0013-7227/01/$03.00/0
Endocrinology
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Vol. 142, No. 6
Printed in U.S.A.

Multidrug Resistance P-Glycoprotein Hampers the Access of Cortisol But Not of Corticosterone to Mouse and Human Brain

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ABSTRACT

In the present study, we investigated the role of the multidrug resistance (mdr) P-glycoprotein (Pgp) at the blood-brain barrier in the control of access of cortisol and corticosterone to the mouse and human brain.

[^{3}H]Cortisol poorly penetrated the brain of adrenalectomized wild-type mice, but the uptake was 3.5-fold enhanced after disruption of Pgp expression in mdr 1a$^{-/-}$ mice. In sharp contrast, treatment with [^{3}H]corticosterone revealed high labeling of brain tissue without difference between both genotypes.

Interestingly, human MDR1 Pgp also differentially transported cortisol and corticosterone. LLC-PK1 monolayers stably transfected with MDR1 complementary DNA showed polar transport of [^{3}H]cortisol that could be blocked by a specific Pgp blocker, whereas [^{3}H]corticosterone transport did not differ between transfected and host cells.

Determination of the concentration of both steroids in extracts of human postmortem brain tissue using liquid chromatography mass spectrometry revealed that the ratio of corticosterone over cortisol in the brain was significantly increased relative to plasma.

In conclusion, the data demonstrate that in both mouse and human brain the penetration of cortisol is less than that of corticosterone. This finding suggests a more prominent role for corticosterone in control of human brain function than hitherto recognized. (*Endocrinology* **142:** 2686–2694, 2001)

THE NATURALLY OCCURRING glucocorticoid in rodents, corticosterone, readily gains access to the brain and accumulates particularly in cell nuclei of limbic brain areas such as the hippocampus, septum, and amygdala (1–4). In these brain areas, corticosterone is retained by mineralocorticoid receptors (MR) that bind corticosterone with a 10-fold higher affinity than glucocorticoid receptors (GR) (5). In contrast, the synthetic glucocorticoid dexamethasone, when administered in tracer doses to adrenalectomized rats or mice, is poorly retained in glucocorticoid target areas in the brain (3, 6–9). Uptake and retention in the anterior pituitary is very high, although both brain and pituitary express similar amounts of GR.

These observations raised the possibility that the blood brain barrier (BBB) limits the access of dexamethasone to the brain (3, 8, 9). Recently, it was indeed demonstrated that the penetration of dexamethasone into the brain is hampered because the multidrug resistance 1a (mdr1a) P-glycoprotein (Pgp) excludes this exogenous compound from mouse brain (10, 11). The drug-transporting Pgp is expressed at the luminal membranes of endothelial cells of the BBB (12, 13). This transmembrane protein is encoded by the *mdr1a* gene in rodents and by the highly homologous *MDR1* gene in humans (14, 15).

Received November 13, 2000.
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Thus, Pgp may explain why moderate amounts of dexamethasone primarily act at the anterior pituitary level to suppress stress-induced ACTH release (3). In contrast, in rodents corticosterone primarily acts on centrally regulated functions underlying behavioral adaptation and the activity of the hypothalamic-pituitary-adrenal axis (16–18). In many other species, cortisol is the principal endogenous glucocorticoid; *e.g.* in human blood, cortisol circulates in 10- to 20-fold higher levels than corticosterone (19–22). As a naturally occurring glucocorticoid, cortisol is commonly accepted to exert similar actions in human brain as corticosterone does in rat and mouse brain. However, although it has a high affinity for MR, a tracer dose of cortisol has been reported to be poorly retained in cell nuclei of rat hippocampi (7). This may not be surprising, because rat and mouse lack the 17α-hydroxylase needed for synthesis of cortisol, which therefore makes this steroid exogenous in these species and hence, as Pgp is known to transport many unrelated but all exogenous compounds (15), a potential target for Pgp-mediated export from the brain.

In the present study, we have first tested the hypothesis that mdr1a Pgp at the mouse BBB limits *in vivo* brain penetration of cortisol. For this purpose we have used adrenalectomized mdr1a null and wild-type mice injected with a tracer dose of [^{3}H]cortisol or [^{3}H]corticosterone, which freely crosses the BBB. In addition, we have investigated whether a species difference exists between the multidrug resistance Pgp of mouse and man, which would allow free entrance of cortisol into the human brain. To explore this possibility, we have examined the corticosteroid transport capabilities of

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monolayers of human MDR1-transfected porcine LLC-PK1 cells compared with nontransfected LLC-PK1 cells. To examine the *in vivo* effect of MDR1 Pgp we have extracted both corticosteroids from human plasma as well as from postmortem human brain material to simultaneously determine cortisol and corticosterone concentrations using liquid chromatography-mass spectrometry (LC-MS).

Materials and Methods

In vivo distribution and autoradiography

The *in vivo* distribution experiments were carried out as described previously (10) with some modifications. Male mdr1a$^{-/-}$ and wild-type FVB mice were bred under specific pathogen-free conditions at TNO (Leiden, The Netherlands). Male mice at the age of 15–20 weeks were used for this study. All experiments were carried out in accordance with the European Communities Council Directive 86/609/EEC and with approval from the animal care committee of the Faculty of Medicine, Leiden University (Leiden, The Netherlands).

After transport, the mice were housed individually at our laboratory at ambient temperature and at a 12-h light, 12-h dark schedule (lights on at 0700 h, lights out at 1900 h) with free access to food and water. To remove the source of endogenous corticosterone, mice were bilaterally adrenalectomized under gas anesthesia (isoflurane) by a dorsal approach. After adrenalectomy (ADX) the animals had free access to 0.9% saline. At the time of the experiment the animals weighed 27 ± 2.7 g (mean ± SD).

Two days after ADX, the animals were sc injected with tritiated steroids (dissolved in 2% ethanol/0.9% saline) for *in vivo* autoradiography. Wild-type (n = 4) and mutant mice (n = 6) were injected with 13 μCi per 10 g (1,2,6,7)-[^{3}H]cortisol (Amersham Pharmacia Biotech, Little Chalfont, UK; specific activity 63 Ci/mmol). As a control for nonspecific retention, one mouse of each genotype was pretreated with a 100-fold excess of unlabeled cortisol. In a separate but similar experiment, mice (n = 7–8) were treated with 2.5 μCi per 10 g (2,4,6,7)-[^{3}H]corticosterone (Amersham Pharmacia Biotech; specific activity 70 Ci/mmol). One hour after injection the animals were decapitated. Trunk blood was collected in EDTA-coated tubes and centrifuged for determination of radioactivity and of remaining corticosterone in the plasma using a ^{125}I-labeled corticosterone RIA kit (ICN Biomedicals, Inc., Costa Mesa, CA). Liver, testis, intestine, and cerebellum were dissected and frozen on dry ice. The pituitary was dissected and mounted on top of the brain (without cerebellum), which were then frozen together in isopentane precooled on dry ice/ethanol. All tissues were stored at −80 C until further use.

All organ tissues studied, except for the brain, were homogenized using Soluene-350 (Packard Bioscience, Groningen, The Netherlands). Hionic-Fluor (Packard Instruments Co., Meriden, CT) was added to tissue homogenates and plasma and radioactivity was determined in a Tricarb β-counter (Packard Instruments Co.). Twelve-micrometer coronal sections of brain were cut on a cryostat and thaw-mounted on poly-L-lysine (Sigma, St. Louis, MO) coated microscopic slides. The slides were put in an x-ray exposure holder (Amersham Pharmacia Biotech) under Ultrofilm (Leica Corp., Heerbrugg, Switzerland) and apposed for 8 weeks. Optical density of radiolabeled steroid retained in pituitary and different brain areas was quantified after subtraction of film background using a computerized Olympus Corp. (Paes, The Netherlands) image analysis system equipped with a Cue CCD camera. From each brain, 3–5 sections were measured by outlining the different brain regions.

