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Research paper

Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats

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Abstract

The uptake of estradiol and progesterone into the cerebrospinal fluid (CSF) after intranasal and intravenous administration in rats was investigated. Each animal received estradiol intranasally (40 μg/rat) and by intravenous infusion (10 μg/rat) into the jugular vein using a vascular access port. Hereafter, the same set of rats was treated with progesterone intranasally (200 μg/rat) and by intravenous infusion (104 μg/rat). Following nasal delivery, both steroid hormones reach C_{max} values in plasma and CSF at 15 min after administration. Intravenous infusion of estradiol and progesterone shows comparable plasma and CSF concentration–time profiles compared to the nasal route. For both hormones the AUC_{CSF}/AUC_{plasma} ratios (mean ± SD) after intranasal delivery (estradiol 2.3 ± 1.1%; progesterone 1.9 ± 0.7%) do not differ significantly from the ratios shown after intravenous infusion (estradiol 2.0 ± 0.6%; progesterone 2.2 ± 0.8%). These results indicate that after nasal delivery estradiol and progesterone are rapidly absorbed into the systemic circulation, from where the non-protein bound hormones probably enter the CSF by crossing the blood–brain barrier. No extra direct nose–CSF transport could be demonstrated.

Keywords: Estradiol; Progesterone; Intranasal delivery; Intravenous delivery; Nose–cerebrospinal fluid drug transport

1. Introduction

Targeting of drugs to the central nervous system is still a difficult task to fulfil. This is due to the tight blood–brain barrier, which prevents the influx of xenobiotic compounds from the systemic circulation into the brain. In order to overcome this barrier, nasal delivery has been explored as an alternative administration route to target drugs directly to the brain via the olfactory neurons [1,2]. However, whether this direct delivery route is effective or not is still under debate.

For direct nose–cerebrospinal fluid (CSF) drug delivery the reported studies by Anand Kumar and co-workers with the female steroid hormones, estradiol and progesterone, are often referred to as evidence of a direct transport route from the nasal cavity into the CSF for such compounds [3–7]. These hormones are involved in the physiological regulation of reproduction and used in contraceptives and as drugs for the treatment of menopausal symptoms. Due to a high first-pass effect in the gastrointestinal tract and liver, both hormones have a low bioavailability after oral administration. Therefore, alternative administration routes such as transdermal [8,9] and intranasal delivery [10,11] have been investigated. The physiological production of estradiol and progesterone is subject to neuro-endocrine regulation in the brain [12], and from this perspective Anand Kumar et al. investigated the uptake of both steroids into the CSF after intranasal and intravenous administration in monkeys [3–7]. From these studies the authors concluded that a direct uptake of both steroid hormones occurred from the nasal cavity into the CSF. The fact that these studies are often cited in literature, show their importance in this research area. However, in the authors' opinion their experimental conditions used for nasal drug delivery were rather aggressive. The steroid hormones were dissolved in either pure propylene glycol (PG) [3] or several mixtures of ethanol, PG and water (1:1:3 and 3:3:4) [4–7]. Moreover, the estradiol and progesterone formulations were administered

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to monkeys using an atomiser, a device which sprayed the formulation into the nasal cavity with a very high pressure (0.25 kg/cm^2) and a gas flow rate of about 62.5 ml/s for a time period of 10–60 s [3,4]. Besides, the AUC_{CSF}/AUC_{serum} ratios after both intranasal and intravenous delivery of the steroids were not measured, 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 [13–18].

In the present study the possibility of direct nose–CSF transport for estradiol and progesterone was investigated by comparing the relative distribution of these drugs over CSF and plasma after both intranasal and intravenous administration in rats. In order to dissolve the lipophilic steroids in saline, randomly methylated β-cyclodextrin (RAMEB) was used as solubiliser. RAMEB is able to form an inclusion complex with lipophilic drugs, thereby enhancing their solubility in aqueous solutions [19]. Nasal delivery in rats was subsequently performed by drop application of the steroid formulations. In order to monitor the distribution of the administered drug a recently developed rat model was used, which enables serial and simultaneous sampling of CSF and plasma [20]. The CSF sampling technique is not terminal for the animals and therefore the same set of rats can be used for both the intranasal and the intravenous routes of drug administration [18].

2. Materials and methods

2.1. Materials

Estradiol (17β-estradiol) and progesterone were purchased from Sigma Chemical (St Louis, MO, USA) and randomly methylated β-cyclodextrin (RAMEB; degree of substitution 1.8) from Wacker-Chemie (Krommenie, The Netherlands). All other reagents were of analytical grade or highest grade commercially available.

