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How Does the Benzamide Antipsychotic Amisulpride get into the Brain?—An *In Vitro* Approach Comparing Amisulpride with Clozapine

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This study evaluated the disposition of the two atypical antipsychotics, amisulpride (AMS) and clozapine (CLZ), and its main metabolite *N*-desmethylclozapine (DCLZ), to their target structures in the central nervous system by applying an *in vitro* blood–brain barrier and blood–cerebrospinal fluid (CSF) barrier based on monolayers of porcine brain microvessel endothelial cells (PMEC) or porcine choroid plexus epithelial cells (PCEC). Permeation studies through PMEC- and PCEC-monolayers were conducted for 60 min at drug concentrations of 1, 5, 10, and 30 μM applied to the donor compartment. PMEC were almost impermeable for AMS (permeation coefficient, $P < 1 \times 10^{-7}$ cm/s) in the resorptive direction, whereas transport in the secretory direction was observed with a P ($\pm$ SD) of $5.2 \pm 3.6 \times 10^{-6}$ cm/s. The resorptive P of CLZ and DCLZ were $2.3 \pm 1.2 \times 10^{-4}$ and $9.6 \pm 5.0 \times 10^{-5}$ cm/s, respectively. For the permeation across PCEC in the resorptive direction, a P of $1.7 \pm 2.5 \times 10^{-6}$ cm/s was found for AMS and a P of $1.6 \pm 0.9 \times 10^{-4}$ and $2.3 \pm 1.3 \times 10^{-5}$ cm/s was calculated for CLZ and DCLZ, respectively. Both, CLZ and DCLZ, could easily pass both barriers with about a five-fold higher permeation rate of CLZ at the PCEC. The permeation of AMS across the BBB was restricted partly due to an efflux transport. It is thus suggested that AMS reaches its target structures via transport across the blood–CSF barrier.
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INTRODUCTION

Amisulpride (AMS), a substituted benzamide, is regarded as an atypical antipsychotic due to the negligible risk of extrapyramidal symptoms during therapy (Coukell *et al*, 1996). It exerts its antipsychotic action by selective blockade of postsynaptic dopaminergic D_2 and D_3 receptors (Martinot *et al*, 1996; Perrault *et al*, 1997; Castelli *et al*, 2001). At low concentrations, a preferential blockade of presynaptic D_2/D_3 receptors was postulated causing a more antidepressive-like action (Perrault *et al*, 1997; Schoemaker *et al*, 1997). Furthermore, AMS preferentially antagonizes D_2/D_3 receptors in the limbic system in comparison to striatal areas (Scatton *et al*, 1997). The predominant action of AMS in extrastriatal areas became especially obvious at low concentrations (Möller, 2001; Xiberas *et al*, 2001). Although it was speculated that at these low dosages mainly D_3 autoreceptors in cortical areas were antagonized (Scatton *et al*, 1997; Pani and Gessa, 2002), the reason for the limbic selectivity of AMS remains unclear. Like its predecessor, sulpiride, it reveals some physicochemical peculiarities. AMS is, besides sulpiride, the most hydrophilic antipsychotic and elimination from the body is mainly accomplished by renal clearance (Dufour and Desanti, 1988). Since AMS undergoes only negligible metabolism, differences in the distribution might mostly determine the availability of AMS at central dopaminergic receptors. Only two studies are published regarding the disposition of AMS between the systemic circulation and peripheral compartments like the central nervous system (CNS) (Umbreit-Luik and Dross, 1983; Dufour and Desanti, 1988). These studies were conducted in rats and reported a differential distribution between brain areas with or without blood–brain barrier (BBB) like the epiphysis. While only less than 10% of the concentration in blood was found in areas protected by BBB, the concentration in, for example, the epiphysis was twice the blood concentration. The relatively high AMS concentration in areas outside the BBB was also found in humans, resulting in a remarkable elevation of plasma prolactin by interfering with the dopaminergic control of