Transepithelial transport and inhibition studies

To examine the interactions of cortisol and corticosterone with the human Pgp we used monolayers of the porcine kidney epithelial cell-line LLC-PK1, and LLC-PK1 cells stably transfected with complementary DNA of the human *MDR1* gene (LLC-PK1:MDR1). Cells obtained from the American Type Culture Collection (Manassas, VA) were kindly provided by the Dutch Cancer Institute (Amsterdam, The Netherlands) (11). Human Pgp has been shown before to be specifically expressed on the apical surface of LLC:PK1:MDR1 cells in these monolayers (23). Therefore, Pgp substrates entering these cells from the basal side will be translocated to the apical compartment, whereas those entering the apical membrane will be pumped back into the medium, thus resulting in polarized transport of substrates. This system models the way Pgp is likely to function in the BBB in excluding drugs from the brain.

Cells were cultured at 37 C in the presence of 5% CO_2 in complete medium, which consisted of DMEM (BioWhittaker Europe, Verviers, Belgium) supplied with 25 mM HEPES and 4.5 g/liter glucose and supplemented with 100.000 U/liter penicillin, 100 mg/liter streptomycin, 2 mM L-glutamine, and 10% (vol/vol) FCS. The LLC-PK1 and LLC-PK1:MDR1 cell lines were subcultured by trypsinization every 3–4 days, and medium was replaced twice a week.

During the experiments complete medium was used. The LLC-PK1 and LLC-PK1:MDR1 cells were seeded on microporous polycarbonate membrane filters (0.4-μM pore size, 12-mm diameter, Transwell; Costar, Cambridge, MA) at a density of 120 × 10^3 cells/cm^2. The cells were grown for 5–6 days in complete medium with one medium replacement at day 3. Two hours before the start of the experiment, the medium was replaced with 800 μl fresh medium at both the apical and basal side of the monolayer. In the inhibition experiments, 1 h later, the potent and selective Pgp blocker LY 335979 (1 μM; kindly provided by Eli Lilly & Co.) or water was added at the basal side. To measure the transepithelial transport from the apical to the basal side or from the basal to the apical side 8 μl of a 100× stock of tritiated steroid ([^{3}H]cortisol, [^{3}H]corticosterone, or (1,2(n))-[^{3}H]cortisone; Amersham Pharmacia Biotech; specific activity 50 Ci/mmol) in ethanol was added in triplicate at the apical or basal side, respectively, at the start of the experiment (0 h). The starting concentrations for each experiment are mentioned in the legends of the appropriate figures. In the dose-response experiment, different concentrations of unlabeled cortisol were used, supplemented with [^{3}H]cortisol. Over the 4 h of study, 75-μl aliquots were taken once every hour from both compartments. Eight microliter samples of the 100× stock, and samples from the compartment opposite that to which activity was added, were counted in a Tricarb β-counter after adding 3 ml Emulsifier Safe (Packard Bioscience). Basal-to-apical and apical-to-basal transport is presented as percentage of total activity added at the beginning of the experiment. Transepithelial electrical resistance was measured before and after the experiments to check the integrity of the monolayers.

Corticosteroid determination in postmortem human brain

Human brain material was collected through the rapid autopsy program of The Netherlands Brain Bank (NBB) (Amsterdam, The Netherlands; coordinator: Dr. R. Ravid). The NBB abides to all local ethical legislation. All tissue has been obtained with informed consent of the donor or next of kin to perform brain autopsy and the subsequent use of brain tissue for scientific purposes; consent is requested in advance together with the permission to use the medical records. Patient tissue was carefully selected; none of the subjects was reported to suffer at the moment of death or to have suffered before from a known neurological or psychiatric disease, or from conditions that might have affected BBB integrity, like transient ischemic attacks, (suspected) prolonged arterial blood pressure changes, prolonged fever, or the presence of multiple brain infarcts. Moreover, none of the subjects had been treated with synthetic steroids or antidepressants at time of death or at any time during life. From every subject, a standard set of brain areas has been carefully investigated (24) by neuropathologists Prof. Dr. D. Troost (Academic Medical Center, Amsterdam, The Netherlands), Prof. Dr. F. C. Stamand, and Dr. W. Kamphorst (both from Free University, Amsterdam, The Netherlands). The final diagnosis was established by relating this neuropathological examination to the outcome of the clinical diagnosis. Following this careful examination, all present subjects were confirmed to be true controls because the tissue was free of any such changes. Postmortem delay was kept as short as possible and was on average 6.75 h. Further clinicopathological details are presented in Table 1. All 11 brain tissue samples used in this study were dissected from superior parietal cortex of male control subjects (mean age 65 ± 5.1), rapidly frozen in liquid nitrogen, and then stored at −80 C until use.

Plasma samples were obtained from 11 male volunteers (mean age 57 ± 6.3).

Samples were prepared for assay by dichloromethane (DCM)/ethanol extraction. The brain samples (weighing about 350 mg) were homogenized in 2 ml 0.1 M perchloric acid with a Potter-Elvehjem tissue homogenizer (Eli Lilly & Co.) (10 times up and down, 1,000 rpm). To

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TABLE 1. Clinicopathological data of the nondemented controls

Case no.	NBB no.	Autopsy no.	Sex	Age	PMD	pH	BW	Cause of death
1	90-090	90/234.3	m	59	4:25	7.23	1409	Myocardial infarction and cardial decompensation
2	94-125	S94/340	m	51	6:00	6.50	1518	Progressive liposarcoma and ileus
3	95-007	S95/019	m	54	9:10	6.89	1335	Bleeding from right A. carotis communicans
4	97-162	S97/387	m	38	10:45	6.71	1618	Wegener's disease, aluminum intoxication
5	98-006	S98/014	m	50	8:30	6.65	1436	Cardiac arrest
6	98-127	S98/235	m	56	5:25	6.55	1522	Cardiac infarction
7	96-085	S96/251	m	84	9:00	6.20	1367	Heart failure, uremia
8	97-157	S97/368	m	69	5:55	6.41	1475	Serious prostate cancer with metastasis
9	98-062	S98/142	m	85	4:35	6.95	1332	Respiratory insufficiency secondary to a metastasized adenocarcinoma
10	98-157	S98/280	m	85	5:13	6.23	1394	Cardiac tamponade
11	98-189	S98/326	m	81	5:20	6.64	1276	Respiratory insufficiency

All tissue was taken from the superior parietal gyrus.
NBB no., NBB identification number; m, male; PMD: post-mortem delay (h); pH, pH of the cerebrospinal fluid; BW, brain weight (g).

check for differences in recovery, 100 ng dexamethasone was added to each sample. The homogenates were transferred with a 4-ml wash of DCM to screw-capped glass tubes. After adding an extra 4 ml DCM, the tubes were shaken on a horizontal reciprocating shaker for 30 min and subsequently centrifuged at 1,000 × *g* at 4 C for 10 min. The DCM layer was transferred to a clean coned tube, rinsed with 1 ml water, and centrifuged at 700 × *g* for 10 min. Then, the DCM-layer was transferred to a long tube and evaporated to dryness in a SpeedVac. To maximize the amount transferred, the extracts were redissolved in 750 μl ethanol and, after transferring to an Eppendorf, evaporated again. The final extracts were resuspended in 100 μl 25% methanol and centrifuged at 13,000 rpm for 5 min. To avoid possible dissimilarities between different extraction methods, the 250-μl plasma samples were extracted in the same way.

