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Evaluation of brain-targeting for the nasal delivery of estradiol by the microdialysis method

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Abstract

The uptake of estradiol into the cerebrospinal fluid (CSF) after intranasal and intravenous administration in rats was investigated to study whether direct nose–CSF transport of estradiol exits or not. Animals received 0.48 mg kg^{-1} estradiol randomly methylated β-cyclodextrin (RAMEB) inclusion complex intranasally and intravenously. Following nasal delivery, estradiol reached a C_{max} value (mean ± S.D.) in plasma (26.70 ± 11.37 ng ml^{-1}) and CSF (54.76 ± 32.84 ng ml^{-1}) after 20 min in each case, while after intravenous infusion, estradiol reached a C_{max} value in plasma (170.08 ± 64.67 ng ml^{-1}) and CSF (26.48 ± 11.34 ng ml^{-1}) at 5 min and 60 min, respectively. The AUC_{CSF}/AUC_{plasma} ratio (1.60 ± 0.67) after intranasal delivery differed significantly from the ratio (0.61 ± 0.16) observed after intravenous infusion ($P < 0.05$). All these results indicate that estradiol is transported into CSF via olfactory neurons, and, hence, there is a direct transport route from the nasal cavity into the CSF for estradiol.

Keywords: Nasal delivery; Intravenous administration; Estradiol; Microdialysis; Olfactory pathway

1. Introduction

The efficacy of hormone replacement therapy in reducing the incidence of menopausal symptoms in women (Kupperman et al., 1953; Steingold et al., 1985) is well established, such as vasomotor disturbances and genitourinary disorders (De Lignieres et al., 1986). In the longer term, hormone replacement therapy (HRT) offers significant prophylaxis against osteoporosis, cardiovascular arteriosclerotic disease, colonic cancer, and Alzheimer's disease. The two most widely used forms of administration of HRT are the oral and transdermal routes (O'Connel, 1995).

In oral administration, a large part of estradiol is transformed into biologically less active estrone and into conjugates by substantial intestinal and hepatic first-pass metabolism (Dada et al., 1978; Lyrenäs et al., 1981; Chetkowski et al., 1986). Although transdermal patches have avoided some of the drawbacks of oral therapy, they present additional problems, including wide interindividual variation in absorption rate (including the presence of poor absorbers) (Stanczyk et al., 1988), the loss of 4–8% of patches due to poor adhesion (Steingold et al., 1985; Amy et al., 1993), and local skin reactions (Frenkel et al., 1994; Grebe et al., 1993).

Besides bypassing hepatic presystemic metabolism, nasal administration offers the benefits of simple dose adjustments, constant absorption, and highly convenient patient administration. Moreover, nasal delivery has been explored as an alternative administration route to target drugs directly to the brain via the olfactory neurons (Illum, 2000; Mathison et al., 1998), providing more opportunities for estradiol to enter the central nervous system and then act on central nervous system (CNS) disorders, especially Alzheimer's disease. However, whether this kind of direct delivery route exists or not is still under debate. In order to clarify of this issue, we investigated the direct nose–cerebrospinal fluid (CSF) drug delivery of estradiol using a microdialysis method and the results showed that, after nasal administration, the concentration of estratiol in CSF is higher than that after intravenous administration at the same dose, thereby proving that estradiol can being transported via the olfactory neurons.

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2. Materials and methods

2.1. Materials

Estradiol (17β-estradiol) was purchased from Xianju Pharmaceutical Factory, China. Randomly methylated β-cyclodextrin (RAMEB) was obtained from Wacker-Chemie, Germany. All other reagents were of analytical grade or the highest grade commercially available.

Microdialysis probes were U-shaped and made of hollow cellulose fiber (DM-22, 200 μm inner diameter and 220 μm outer diameter, EICOM CORP, Japan) and were used for both the in vitro and in vivo studies. The membrane was 4 mm in length with a molecular weight cut-off of 5000 Da. Artificial CSF composed of 128 mM NaCl, 2.6 mM KCl, 1.26 mM $CaCl_2$ and 2 mM $MgCl_2$ was prepared using deionized distilled water. The solution was filtered through a 0.47 μm nylon filter before use.

Male Sprague–Dawley rats weighing 250–300 g were provided by the animal house of Shenyang Pharmaceutical University, China. These animals were allowed to acclimatize in environmentally controlled quarters (24 ± 1 °C and 12:12 h light–dark cycle) for at least 5 days before being used for experiments.