2.2. Drug formulations

Estradiol and progesterone were dissolved in ethanol with RAMEB (molar ratio 1:2) to form inclusion complexes [21]. Ethanol was evaporated under a mild nitrogen stream (35 °C) and the inclusion complexes were dissolved in sterile saline to obtain the final estradiol and progesterone formulations. The estradiol formulations contained the following estradiol and RAMEB concentrations: 2 mg/ml and 2% (w/v) for nasal delivery and 0.01 mg/ml and 0.01% (w/v) for intravenous infusion. For the progesterone (mg/ml) formulations these concentrations were 10 mg/ml and 9% (w/v) for nasal delivery and 0.1 mg/ml and 0.09% (w/v) for intravenous infusion.

2.3. Animals

Male Wistar rats (Charles River, Someren, The Netherlands) weighing 250–340 g at the start of the experiments were used. The animals ($n = 7$) were housed separately with free access to food and water with a 12-h light/dark cycle. All animal experiments were approved by the Ethical Committee for Animal Experiments (Leiden University).

2.4. Implantation of vascular access port (VAP)

The animals were provided with a VAP as described before [18]. Briefly, the rats were anaesthetised with Hypnorm® (0.5 ml/kg) and Dormicum® (0.5 ml/kg) intramuscularly. Two incisions were made, one at the level of the lower ribs to create a pocket for inserting the VAP (Access Technologies, Skokie, IL, USA) and one in the neck to cannulate the jugular vein. The VAP, attached to a silicone catheter (ID 0.5 mm, OD 1.0 mm), was filled with heparin solution (400 IU/ml) using a Huberpoint needle (Access Technologies, Skokie, IL, USA) and fitted into the pocket. The catheter was tunnelled underneath the skin from the pocket to the second incision in the neck and inserted into the jugular vein. As post-operative care, Temgesic® (0.3 ml/kg, intramuscularly) was given for pain relief. The rats were allowed to recover 1 week before starting the experiments. To avoid blockage of the catheter, the VAP was flushed weekly with heparin solution (400 μl; 400 IU/ml).

2.5. Nasal and intravenous delivery

Estradiol was delivered intranasally (40 μg/20 μl per rat) into the left nostril using a polyvinylchloride (PVC) tube attached to a Hamilton syringe, while the animal was fixed in a stereotaxic frame in the supine-70° angle position [20]. Intravenous infusion of estradiol (67 μl/min for 15 min (10 μg/rat)) was performed into the jugular vein using the VAP as previously described [18]. In between experiments the rats were allowed to recover for 1 week. Hereafter the same set of rats was treated with progesterone intranasally (200 μg/20 μl per rat) and by intravenous infusion (69 μl/min for 15 min (104 μg/rat)) following the same methods and time schedule.

The intravenous infusion rates for estradiol and progesterone were chosen in such a way as to simulate the observed maximal steroid plasma levels after intranasal delivery. This infusion rate was determined by giving the rats ($n = 3$) an intravenous bolus injection of the steroid hormone as described previously [18].

Prior to and following drug delivery, blood and CSF samples were taken until 120 min after each administration. Each rat received the nasal and intravenous treatment for both estradiol and progesterone.

2.6. Blood and CSF sampling

Blood samples (200 μl) were taken from the tail vein using heparinised tubes (Microvette® CB 100/200, Sarstedt, Nümbrecht, Germany). Samples were centrifuged (15 min at 14,000 rpm; ambient temperature) and the plasma obtained was stored at 4 °C until analysis.

For CSF sampling a cisternal puncture was performed as described before [20]. Briefly, rats were anaesthetised as mentioned above and fixed in a stereotaxic frame using the supine-70° angle position. The cisternal puncture was performed 5.2–6.5 mm ventrally from the occipital crest, dependent on the rat's weight. After the puncture, one drop of CSF was microscopically examined for erythrocyte content; the experiment was continued when the erythrocyte contamination was less than 500 cells/μl (<0.01% of normal blood content). Following intranasal or intravenous drug administration, CSF samples (30–40 μl) were taken and directly collected in pre-weighed antibody-coated radioimmunoassay tubes and stored at 4 °C until analysis.

2.7. Estradiol and progesterone analysis

Plasma and CSF samples were analysed for estradiol or progesterone by radioimmunoassay (Coat-A-Count® Estradiol and Progesterone RIA kits, DPC, Los Angeles, CA, USA) with detection limits of 8 and 20 pg/ml, respectively. The analyses were performed according to the manufacturer's protocol. When calculating the estradiol and progesterone concentrations for the CSF samples, the different sample volumes were taken into account.

2.8. Data analysis

The area under the plasma or CSF concentration–time curve (AUC) values (0–120 min) were calculated using the trapezoidal rule. All AUC values and AUC_{CSF}/AUC_{plasma} ratios were calculated for each individual animal before determining mean values. Data were analysed according to the paired Student's *t*-test, using the computer program SPSS version 8.0 for Windows.

