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In-vivo and In-vitro Evidence of a Carrier-mediated Efflux Transport System for Oestrone-3-sulphate across the Blood–Cerebrospinal Fluid Barrier

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Abstract

The efflux transport of oestrone-3-sulphate, a steroid hormone sulphate, across the blood–cerebrospinal fluid barrier has been examined following its intracerebroventricular administration. [^{3}H]Oestrone-3-sulphate was eliminated from cerebrospinal fluid (CSF) with an apparent efflux clearance of 205 μL min^{-1} per rat. There was 25% of unmetabolized [^{3}H]oestrone-3-sulphate in the plasma 5 min after intracerebroventricular administration, indicating that at least a part of [^{3}H]oestrone-3-sulphate is transported from CSF to the circulating blood across the blood–CSF barrier. This efflux transport was inhibited by co-administration of excess oestrone-3-sulphate (25 mM10 μL = 0·25 μmol) into rat cerebral ventricle.

To characterize the oestrone-3-sulphate transport process, an in-vitro uptake experiment was performed using isolated rat choroid plexus. Oestrone-3-sulphate uptake by isolated rat choroid plexus was found to be a saturable process with a Michaelis-Menten constant (K_m) of 18·1 ± 6·3 μM, and a maximum uptake rate (V_{max}) of 48·0 ± 15·1 pmol min^{-1} μL^{-1} of tissue. The oestrone-3-sulphate transport process was temperature dependent and was inhibited by metabolic inhibitors such as 2,4-dinitrophenol and rotenone, suggesting an energy dependence. This uptake process was also inhibited by steroid hormone sulphates (1 mM dehydroepiandrosterone sulphate and 1 mM oestrone sulphate), bile acids (1 mM taurocholic acid and 1 mM cholic acid) and organic anions (1 mM sulphobromophthalein and 1 mM phenolsulphonphthalein), whereas 1 mM *p*-aminohippuric acid, 1 mM *p*-nitrophenol sulphate, 0·1 mM methotrexate and the cardiac glycoside, 2·5 μM digoxin, had little effect.

In conclusion, these results provide evidence that oestrone-3-sulphate is transported from CSF to the circulating blood across the blood–CSF barrier via a carrier-mediated efflux transport system.

Oestrone-3-sulphate, the sulphate conjugate of oestrone, is the most abundant oestrogen in plasma, at a concentration of 0·3–0·9 μg mL^{-1} (Kishimoto 1973). Although the physiological role of oestrone-3-sulphate in the brain is still unknown, it has been used to treat senile dementia of the Alzheimer's type as a form of oestrogen replacement therapy (Honjoh et al 1989). It is important to clarify the disposition of oestrone-3-sulphate in the brain in order to understand the mechanism of oestrogen replacement therapy. Oestrone-3-sulphate undergoes very limited distribution to the brain compared with oestrone (Kishimoto 1973; Steingold et al 1986), and the percentage of oestrone and oestrone-3-sulphate passing into the brain, estimated by the brain uptake index method, has been found to be about 100% and 6·5%, respectively (Steingold et al 1986). This evidence suggests that the blood–brain barrier and blood–cerebrospinal fluid (CSF) barrier may hinder oestrone-3-sulphate transport from the circulating blood to the brain. This has

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been investigated by developing the brain efflux index method (Kakee et al 1996) and it has been shown that the blood-brain barrier has several efflux transport systems for P-glycoprotein (Tsuji et al 1992) and organic anions such as *p*-aminohippuric acid (Kakee et al 1997), 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (DDI) (Takasawa et al 1997a) and taurocholic acid (Kitazawa et al 1998). Moreover, there are active organic anion efflux transport systems from CSF to blood, located in the choroid plexus, for benzylpenicillin (Suzuki et al 1987), *p*-aminohippuric acid (Holloway & Cassin 1972), AZT, DDI (Takasawa et al 1997b) and quinolone antibiotics (Ooie et al 1996). This supports the hypothesis that the choroid plexus does not act as a static wall but as an efflux pump for transporting organic anions from CSF to blood. Based on the distribution of oestrone-3-sulphate in the brain, therefore, it is important to investigate the mechanism of oestrone-3-sulphate transport across the blood–CSF barrier since this may play a role in the influx and efflux of both endogenous and exogenous substrates.

The purpose of the present study is to characterize the transport of oestrone-3-sulphate at the blood–CSF barrier in-vivo and using isolated choroid plexus.