2.2. Preparation of RAMEB–estradiol inclusion complexes

Estradiol and RAMEB were dissolved in 95% (w/w) ethanol in a molar ratio of 1:2 to form inclusion complexes (Hermens et al., 1990) by the saturated solution method. RAMEB was used as a solubilizer to enhance the solubility of estradiol by inclusion complex formation. Ethanol was evaporated under a mild nitrogen stream (50 °C) and the inclusion complexes were dissolved in sterile saline to obtain the final estradiol formulations. The estradiol formulations had the following estradiol and RAMEB concentrations: 2 mg ml^{-1} estradiol and 2% (w/v) RAMEB for nasal delivery, and 0.01 mg ml^{-1} and 0.01% (w/v) for intravenous delivery, respectively.

2.3. Animal experiments

2.3.1. Nasal cavity isolation and jugular vein cannulation

The male Sprague–Dawley rats were anaesthetized with an intraperitoneal injection of urethane (1.2 g kg^{-1}). During the experiment, body temperature was maintained at 37 °C under an infrared lamp. The nasal cavity was isolated from the respiratory and gastrointestinal tracts using a procedure described by Hirai et al. (1981) and Huang et al. (1985). Briefly, after an incision was made in the neck, the trachea was cannulated with a polyethylene tube to maintain respiration. Another PE-200 tube was inserted through the esophagus toward the posterior part of the nasal cavity and ligated. The passage of the nasopalatine tract was sealed with an adhesive agent to prevent drainage of the solution from the nasal cavity to the mouth. A polyethylene tube was inserted into the jugular vein for intravenous injection and blood sampling.

2.3.2. Estradiol administration and collection of biological samples

For intranasal administration, the estradiol inclusion complex solution was instilled into the right nostril at a dose of 0.48 mg kg^{-1} via a microsyringe which was attached to a blunt needle. Blood samples (0.3 ml) were drawn from the tube in the jugular vein into heparin stabilized test-tubes at different times: 0, 5, 10, 20, 30, 45, 60, 90, 120, 150, 180 min. After each blood withdrawal, the same volume of sterile normal saline was put back into the circulation to maintain total blood volume. CSF perfusate samples were collected at intervals of 20 min for 4 h. Plasma was separated by centrifugation at 3000 rpm for 15 min and kept frozen at −20 °C together with CSF perfusate for subsequent analysis.

Intravenous administration was also carried out by injecting a bolus dose of 0.48 mg kg^{-1} through an indwelling jugular vein cannula. The method of sample collection was the same as above.

Both nasal and intravenous administration should be performed following the successful implantation of a microdialysis probe and stabilization for 1 h with artificial CSF.

2.4. Determination of estradiol concentration in cerebrospinal fluid (CSF) by the microdialysis method

The concentrations of the drug in the dialysate reflect the concentrations in the (extracelluar) fluid around the semipermeable part of the probe. However, as the dialysis procedure is not performed under equilibrium conditions, the concentration in the dialysate will be different from that in the periprobe fluid. The term recovery is used to describe this relationship.

2.4.1. In vitro relative recovery of the microdialysis probe determined by the zero-net flux method (ZNF)

In this study, the microdialysis probe was immersed in artificial CSF solution containing estradiol (50.20 ng ml^{-1}) as a dialysis medium and perfused at 4 μl min^{-1} with artificial CSF solution containing different concentrations of estradiol (5.02, 25.10, 50.20, 75.30, 100.40 ng ml^{-1}, C_p). Microdialysate samples (C_d, 80 μl) were collected for each concentration ($n = 3$) of perfusion solution. The concentration difference between microdialysate samples and perfusion solution ($C_d - C_p$) was plotted against the concentration in the perfusion solution (C_p). The recovery was determined from the slope of the linear regression, while the abscissa intercept represented the concentration in the medium outside the probe (Lonnroth et al., 1987).

2.4.2. Probe implantation

The rats had their skulls shaved and were placed in a stereotaxic apparatus after being anesthetized. A midline incision of approximately 2 cm was made parallel to the sagittal suture. The bregma was located and used as the reference point for positioning the microdialysis probe. A microdialysis probe was stereotaxically inserted through a cranial burr hole made by a dental drill to a depth of 3.1 mm, using the following coordinates, in relation to the bregma: 1.5 mm lateral, 0.9 mm posterior, and the probe was attached to the skull with dental cement.

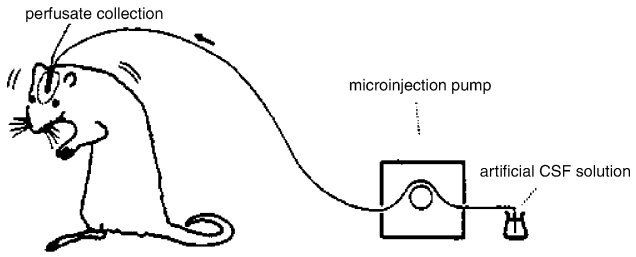

Fig. 1. Microdialysis experimental device.