3. Results

To determine whether or not estradiol and progesterone are transported from the nasal cavity into the CSF via the olfactory neurons, these steroid hormones were administered intranasally and intravenously in the same set of rats. Both estradiol and progesterone reached a C_{max} (mean ± SD) in plasma at 15 min after intranasal administration (17.3 ± 2.2 and 63.0 ± 12 ng/ml, respectively; Figs. 1a and 2a). In CSF the C_{max} values for estradiol and progesterone were about 50-fold lower (0.32 ± 0.15 and 1.16 ± 0.39 ng/ml at $t = 15$ min, respectively) compared to the observed plasma levels (Figs. 1b and 2b).

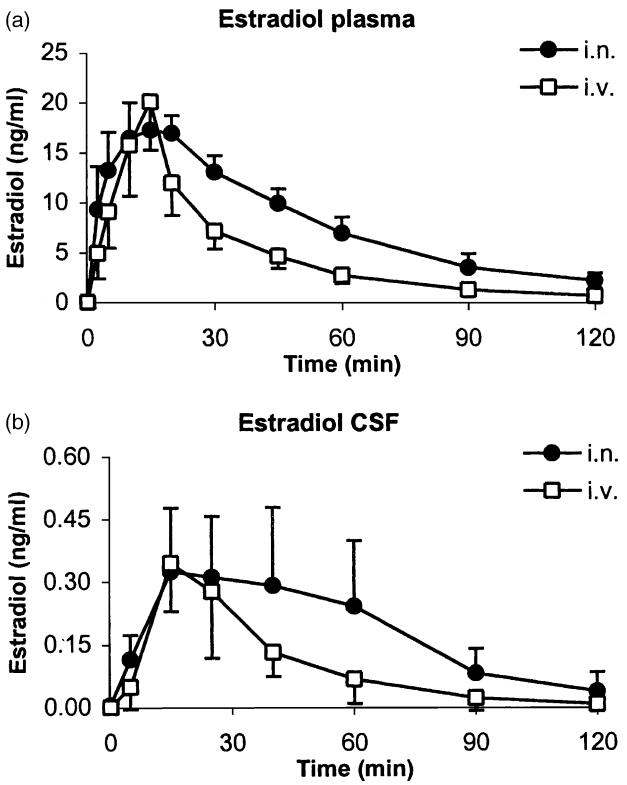

Fig. 1. Estradiol plasma (a) and CSF (b) concentrations after intranasal (i.n.; 40 μg/rat) and intravenous (i.v.; 10 μg/rat) administration in rats. Data are presented as mean ± SD of seven animals.

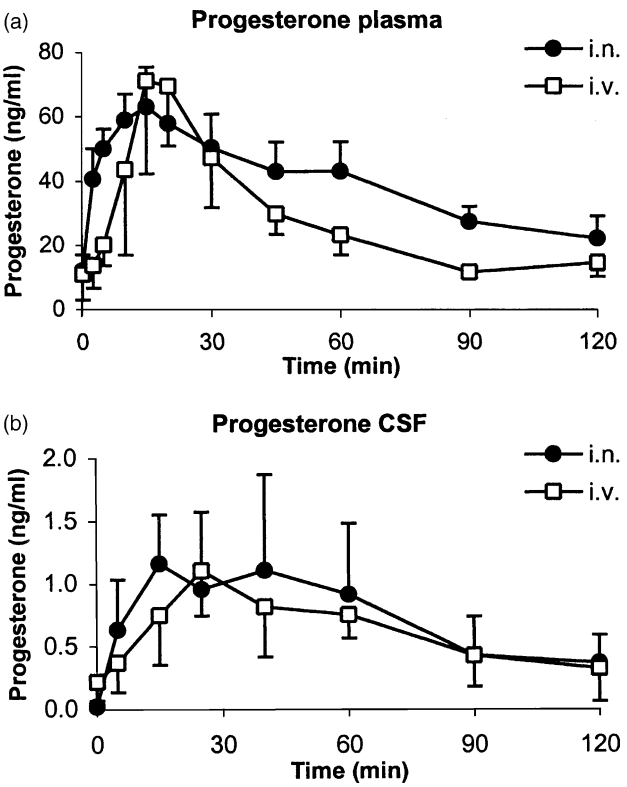

Fig. 2. Progesterone plasma (a) and CSF (b) concentrations after intranasal (i.n.; 200 μg/rat) and intravenous (i.v.; 104 μg/rat) administration in rats. Data are presented as mean ± SD of seven animals.