LC-MS was the method of choice to measure the levels of cortisol and corticosterone in the supernatants of the extracts as it allowed the simultaneous measurements of both hormones in small samples with dexamethasone as internal standard. The assays were performed on a Triple Stage Quadrupole mass spectrometer (Finnigan MAT TSQ-700, San Jose, CA) with a custom-made atmospheric pressure chemical ionization interface. A modification of the method of Van der Hoeven *et al.* (25) was used. The analysis was performed in negative ionization mode using selective ion monitoring of $[M+CH_3COO]^-$ of cortisol, corticosterone, and dexamethasone, alternately scanning mass/charge (*m/z*) 421, 406, and 452. The ion-source temperature and the nebulization heater were kept at 200 and 400 C, respectively. The voltages on the corona needle and on the electron-multiplier were set at −3200 and −1800 V, respectively. Each experiment, a new calibration series was made in 25% methanol with eight concentrations ranging from 5–500 ng/ml of both cortisol and corticosterone. Dexamethasone (1 μg/ml) was used as an internal standard. An alkyl-diol-sianol C_{18} column was used to separate the steroids. After injection of 20 μl of the calibration or extraction samples, the column was washed with acetonitrile-water (40/60%, vol/vol) containing 1 g/liter acetic acid at a flow rate of 0.5 ml/min. The detection limit of this assay was 5 ng/ml. Corticosteroid concentrations were calculated from a standard plot of area under the curve *vs.* concentration. Presented data are corrected for recovery of dexamethasone, which was in the order of 20–40%.

Statistical analysis

Human and mouse data were evaluated by Student's *t* test. The results of the monolayer experiments were analyzed by repeated measures ANOVA. Significance was taken at *P* less than 0.05.

Results

Differences in brain uptake and retention of [^{3}H]corticosterone and [^{3}H]cortisol

At 1 h after administration of [^{3}H]cortisol to ADX mice, the uptake of radioactivity in brain showed a clear difference between mdr1a$^{-/-}$ mutant mice and wild-type mice. The amount of cortisol in cerebellum homogenates was 3.5-fold higher in mdr1a knockouts than in mdr1a$^{+/+}$ mice (Fig. 1). In contrast, the amount of [^{3}H]corticosterone in cerebellum did not differ between the two genotypes. For both corticosteroids the presence or absence of the *mdr1a* gene did not affect their concentration in plasma, liver, testis, and intestine (Fig. 1 and Table 2). The autoradiograms (Figs. 2 and 3) extend these results to the regional distribution of the [^{3}H]-steroids in the brain. The mdr1a$^{+/+}$ animals showed hardly any labeling of brain tissue after administration of [^{3}H]cortisol (Fig. 2A). Labeling in brain sections was restricted to the cerebral ventricles. However, the mdr1a$^{-/-}$ mutants showed increased labeling of whole brain (Fig. 2B). In particular, radioactivity was retained in hippocampal cell fields and to a lesser extent the amygdala. In contrast, after treatment with

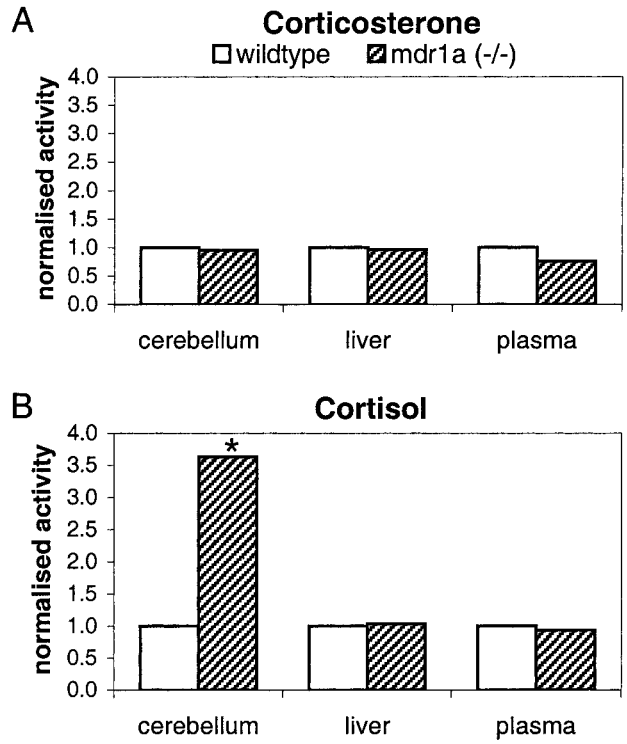

FIG. 1. Radioactivity of [^{3}H]corticosterone (A) and [^{3}H]cortisol (B) in cerebellum and liver homogenates and plasma of wild-type and mdr1a$^{-/-}$ mice (n = 4–8). Data are presented relative to wild-type set at 1.0. *, $P < 0.01$, *t* test on untransformed data.

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TABLE 2. Uptake of radioactivity in tissue homogenates and blood 1 h after administration of [^{3}H]steroid

	Wild-type	mdr1a$^{(-/-)}$
Cortisol		
Dose (μg/kg)	8	8
Cerebellum (nCi/mg)	0.09 ± 0.00	0.33 ± 0.03[a]
Plasma (nCi/μl)	0.48 ± 0.05	0.45 ± 0.05
Liver (nCi/mg)	8.01 ± 0.47	8.27 ± 0.58
Testis (nCi/mg)	0.21 ± 0.01	0.23 ± 0.02
Intestine (nCi/mg)	1.91 ± 0.93	2.12 ± 0.82
Brain to blood ratio	0.20 ± 0.02	0.77 ± 0.05[a]
Corticosterone		
Dose (μg/kg)	1.5	1.5
Cerebellum (nCi/mg)	0.11 ± 0.01	0.10 ± 0.01
Plasma (nCi/μl)	0.26 ± 0.02	0.20 ± 0.02
Liver (nCi/mg)	1.77 ± 0.05	1.70 ± 0.07
Testis (nCi/mg)	0.12 ± 0.02	0.13 ± 0.01
Intestine (nCi/mg)	1.07 ± 0.34	1.74 ± 0.90
Brain to blood ratio	0.39 ± 0.03	0.52 ± 0.05

[a] $P < 0.01$, compared with wild-type.

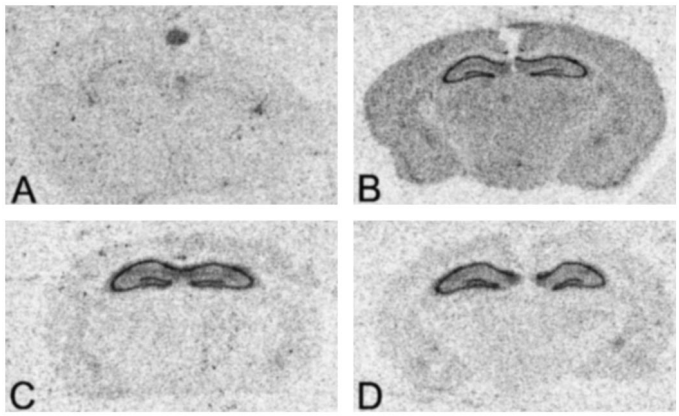

FIG. 2. Representative autoradiograms of 12-μm coronal sections of the brain of wild-type (A, C) and mdr1a$^{-/-}$ mice (B, D) at hippocampus level. Autoradiograms show labeling with [^{3}H]cortisol (A, B) or [^{3}H]corticosterone (C, D). Note the pituitary mounted on top of the brain. The *dark spots* in (A) represent transverse sectioning of the cerebroventricular space and adjacent ventricular walls. Also note that even a lower dose of [^{3}H]corticosterone more heavily labels the hippocampus compared with [^{3}H]cortisol.