2.4.3. Microdialysis procedure

The inflow to the microdialysis probe was driven by a microinjection pump (S200, KD Scientific Company, USA), perfused with artificial CSF, and the outflow was collected in small polypropylene tubes. Before and during the implantation procedure the probe was filled with artificial CSF solution at a rate of 10 μl min^{-1}. Then, 5 min after implantation of the probe, the flow rate was reduced to 4 μl min^{-1} and was maintained at this level throughout the experiment. The microdialysis experimental device is shown in Fig. 1.

A recovery time of 1 h was allowed after the insertion of the sampling probe prior to drug administration.

2.4.4. In vivo recovery

However, the in vitro simulated experiment cannot actually represent the in vivo recovery. In order to calculate the exact estradiol concentration in CSF, the in vivo recovery was measured using the retrodialysis method. Three different concentrations (5, 50, 100 ng ml^{-1}) were studied in this experiment.

2.5. Analytical method

2.5.1. Disposition of biological samples

Briefly, 0.2 ml plasma was spiked with 5 μl internal standard (ethyl hydroxybenzoate, 12.5 μg ml^{-1}) solution. The sample was then vortexed with ether (5 ml) for 5 min, and centrifuged for 5 min at 3000 rpm before the supernatant was transferred to a new glass tube and evaporated to dryness in a water bath at 40 °C under N_2 flow. The residue was dissolved in 200 μl methanol, then 50 μl was injected onto the HPLC column.

CSF perfusate samples were injected directly into the HPLC system for estradiol analysis without any pre-treatment.

2.5.2. RP-HPLC fluorescence analysis of estradiol

An HPLC method was developed and validated for estradiol assay in rat plasma samples. The HPLC equipment consisted of a HITACHI L-7110 Intelligent HPLC pump, and a HITACHI L-7200 Intelligent HPLC Autosampler, a HITACHI L-7420 Intelligent HPLC Detector, and a ANASTAR Chromatography Data System. Separation was achieved at 30 °C on a Kromasil C_{18} column (200 mm × 4.6 mm, particle size 5 μm, Zirchrom Company). The mobile phase consisted of acetonitrile–water (40:60 for plasma samples, and 50:50 for CSF samples), filtered and degassed under reduced pressure, prior to use. A guard column was used to prevent column clogging. Eluent was monitored by a fluorescence detector set at 267 nm (λ_{ex}) and 302 nm (λ_{em}), and its flow rate was 1.0 ml min^{-1}.

2.6. Data analysis

Absolute concentrations in CSF were calculated from the concentrations in the dialysates using the following equation: $C = C_d/R$, where R is the in vivo relative recovery. The area under the plasma concentration–time curve (AUC) value (0–180 min) and CSF concentration–time curve (AUC) value (0–240 min) were calculated using the trapezoidal rule. All AUC values and AUC_{CSF}/AUC_{plasma} ratios were calculated for each individual animal before the paired Student's *t*-test, using the computer program SPSS version 8.0 for Windows. Data were presented as mean ± S.D. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Analytical method

Due to the low estradiol levels in blood or, of course, in CSF, almost all of the studies (Tomas et al., 2000; Elisabeth

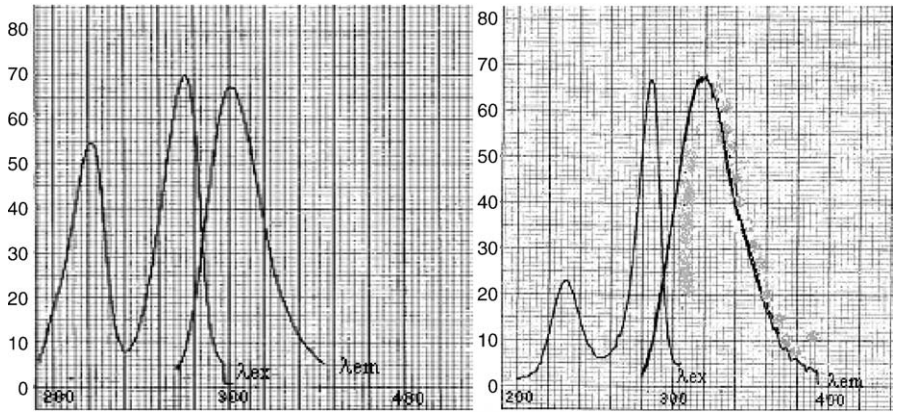

Fig. 2. The fluorescence scanning spectrum of 10 μg ml^{-1} estradiol solution (left) and ethyl hydroxybenzoate solution (right).