Materials and Methods

Animals and reagents

Male Wistar rats weighing 250–300 g were purchased from Charles River (Yokohama, Japan). The present study was approved by the Tohoku University Animal Care Committee. [6,7-^{3}H(N)] Oestrone-3-sulphate ammonium salt ([^{3}H]oestrone-3-sulphate, 53 Ci mmol^{-1}) and [carboxyl-^{14}C]inulin ([^{14}C]inulin, 2·3 mCi g^{-1}) were purchased from DuPont NEN (Boston, MA). [^{14}C]butanol (2 mCi mmol^{-1}) was from American Radiolabeled Chemicals (St Louis, MO). Oestrone, sodium taurocholate, sodium cholate, *p*-aminohippuric acid and 2,4-dinitrophenol were purchased from Wako Pure Chemical (Osaka, Japan); sulphobromophthalein sodium hydrate was from Nacalai Tesque Inc. (Kyoto, Japan); phenolsulphonphthalein was from Tokyo Kasei (Tokyo, Japan); oestrone-3-sulphate, digoxin, rotenone and xylazine hydrochloride were from Sigma Chemical Co. (St Louis, MO); Ketaral 50 (ketamine hydrochloride) was used as an anaesthetic and obtained from Sankyo Co. (Tokyo, Japan). All other chemicals were of reagent grade available commercially.

Efflux of oestrone-3-sulphate from the CSF after intracerebroventricular administration

The elimination of [^{3}H]oestrone-3-sulphate after intracerebroventricular administration was studied by the method described previously (Takasawa et al 1997b). Male Wistar rats were anaesthetized by intramuscular administration of ketamine hydrochloride (235 mg kg^{-1}) and xylazine (2·3 mg kg^{-1}) and their heads were fixed in a stereotaxic apparatus (Narishige Co., Tokyo, Japan). An 18-gauge needle connected to silastic tubing was inserted into the left lateral ventricle through a hole drilled in the skull. [^{3}H]Oestrone-3-sulphate (0·520 μCi) and [^{14}C]inulin (0·0129 μCi) were dissolved in 10 μL of ECF buffer containing (in mM): 122 NaCl, 25 $NaHCO_3$, 3 KCl, 0·4 K_2HPO_4, 1·4 $CaCl_2$, 1·2 $MgCl_2$, 10 D-glucose and 10 HEPES at pH 7·4 and administered through the cannula. CSF (50–100 μL) was withdrawn by cisternal puncture at 2, 10, 15 and 20 min after administration. The kinetic parameters for the elimination of [^{3}H]oestrone-3-sulphate and [^{14}C]inulin were determined from equation 1 (Ogawa et al 1994) using the nonlinear least-square regression analysis program, MULTI (Yamaoka et al 1981).

$$C_{CSF}(t) = dose/Vd \times \exp(-k_{el} \times t) \qquad (1)$$

where C_{CSF} (t), Vd and k_{el} are the CSF concentration at time t, the volume of distribution and the elimination rate constant, respectively, of either [^{3}H]oestrone-3-sulphate or [^{14}C]inulin. Apparent elimination clearance value was determined to multiply k_{el} by Vd ($k_{el} \times Vd$). The radioactivities due to [^{3}H]oestrone-3-sulphate and [^{14}C]inulin in the CSF was determined using a liquid scintillation counter (LSC 5000, Aloka, Tokyo, Japan).

Metabolism of [^{3}H]oestrone-3-sulphate after intracerebroventricular administration

The amount of [^{3}H]oestrone-3-sulphate metabolite in the plasma after 5 min intracerebroventricular administration of [^{3}H]oestrone-3-sulphate (8 μCi 10 μL^{-1}) was determined by high-performance liquid chromatography (HPLC). Blood samples were collected via the ipsilateral jugular vein. Plasma (1 mL) was mixed with 2·5 mL of tetrahydrofuran and 1 g of NaCl and then centrifuged for 15 min at 310 × *g* at 4°C. The supernatant was evaporated under a stream of nitrogen gas and reconstituted in 3 mL mobile phase. A portion (250 μL) of each sample was injected into the HPLC system, which consisted of a pump (LC-6A, Shimadzu, Kyoto, Japan), a controller (SCL-6A, Shimadzu) and a UV detector (SPD-6A, Shi-

madzu). A reversed-phase column, TSK gel ODS-80 (15·0 cm × 4·6 mm i.d., Toso, Tokyo, Japan) was used. The mobile phase was $OHCH_3$0·1% trifluoroacetic acid (55 : 45 (v/v)) and pumped at a flow rate of 1·0 mL min^{-1}. Both unlabelled oestrone-3-sulphate and oestrone were monitored at 267 nm. The radioactivity in the eluent (500 μL) was assayed as described above.