Table 1
AUC_{CSF}/AUC_{plasma} ratios of estradiol and progesterone after intranasal delivery (i.n.) and intravenous infusion (i.v.) in rats

	Estradiol		Progesterone	
	i.n.	i.v.	i.n.	i.v.
AUC_{CSF} (ng min/ml)	22.1 ± 10.6	12.0 ± 5.5	94.8 ± 44.9	74.3 ± 27.4
AUC_{plasma} (ng min/ml)	975 ± 91	583 ± 113	4739 ± 790	3333 ± 544
AUC_{CSF}/AUC_{plasma} (%)	2.3 ± 1.1	2.0 ± 0.6	1.9 ± 0.7	2.2 ± 0.8

Data are presented as mean ± SD ($n = 7$).

Intravenous infusion of estradiol and progesterone showed comparable plasma and CSF concentration–time profiles compared to the nasal route of administration for these steroid hormones.

For both hormones the AUC_{CSF}/AUC_{plasma} ratios after intranasal delivery (estradiol: 2.3 ± 1.1%; progesterone: 1.9 ± 0.7%) did not differ significantly from the ratios observed after intravenous infusion (estradiol: 2.0 ± 0.6%; progesterone: 2.2 ± 0.8%; Table 1).

4. Discussion

In the present study similar AUC_{CSF}/AUC_{plasma} ratios are observed for estradiol and progesterone after nasal and intravenous administration to rats, which demonstrates that there is no additional uptake of these hormones from the nasal cavity into the CSF. This finding is supported by a study investigating nasal estradiol in monkeys measuring plasma and CSF profiles [22]. However, this is in contrast to the observations in monkeys by the research group of Anand Kumar [3–7]. A remarkable feature in their studies is the composition of the nasal formulations and the used delivery method. The formulations contained mixtures of ethanol, PG and water (1:1:3 and 3:3:4) [4–7] or even pure PG [3] to dissolve the steroid hormones. PG and ethanol are widely used as solvents and drug excipients [23]. However, PG may also cause local irritation of the mucous membranes. The atomiser, used as delivery device, blows the formulation into the nose with a great force (0.25 kg/cm^2) which is continued for a time period of 10–60 s [3,4]. Furthermore, experimental groups of only 2–3 animals were used [3,4,6,7], which is too few to prove significant differences between groups. The transport of progesterone via the nose–CSF pathway in monkeys was based on increased AUC_{CSF} and AUC_{serum} values, following intranasal delivery compared to intravenous administration [4,5,7]. In our view simultaneously elevated AUC values in blood and in CSF after nasal delivery is an indication that the uptake of progesterone into CSF occurs via the blood–brain barrier, rather than via direct transport between the nasal cavity and the CSF. Finally, the concentration (C) ratio C_{CSF}/C_{serum} was reported for only a few time points [3,6], not offering a representative drug distribution profile after nasal or intravenous delivery. Taken together, the aggressive delivery method and the experimental design of these studies are responsible for the so-called nose–CSF transport of estradiol and progesterone in monkeys.

In the present study the distribution of estradiol and progesterone over plasma and CSF was determined using a previously described rat model [18], which has the advantage of simultaneous and serial sampling of blood and CSF. In order to diminish the inter-animal variability, the steroid hormones were delivered both intranasally and intravenously in the same animal. The observed AUC values and AUC_{CSF}/AUC_{plasma} ratios (Table 1) show that the distribution of these drugs over CSF and plasma are similar after both routes of administration. This indicates that after nasal delivery estradiol and progesterone are rapidly absorbed into the systemic circulation, from where the non-protein bound hormones probably subsequently enter the CSF by crossing the blood–brain barrier. Moreover, nasal estradiol and progesterone formulations containing RAMEB in similar concentrations as described here, are not toxic for ciliary movement [24]. Also in humans during a treatment period of 1 year ($n > 1400$) the safety of a RAMEB-estradiol formulation has been demonstrated [25]. This indicates that the nasal estradiol and progesterone formulations used in the present study are not aggressive.

A transport to the CSF via the blood–brain barrier has also been reported for low molecular weight and lipophilic compounds such as a cognition enhancing drug [13], a serotonin antagonist [16] and the steroid hormone hydrocortisone [18], and for the high molecular weight and hydrophilic vitamin B_{12} analogue hydroxocobalamin [26]. Nevertheless, other reports claim a direct nose–CSF pathway for lipophilic (lidocaine [14] and the antihistamine hydroxyzine [27]) and hydrophilic (zidovudine [28], L-dopa prodrugs [29], cephalexin [30]) low molecular weight drugs. This demonstrates that there is still no unambiguous answer to the question whether or not a direct nose-to-CSF/brain transport route exists.

In conclusion, the present study examined the uptake of estradiol and progesterone into the CSF after nasal and intravenous delivery in rats. No significant differences in AUC_{CSF}/AUC_{plasma} ratios were found after both routes of administration. This proves there is no direct nose-to-CSF transport of these steroid hormones in rats after nasal administration.

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