[^{3}H]corticosterone the null and wild-type ADX mice did not differ in their strong labeling of hippocampal neurons or of any other part of the brain (Fig. 2, C and D). In both mutants and controls, [^{3}H]cortisol labeling of the pituitary, which lies outside the BBB, was not affected by the absence of the mdr1a Pgp. Pretreatment with excess unlabeled cognate steroid to block specific labeling to receptors resulted in loss of labeling of hippocampal neuronal fields and amygdala, but not of the rest of the brain (data not shown). Moreover, hippocampal optical density showed an inverse correlation with residual levels of endogenous corticosterone, illustrating that the signal represents specific receptor-bound steroid (data not shown). The cortex and the pituitary lack this correlation, but cortex labeling showed a clear effect of disruption of the *mdr1a* gene (Fig. 3). These data evidently demonstrate that the presence of mdr1a Pgp in the BBB hampers the access of cortisol to the mouse brain, but does not have any effect on the access of the endogenous glucocorticoid corticosterone.

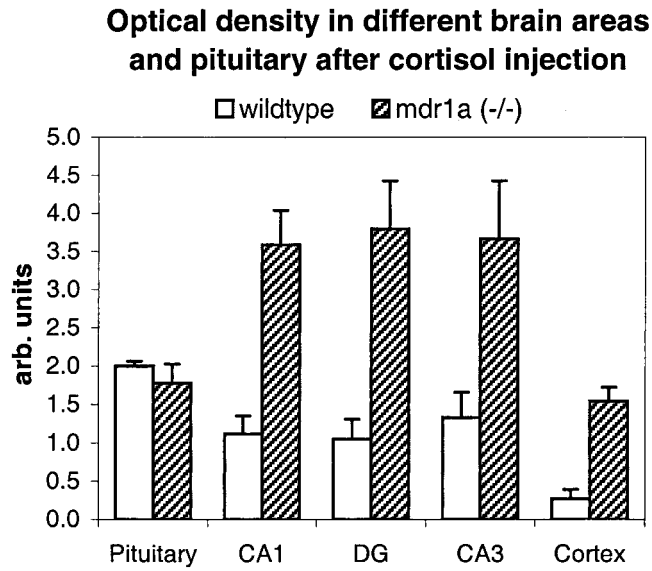

FIG. 3. Quantification of the autoradiograms of [^{3}H]cortisol in wild-type (n = 3) and mdr1a$^{-/-}$ (n = 5) mice. There are no differences between wild-type and mutants for pituitary. Differences in cortex and hippocampal areas CA1 and DG are significant at P less than 0.05. The values for CA3 are not significantly different ($P = 0.07$). Three sections per animal were measured.

Transepithelial transport of steroids in monolayers of LLC-PK1 and LLC-PK1:MDR1 cells

Corticosterone transport in the monolayers of LLC-PK1 cells stably transfected with the human *MDR1* gene was not different from transport in monolayers of its control cell line (Fig. 4B), although some polar transport was observed in both cell lines in all our experiments (Figs. 4B and 5B). Nonetheless, this demonstrates the absence of human MDR1 Pgp-mediated transport of corticosterone. However, cortisol was transported in a polarized fashion in the MDR1-transfected monolayers, but not in the host cells (Fig. 4A). Polarized transport in MDR1 monolayers of cortisol was abolished in presence of LY335979, a potent and selective Pgp blocker (26, 27), resulting in similar fractions transported as in untransfected cells (Fig. 5A). This confirms that cortisol transport is mediated by human Pgp. LY335979 did not change the fraction of corticosterone translocated through the membrane (Fig. 5B). We also examined transepithelial transport of different concentrations of cortisol, ranging from 5 to 625 nM, but did not demonstrate any saturation at higher dose. At all concentrations tested, about 3.5 times more [^{3}H]cortisol had been transported from basal to apical sides than from apical to basal sides after 4 h (data not shown). Interestingly, these data show that human MDR1 Pgp is also able to transport cortisol, whereas corticosterone passage remains unchanged.

A potential limitation of our assay is the use of radiolabeled glucocorticoids and, consequently, the possibility that the transport of metabolites has been measured rather than the unmetabolized compounds. Because of the presence of 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2 in LLC-PK1 cells (28), the main probable metabolites are the inactive forms of the glucocorticoids, *i.e.* cortisone in case of cortisol and 11-dehydrocorticosterone in case of corticosterone. Therefore, we first tested cortisone transport in our

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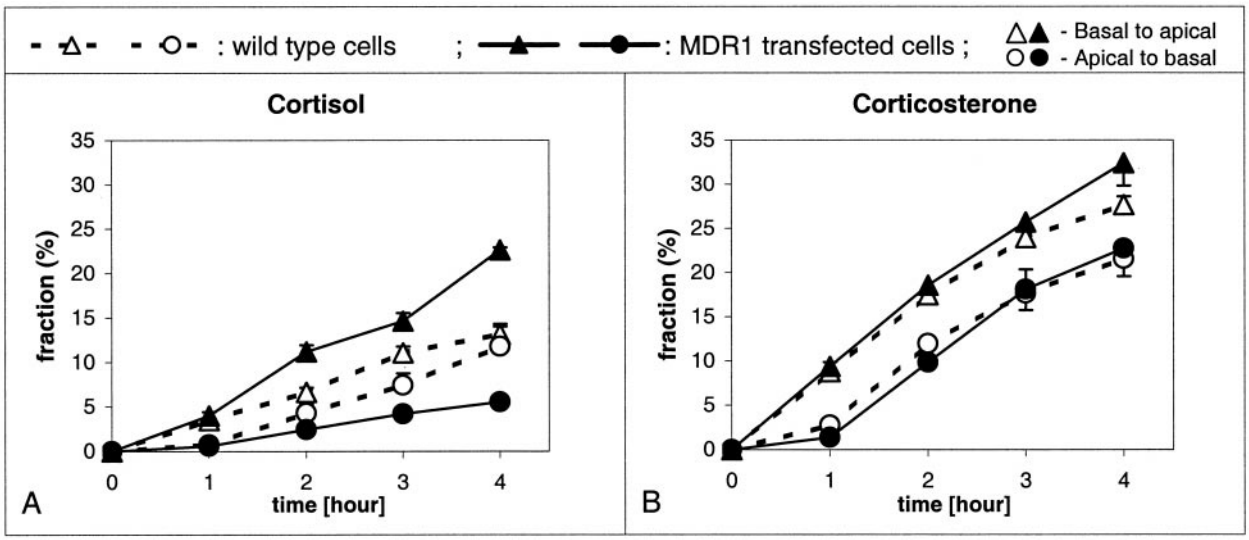

FIG. 4. Activity of [^{3}H]cortisol (A) and [^{3}H]corticosterone (B) present in medium at different timepoints after adding [^{3}H]steroid to the opposite compartment at 0 h. Transepithelial transport from basal-to-apical (△, ▲) and from apical-to-basal (○, ●) compartment was measured in wild-type LLC-PK1 (*broken line*) or MDR1-transfected LLC-PK1 (*solid line*) monolayers. Presented is the fraction of the dose of radioactivity, which is 9 nM for both steroids, added to the respective compartment. Each *point* represents the mean of three monolayers ± SEM. Repeated measures ANOVA showed a significant interaction of time∗ cell type∗ transport for cortisol ($P = 0.00$) but not for corticosterone.