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the hypophyseal hormones (Grunder *et al*, 1999). Different distributions in different brain areas have also been reported in a study assessing the local cerebral glucose use in rats after AMS compared with haloperidol. For AMS, the glucose utilization was much higher in the temporal cortex compared with, for example, the nucleus accumbens (Cudennec *et al*, 1997). Striatal dopamine D_2 receptor occupancy in man was recently assessed by positron emission tomography (PET) (Martinot *et al*, 1996, Xiberas *et al*, 2001). It should be emphasized here that the striatum might be not the best region to elucidate the therapeutic action of AMS, which is suggested to be more active in extrastriatal areas (Perrault *et al*, 1997; Schoemaker *et al*, 1997; Pani and Gessa, 2002). This was corroborated by the study of Xiberas *et al* (2001), who revealed an accelerated occupancy of dopamine receptors in the extrastriatal compared with striatal areas at relatively low serum concentrations of AMS. These studies in humans are in agreement with the findings in rat (see above). The reason for the regioselectivity is, however, still obscure. Thus, the primary purpose of this study was to evaluate the overall disposition of AMS to its target structures in the CNS by applying an *in vitro* BBB- and blood–cerebrospinal fluid (CSF) barrier (BCB)-model based on monolayers of porcine brain microvessel endothelial cells (PMEC) or porcine choroid plexus epithelial cells (PCEC). Although this study mainly focused on the permeation of AMS across BBB and BCB, it was also aimed at the comparison of the permeation of AMS with the permeation properties of clozapine (CLZ), another atypical antipsychotic. In contrast to AMS, CLZ is highly lipophilic and was found to be readily and equally distributed to the CNS. In rats, a more than 15-fold higher concentrations in the brain compared with blood was detected (Wilk and Stanley, 1978; Weigmann *et al*, 1999). In humans, a PET study by Nordstrom *et al* (1995) revealed rather high serotonin subtype 5-HT_{2C} receptor occupancy even at low CLZ serum concentrations. A second purpose of the study was therefore to evaluate possible different behavior of BBB and the BCB with respect to these different physicochemical substrates.

METHODS

Chemicals

Racemic AMS was kindly donated by Sanofi-Synthelabo (Quetigny, France). CLZ and its main metabolite *N*-desmethylclozapine (DCLZ) and verapamil were purchased from Sigma (Taufkirchen, Germany). (−)-[Methoxy-^{3}H]-sulpiride (^{3}H-SUL), specific activity 77 Ci/mmol, and [^{14}C]mannitol (specific activity 50–63 mCi/mmol) were purchased from Perkin-Elmer (Boston, MA, USA). Other chemicals for analytical purpose were of the highest quality commercially available. Chemicals for the preparation of cell culture- and assay-media are given in the text.

High-Performance Liquid Chromatography (HPLC) Analysis

Quantitative analysis of racemic AMS, CLZ, and DCLZ was performed mainly according to a method recently established for the analysis of sulpiride in human serum (Müller *et al*, 2001). In brief, 150 µl of the sample in assay medium had to be supplemented with 50 µl acetonitrile to obtain a UV-signal and a final injection volume of 150 µl was injected by a Gilson 231 autoinjector (Abimed-Analysentechnik, Langenfeld, Germany) on a Lichrospher-CN analytical column (125 × 4.0 mm, 5 µm particle size; MZ-Analysentechnik, Mainz, Germany) coupled to a UV-detector (LC-10, Shimadzu, Duisberg, Germany) set at 210 nm. The mobile phase consisted of 50% acetonitrile and 50% 0.008 M phosphate buffer, pH 6.4 (vol/vol). Calibration samples were prepared in PBS buffer in a concentration range between 10 and 500 µg/l AMS (0.027–1.35 µM) and 20–5000 µg/l CLZ and DCLZ (0.061–15.2 and 0.064–16.0 µM, respectively). The limit of quantification was in the range of the lowest calibration sample and the calibration curves were always linear in the whole calibration range with a correlation coefficient (r^2) always exceeding 0.99.

Determination of the Partition Coefficient (log *P*) and the Apparent Partition Coefficient (log $D_{(7.4)}$)

Log *P* values were determined by potentiometric titration using a PCA 200 instrument (Sirius analytical instruments, Forrest Row, UK) according to the equation $\log P = log(10^{(pk_a - pk_{a(o)})} - 1) - \log(r)$, where $pk_{a(o)}$ is the pk_a in an octanol/water mixture and (r) is the volume ratio octanol/water (Takacs-Novak and Avdeef, 1996; Avdeef *et al*, 1999). Log $D_{(7.4)}$ was calculated from $\log D_{(7.4)} = \log P - \log(1 + 10^{pk_a - pH})$ and assessed by the shake-flask technique (Leo, 1987) by measuring the distribution of the ionized + nonionized form between 1-octanol and an HBSS/HEPES buffer (pH 7.4).