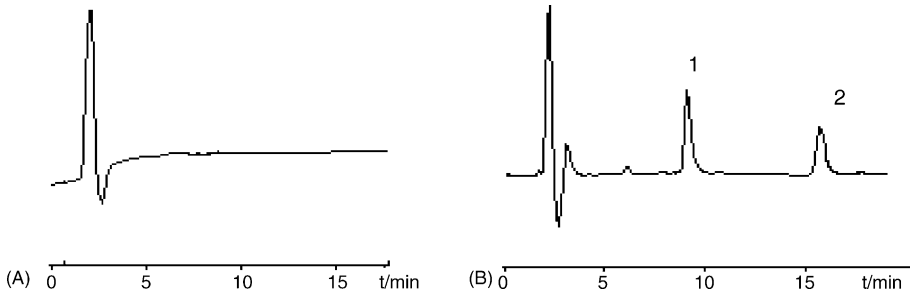

Fig. 3. HPLC chromatogram of estradiol in rat plasma. (A) Blank plasma; (B) plasma sample (1, estradiol; 2, ethyl hydroxybenzoate).

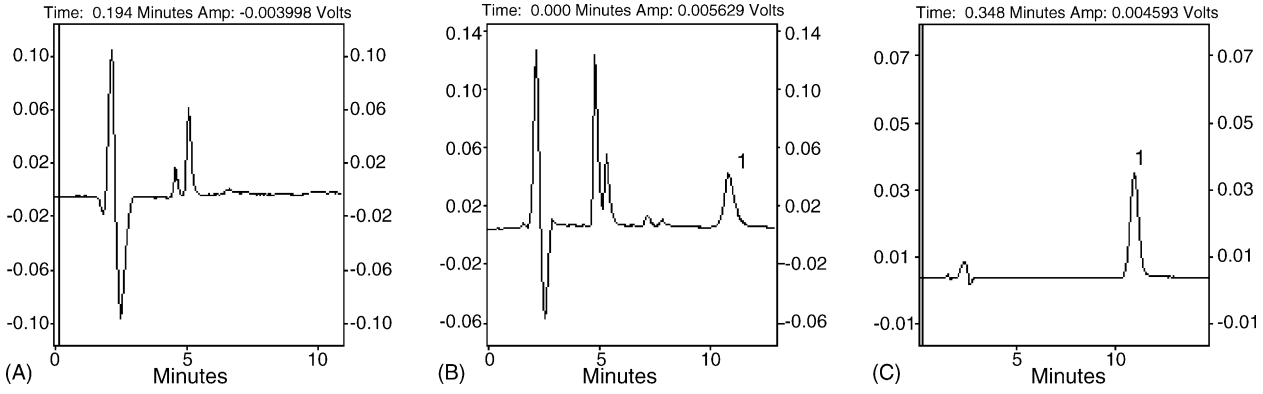

Fig. 4. HPLC chromatogram of estradiol in CSF. (A) Blank CSF; (B) sample; (C) estradiol peaks: 1, estradiol.

et al., 1999; Asko et al., 2001; Asko et al., 2000; Mascha et al., 2004) have used radioimmunoassay (RIA) to determine the estradiol concentration in biological samples. However, an RP-HPLC with fluorescence detection was used in this study, due to the characteristic fluorescence absorption of estradiol.

3.1.1. Determination of detection wavelength

The fluorescence scanning spectrum (Fig. 2) show that estradiol exhibits fluorescence absorption, and the wavelengths of excitation and emission were 267 nm and 302 nm, respectively, while ethyl hydroxybenzoate (IS) has a maximal absorption at corresponding wavelengths.

3.1.2. Representative chromatograms of estradiol in plasma and CSF

As shown in Figs. 3 and 4, estradiol in plasma and CSF can be detected sensitively without any interference, confirming that the analytical method used was suitable.

3.2. In vitro recovery of microdialysis probe

According to Table 1, the concentration difference between microdialysate samples and perfusion solution ($C_d - C_p$) was plotted against the concentration in the perfusion solution (C_p), shown in Fig. 5.

The linear regression function was: $C_d - C_p = -0.6164C_p + 32.153$, $r = 0.9979$. The in vitro recovery was 61.64%, determined from the slope of the linear regression, while the abscissa intercept concentration (estradiol in the medium outside the probe) was 52.16 ng ml^{-1}, in agreement with the concentration of estradiol in the dialysis medium (50.20 ng ml^{-1}).