Choroid plexus uptake study

The uptake of [^{3}H]oestrone-3-sulphate by the isolated rat choroid plexus was examined using the centrifugal filtration method described previously in detail (Suzuki et al 1987). The rats were decapitated and the choroid plexus was isolated from the lateral ventricles. The isolated choroid plexus was incubated in a vial containing 300 μL of the ECF buffer at 37°C as described previously (Suzuki et al 1987). After pre-incubation for 1 min at 37°C, [^{3}H]oestrone-3-sulphate and [^{14}C]butanol, in the presence or absence of inhibitors, were added simultaneously to initiate the experiment. The final concentration of [^{3}H]oestrone-3-sulphate and [^{14}C]butanol was 1·60 μCi mL^{-1} and 0·40 μCi mL^{-1}, respectively. The temperature dependence was examined at 4°C. Na^+-free ECF buffer was prepared by equimolar replacement of NaCl and $NaHCO_3$ with choline chloride and choline bicarbonate, respectively. Cl^--free ECF buffer was prepared by equimolar replacement of NaCl, KCl, $CaCl_2$ and $MgCl_2$ with sodium gluconate, potassium gluconate, calcium gluconate and $MgSO_4$, respectively. The tissue-to-medium (T/M) ratio of [^{3}H]oestrone-3-sulphate was calculated using [^{14}C]butanol as a cell volume marker because butanol is quickly distributed into choroid plexus (the mean cell volume of choroid plexus was 1·79 ± 0·08 μL per choroid plexus). The adherent water space of [^{3}H]oestrone-3-sulphate was corrected by using [^{14}C]inulin; the T/M ratio of inulin is 0·1. The T/M ratio of [^{3}H]oestrone-3-sulphate was calculated by equation 2 (Suzuki et al 1987).

$$\text{T/M ratio} = (\text{apparent T/M ratio} - 0{\cdot}1)/(1 - 0{\cdot}1) \quad (2)$$

The purity of [^{3}H]oestrone-3-sulphate in the incubation medium was determined by means of HPLC as described above and was greater than 97% throughout the experiments.

Kinetic analysis

For kinetic studies of oestrone-3-sulphate uptake by the choroid plexus, the Michaelis-Menten constant (K_m), maximal uptake rate (V_{max}) and the non-saturable uptake clearance (P_{non}), including adsorption to the choroid plexus, were calculated from equation 3 using MULTI (Yamaoka et al 1981)

$$\text{T/M ratio at 3 min} = \{V_{max}/(K_m + C) + P_{non}\} \times 3\ \text{min} \quad (3)$$

where C is the concentration of oestrone-3-sulphate in the incubation medium.

An unpaired, two-tailed Student's *t*-test was used to determine the significance of differences between two group means. Statistical significance among means of more than two groups was determined by one-way analysis of variance followed by modified Fisher's least-squares difference method.

Results

Efflux of [^{3}H]oestrone-3-sulphate from the CSF after intracerebroventricular administration

Figure 1 shows the time-courses of the percentage dose of [^{3}H]oestrone-3-sulphate and [^{14}C]inulin remaining in the CSF after intracerebroventricular administration. The k_{el} of [^{3}H]oestrone-3-sulphate and [^{14}C]inulin was 0·177 ± 0·045 and 0·0143 ± 0·0060 min^{-1}, and Vd was 1308 ± 743 and 350 ± 28·4 μL per rat, respectively. The apparent elimination clearance of [^{3}H]oestrone-3-sulphate and [^{14}C]inulin from the cerebral ventricle was estimated to be 205 and 5·63 μL min^{-1} per rat, respectively. Following co-administration of unlabelled oestrone-3-sulphate (10 μL × 25 mM oestrone-3-sulphate) into rat ventricle, the CSF concentration of [^{3}H]oestrone-3-sulphate at 20 min was 102·7 ± 15·7 (% dose) mL^{-1} and significantly increased compared with a tracer dose of [^{3}H]oestrone-3-sulphate (3·80 ± 2·73 (% dose) mL^{-1}) ($P < 0{\cdot}01$), indicating the saturation of efflux for [^{3}H]oestrone-3-sulphate.