Permeation Across Caco-2 Cells

P-gp-expressing Caco-2 cells (American Type Culture Collection, Rockville, MD) were cultivated (21–29 days) on polycarbonate filter membranes using 1.13 cm^2 Transwell™ cell culture inserts (0.4 µm pore size, polycarbonate membrane 12 mm filter, Corning Costar, Bodenheim, Germany). During this time, the development of the monolayers was monitored by transepithelial electrical resistance (TEER) measurements using a Milicell™ ERS and chopstick electrodes (Milicel ERS, Milipore, Bedford, USA) and visual control under a light microscope. As transport medium, Hank's Balanced Salt Solution (HBSS) supplemented with 10 mM MES adjusted to pH 7.4 was used throughout the experiments. Prior to the experiments, the cells were preincubated in transport buffer at 37°C at 5% CO_2 and 90% relative humidity for 15 min. Each experiment was performed in triplicate at 37°C under continuous stirring. Drug (AMS, CLZ, DCLZ) solutions (0.1 mM) were added to the donor side of the monolayers either in the absence or in the presence of verapamil (0.5 mM). Samples were taken from the acceptor side at the beginning, after 30, 60, 90, and 120 min (end of the experiment). Samples were analyzed by HPLC and [^{14}C]mannitol was applied to assess the cell monolayer integrity at the end of the experiment.

PMEC Cell Culture

Primary brain endothelial cells were isolated from porcine brain homogenate by dispase digestion followed by a

dextran density gradient centrifugation. Endothelial cells were plated onto collagen-coated culture surfaces, subcultivated after 3 days and plated in a culture medium, consisting of Medium M199 (Biochrom, Berlin, Germany), Earle's salts, 0.7 mM glutamine, antibiotics, and 10% ox serum (v/v), on rat tail collagen-coated Transwell™ filter inserts (Costar, Bodenheim, Germany). On the third day after passage, the culture medium was replaced by serum-free assay medium (Hoheisel *et al*, 1998) consisting of medium DME/Ham's F12 (Biochrom, Berlin, Germany) with 0.7 mM glutamine (Biochrom), antibiotics (Sigma), and 550 nM hydrocortisone (Sigma). The tightness of the monolayer was assessed directly prior to permeability studies by determination of the transendothelial electrical resistance (TEER) using an ENDOHM-24™ chamber and the EVOHM™ voltmeter (World Precision Instruments, Berlin, Germany) and by evaluating the paracellular diffusion of ^{14}C-sucrose after the last permeability experiment was conducted. The preparation procedure is given in detail by Franke *et al* (2000). The mean ($\pm$ SD) TEER was $1230 \pm 139\,\Omega\,cm^2$ ($N=12$) for AMS, for CLZ and $1044 \pm 211\,\Omega\,cm^2$ ($N=12$) for DCLZ. The ^{14}C-sucrose permeation was always in the range of 1×10^{-6} cm/s.

PCEC Cell Culture

PCEC from porcine choroid plexus were obtained by a preparation basically described by Crook *et al* (1981), slightly modified by Gath *et al* (1997), and described in detail by Hakvoort *et al* (1998) and Haselbach *et al* (2001). In brief, porcine choroid plexus tissue was digested by trypsin, released cells were centrifuged and thereafter resuspended in DME/HAM's F12 medium supplemented with 10% fetal bovine serum (Biochrom), 4 mM L-glutamin, 5 μg/ml insulin (Sigma), 20 μM cytosine arabinoside (Sigma), and 100 μg/ml antibiotics (Sigma). After 5 days, cells were seeded on laminin (Sigma)-coated permeable membranes (Costar, Cambridge, MA, USA). After reaching confluence (7–9 days), cells were washed and incubated with a serum-free medium (DMA/HAM's F-12 medium (1 : 1) supplemented with 4 mM L-glutamine and 5 μg/ml insulin). The mean ($\pm$ SD) TEER was $1819 \pm 415\,\Omega\,cm^2$ ($N=12$) for AMS, $2022 \pm 403\,\Omega\,cm^2$ ($N=12$) for CLZ, and $1768 \pm 330\,\Omega\,cm^2$ ($N=12$) for DCLZ.