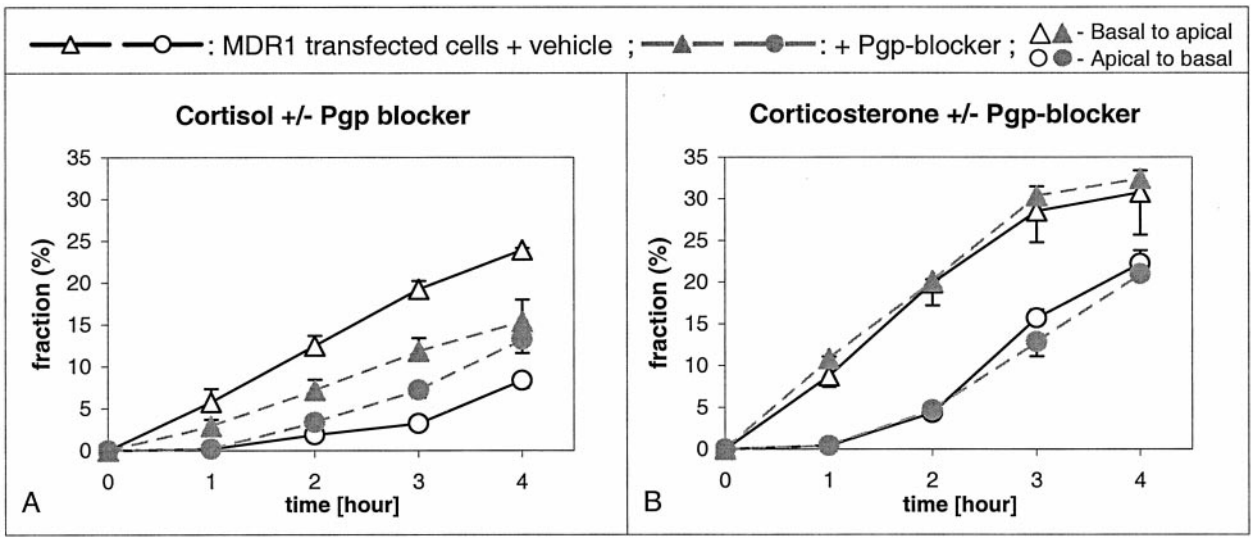

FIG. 5. Activity of [^{3}H]cortisol (A) and [^{3}H]corticosterone (B) present in medium at different time points after adding [^{3}H]steroid to the opposite compartment at 0 h and 1 μM LY335979 (*broken line*) or water (*solid line*) 1 h before. Transepithelial transport from basal-to-apical (△, ◬) and from apical-to-basal (○, ◍) compartment was measured in MDR1-transfected LLC-PK1 monolayers. Presented is the fraction of the dose of radioactivity, which is 8 nM for cortisol and 28 nM for corticosterone, added to the respective compartment. Each *point* represents the mean of three monolayers ± SEM. Repeated measures ANOVA showed a significant interaction of time∗ cell type∗ transport for cortisol ($P = 0.00$), but not for corticosterone.

monolayers. Cortisone showed polarized transport in LLC-PK1:MDR1 monolayers, which could be blocked by LY335979 (Fig. 6). To subsequently exclude the possibility that we have measured the metabolites we further examined the transport of [^{3}H]cortisol or [^{3}H]corticosterone in presence of carbenoxolone (10^{-6} and 10^{-5} M). The addition of this 11β-HSD inhibitor did not change the transport properties of LLC-PK1 or LLC-PK1:MDR cells in any way (data not shown).

These data suggest that Pgp in human BBB as in mice limits the access of cortisol to the brain, but does not affect the penetration of corticosterone. Accordingly, we expected more corticosterone relative to cortisol in human brain than in human plasma.

Corticosteroid levels in human brain

To test whether MDR1 Pgp in human BBB increases the ratio of corticosterone over cortisol in brain, we determined the concentrations of both glucocorticoids in human brain samples (Table 3). Thus, we were able to establish the brain corticosterone to cortisol ratio of 11 subjects, which was

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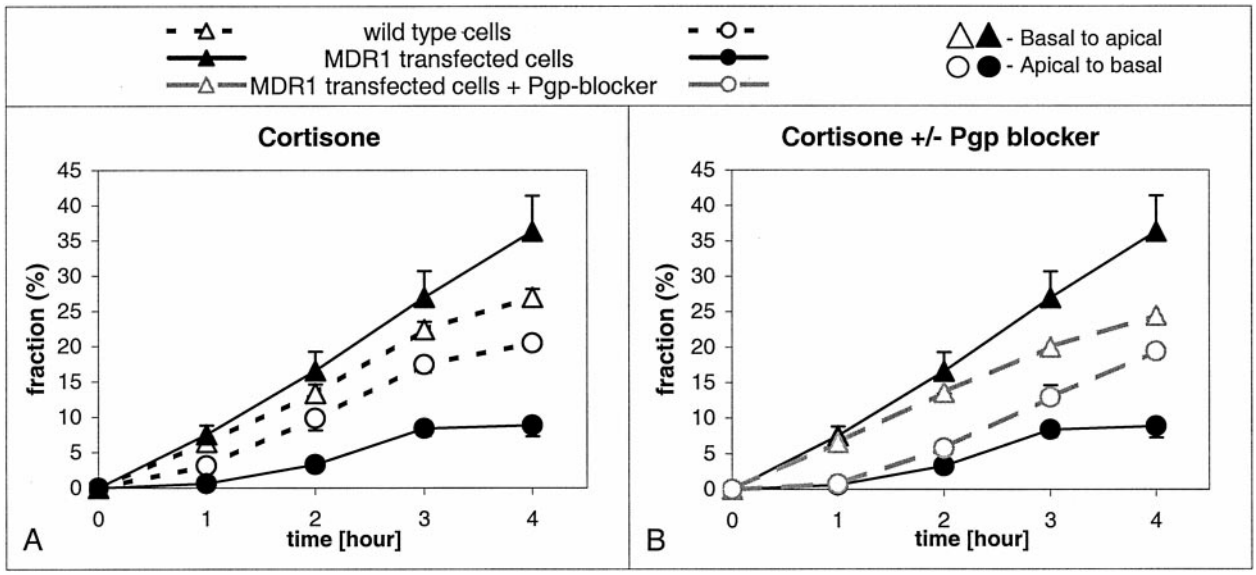

FIG. 6. A, Activity of [^{3}H]cortisone present in medium at different time points after adding [^{3}H]cortisone to the opposite compartment at 0 h. Transepithelial transport from basal-to-apical (△, ▲) and from apical-to-basal (○, ●) was measured in wild-type LLC-PK1 (*broken line*) or MDR1-transfected LLC-PK1 (*solid line*) monolayers. B, Transepithelial transport of [^{3}H]cortisone was measured in MDR1-transfected LLC-PK1 monolayers after adding 1 μM LY335979 (*broken line*) or water (*solid line*) 1 h before start of the experiment. Presented is the fraction of the dose of radioactivity, which is 9 nM, added to the respective compartment. Each *point* represents the mean of three monolayers ± SEM. Repeated measures ANOVA showed a significant interaction of time∗ cell type∗ transport for cortisone in both A and B ($P = 0.00$).

TABLE 3. Corticosterone and cortisol levels in extracts of human brain tissue and plasma

Brain (case no.)	NBB no.	Corticosterone (ng/mg)	Cortisol (ng/mg)	Ratio
1	90-090	81.28	291.48	0.28
2	94-125	32.20	201.62	0.16
3	95-007	35.07	121.88	0.29
4	97-162	11.23	33.08	0.34
5	98-006	14.18	46.49	0.31
6	98-127	55.83	176.90	0.32
7	96-085	83.41	507.46	0.16
8	97-157	122.76	442.02	0.28
9	98-062	84.17	265.42	0.32
10	98-157	280.57	443.14	0.63
11	98-189	24.10	70.25	0.34
	AVG	74.98	236.34	0.31
	SEM	23.14	50.92	0.04
Plasma (case no.)	Age	(ng/ml)	(ng/ml)	
1	27	5.77	306.49	0.02
2	20	8.14	286.48	0.03
3	32	4.87	224.65	0.02
4	62	12.57	78.50	0.16
5	83	3.89	57.70	0.07
6	70	11.42	126.29	0.09
7	76	4.18	66.68	0.06
8	69	4.38	84.18	0.05
9	63	2.21	71.03	0.03
10	68	4.11	54.22	0.08
11	56	0.95	39.22	0.02
	AVG	5.68	126.86	0.06
	SEM	1.09	29.53	0.01