Metabolism of [^{3}H]oestrone-3-sulphate after intracerebroventricular administration

Figure 2 illustrates the HPLC chromatogram of [^{3}H]oestrone-3-sulphate in ipsilateral jugular vein plasma 5 min after intracerebroventricular administration. The metabolite of [^{3}H]oestrone-3-sulphate was present in the ipsilateral jugular vein plasma. The percentage of unmetabolized [^{3}H]oestrone-3-sulphate found in ipsilateral jugular vein plasma 5 min after intracerebroventricular administration was 25%, indicating that at least part of the [^{3}H]oestrone-3-sulphate was transported

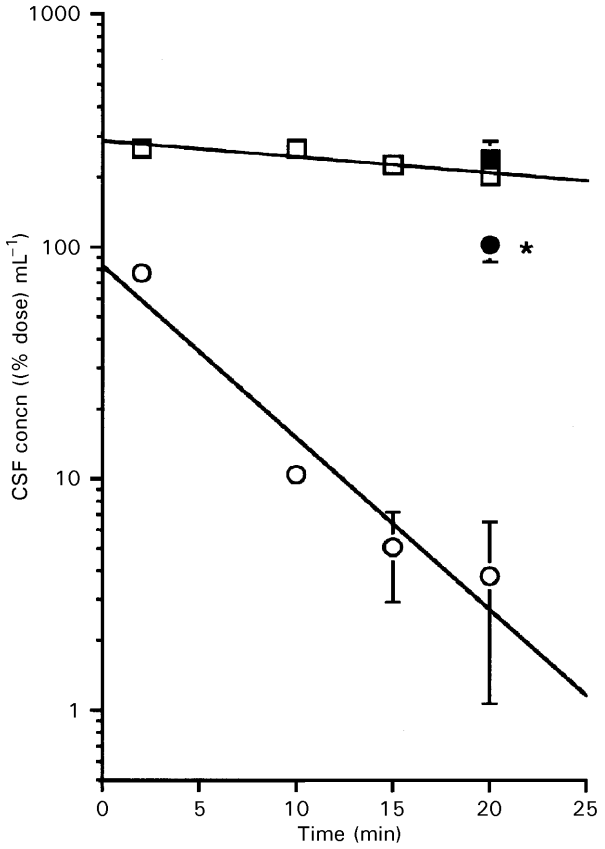

Figure 1. Cerebrospinal fluid concentration profiles of [^{3}H]oestrone-3-sulphate and [^{14}C]inulin after intracerebroventricular administration to rats. Each point represents the mean ± s.e.m. of 3–4 rats. A tracer dose of [^{3}H]oestrone-3-sulphate (○: 0·520 μCi per head) and [^{14}C]inulin (□: 0·0129 μCi per head) was administered into the rat ventricle. ●, ■: [^{3}H]oestrone-3-sulphate and [^{14}C]inulin, respectively, in the case of co-administration of unlabelled oestrone-3-sulphate (10 μL × 25 mM) into the rat ventricle. *$P < 0·01$, compared with a tracer dose of [^{3}H]oestrone-3-sulphate.

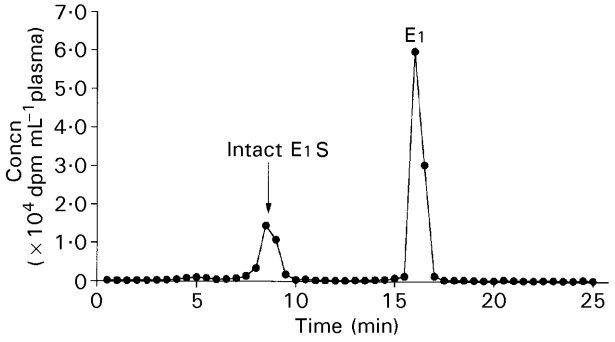

Figure 2. Typical HPLC chromatogram of the ipsilateral jugular vein plasma 5 min after intracerebroventricular administration of [^{3}H]oestrone-3-sulphate (8 μCi per head) to rats. E_1S, oestrone-3-sulphate; E_1, oestrone.