Permeability Studies

The experimental setup for permeation studies across PMEC is described in detail by Franke *et al* (1999, 2000). AMS, CLZ, or DCLZ were applied to the apical (luminal) side, representing the lumen of a microvessel, and samples of 150 μl were taken from the basolateral acceptor compartment after 15, 30, 45, and 60 min. The sample volume was always replaced by an equal volume of fresh medium. Each drug was tested at four different concentrations (1, 5, 10, and 30 μM) and three different filters/concentration were used. The concentrations were chosen to resemble utmost the concentrations under therapeutic conditions in humans. Only AMS (30 μM) was tested in the opposite direction from basolateral to apical, representing the efflux from CNS into the blood. To determine active transport, ^{3}H-SUL, which is structurally related to AMS, was applied to both sides (apical and basolateral) in equimolar concentrations (1, 5, 10, and 30 μM). Radioactivity at each side was measured after 0.5, 22.5, 29, 47, and 53 h in a beta-counter. For permeation studies across PCEC, drugs were applied to the basolateral side, which represents the blood side, while samples were taken from the apical side, which represents the cerebrospinal fluid. The concentrations of drugs and sampling times were the same as described for PMEC permeation. All transport experiments were conducted at 37°C. To correct for possible additional contribution of the coated filters to the total permeation, diffusion of drugs (only AMS and CLZ) through coated filters without cell monolayers was tested (1, 5, 10, and 30 μM; samples taken after 5, 10, 15, and 20 min).

Calculation

Permeability coefficients (P) were calculated according to Pardridge *et al* (1990) using the equation

$$P(\mathrm{cm/s}) = (\mathrm{d}Q/\mathrm{d}t \times V)/(A \times C_0)$$

where $\mathrm{d}Q/\mathrm{d}t$ is the translocation rate, V is the volume of the acceptor compartment, A is the filter surface (1.13 cm^2), and C_0 is the initial concentration.

To correct for the filter contribution, the total endothelial permeability coefficient (P) was divided by the blank filter diffusion (P_f) and given as percentage of unrestricted diffusion through blank filter.

If no concentration dependency was detectable, P was calculated as mean $\pm$ SD from all concentrations after 60 min of incubation. Kinetic analyses were performed by means of nonlinear least square curve fitting using the GraFit program (version 4.03, Erithacus Software Ltd, Staines, UK).

RESULTS

Log *P* and log $D_{(7.4)}$ Values

The potentiometric method and the shake-flask method gave comparable results for all drugs tested. The exact log P and log $D_{(7.4)}$ values are given in Table 1. While the difference between log P and log $D_{(7.4)}$ was only negligible in the case of CLZ, the difference was almost 10-fold for DCLZ and about 50-fold for AMS.

Permeation Through Coated Blank Filter

Diffusion through coated blank filter was about 10-fold lower for AMS compared with CLZ (2.18×10^{-5} *vs* 3.75×10^{-4} cm/s and 3.6×10^{-5} *vs* 4.7×10^{-4} cm/s for PMEC and PCEC, respectively).

Permeation Across Caco-2 Cells

The permeation of AMS showed a 2.68 higher secretory flux (basolateral to apical, b–a) compared to the resorptive flux (apical to basolateral, a–b), as shown in Table 2. Furthermore, addition of 0.5 mM verapamil increased the flux in the resorptive direction from 0.22 ± 0.02 to $0.46 \pm 0.03 \times 10^{-5}$ cm/s (Table 2). While the flux in the secretory direction was slightly higher than in the resorptive

Table 1 pk_a, log P, and log $D_{(7.4)}$ Values of AMS, CLZ, and DCLZ

Compound	pk_a mean[a] ± SD (N)	log P mean ± SD (N)	log $D_{(7.4)}$ potentiometric method	log $D_{(7.4)}$ [b] shake flask
Amisulpride	9.08 ± 0.01 (3)	1.402 ± 0.02 (2)	−0.265	−0.265
Clozapine	7.41 ± 0.01 (3)	3.642 ± 0.02 (3)	3.281	2.735
DCLZ	8.50 ± 0.01 (3)	2.782 ± 0.03 (3)	1.839	2.379

[a]pk_a values were determined from various methanol–water mixtures at 25°C with an ionic strength of 0.15 M KCl using the Yasuda–Shedlovsky extrapolation method.
[b]At pH of 7.4.

