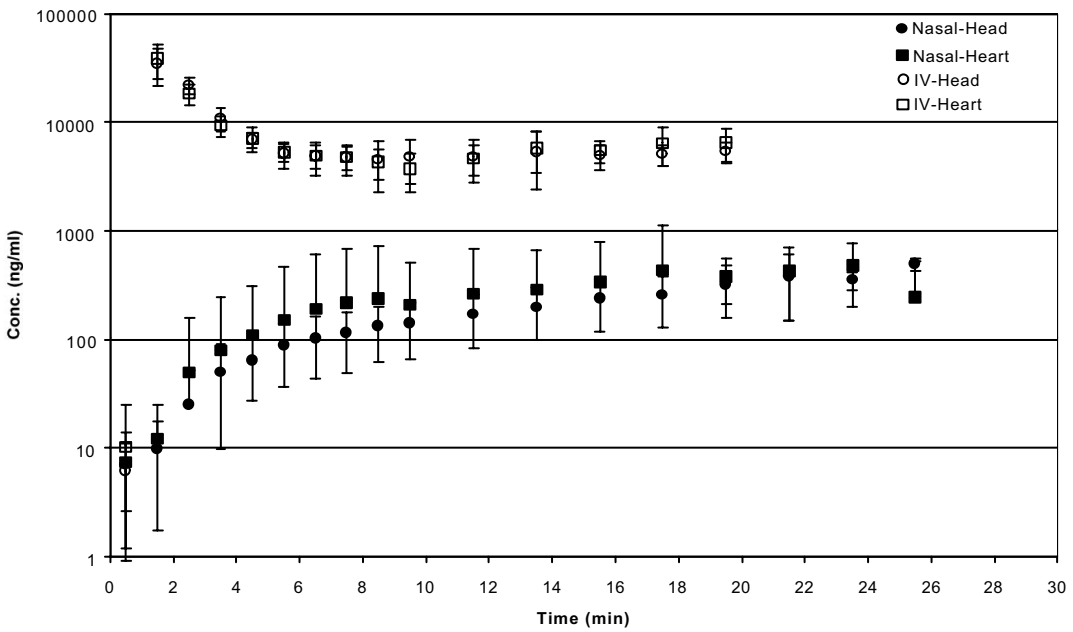

Figure 1. 'Head' and 'Heart' plasma concentrations (mean ± S.D.) of sumatriptan in rats following an intranasal or intravenous dose (10 mg/kg) of sumatriptan to groups of 10 (intravenous) or 20 (intranasal) rats