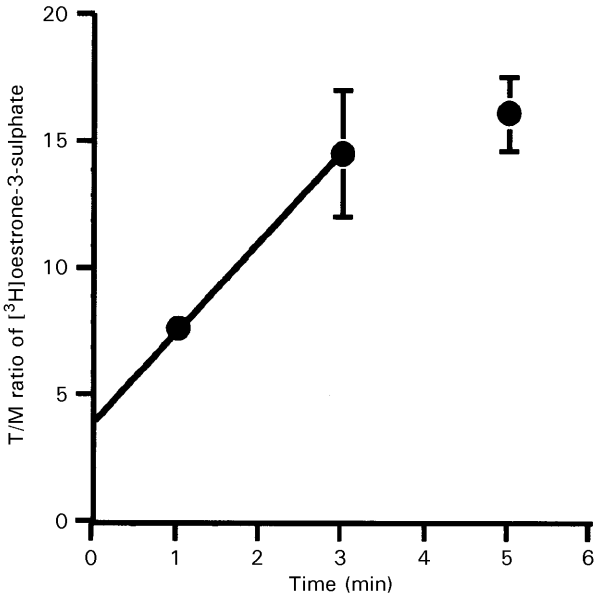

Figure 3. Time-course of [^{3}H]oestrone-3-sulphate uptake by isolated rat choroid plexus. T/M ratio = tissue-to-medium ratio. Choroid plexus was incubated at 37°C in medium containing [^{3}H]oestrone-3-sulphate in the presence of 0·03 μM oestrone-3-sulphate. Each point represents the mean ± s.e.m. of 3 experiments.

from CSF to the circulating blood across the blood–CSF barrier.

Uptake of [^{3}H]oestrone-3-sulphate by rat choroid plexus

To characterize oestrone-3-sulphate efflux transport process across the blood–CSF barrier in-vitro, [^{3}H]oestrone-3-sulphate uptake by isolated rat choroid plexus was investigated. The time-course of [^{3}H]oestrone-3-sulphate uptake at 1, 3 and 5 min is shown in Figure 3. There was no significant difference between the T/M ratio of [^{3}H]oestrone-3-sulphate at 3 min and 5 min. Thus, the initial uptake clearance of oestrone-3-sulphate was estimated from the slope of the T/M ratio of [^{3}H]oestrone-3-sulphate at 1 min and 3 min and was $3·44 \pm 2·53$ μL min^{-1} (μL tissue)$^{-1}$. Extrapolating this line to zero yielded a positive intercept, which indicates the initial adsorption of [^{3}H]oestrone-3-sulphate to the choroid plexus, which was determined to be $4·25 \pm 3·34$. The extent of the initial adsorption was lower than the T/M ratio at 3 min.

Inhibitory effect of oestrone-3-sulphate on [^{3}H]oestrone-3-sulphate uptake

Figure 4 shows the inhibitory effect of oestrone-3-sulphate on [^{3}H]oestrone-3-sulphate uptake at 3 min (i.e., concentration-dependence of the [^{3}H]oestrone-3-sulphate uptake). The T/M ratio of [^{3}H]oestrone-3-sulphate uptake at 3 min decreased

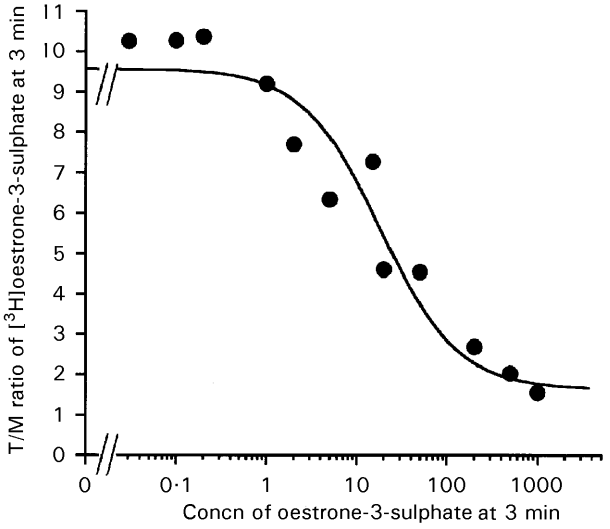

Figure 4. Inhibitory effect of oestrone-3-sulphate on the [^{3}H]oestrone-3-sulphate uptake by isolated rat choroid plexus. Each point represents a single experiment. The solid line was estimated from equation 3 using nonlinear least-square regression analysis program. The K_m, V_{max} and P_{non} value were $18{\cdot}1 \pm 6{\cdot}3\ \mu$M, $48{\cdot}0 \pm 15{\cdot}1$ pmol min^{-1} (μL tissue)$^{-1}$, and $0{\cdot}545 \pm 0{\cdot}074\ \mu$L min^{-1} ($\mu$L tissue)$^{-1}$, respectively.