0.31 ± 0.04 (mean ± SEM) (Fig. 7). In contrast, we measured a corticosterone to cortisol ratio in plasma samples of age-matched males of 0.06 ± 0.01 (Fig. 7). Statistical analysis showed that the difference between the brain and plasma

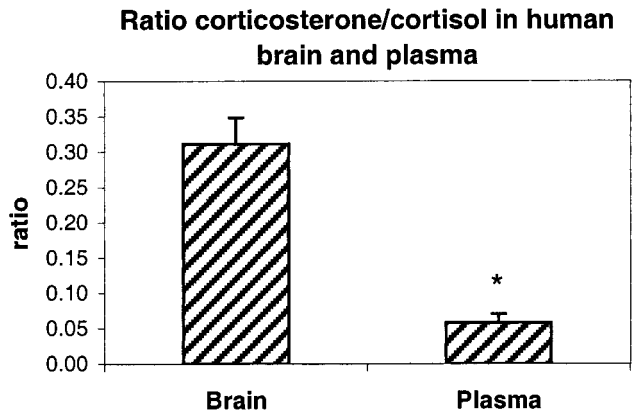

FIG. 7. Ratio of corticosterone over cortisol in extracts of human brain and plasma. Data are presented as mean ± SEM. ∗, $P < 0.01$.

ratios was significant ($t(1,20) = 6.444$, $P < 0.01$). Thus, corticosterone appears to penetrate more easily in the human brain than cortisol, resulting in a higher ratio of corticosterone over cortisol present in brain compared with plasma.

Discussion

The present study indicates that Pgp at the level of the BBB is of importance with respect to the degree of brain exposure to the naturally occurring glucocorticoids cortisol and corticosterone. Our data show that the mdr1a Pgp present in BBB hampers the penetration of cortisol into the mouse brain, whereas corticosterone uptake is not affected. Interestingly, our results with monolayers of human MDR1-transfected LLC-PK1 cells suggest that Pgp exports cortisol and not corticosterone from human brain as well. This is consistent, at least, with the accumulation of corticosterone over cortisol

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in the samples of human postmortem brain relative to plasma, as determined in our LC-MS experiments.

Previous studies had already established *in vivo* the low cell nuclear retention of cortisol in rat brain (7). In rats, the first pass uptake in brain after a carotid injection of [^{3}H]cortisol appeared to be negligible in contrast to uptake of [^{3}H]corticosterone, whereas uptake of the labeled steroids in liver after portal injection was not different (29). A tracer dose of corticosterone is known to label only the high affinity hippocampal MR, leaving the lower affinity GR undetectable. The uptake of this steroid is not affected by disruption of the *mdr1a* gene (Ref. 10 and this study). Cortisol also binds with a rather high affinity to MR (16). In fact, our autoradiography study revealed a pattern of cortisol labeling in the mdr1a$^{-/-}$ mouse hippocampus reminiscent of that of corticosterone. In the present study, the effect of mdr1a ablation on specific binding of cortisol to the low capacity MR in the hippocampus is less pronounced than its effect on uptake in whole brain. This is probably due to the lower affinity of cortisol for MR in rodent brain (30, 31). Anyhow, our data convincingly demonstrate that the mouse mdr1a Pgp hampers the brain uptake of cortisol but not of corticosterone.

As a model for Pgp function in human BBB, we have used monolayers of pig kidney epithelial LLC-PK1 cells stably transfected with the *MDR1* gene to measure transport of steroids by human Pgp. Such monolayers of epithelial cells are a plausible model for Pgp-mediated transport at the BBB, given the apical localization of Pgp forming a barrier between the two compartments. A confounding factor is that LLC-PK1 host cells contain low levels of porcine Pgp (23, 32, 33). Thus, in theory, porcine Pgp might be responsible for polar transport of corticosterone seen in both cell lines, although any effect after application of the potent and selective Pgp-blocker was absent. LLC-PK1 cells also have endogenous 11β-hydroxysteroid dehydrogenase type 2 activity, able to inactivate cortisol and corticosterone (28). Because we used radiolabeled glucocorticoids it is possible that we have actually measured transport of labeled metabolites rather than the parent hormone. However, the transport of [^{3}H]cortisol and [^{3}H]corticosterone did not change in the presence of the 11β-HSD inhibitor carbenoxolone, indicating that 11β-HSD activity did not interfere. Therefore, we conclude that our monolayers are a suitable model of Pgp function in human BBB.

Our data corroborate several studies on transport of cortisol and corticosterone by Pgp (23, 34–39). Differential transport of these two steroids by murine Pgp has been observed in several drug-resistant cell lines, using steroid induced apoptosis (35, 40) or steroid accumulation (38, 39) as readouts. The murine mdr1b Pgp has some capacity to transport corticosterone (38), but this second murine multidrug resistance Pgp is not expressed at the BBB. In view of the lack of corticosterone transport that we have observed in cells stably transfected with the human *MDR1* gene, the corticosteroid transport capabilities of the human MDR1 Pgp apparently correspond to that of murine mdr1a rather than to that of mdr1b Pgp. Using comparable monolayers to those in this study, Ueda *et al.* (23) have already demonstrated that cortisol is transported by the human MDR1 Pgp, but corticosterone was not included in their assay. In human colon carcinoma cells the amount of accumulated [^{3}H]cortisol is lower than of corticosterone (39), whereas both steroids equally increase accumulation of the Pgp substrate [^{3}H]vinblastine, exemplifying a difference between actual transport by and binding of steroids to the pump.

The difference in interaction of Pgp with cortisol and corticosterone is remarkable considering their large similarity in molecular structure. Pgp is an efflux transporter with a surprisingly broad substrate spectrum (41), but corticosterone only differs from cortisol in the lack of the 17-hydroxyl group. However, there are indications that both the 17-hydroxyl and the 11-hydroxyl group determine the ability of steroids to be transported by Pgp (40). Pgp transports steroids having both these hydroxyl-groups whereas steroids lacking one of these groups are probably minimally if at all transported. A caveat is that these indications are based on the extent of glucocorticoid resistance, which also depends on GR affinity. It is difficult to assess the influence of the 11-hydroxyl group because steroids lacking this group do also have a low affinity for GR. Therefore, cortisone could not be identified as a substrate of Pgp in the previous study; however, using LLC-PK1:MDR1 monolayers, we demonstrated that cortisone is also transported by Pgp.

Our study with the mdr1a null mice is the first to directly show the involvement of Pgp in excluding a naturally occurring glucocorticoid from the brain. Previous studies have demonstrated that access of the synthetic glucocorticoid dexamethasone to the brain was also enhanced in the mdr1a$^{-/-}$ mouse (10, 11). *In vitro* studies have confirmed that dexamethasone is a Pgp substrate (23, 40, 42). In fact, in our stably MDR1-transfected LLC-PK1 monolayers, dexamethasone behaved very similar to cortisol (43). Thus, human MDR1 Pgp, like mouse mdr1a Pgp, transports both cortisol and dexamethasone, but not corticosterone.

Our *in vitro* results using monolayers of stably MDR1-transfected LLC-PK1 cells show that the endogenous presence in a species of a naturally occurring glucocorticoid is not a prerequisite to exclude transport by Pgp. We have clearly demonstrated that human MDR1 Pgp is able to discriminate between cortisol and corticosterone. Both glucocorticoids are present in human plasma, although cortisol circulates in about 10–20 times higher levels than corticosterone (19–22, 44). The data strongly suggest that corticosterone rather than cortisol can freely gain access to the human brain. Alternatively, BBB passage of cortisone and subsequent conversion of cortisone to cortisol by 11β-HSD type 1 present in brain (45), might regenerate cortisol in brain. The fact that MDR1 Pgp also transports cortisone, argues against the possibility that cortisol would be able to circumvent Pgp in the BBB through this route, because less cortisone would also be available for 11β-HSD type 1 conversion in brain. Therefore, the limited access of cortisol and cortisone is likely to result in overall lower brain levels of glucocorticoids and in an increase of corticosterone relative to cortisol in human brain compared with plasma.