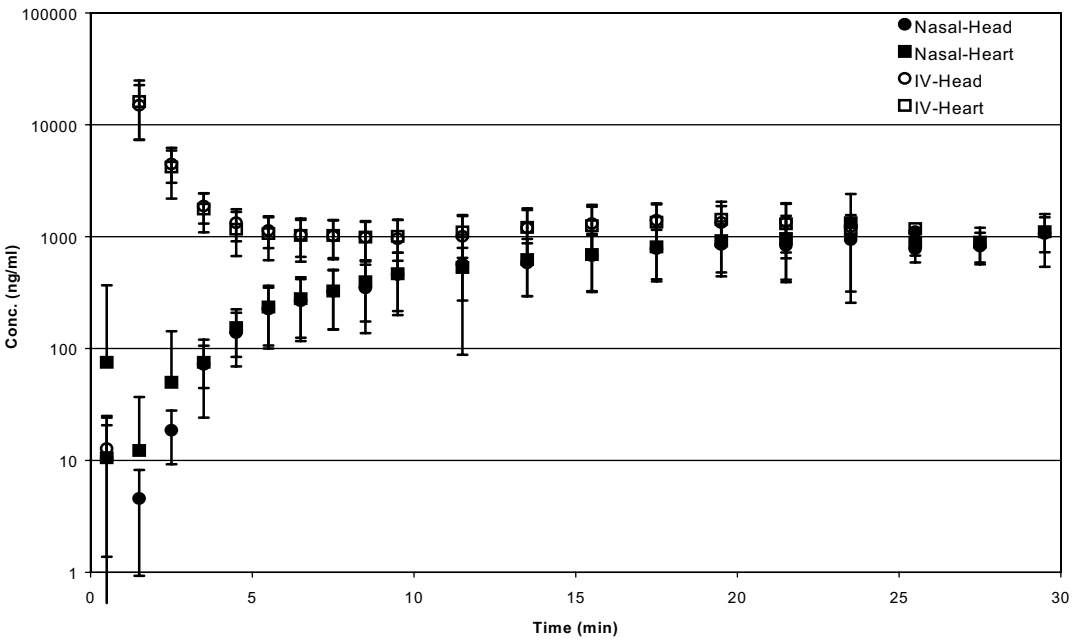

Figure 2. 'Head' and 'Heart' plasma concentrations (mean ± S.D.) of naratriptan in rats following an intranasal (6 mg/kg) or intravenous (4 mg/kg) dose of naratriptan to groups of 10 (intravenous) or 20 (intranasal) rats

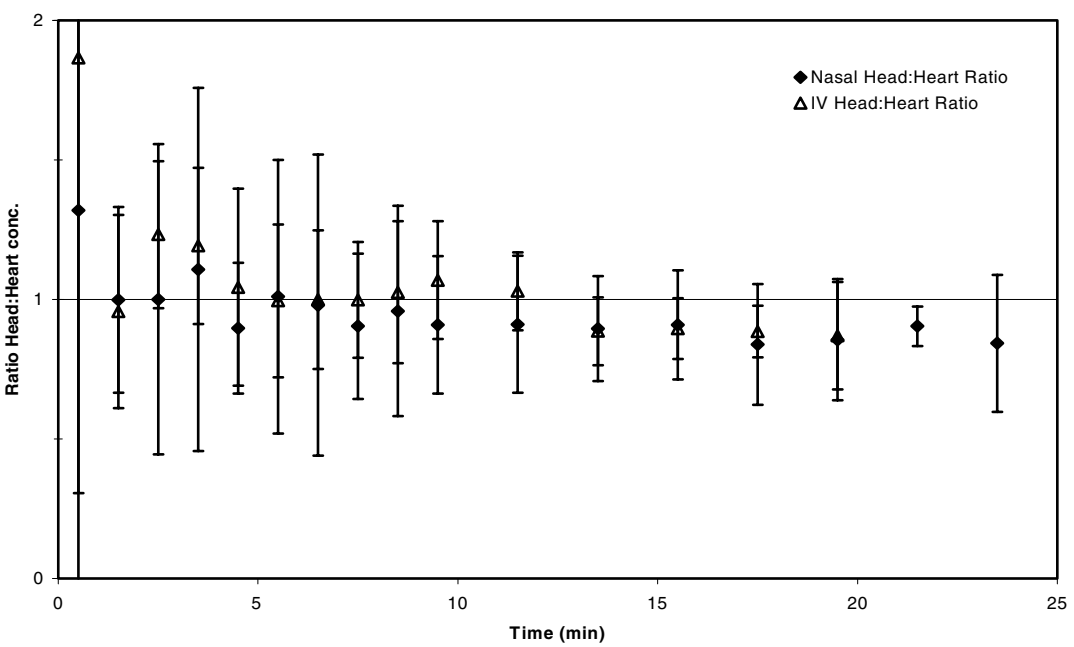

Figure 3. The ratio between 'Head' and 'Heart' plasma concentrations (mean ± S.D.) of sumatriptan after nasal and intravenous administration to groups of 10 (intravenous) or 20 (intranasal) rats