3.3. In vivo recovery

The results are shown in Table 2. Obviously, the recovery in vivo is much lower than that in vitro. Using the recovery in vitro will lead to incorrect results.

Table 1
E_2 concentration in the microdialysis solution ($n = 3$)

Probe number	E_2 concentration (ng ml^{-1}, C_p)				
	5.02	25.10	50.20	75.30	100.40
1	34.96 ± 0.90	41.51 ± 0.62	48.15 ± 1.00	61.00 ± 0.94	71.27 ± 0.69
2 (C_d)	35.54 ± 0.70	41.05 ± 1.32	48.58 ± 0.90	62.82 ± 0.85	71.42 ± 0.86
3	35.35 ± 1.13	42.81 ± 1.84	49.58 ± 1.07	61.29 ± 0.94	71.57 ± 0.60
Mean	35.28	41.79	48.77	61.71	71.42
S.D.	0.30	0.91	0.73	0.98	0.15

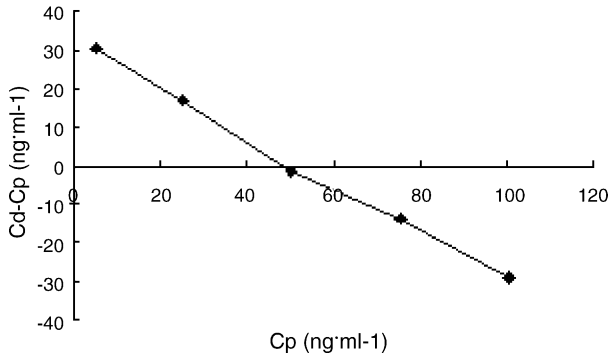

Fig. 5. The curve of $C_d - C_p$ and C_p ($n = 3$).

Table 2
In vivo recovery of E_2 using retrodialysis method ($n = 3$)

Rat number	E_2 concentration (ng ml^{-1}, C_p)		
	5.02	50.20	100.40
1	41.06 ± 9.87	41.05 ± 12.34	41.97 ± 7.46
2	43.19 ± 10.14	39.87 ± 10.11	40.96 ± 9.53
3	42.11 ± 8.75	39.41 ± 9.47	45.68 ± 8.37
Mean	42.12 ± 7.64	40.11 ± 10.04	42.87 ± 7.44

3.4. In vivo absorption studies

To determine whether or not estradiol is transported from the nasal cavity into the CSF via the olfactory neurons, estradiol was administered intranasally and intravenously in the same set of rats. Estradiol reached a C_{max} (mean ± S.D.) at 20 min in plasma after intranasal administration (26.70 ± 11.37 ng ml^{-1}; Fig. 6), while in CSF, estradiol had a C_{max} of 54.76 ± 32.84 ng ml^{-1} (Fig. 7) at 20 min. For intravenous delivery, the C_{max} (170.08 ± 64.67 ng ml^{-1}) were reached at 5 min in plasma (Fig. 6) but delayed to 60 min in CSF (26.48 ± 11.34 ng ml^{-1}; Fig. 7).

Table 3 summarizes the CSF-to-plasma estradiol AUC ratios following intravenous and intranasal administration. The AUC_{CSF}/AUC_{plasma} ratio (1.60 ± 0.67; Table 3) after intranasal delivery differed significantly from the ratio (0.61 ± 0.16; Table 3) observed after intravenous infusion ($P < 0.05$).

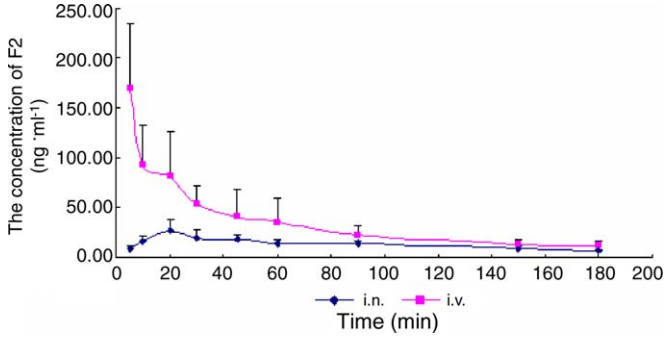

Fig. 6. Mean plasma concentration–time curves of estradiol in rats after i.n. and i.v. administration of estradiol at the dose of 0.48 mg kg^{-1} ($n = 5$).