Table 2 Permeability Coefficients (*P*) of AMS (0.1 mM), CLZ (0.1 mM), and DCLZ (0.1 mM) Across Caco-2 Cell Monolayers and Effect of Verapamil (+V, 0.5 mM) Using Transport Studies in the Absorptive-, Apical-to-Basolateral (a–b), and Secretory-, Basolateral-to-Apical (b–a) Directions

Drug	Flux direction	*P* (10^{-5} cm/s)	Ratio: *P* (b–a)/*P* (a–b)	Ratio: $P_{+verapamil}$ (a–b)/*P* (a–b)
AMS	a–b	0.22 ± 0.02	2.68	2.09
AMS	b–a	0.59 ± 0.07		
AMS+V	a–b	0.46 ± 0.03		
CLZ	a–b	3.11 ± 0.12	1.09	0.99
CLZ	b–a	3.42 ± 0.60		
CLZ+V	a–b	3.09 ± 0.16		
DCLZ	a–b	2.10 ± 0.48	1.58	1.25
DCLZ	b–a	3.32 ± 0.15		
DCLZ+V	a–b	2.63 ± 0.22		

DATA are presented as means ($n = 2$ or 3 measurements) ± SD.

direction ($2.1 \pm 0.5 \times 10^{-5}$ *vs* $3.3 \pm 0.2 \times 10^{-5}$ cm/s) for DCLZ, there was no difference between the resorptive and the secretory flux for CLZ with $3.11 \pm 0.1 \times 10^{-5}$ and $3.42 \pm 0.6 \times 10^{-5}$, respectively.

Permeation Across PMEC

AMS was not able to permeate PMEC in considerable concentrations in the resorptive direction. Only with one of three preparations, measurable amounts were found in the acceptor compartment at the highest concentration of 30 μM and after 60-min incubation time. The approximated *P* ($<10^{-7}$ cm/s) was even lower than that of sucrose (about 10^{-6} cm/s). On the other hand, a considerable permeation in the secretory direction (basolateral to apical) was found with a *P* (mean ± SD) of $5.2 \pm 3.6 \times 10^{-6}$ cm/s. The *P* of CLZ and DCLZ in the resorptive direction was more than a magnitude higher with $P = 2.3 \pm 1.2 \times 10^{-4}$ and $0.96 \pm 0.5 \times 10^{-4}$ cm/s, respectively (Figure 1). Concentration dependence became only visible for DCLZ (Figure 1) pointing to a saturable process at concentrations higher than 1 μM. At maximum, 50% of the applied CLZ dose was found at the basolateral side, while only 12% were measurable for DCLZ. Compared with the permeation through the blank filter, the permeation through PMEC made up 62 ± 5.4% for CLZ but <1% for AMS. Permeation kinetics of CLZ and DCLZ over time were best described by a quadratic function (best fit of the data), with $y = 0 \quad 0.0061x^2 + 0.227x + 0.1032$ ($R^2 = 0.9995$) and $y = 0.001x^2 + 0.055x + 0.1427$ ($R^2 = 0.9999$) for CLZ and DCLZ, respectively, assuming slightly nonlinear kinetics with accelerated permeation rates at higher concentrations (Figure 1). No active transport was found for ^{3}H-SUL (Figure 2).