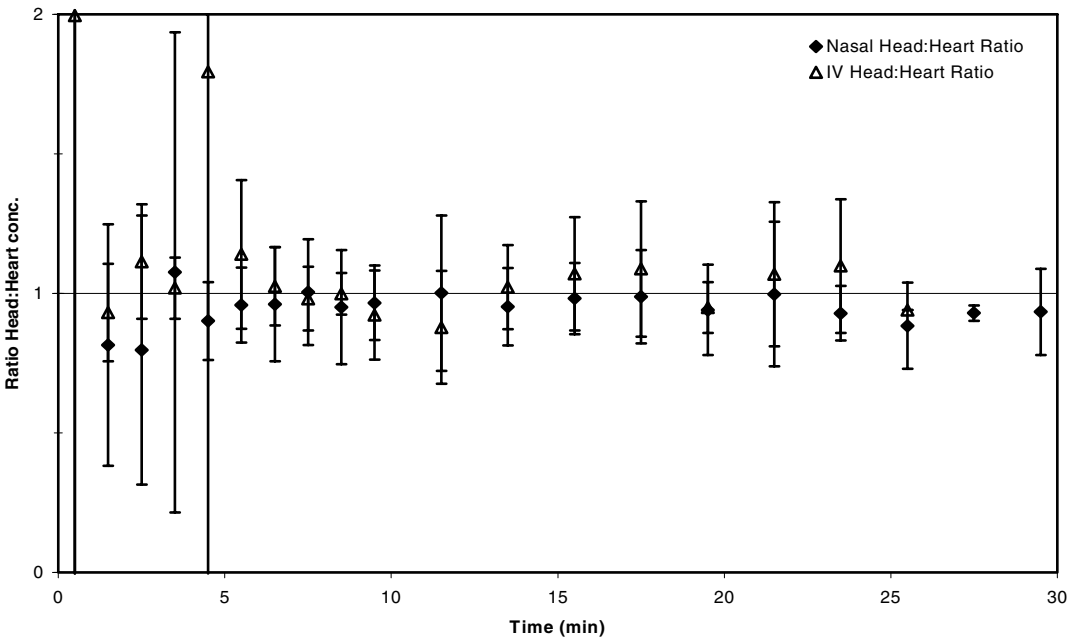

Figure 4. The ratio between 'Head' and 'Heart' plasma concentrations (mean ± S.D.) of naratriptan after nasal and intravenous administration to groups of 10 (intravenous) or 20 (intranasal) rats

ratio of 'head':'heart' plasma concentrations obtained after intravenous administration was close to 1.00.

The results supplement previous investigations in rats, where peak levels of sumatriptan were achieved at 30 min after an intranasal dose (mean concentration was 195 ng/ml for a 5 mg/kg dose) [10]. The absolute bioavailability of an intranasal dose was estimated to be approximately 30%. In this current study, plasma concentrations can be seen to increase from the first minute after intranasal dosing onward upto the last time points (25–30 min), where concentrations reached approximately 400 ng/ml for a 10 mg/kg dose. The absorption pattern is consistent with the clinical onset of efficacy of sumatriptan after an intranasal dose which occurs as early as 15 min post dose [11,12].

The plasma concentrations of both substances increased exponentially in our experiment, indicating a first-order type of absorption. The curve form was similar to that of cocaine, but different from that of propanol, tyrosine, and diazepam, where the uptake from the nasal mucosa was very fast [6,7]. The plasma concentrations obtained after a nasal administration of the three last substances were similar to those obtained after i.v. administration. All three were transferred between the blood vessels in the head in the rat model, while cocaine (like sumatriptan and naratriptan) was not [6,7]. This may be a result of the vasoactive properties of these compounds (although it is uncertain whether $5HT_1$ receptors are located in the nasal vasculature of rats). Alternatively, the results may point to a limitation of the present rat model: transfer may only be documented if a very rapid nasal uptake is present. In the case of a somewhat slower absorption, the locally increased concentration in the carotid artery may be obscured by a combination of the time delay of the transfer and the steadily increasing concentrations in both brain and general arterial blood. The lack of documented local transfer of naratriptan and sumatriptan may thus be an experimental artifact.