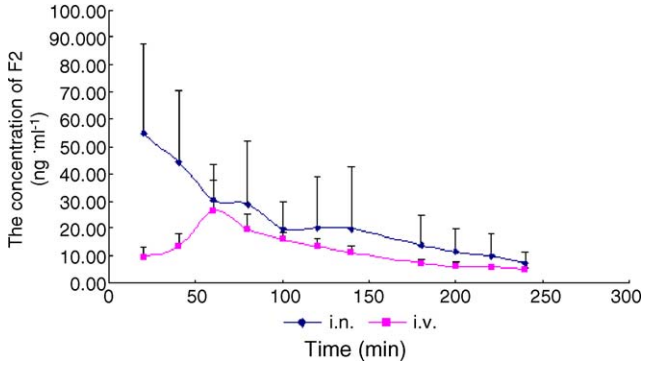

Fig. 7. Mean CSF concentration–time curves of estradiol in rats after i.n. and i.v. administration of estradiol at the dose of 0.48 mg kg^{-1} ($n = 5$).

Table 3
AUC_{CSF}/AUC_{plasma} ratios of estradiol after intranasal delivery (i.n.) and intravenous administration (i.v.) in rats (mean ± S.D., $n = 5$) ($P < 0.05$)

	Estradiol	
	i.n.	i.v.
AUC_{CSF} (ng min ml^{-1})	6573.76 ± 3506.22	4771.03 ± 1037.51
AUC_{plasma} (ng min ml^{-1})	4117.24 ± 602.50	7844.61 ± 2783.13
AUC_{CSF}/AUC_{plasma}	1.60 ± 0.67	0.61 ± 0.16

4. Discussion

Most of literatures describes an RIA method for the determination of estradiol concentrations in biological samples, including blood and CSF samples (Tomas et al., 2000; Elisabeth et al., 1999; Asko et al., 2000, 2001; Mascha et al., 2004). Although immunoassays are highly sensitive, the assay itself is time-consuming, and the antibodies often exhibit undesirable cross-reactivity, leading to erroneous results in some matrices. In addition, the RIAs require disposal of radioactive material. Compared with RIA, the RP-HPLC with fluorescence detector method, described in this paper, is much more suitable for estradiol studies because of its speed, low cost and safety. The method was validated in terms of linearity and assay range, accuracy, recovery, precision and stability of biological samples.

Microdialysis was originally developed to measure concentrations of endogenous substances in the extracellular fluid (ECF) of normal brain (Bito et al., 1966), particularly neurotransmitters (Westerink, 1995), and it has since become an important tool to investigate the disposition of many classes of drugs (Elmquist and Sawchuk, 1997; Hansen et al., 1999).

The two CSF sampling methods used most often are cisternal puncture (Waynforth and Flecknell, 1980; Chou and Levy, 1981; Chou and Donovan, 1997) and cisternal cannulation method (Sarna et al., 1983; Kornhuber et al., 1986; Westergren and Johansson, 1991), which are invasive methods. Both approaches sample CSF from the cisterna magna, the largest CSF compartment lying between the cerebellum and the upper vertebrae. The former method is terminal for the animal, while the second has the advantage that anaesthesia is not required for CSF sampling. However, the cannulation method is highly susceptible to infections and cannulas are easily blocked. Another disadvantage of

both methods is the high risk of contamination of the CSF samples with red blood cells. The microdialysis technique, on the other hand, eliminates the removal of significant CSF volumes during the experiment, and the implantation of microdialysis probes is less invasive.

The effects of flow rate and concentration surrounding the dialysis probe on the in vitro relative recovery of estradiol were investigated. It can be seen that the relative recovery of estradiol decreased as the flow rate increased, while it remained constant with fluctuations in drug concentration. After thorough consideration of the recovery and sample volume collected, we chose $4\,\mu l\,min^{-1}$ as the perfusion rate, and 20 min as the sampling interval.

Anand Kumar et al. (1974) have reported a direct transport route from the nasal cavity into the CSF for estradiol when they investigated the uptake of estradiol into the CSF after intranasal and intravenous administration in monkeys. From these studies the authors concluded that direct uptake of estradiol occurred from the nasal cavity into the CSF. Estradiol was dissolved in pure propylene glycol (PG) (Anand Kumar et al., 1974), however, the estradiol RAMEB inclusion complex was used in the present study. Unfortunately, the AUC_{CSF}/AUC_{plasma} ratios after both intranasal and intravenous delivery of the steroid were not measured in their study, although this is a well-accepted approach to determine an additional contribution of the nose–CSF pathway to the uptake of drugs into the CSF after nasal delivery in comparison with intravenous administration (Hussain et al., 1990; Chou and Donovan, 1998; Chow et al., 1999; Dahlin and Björk, 2000; Chow et al., 2001). In the present study, the AUC_{CSF}/AUC_{plasma} ratio after intranasal delivery differed significantly from the ratio observed after intravenous infusion ($P < 0.05$), which is in contrast to the results of Mascha (Mascha et al., 2004), probably resulting from different methods of sampling the CSF. This indicates that a large fraction of the estradiol dose is rapidly absorbed into the CSF via olfactory neurons instead of being absorbed into the systemic circulation.