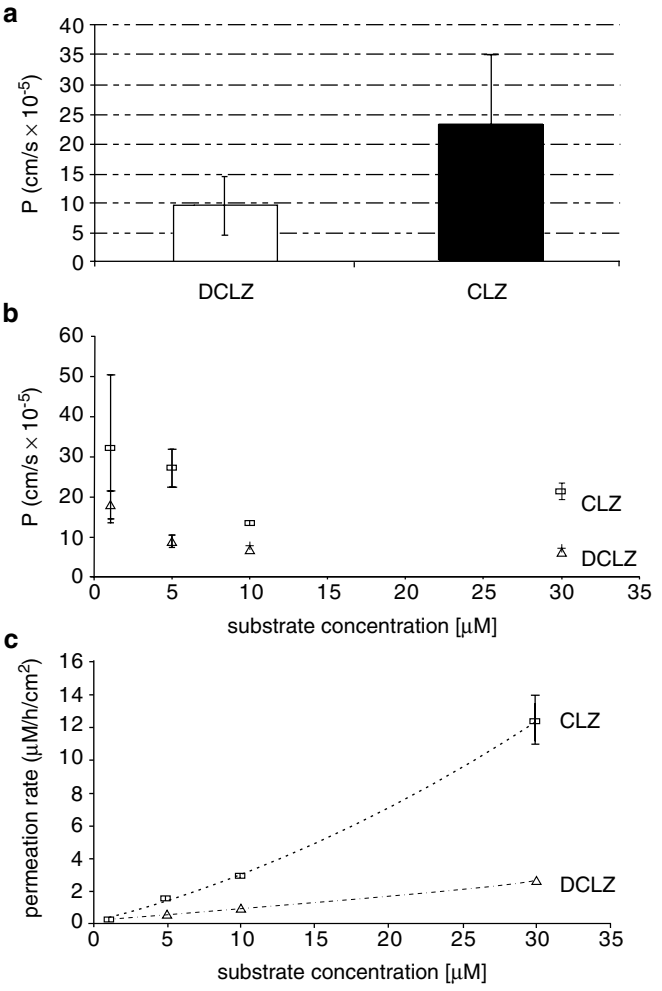

Figure 1 Upper panel (a) Permeability of CLZ and DCLZ through porcine microvessel endothelial cell monolayer (PMEC) in the resorptive direction (apical–basolateral). The mean permeability coefficients (*P*) ± SD of four different concentrations (1, 5, 10, and 30 μM) through three PMEC different preparations each are given. Middle panel (b) Concentration dependence of the permeability coefficients (*P*) for the apical to basolateral transport through PMEC for CLZ (squares) and DCLZ (triangles). Lower panel (c) Permeation kinetics (permeation rate *vs* substrate concentration) of CLZ (squares) and NDCLZ (triangles) across PMEC. Kinetics was best described by a quadratic function with $y = 0.0061x^2 + 0.227x + 0.1032$ ($R^2 = 0.9995$) and $y = 0.001x^2 + 0.055x + 0.1427$ ($R^2 = 0.9999$) for CLZ and DCLZ, respectively.

Permeation Across PCEC

All drugs tested were able to permeate plexus epithelial cells from the basolateral (blood) to the apical (plexus) side. The *P* values were, however, rather different with $1.7 \pm 2.5 \times 10^{-6}$ cm/s for AMI, $2.3 \pm 1.2 \times 10^{-5}$ cm/s for DCLZ, and $1.6 \pm 0.95 \times 10^{-4}$ cm/s for CLZ (Figure 3). Permeation across PCEC compared with blank filter diffusion was 9.3 ± 6.6 and 62 ± 5.4% for AMS and CLZ,

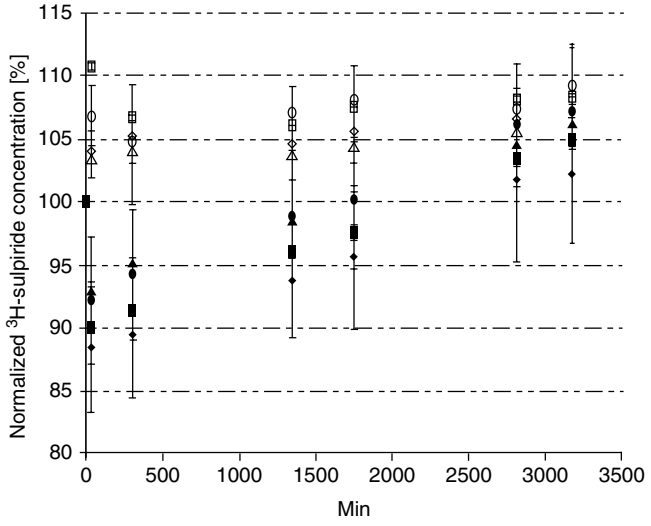

Figure 2 Time-dependent permeation of [^{3}H]sulpiride across porcine microvessel endothelial cells over time (up to 53 h) from apical to basolateral (resorptive, filled symbols) or from basolateral to apical (efflux, open symbols). Each datapoint represents the mean ($\pm$ SD) of $N = 3$ measurements. Measured concentrations were normalized to the concentration in the apical compartment at the beginning = 100%. Different concentrations are depicted as diamond (1 μM), triangle (5 μM), circle (10 μM), and square (30 μM). No active transport in either direction was detectable.

respectively (Figure 3). The permeation kinetics was mostly linear over the entire concentration range for all substances (Figure 3).

DISCUSSION

This study was the first that investigated the permeation of two atypical antipsychotics through biomembranes that restrict free access to the CNS. The study revealed tremendous differences between the two atypical antipsychotics, CLZ and AMS. As these are structurally unrelated compounds—the former is a highly lipophilic dibenzazepine with a pk_a of about 7.4, AMS is a more hydrophilic substituted benzamide with a pk_a of about 9.08—differences had been expected (Doan *et al*, 2002). Interestingly, while the partition between octanol and buffer expressed as log *P* was 175-fold higher for CLZ, the apparent partition ($\log D_{(7.4)}$) at a more physiological pH of 7.4 was almost 4000-fold higher for CLZ. As the permeation experiments were conducted at physiological pH, $\log D_{(7.4)}$ seemed to correlate much better with the permeation through microvessel endothelial cells than log *P*. Both drugs have to pass the BBB to exert their pharmacological effects. It was thus unexpected that AMS obviously did not penetrate the BBB model. It should be mentioned that the permeability of AMS through the cell-free filter was about 10-fold lower compared with CLZ. The difference in the permeation of AMS through PMEC could, however, not be solely explained by this methodological issue since the difference was more than 1000-fold comparing CLZ and AMS in the resorptive direction. The poor permeation in the resorptive direction was even more restricted by a remarkable transport in the secretory direction. This prominent flux in the secretory direction also became visible when the Caco-2 model was