An *in vivo* cell nuclear retention study in ADX rhesus monkeys, which have cortisol as their main glucocorticoid, showed a similar regional pattern for both corticosteroids, but the amount of cortisol radioactivity was lower than that of corticosterone (46). This observation substantiates that

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even in an animal that normally produces cortisol, this glucocorticoid less efficiently penetrates into the brain than corticosterone. We demonstrated a shift in the corticosterone to cortisol ratio in favor of corticosterone in human autopsy brain samples compared with plasma samples. These results support data reported by Brooksbank *et al.* (47), who also demonstrated that corticosterone is accumulated in the brain to a substantially greater extent than cortisol. They found a ratio of corticosterone to cortisol of about 0.4. Earlier, Fazekas and Fazekas (48) also determined corticosteroid levels in human brain using paper chromatography and similarly reported high levels of corticosterone relative to cortisol.

The privileged uptake of corticosterone in the brain is also expected to promote its receptor occupancy relative to cortisol. There are indications that corticosterone might have a higher affinity for the MR than cortisol. At least this is the case for the rat MR (30, 31), but data presented by Arriza *et al.* (49) also suggests that corticosterone is the more potent competitor at human MR. Furthermore, transactivation of human MR in response to cortisol and corticosterone indicates that corticosterone is more effective than cortisol (50). Thus, besides the hampered uptake in human brain, cortisol might also less effectively mediate the human brain MR response. Should it indeed be confirmed that levels of GR are relatively low in the human hippocampus, as was recently claimed for the rhesus monkey (51), glucocorticoid-mediated effects on hippocampal functioning might then mainly reflect corticosterone acting through MR rather than cortisol. At least, our data suggest that the human glucocorticoid feedback system might be more complex than the rodent system in view of the potential different roles for cortisol and corticosterone.

The influence of cortisol on brain functioning and its role as main corticosteroid in glucocorticoid feedback to the human brain is commonly accepted. However, in contrast to rodents where corticosterone readily enters the brain, the main glucocorticoid in human appears to be partially excluded from the brain. It would be interesting to know how much either corticosterone and cortisol contributes to stabilization of neuronal excitability (52), maintenance of neuronal integrity (53), suppression of hypothalamic-pituitary-adrenal activity (18), and facilitation of behavioral adaptation (54). The preferential uptake of corticosterone in human brain may further be used as a lead toward the development of novel selective steroids for treatment of stress-related brain disorders.

In conclusion, we have demonstrated the involvement of Pgp in hampering the access of the naturally occurring glucocorticoid cortisol rather than corticosterone to both mouse and human brain. Therefore, the data suggest that corticosterone may play a more prominent role in the modulation of human brain function than hitherto recognized.

Acknowledgments

We gratefully acknowledge Marc Fluttert, Sergiu Dalm, and Dirk-Jan van den Berg for animal handling and technical assistance. We are grateful to Margret Blom for assistance with cell cultures and Barry Karabatak and Bertil Hofte for technical assistance at the LC-MS. We thank The Netherlands Brain Bank (coordinator: Dr. R. Ravid) for provision of the human brain tissue and Mr. A. Holtrop for assistance with the tissue selection. We thank Dr. Eef Lentjes for help with plasma samples.

References

1. **McEwen BS, Weiss JM, Schwartz LS** 1968 Selective retention of corticosterone by limbic structures in rat brain. Nature 220:911–912
2. **McMurry L, Hastings JW** 1972 Rat brain binds adrenal steroid hormone: radioautography of hippocampus with corticosterone. Science 175:1133–1136
3. **De Kloet ER, Wallach G, McEwen BS** 1975 Differences in corticosterone and dexamethasone binding to rat brain and pituitary. Endocrinology 96:598–609
4. **Coutard M, Duval D, Osborne-Pellegrin MJ** 1987 *In vivo* competitive autoradiographic study of [^{3}H]corticosterone and [^{3}H]aldosterone binding sites within mouse brain hippocampus. J Steroid Biochem 28:29–34
5. **Reul JM, De Kloet ER** 1985 Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117: 2505–2511
6. **De Kloet ER, Van der Vies J, De Wied D** 1974 The site of the suppressive action of dexamethasone on pituitary-adrenal activity. Endocrinology 94:61–73
7. **McEwen BS, De Kloet ER, Wallach G** 1976 Interactions *in vivo* and *in vitro* of corticoids and progesterone with cell nuclei and soluble macromolecules from rat brain regions and pituitary. Brain Res 105:129–136
8. **Rees HD, Stumpf WE, Sar M** 1975 Autoradiographic studies with ^{3}H-dexamethasone in the rat brain and pituitary. In: Stumpf WE, Grant L (eds) Anatomical Neuroendocrinology. S. Karger, Basel, pp 262–269
9. **Coutard M, Osborne-Pellegrin MJ, Funder JW** 1978 Tissue distribution and specific binding of tritiated dexamethasone *in vivo*: autoradiographic and cell fractionation studies in the mouse. Endocrinology 103:1144–1152
10. **Meijer OC, De Lange ECM, Breimer DD, De Boer AG, Workel JO, De Kloet ER** 1998 Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. Endocrinology 139:1789–1793
11. **Schinkel AH, Wagenaar E, Van Deemter L, Mol CAAM, Borst P** 1995 Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Invest 96:1698–1705
12. **Cordon-Cardo C, O'Brien JP, Casals D, Rittman GL, Biedler JL, Melamed MR, Bertino JR** 1989 Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc Natl Acad Sci USA 86:695–698
13. **Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC** 1989 Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein P170: evidence for localization in brain capillaries and crossreactivity of one antibody with a muscle protein. J Histochem Cytochem 37:159–164
14. **Jette L, Pouliot JF, Murphy GF, Béliveau R** 1995 Isoform I (mdr3) is the major form of P-glycoprotein expressed in mouse brain capillaries. Evidence for cross-reactivity of antibody C219 with an unrelated protein. Biochem J 305:761–766
15. **Van de Vrie W, Marquet RL, Stoter G, De Bruijn EA, Eggermont AM** 1998 *In vivo* model systems in P-glycoprotein-mediated multidrug resistance. Crit Rev Clin Lab Sci 35:1–57
16. **De Kloet ER** 1991 Brain corticosteroid receptor balance and homeostatic control. Front. Neuroendocrinol 12:95–164
17. **De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M** 1998 Brain corticosteroid receptor balance in health and disease. Endocr Rev 19:269–301
18. **Dallman MF, Akana SF, Scribner KA, Bradbury MJ, Walker CD, Strack AM, Cascio CS** 1992 Stress, feedback and facilitation in the hypothalamopituitary-adrenal axis. J Neuroendocrinol 4:517–526
19. **Nishida S, Matsumura S, Horino M, Oyama H, Tenku A** 1977 The variations of plasma corticosterone/cortisol ratios following ACTH stimulation or dexamethasone administration in normal men. J Clin Endocrinol Metab 45:585–588
20. **Underwood RH, Williams GH** 1972 The simultaneous measurement of aldosterone, cortisol, and corticosterone in human peripheral plasma by displacement analysis. J Lab Clin Med 79:848–862
21. **West CD, Mahajan DK, Chavre VJ, Nabors CJ, Tyler FH** 1973 Simultaneous measurement of multiple plasma steroids by radioimmunoassay demonstrating episodic secretion. J Clin Endocrinol Metab 36:1230–1236
22. **Kage A, Fenner A, Weber B, Schoneshofer M** 1982 Diurnal and ultradian variations of plasma concentrations of eleven adrenal steroid hormones in human males. Klin Wochenschr 60:659–666
23. **Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T, Hori R** 1992 Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. J Biol Chem 267:24248–24252
24. **Ravid R, Swaab DF, Van Zwieten EJ, Salehi A** 1995 Controls are what make a brain bank go round. In: Cruz-Sanchez FF, Cuzner ML, Ravid R (eds) Neuropathological Diagnostic Criteria for Brain Banking, Biomedical and Health Research, vol 10. IOS Press, Amsterdam, pp 4–13
25. **Van der Hoeven RA, Hofte AJ, Frenay M, Irth H, Tjaden UR, Van der Greef J, Rudolphi A, Boos KS, Marko Varga G, Edholm LE** 1997 Liquid chromatography-mass spectrometry with on-line solid-phase extraction by a restrict-