Initially, a hypothesis was proposed that, to exert CNS activity following nasal drug application, 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 estradiol in the CSF following nasal administration. It can be seen from Fig. 7 that the t_{max} in the CSF was much shorter after nasal administration than after intravenous infusion (20 min, i.n.; 60 min, i.v.), which suggests that this hypothesis is incorrect, and there is a direct nasal to brain pathway for estradiol. Moreover, the C_{max} in CSF was two-fold higher than that after intravenous administration, showing that after nasal delivery a large fraction of the estradiol dose reaches the CSF instead of the systemic circulation. Again, this confirms the existence of a direct transport route from the nasal cavity into the CSF for estradiol.

References

Amy, J.J., Balmer, J.A., Baumarten, K., et al., 1993. A randomized study to compare the effectiveness, tolerability and acceptability of two different transderma; estradiol replacement therapies: the transdermal HRT Investigators Group. Int. J. Fertil. Menopausal. Stud. 38, 5–11.

Anand Kumar, T.C., David, G.F.X., Umberkoman, B., Saini, K.D., 1974. Uptake of radioactivity by body fluids and tissues in rhesus monkeys after intravenous injection or intranasal spray of tritium-labelled oestradiol and progesterone. Curr. Sci. 43, 435–439.

Asko, Järvinen, Granander, Marjo, Laine, Tarmo, et al., 2000. Effect of dose on the absorption of estradiol from a transdermal gel. Maturitas 35, 51–56.

Asko, Järvinen, Bäckström, Ann-Christine, Elfström, Charlotta, et al., 2001. Comparative absorption and variability in absorption of estradiol from a transdermal gel and a novel matrix-type transdermal patch. Maturitas 38, 189–196.

Bito, L., Davson, H., Levin, E., Murray, M., Snider, N., 1966. The concentrations of free amino acids and other electrolytes in cerebrospinal fluid, in vivo dialysate of brain, and blood plasma of the dog. J. Neurochem. 13, 1057–1067.

Chetkowski, R.J., Meldrum, D.R., Steingold, K.A., et al., 1986. Biologic effects of transdermal estradiol. N. Engl. J. Med. 314, 1615–1620.

Chou, K.J., Donovan, M.D., 1997. Distribution of antihistamines into the CSF following intranasal delivery. Biopharm. Drug Dispos. 18, 335–346.

Chou, K.J., Donovan, M.D., 1998. Lidocaine distribution into the CNS following nasal and arterial delivery: a comparison of local sampling and microdialysis techniques. Int. J. Pharm. 171, 53–61.

Chou, R.C., Levy, G., 1981. Effect of heparin or salicylate infusion on serum protein binding and on concentrations of phenytoin in serum, brain and cerebrospinal fluid of rats. J. Pharmacol. Exp. Ther. 219, 42–48.

Chow, H.H.S., Chen, Z., Matsuura, G.T., 1999. Direct transport of cocaine from the nasal cavity to the brain following intranasal cocaine administration in rats. J. Pharm. Sci. 88, 754–758.

Chow, H.H.S., Anavy, N., Villalobos, A., 2001. Direct nose–brain transport of benzoylecgonine following intranasal administration in rats. J. Pharm. Sci. 90, 1729–1735.

Dada, O.A., Laumas, V., Landgren, B.-M., Cekan, S.Z., Diczfalusy, E., 1978. Effect of graded oral doses of oestradiol on circulating hormonal levels. Acta Endocrinol. 88, 754–767.

Dahlin, M., Björk, E., 2000. Nasal absorption of (S)-UH-301 and its transport into the cerebrospinal fluid of rats. Int. J. Pharm. 195, 197–205.

De Lignieres, B., Basdevant, A., Thomas, G., Thalabard, J.C., Mercier-Bodard, C., Conard, J., 1986. Biological effects of estradiol-17β in postmenopausal women: oral versus percutaneous administration. J. Clin. Endocrinol. Metab. 62, 536–541.

Elisabeth, S., Westlund, P., Landgren, B.-M., et al., 1999. Bioavailability of norethisterone acetate alone and in combination with estradiol administered in single or multiple oral doses to postmenopausal women. Maturitas 33, 59–69.

Elmquist, W.F., Sawchuk, R.J., 1997. Application of microdialysis in pharmacokinetic studies. Pharm. Res. 14, 267–288.