Clinical effects after *in vivo* egg implantation were higher than that predicted from peripheral plasma concentrations after vaginal administration of progesterone [13], so the theory that local

concentrations relative to general systemic concentrations may be increased by local transfer of drug to the blood could be important for drugs with adverse side effects. There are no published data relating the possibility of a local transfer between the blood in the cavernous sinus and the carotid artery to the effects of naratriptan or sumatriptan in man. This is understandable since the documentation of a counter current transfer of drugs in the cavernous sinus-carotid artery complex so far has been limited to the rat model. Local transfer of a drug after nasal administration in man would offer the advantage of a (partly) selective pharmacological treatment of the cranial arteries offering more effect and less side effects. Undocumented local transfer of sumatriptan may thus contribute to the clinical experience that the substance is well tolerated with an incidence of adverse events similar to placebo [11,14].

References

1. Einer-Jensen N, Hunter RHF. Local regulation of physiological events of and the potential for local treatment of the ovaries, fallopian tubes and uterus. *Hum Reprod Update* 2000; **6**: 132–138.
2. Baker MA. Brain cooling in endotherms in heat and exercise. *Annu Rev Physiol* 1982; **44**: 85–96.
3. Einer-Jensen N, Khorooshi MH. Cooling of the brain through oxygen flushing of the nasal cavities in intubated rats: an alternative model for treatment of brain injury. *Exp Brain Res* 2000; **130**: 244-247.
4. Skipor J, Grzegorzewski W, Wasowska B, Krzymowski T. Counter current transfer of beta-endorphin in the perihypophyseal cavernous sinus-carotid rete vascular complex of sheep. *Exp Clin Endocrinol Diabetes* 1997; **105**: 308–313.
5. Stefanczyk-Krzymowska S, Krzymowski T, Grzegorzewski W, Wasowska B, Skipor J. Humoral pathway for local transfer of the priming pheromone androstenol from the nasal cavity to the brain and hypophysis in anaesthetized gilts. *Exp Physiol* 2000; **85**: 801–809.
6. Einer-Jensen N, Larsen L. Transfer of tritiated water, tyrosine, and propanol from the nasal cavity to cranial arterial blood in rats. *Exp Brain Res* 2000; **130**: 216–220.
7. Einer-Jensen N, Larsen L. Local transfer of diazepam, but not of cocaine, from the nasal cavities to the brain arterial blood in rats. *Pharmacol Toxicol* 2000; **87**: 276–278.
8. Humphrey PPA, Feniuk W, Perren MJ, Connor HE, Oxford AW. The pharmacology of the novel 5-HT_1-like receptor agonist, GR43175. *Cephalalgia* 1989; **9 (Suppl. 9)**: 23–33.
9. Connor HE, Feniuk W, Beattie DT, North PC, Oxford AW, Saynor DA, Humphrey PA. Naratriptan: biological profile in animal models relevant to migraine. *Cephalalgia* 1997; **17**: 145–52.
10. Ayres DW, Barrow A, Scully NL, Curtis GC, Hughes HM. Absorption, pharmacokinetics and metabolism of ^{14}C-sumatriptan following intranasal administraion to the rat. *Xenobiotica* 1996; **26**: 1273–1282.
11. Dahlof C. Sumatriptan nasal spray in the acute treatment of migraine: a review of clinical studies. *Cephalalgia* 1999; **19**: 769–778.
12. Duquesnot C, Mamet JP, Sumner D., Fuseau E. Comparative clinical pharmacokinetics of single doses of sumatriptan following subcutaneous, oral, intranasal administration. *Eur J Pharm Sci* 1998; **6**: 99–104.
13. Cicinelli E, Cignarelli M, Sabatelli S, Romano F, Schonauer LM, Padovano R, Einer-Jensen N. Plasma concentrations of progesterone are higher in the uterine artery than in the radial artery after vaginal administration of micronized progesterone in an oil-based solution to postmenopausal women. *Fertil Steril* 1998; **69**: 471–473.
14. Peikert A, Becker WJ, Ashford EA, Dahlof C, Hassani H, Salonen RJ. Sumatriptan nasal spray: a dose-ranging study in the acute treatment of migraine. *Eur J Neurol* 1999; **6**: 43–49.