on increasing the amount of unlabelled oestrone-3-sulphate in the incubation medium, indicating a concentration-dependent behaviour. Nonlinear least-squares regression analysis provided a K_m of $18{\cdot}1 \pm 6{\cdot}3\ \mu$M, a V_{max} of $48{\cdot}0 \pm 15{\cdot}1$ pmol min^{-1} (μL tissue)$^{-1}$ and a P_{non} of $0{\cdot}545 \pm 0{\cdot}074\ \mu$L min^{-1} ($\mu$L tissue)$^{-1}$.

Effect of various conditions on [^{3}H]oestrone-3-sulphate uptake

The effect of metabolic inhibitors, incubation temperature, and Na^+-free and Cl^--free conditions on [^{3}H]oestrone-3-sulphate uptake by isolated rat choroid plexus is summarized in Table 1. Conditions involving 2,4-dinitrophenol at 1 mM, rotenone at 0·025 mM and a temperature of 4°C reduced [^{3}H]oestrone-3-sulphate uptake by 38·2% ($P < 0{\cdot}05$), 73·5% ($P < 0{\cdot}01$) and 79·3% ($P < 0{\cdot}01$), respectively, whereas both Na^+-free and Cl^-- free conditions had no effect on [^{3}H]oestrone-3-sulphate uptake.

Table 1. Effect of various conditions on [^{3}H]oestrone-3-sulphate uptake by rat isolated choroid plexus.

Condition	% of control
Control	100·0 ± 4·0
2,4-Dinitrophenol (1 mM)	61·8 ± 2·2*
Rotenone (0·025 mM)	26·5 ± 2·0**
4°C	20·7 ± 4·1**
Na^+-free†	82·8 ± 7·3
Cl^--free†	80·8 ± 12·8

Choroid plexus was incubated with [^{3}H]oestrone-3-sulphate (0·03 μM) for 3 min at 37°C in the presence or absence of inhibitors. Each value represents the mean ± s.e.m. of 3–4 experiments. †Na^+-free and Cl^--free conditions were prepared by replacement with choline and gluconate, respectively. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, compared with control.

Effect of several inhibitors on [^{3}H]oestrone-3-sulphate uptake

The steroid hormone sulphate, dehydroepiandrosterone sulphate (DHEAS; 1 mM), reduced [^{3}H]oestrone-3-sulphate uptake by 85·3% ($P < 0{\cdot}001$; Table 2). Unlabelled oestrone-3-sulphate (1 mM) reduced it by 84·2% ($P < 0{\cdot}01$). Oestrone (0·1 mM) in 0·05% DMSO reduced oestrone-3-sulphate uptake by 44% compared with the [^{3}H]oestrone-3-sulphate control in 0·05% DMSO ($P < 0{\cdot}05$). Bile acids (1 mM), such as sodium taurocholate and sodium cholate, reduced oestrone-3-sulphate uptake by 63·6% ($P < 0{\cdot}05$) and 70·1% ($P < 0{\cdot}01$), respectively. Organic anions (1 mM), such as sulfobromophthalein and phenolsulfonphthalein, also reduced oestrone-3-sulphate uptake by 79·3% ($P < 0{\cdot}01$) and 67·8% ($P < 0{\cdot}05$), respectively. Other organic anions such as *p*-aminohippuric acid and *p*-nitrophenol sulphate (1 mM), and methotrexate (0·1 mM), did not affect the oestrone-3-sulphate uptake. The cardiac glycoside, digoxin (0·0025 mM), reduced oestrone-3-sulphate uptake by 28%, but this effect was not significantly different from that of the control ($P > 0{\cdot}05$). The volume for choroid plexus estimated by [^{14}C]butanol in the presence of inhibitors was not significantly different from that of control (data not shown), indicating that the inhibitors did not affect the integrity of the choroid plexus.

Table 2. Effect of steroid hormone sulphate, bile acids, organic anions and cardiac glycosides on [^{3}H]oestrone-3-sulphate uptake by rat isolated choroid plexus.