Frenkel, Y., Kopernik, G., Lazer, S., et al., 1994. Acceptability and skin reactions to transdermal estrogen replacement therapy in relation to climate. Maturitas 20, 31–36.

Grebe, S.K., Adams, J.D., Feek, C.M., 1993. Systemic sensitization to ethanol by transdermal estrogen patches. Arch. Dermatol. 129, 379–380.

Hansen, D.K., Davies, M.I., Lunte, S.M., Lunte, C.E., 1999. Pharmacokinetic and metabolism studies using microdialysis sampling. J. Pharm. Sci. 88, 14–27.

Hermens, W.A.J.J., Deurloo, M.J.M., Romeijn, S.G., Verhoef, J.C., Merkus, F.W.H.M., 1990. Nasal absorption enhancement of 17-b-oestradiol by dimethyl-β-cyclodextrin in rabbits and rats. Pharm. Res. 7, 500–503.

Hirai, S., Yashiki, T., Matsuzawa, T., Mima, H., 1981. Absorption of drugs from the nasal mucosa. Int. J. Pharm. 7, 317–325.

Huang, C.H., Kimura, R., Bawarshi-Nassar, R., Hussain, A., 1985. Mechanism of nasal absorption of drugs. I. Physicochemical parameters influencing the rate of in situ nasal absorption of drugs in rats. J. Pharm. Sci. 74, 608–611.

Hussain, M.A., Rakestraw, D., Rowe, S., Aungst, B.J., 1990. Nasal administration of a cognition enhancer provides improved bioavailability but not enhanced brain delivery. J. Pharm. Sci. 79, 771–772.

Illum, L., 2000. Transport of drugs from the nasal cavity to the central nervous system. Eur. J. Pharm. Sci. 11, 1–18.

Kornhuber, M.E., Kornhuber, J., Cimniak, U., 1986. A method for repeated CSF sampling in the freely moving rat. J. Neurosci. Meth. 17, 63–68.

Kupperman, H.S., Blatt, M.H.G., Wiesbader, H., Filler, W., 1953. Comparative clinical evaluation of estrogenic preparations by the manopausal and amenorrheal indices. J. Clin. Endocrinol. 13, 688–703.

Lonnroth, P., Jansson, P.A., Smith, U., 1987. A microdialysis method allowing characterization of intercellular water space in humans. Am. J. Physiol. 253, 228–231.

Lyrenäs, S., Carlström, K., Bäckström, T., von Schoultz, B., 1981. A comparison of serum oestrogen levels after percutaneous and oral administration of oestradiol-17b. Br. J. Obstet. Gynecol. 88, 181–187.

Mascha, P., van den Berg, J., Coos Verhoef, Romeijn, Stefan G., et al., 2004. Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats. Eur. J. Pharm. Biopharm. 58, 131–135.

Mathison, S., Nagilla, R., Kompella, U.B., 1998. Nasal route for direct delivery of solutes to the central nervous system: fact or fiction? J. Drug Target. 5, 415–441.

O'Connel, M.B., 1995. Pharmacokinetic and pharmacologic variation between different estrogen products. J. Clin. Pharmacol. 35, 18–24.

Sarna, G.S., Hutson, P.H., Tricklebank, M.D., Curzon, G., 1983. Determination of brain 5-hydroxytryptamine turnover in freely moving rats using repeated sampling of cerebrospinal fluid. J. Neurochem. 40, 383–388.

Stanczyk, F.Z., Shoupe, D., Nunez, V., Macias-Gonzales, P., Vijod, M.A., Lobo, R.A., 1988. A randomized comparison of nonoral estradiol delivery in postmenopausal women. Am. J. Obstet. Gynecol. 159, 1540–1546.

Steingold, K.A., Laufer, L., Chetkowski, J., et al., 1985. Treatment of hot flushes with transdermal estradiol administration. J. Clin. Endocrinol. Metab. 61, 627–632.

Tomas, L.G., Andersson, Stehle, Brigitte, Davidsson, Bertil, et al., 2000. Bioavailability of estradiol from two matrix transdermal delivery systems: Menorest® and Climara®. Maturitas 34, 57–64.

Waynforth, H.B., Flecknell, P.A., 1980. Cisternal puncture and Intracisternal Injection. Academic Press, London, pp. 59–61.

Westergren, I., Johansson, B.B., 1991. Changes in physiological parameters of rat cerebrospinal fluid during chronic sampling: evaluation of two sampling methods. Brain Res. Bull. 27, 283–286.

Westerink, B.H., 1995. Brain microdialysis and its application for the study of animal behaviour. Behav. Brain Res. 70, 103–124.