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ed-access C18 precolumn for direct plasma and urine injection. J Chromatogr A 762:193–200
26. **Dantzig AH, Shepard RL, Law KL, Tabas L, Pratt S, Gillespie JS, Binkley SN, Kuhfeld MT, Starling JJ, Wrighton SA** 1999 Selectivity of the multidrug resistance modulator, LY335979, for P-glycoprotein and effect on cytochrome P-450 activities. J Pharmacol Exp Ther 290:854–862
27. **Starling JJ, Shepard RL, Cao J, Law KL, Norman BH, Kroin JS, Ehlhardt WJ, Baughman TM, Winter MA, Bell MG, Shih C, Gruber J, Elmquist WF, Dantzig AH** 1997 Pharmacological characterization of LY335979: a potent cyclopropyldibenzosuberane modulator of P-glycoprotein. Adv Enzyme Regul 37:335–347
28. **Leckie C, Chapman KE, Edwards CR, Seckl JR** 1995 LLC-PK1 cells model 11 β-hydroxysteroid dehydrogenase type 2 regulation of glucocorticoid access to renal mineralocorticoid receptors. Endocrinology 136:5561–5569
29. **Pardridge WM, Mietus LJ** 1979 Transport of steroid hormones through the rat blood-brain barrier. Primary role of albumin-bound hormone. J Clin Invest 64:145–154
30. **De Kloet ER, Veldhuis HD, Wagenaars JL, Bergink EW** 1984 Relative binding affinity of steroids for the corticosterone receptor system in rat hippocampus. J Steroid Biochem 21:173–178
31. **Myles K, Funder JW** 1994 Type I (mineralocorticoid) receptors in the guinea pig. Am J Physiol 267:E268–E272
32. **Horio M, Pastan I, Gottesman MM, Handler JS** 1990 Transepithelial transport of vinblastine by kidney-derived cell lines. Application of a new kinetic model to estimate in situ Km of the pump. Biochim Biophys Acta 1027:116–122
33. **Decorti G, Peloso I, Favarin D, Klugmann FB, Candussio L, Crivellato E, Mallardi F, Baldini L** 1998 Handling of doxorubicin by the LLC-PK1 kidney epithelial cell line. J Pharmacol Exp Ther 286:525–530
34. **Yang CP, DePinho SG, Greenberger LM, Arceci RJ, Horwitz SB** 1989 Progesterone interacts with P-glycoprotein in multidrug-resistant cells and in the endometrium of gravid uterus. J Biol Chem 264:782–788
35. **Gruol DJ, Bourgeois S** 1994 Expression of the mdr1 P-glycoprotein gene: a mechanism of escape from glucocorticoid-induced apoptosis. Biochem Cell Biol 72:561–571
36. **Van Kalken CK, Broxterman HJ, Pinedo HM, Feller N, Dekker H, Lankelma J, Giaccone G** 1993 Cortisol is transported by the multidrug resistance gene product P-glycoprotein. Br J Cancer 67:284–289
37. **Orlowski S, Mir LM, Belehradek J, Garrigos M** 1996 Effects of steroids and verapamil on P-glycoprotein ATPase activity: progesterone, desoxycorticosterone, corticosterone and verapamil are mutually non-exclusive modulators. Biochem J 317:515–522
38. **Wolf DC, Horwitz SB** 1992 P-glycoprotein transports corticosterone and is photoaffinity-labeled by the steroid. Int J Cancer 52:141–146
39. **Barnes KM, Dickstein B, Cutler GB, Fojo T, Bates SE** 1996 Steroid transport, accumulation, and antagonism of P-glycoprotein in multidrug-resistant cells. Biochemistry 35:4820–4827
40. **Bourgeois S, Gruol DJ, Newby RF, Rajah FM** 1993 Expression of an *mdr* gene is associated with a new form of resistance to dexamethasone-induced apoptosis. Mol Endocrinol 7:840–851
41. **Schinkel AH, Smit JJ, Van Tellingen O, Beijnen JH, Wagenaar E, Van Deemter L, Mol CAAM, Van der Valk MA, Robanus Maandag EC, Te Riele HP, Berns AJM, Borst P** 1994 Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 77:491–502
42. **Gruol DJ, Vo QD, Zee MC** 1999 Profound differences in the transport of steroids by two mouse P-glycoproteins. Biochem Pharmacol 58:1191–1199
43. **Karssen AM, Meijer OC, De Boer AG, Van der Sandt ICJ, De Lange ECM, De Kloet ER,** The multidrug resistance 1a p-glycoprotein affects the penetration of glucocorticoids, except corticosterone, in the brain. Program of the 30th Annual Meeting Society for Neuroscience, New Orleans, LA, 2000, p 419 (Abstract 152.17)
44. **Seckl JR, Campbell JC, Edwards CR, Christie JE, Whalley LJ, Goodwin GM, Fink G** 1990 Diurnal variation of plasma corticosterone in depression. Psychoneuroendocrinology 15:485–488
45. **Seckl JR** 1997 11β-Hydroxysteroid dehydrogenase in the brain: a novel regulator of glucocorticoid action? Front Neuroendocrinol 18:49–99
46. **Gerlach JL, McEwen BS, Pfaff DW, Moskovitz S, Ferin M, Carmel PW, Zimmerman EA** 1976 Cells in regions of rhesus monkey brain and pituitary retain radioactive estradiol, corticosterone and cortisol differentially. Brain Res 103:603–612
47. **Brooksbank BW, Brammall MA, Shaw DM** 1973 Estimation of cortisol, cortisone and corticosterone in cerebral cortex, hypothalamus and other regions of the human brain after natural death and after death by suicide. Steroids Lipids Res 4:162–183
48. **Fazekas IG, Fazekas AT** 1967 Corticosteroid fractions of the human brain. Endokrinologie 51:183–210
49. **Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM** 1987 Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. Science 237:268–275
50. **Lombes M, Kenouch S, Souque A, Farman N, Rafestin-Oblin ME** 1994 The mineralocorticoid receptor discriminates aldosterone from glucocorticoids independently of the 11 β-hydroxysteroid dehydrogenase. Endocrinology 135:834–840
51. **Sanchez MM, Young LJ, Plotsky PM, Insel TR** 2000 Distribution of corticosteroid receptors in the rhesus brain: relative absence of glucocorticoid receptors in the hippocampal formation. J Neurosci 20:4657–4668
52. **Joëls M, De Kloet ER** 1994 Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. Prog Neurobiol 43:1–36
53. **McEwen BS, Cameron H, Chao HM, Gould E, Magarinos AM, Watanabe Y, Woolley CS** 1993 Adrenal steroids and plasticity of hippocampal neurons: toward an understanding of underlying cellular and molecular mechanisms. Cell Mol Neurobiol 13:457–482
54. **Oitzl MS, Van Haarst AD, De Kloet ER** 1997 Behavioral and neuroendocrine responses controlled by the concerted action of central mineralocorticoid (MRS) and glucocorticoid receptors (GRS). Psychoneuroendocrinology [Suppl 1] 22:S87–S93

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