Inhibitor	Concn (mM)	% of control
Control		100·0 ± 4·0
Oestrone-3-sulphate	1	15·8 ± 2·6***
Dehydroepiandrosterone sulphate	1	14·7 ± 1·3***
Oestrone (in 0·05% DMSO)	0·1	56·0 ± 8·7*†
Sodium taurocholate	1	36·4 ± 2·8*
Sodium cholate	1	29·1 ± 3·6**
Sulfobromophthalein	1	20·7 ± 3·2**
Phenolsulfophthalein	1	32·2 ± 9·1**
p-Aminohippuric acid	1	98·2 ± 19·7
p-Nitrophenol sulphate	1	102·5 ± 2·5
Methotrexate	0·1	83·6 ± 2·6
Digoxin	0·0025	71·9 ± 23·6

Choroid plexus was incubated with [^{3}H]oestrone-3-sulphate (0·03 μM) for 3 min at 37°C in the presence or absence of inhibitors. Each value represents the mean ± s.e.m. of 3–4 experiments. †Control experiments were performed using 0·05% DMSO. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$, compared with control.

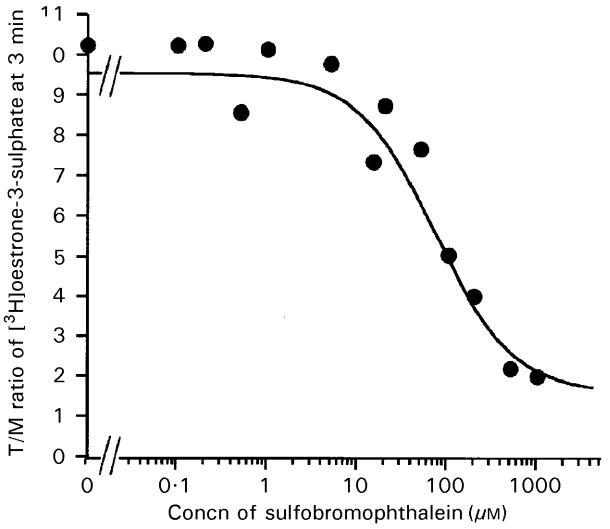

Figure 5. Inhibitory effect of sulfobromophthalein on [^{3}H]oestrone-3-sulphate uptake by isolated rat choroid plexus. T/M ratio = tissue-to-medium ratio. Each point represents a single experiment. The IC50 value was $71{\cdot}8 \pm 9{\cdot}8\ \mu$M.

The T/M ratio of [^{3}H]oestrone-3-sulphate at 3 min fell on increasing the unlabelled sulfobromophthalein concentration, with a 50% inhibition concentration (IC50) of $71{\cdot}8 \pm 9{\cdot}8\ \mu$M (Figure 5).

Discussion

This study demonstrates that oestrone-3-sulphate is transported by a carrier-mediated efflux transport process from the CSF to the circulating blood across the blood–CSF barrier. [^{3}H]Oestrone-3-sulphate was eliminated from CSF with a half-life of 4·1 min after intracerebroventricular administration (Figure 1). The maximal efflux clearance of [^{3}H]oestrone-3-sulphate across the blood–CSF barrier was $232\ \mu$L min^{-1} per rat, 46·3-fold greater than that of [^{14}C]inulin ($5{\cdot}01\ \mu$L min^{-1} per rat), reflecting the bulk flow rate of CSF (Figure 1). Recently, Shimada et al (1999) were able to show that oestrone is present in rat brain using gas chromatography-tandem mass spectrometry. Although it has not been determined yet whether oestrone-3-sulphate is present in the brain, one possibility is that oestrone-3-sulphate concentration in the brain remains very low due to the efflux process at the blood-CSF barrier (Figure 1). About 25% of oestrone-3-sulphate was detected in intact form in ipsilateral jugular vein blood after intracerebroventricular administration of [^{3}H]oestrone-3-sulphate (Figure 2). The efflux clearance of [^{3}H]oestrone-3-sulphate is 11·6-fold greater than that of [^{14}C]inulin. The remaining 75% was in the form of metabolite, oestrone, which is a desulphated product of oestrone-3-sulphate. This finding can be explained by the fact that oestrone sulphatase, which converts oestrone-3-sulphate to oestrone, is located in choroid plexus (Kawano & Aikawa 1987). In addition, our data showed that [^{3}H]oestrone-3-sulphate efflux from CSF was saturated by excess cold oestrone-3-sulphate ($10\ \mu$L × 25 mM oestrone-3-sulphate). The concentration of cold oestrone-3-sulphate in CSF is estimated to be about 1 mM due to the dilution in the CSF (25 mM injection volume ($10\ \mu$L)/volume of CSF ($250\ \mu$L) = 1 mM) (Figure 1). These results support the hypothesis that oestrone-3-sulphate is transported by a carrier-mediated efflux transport process in the choroid plexus. To characterize the oestrone-3-sulphate efflux transport process via the blood-CSF barrier, a [^{3}H]oestrone-3-sulphate uptake study was performed using freshly isolated rat choroid plexus. The initial uptake rate of [^{3}H]oestrone-3-sulphate was $3{\cdot}44 \pm 2{\cdot}53\ \mu$L min^{-1} ($\mu$L tissue)$^{-1}$ in the choroid plexus (Figure 3). Thus, the clearance in-vivo was estimated to be $20{\cdot}6\ \mu$L min^{-1} per rat ($3{\cdot}44\ \mu$L min^{-1} (μL tissue)$^{-1}$ × $6\ \mu$L per rat, since the volume of total rat choroid plexus is $6\ \mu$L per rat in the lateral, third and fourth cerebral ventricles) (Ogawa et al 1994). This was 10% of the total elimination ($205\ \mu$L min^{-1} per rat) in-vivo.