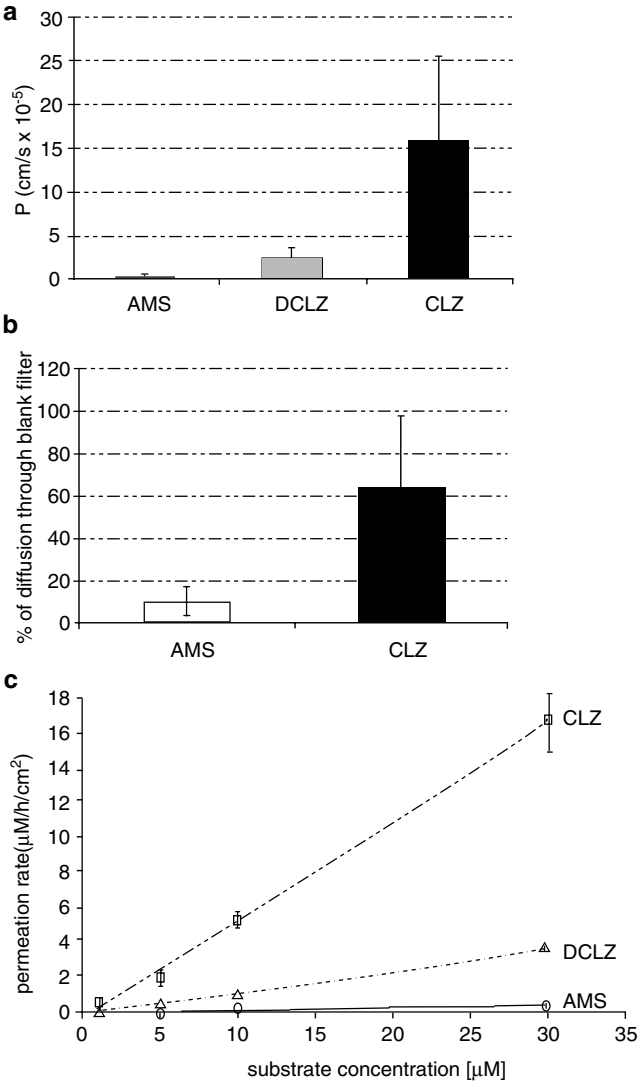

Figure 3 Upper left panel (a) Permeability of AMS, DCLZ, and CLZ across PCEC in the resorptive direction (basolateral to apical). The mean permeability coefficients (*P*) $\pm$ SD of four different concentration (1, 5, 10, and 30 μM) through three different PCEC preparations each are given. Upper right panel (b) Permeation of AMS and CLZ across PCEC in comparison to the unrestricted diffusion through coated filters. Lower panel (c) Permeation kinetics (permeation rate *vs* substrate concentration) of AMS (circles), CLZ (squares), and DCLZ (triangles) across PCEC. Kinetics was linear over the concentration range with $y = 0.572x - 0.35$ ($R^2 = 0.998$) for CLZ, $y = 0.122x - 0.046$ ($R^2 = 0.998$) for DCLZ, and $y = 0.0124x + 0.01$ ($R^2 = 0.990$) for AMS.

applied. A ratio >2 of the apical to basolateral permeability (resorptive direction) *vs* apical to basolateral permeability (secretory direction) across Caco-2 monolayers was reported to be indicative of P-glycoprotein (Pgp) substrates (Polli *et al*, 2001). We would, therefore, regard AMS as a Pgp substrate, while CLZ and DCLZ did not fulfill the criteria of a Pgp-sensitive drug. DCLZ had a lower permeability rate than CLZ, which was in good agreement with our findings in rats after oral administration of CLZ (Weigmann *et al*, 1999). In contrast to PMEC, permeation of AMS across plexus epithelial cells was higher and more comparable to the permeation of CLZ and DCLZ. The difference between CLZ and DCLZ was even more