The oestrone-3-sulphate uptake by choroid plexus was temperature, energy (Table 1) and concentration dependent with a K_m of $18{\cdot}1\ \mu$M (Figure 4), suggesting carrier-mediated active transport. The K_m value in this study was identical to the K_m for oestrone-3-sulphate uptake by isolated rat hepatocytes ($16\ \mu$M) (Hassen et al 1996). OATP- and oatp-cRNA, which are human and rat organic anion transporting polypeptide (Jacquemin et al 1994), respectively, when injected into *Xenopus laevis* oocytes, mediated oestrone-3-sulphate uptake with a K_m of $59\ \mu$M and $4{\cdot}5\ \mu$M, respectively (Bossuyt et al 1996a, b). The [^{3}H]oestrone-3-sulphate uptake by the isolated rat choroid plexus was significantly inhibited by 1 mM TCA, CA, BSP and DHEAS (Table 2). This type of inhibition was in good agreement with that of oatp- and OATP-mediated steroid hormone transport in the liver (Hagenbuch & Meier 1996; Kanai et al 1996; Kullak-Ublick et al 1998). Based on kinetic inhibition studies, the IC50 value of sulphobromophthalein for oestrone-3-sulphate uptake by isolated choroid plexus was $71{\cdot}8\ \mu$M (Figure 5) and similar to the K_m for sulphobromophthalein uptake in the OATP-cRNA-injected *Xenopus laevis* oocytes ($20\ \mu$M) (Kullak-Ublick et al 1995), since sulphobromophthalein is a better substrate for oatp

rather than other organic anion transporters (Jacquemin et al 1994). The Na^+- and Cl^--independent oestrone-3-sulphate uptake process in the rat choroid plexus also suggested an oatp-mediated transport process (Table 1) since oatp has been demonstrated to act as an organic anion/glutathione-exchanger in a Cl^--independent process (Li et al 1998). Moreover, confocal microscopy has showed that oatp is localized in the brush border side of the rat choroid plexus (Angeletti et al 1997). The participation of oatp2 (Noé et al 1997), which is also located in the rat choroid plexus (Abe et al 1998), cannot be ruled out entirely since digoxin partially inhibited oestrone-3-sulphate uptake (Table 2). However, other organic anion transporters such as OAT1 (Sekine et al 1997), *p*-nitrophenol sulphate transport system (Sakuma-Sawada et al 1997) and OAT-K1 (Saito et al 1996) may not involve oestrone-3-sulphate efflux transport in the rat choroid plexus due to the lack of inhibition by their respective substrates such as *p*-aminohippuric acid, *p*-nitrophenol sulphate and methotrexate, respectively (Table 2).

In conclusion, there is a significant oestrone-3-sulphate efflux transport process in the choroid plexus which most likely involves an oatp-mediated active transport process. This efflux process in the choroid plexus may involve pumping steroid hormone sulphate from the CSF into the blood.

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