pronounced at the PCEC-monolayer with about a 10-fold higher *P* of CLZ. The generally higher permeation rates of CLZ might be also caused by an active transport through these barriers as it had been shown to play a role for the synthetic opioid fentanyl (Henthorn *et al*, 1999). The lack of transport of AMS across this BBB model was a rather surprising result. It seems to be caused by the additive effects of disadvantageous physicochemical properties and an efflux transport. The latter might be catalyzed by Pgp, which has been detected at the luminal side of porcine microvessel endothelial cells (Miller *et al*, 2000). In a recent study, the structurally related benzamide SUL was suggested to be a substrate of Pgp (Baluom *et al*, 2001). Our own experiments, however, did not provide any evidence for an active transport of SUL at the PMEC and at a concentration of 10 μM, SUL was able to permeate PMEC with a *P* of 2.2×10^{-6} cm/s. This pointed to different properties of these chemically related drugs with respect to the CNS disposition. Pgp could also be the reason for the accelerated transport across PCEC, since it was found to be expressed at the luminal side of plexus epithelial cells (Rao *et al*, 1999). Besides Pgp, multidrug resistance associated protein (MRP2) was detected at the luminal side of PMEC (Miller *et al*, 2000) and at the basolateral (blood-) side of PCEC (MRP1) (Gao and Meier, 2001). Quite recently, the so-called brain multidrug resistance protein (BMDP), a new member of the ABCG subfamily, was found to be expressed in high rates in PMEC (Eisenblätter and Galla, 2002; Eisenblätter *et al*, 2003). It might participate in drug efflux at the BBB and needs to be considered. While AMS is likely to be a substrate of Pgp, it is still unknown if one of the drugs tested might be a substrate of other transporters like MRP, BMDP, or the organic anion transporter protein (OATP), which is expressed especially at the BCB (Angeletti *et al*, 1997). Furthermore, this is only an *in vitro* model of the BBB and BCB and conclusions from our results on the *in vivo* situation in humans should be drawn rather cautiously. Limitations of the applied BBB model includes the fact that porcine cells were used that do not necessarily express the same transporter proteins at the same rate as in human tissue. Secondly, it is an artificial system and thus even though the expressed proteins are highly conserved, the expression rate might differ substantially from the *in vivo* situation (Miller *et al*, 2000; Gutmann *et al*, 1999). The most important structural difference, however, is related to the fact that we have no brain tissue behind the acceptor side. Brain tissue might act as a lipophilic sponge by increasing the flux rates, especially of lipophilic drugs that are not a substrate of an efflux transporter. Despite these limitations, it can be concluded that AMS is evidently not able to permeate the BBB to a considerable extent. It is thus worth hypothesizing about the route of AMS to its targets in the brain. CSF functions probably not only include mechanical protection of the brain and a sink action but also nutrient supply and even drug delivery to the brain and from the brain to the periphery (Ghersi-Egea and Strazielle, 2001; King *et al*, 2001). Results of our study suggest that AMS might be at least partially distributed to its targets via the ventricular CSF. This would explain the higher concentrations measured in brain areas more close to the ventricles like the hypothalamus compared with structures like the thalamus and might be one reason for the proposed preferential extrastriatal action of AMS. It would be an interesting new aspect in the development of psychotropic drugs if certain drugs can actually enter the CNS via the CSF. However, the hypothesis needs to be proven in future experiments.

If we focus on the chemically related benzamide antipsychotics sulpiride and AMS, the latter had about a 10-fold higher affinity to dopamine D_2 and D_3 receptors than sulpiride (*in vitro* dissociation constants, K_i, at the D_2 receptor 1.3 *vs* 10 nmol/l for AMS and sulpiride, respectively; for reference, see Coukell *et al*, 1996). Moreover, AMS was so far suggested to permeate better into brain tissue due to its slightly higher lipophilicity. However, from *in vivo* radioligand binding studies using [^{3}H]raclopride, sulpiride and AMS displaced [^{3}H]raclopride binding with a comparable ED_{50} for limbic structures (14.6 *vs* 17.3 mg/kg i.p.) and for the striatum (45.5 *vs* 43.6 mg/kg i.p.) (Schoemaker *et al*, 1997). This discrepancy between the *in vitro* and *in vivo* properties can be easily explained by the restricted permeation of AMS across the BBB as shown in our experiments. The higher *in vitro* affinity of AMS to its target receptor is thus obviously overshadowed by the poor CNS permeability. This is another example of the importance of evaluating the CNS permeability of psychotropic drugs to assess their actual therapeutic efficacy.

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