Effects of Chronic Renal Failure on Brain Drug Transporters in Rats

Judith Naud, Louis-Philippe Laurin, Josée Michaud, Stéphanie Beauchemin, Francois A. Leblond, and Vincent Pichette

Service de Néphrologie et Centre de Recherche de l’Hôpital Maisonneuve-Rosemont (J.N., L.-P.L., J.M., S.B., F.A.L., V.P.) and Département de Pharmacologie (J.N., J.M., V.P.), Faculté de Médecine, Université de Montréal, Québec, Canada

ABSTRACT:

Studies demonstrated that chronic renal failure (CRF) affects the expression and activity of intestinal, hepatic, and renal drug transporters. Such drug transporters are expressed in brain cells and at the blood-brain barrier (BBB), where they limit the entry and distribution of drugs in the brain. Perturbations in brain drug transporter equilibrium by CRF could lead to central drug toxicity. This study evaluates how CRF affects BBB drug transporters using a 5/6 nephrectomized rat model. Protein and mRNA levels of Bcrp, Mrp2 to -4, Oat3, Oatp2 and -3, and P-gp in CRF rat brain biopsies, as well as in astrocytes and RBECs incubated with CRF serum. These decreases did not correlate with in vivo changes because BBB permeability of benzylpenicillin was decreased by 30% in CRF rats, whereas digoxin, doxorubicin, and verapamil permeabilities were unchanged. It thus seems that even with decreased drug transporters, BBB integrity and function is conserved in CRF.

INTRODUCTION

In recent years, many studies have demonstrated that chronic renal failure (CRF) significantly affects the expression and activity of intestinal, hepatic, and renal drug transporters via uremic mediators, thus altering the pharmacokinetics of many drugs (Huang et al., 2000; Laouari et al., 2001; Sun et al., 2004, 2006; Naud et al., 2007, 2008, 2011). Such drug transporters are also expressed in brain cells and at the blood-brain barrier (BBB) where they limit the entry and distribution of drugs in the brain (Fig. 1) (Löscher and Potschka, 2005a,b,c; Ohtsuki and Terasaki, 2007). Two major drug transporter families have been identified: ATP-binding cassette (ABC) transporters and solute carriers. The most important ABC transporters at the BBB are ATP-binding cassette (ABC) transporters and solute carriers. The most important transporters at the BBB are ABC transporters, which mediate the efflux of molecules from the capillary cell into the blood (Kusuhara and Sugiyama, 2005; Löscher and Potschka, 2005c; Deeken and Löscher, 2007; Oswald et al., 2007; Eyal et al., 2009). Mrp4 was also found on the abluminal side of bovine brain capillaries, where it is thought to mediate the efflux of molecules from the capillary cell into the brain, but this was not reported in rat and human brain (Kusuhara and Sugiyama, 2005; Löscher and Potschka, 2005c; Deeken and Löscher, 2007; Oswald et al., 2007). For solute carriers, the most important at the BBB are the organic anion transporter 3 (Oat3) and the organic anion-transporting polypeptides 2 and 3 (Oatp2–3). Oat3 is located on both membranes and mediates drug efflux from the brain into the blood (Kikuchi et al., 2003; Mori et al., 2003, 2004; Löscher and Potschka, 2005c; Deeken and Löscher, 2007; Oswald et al., 2007). Furthermore, it was recently hypothesized that Oat3 is also implicated in the influx of drugs from the blood to the brain (Ose et al., 2009). Oatp2 is expressed at the luminal and abluminal membranes, whereas Oatp3 was found only at the luminal membrane of brain capillary cells, and they both mediate bidirectional transport of drugs (Löscher and Potschka, 2005c; Deeken and Löscher, 2007; Ohtsuki and Terasaki, 2007; Ose et al., 2010). Perturbations in brain drug transporter equilibrium caused by CRF could lead to central toxicity of drugs.

This work was supported by La Fondation Hôpital Maisonneuve-Rosemont (La Néphrologie et son Impact), V. Pichette was supported by a career award from Fonds de Recherche en Santé du Québec.

ABBREVIATIONS: CRF, chronic renal failure; Bcrp, breast cancer resistance protein; CT, cycle threshold. Oat, organic anion transporters; Oatp, organic anion-transporting polypeptide; PCR, polymerase chain reaction; P-gp, P-glycoprotein; PMSF, phenylmethylsulfonylfluoride; BBB, blood-brain barrier; IL, interleukin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PAH, p-aminohippuric acid; IS, indoxyl sulfate; CMPF, 3-carboxyl-4-methyl-5-propyl-2-furonapropionate; RBEC, rat brain endothelial cell; ECBM, endothelial cell basal medium; ABC, ATP-binding cassette; Crt, cycle threshold.
Animals were allowed an acclimatization period of at least 7 days before the Research Center animal care facility and maintained on Harlan Teklad rodent Laboratories, Portage, MI, weighing 176 to 225 g, were housed in the rats; and 3) the in vivo intracerebral accumulation of [14C]benzylpenicillin, [3H]digoxin, [14C]doxorubicin, and [3H]verapamil for sub-

Experimental Model. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI), weighing 176 to 225 g, were housed in the Research Center animal care facility and maintained on Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, QC, Canada) and water ad libitum. Animals were allowed an acclimatization period of at least 7 days before the first nephrectomy was performed. All the experiments were conducted according to the Canadian Council on Animal Care guidelines for the care and use of laboratory animals and under the supervision of our local animal care committee.

Experimental Protocol. Studies were performed in two groups of animals: control pair-fed and CRF. Drug transporter protein expression and mRNA levels were measured in at least six rats per group. Drug transporter activity was measured in vivo through the cerebral accumulation of [14C]benzylpenicillin, [3H]digoxin, [14C]doxorubicin, and [3H]verapamil in at least three rats per group, per experiment.

Chronic renal failure was induced by two-stage 5/6 nephrectomy as described previously (Leblond et al., 2001). Every animal had ad libitum access to water, but to limit the effects of CRF-induced malnutrition, control pair-fed rats were fed the same amount of chow that CRF rats ate on the previous day. We have previously demonstrated that the caloric reduction in control animals induced by pair-feeding did not influence the expression of drug transporters (our unpublished data). At day 41 after nephrectomy, the rats were housed in metabolic cages, and urine was collected for 24 h to determine the clearance of creatinine. Rats were sacrificed by decapitation 42 days after nephrectomy for organ and blood collection or underwent a radiolabeled drug accumulation study. After the rats were sacrificed, the brain was immediately excised, rinsed in ice-cold saline, and flash-frozen in liquid nitrogen. Samples were stored at −80°C until membrane preparation or mRNA extraction. Blood was collected for the measurement of serum creatinine and urea and for bioassays with cultured RBECs and astrocytes.

Preparation of Crude Brain Membranes. Frozen rat brain biopsies (500 mg) were homogenized in 3 ml of homogenizing buffer (250 mM sucrose, 10 mM HEPES, and 10 mM Tris-HCl, pH 7.4) containing 0.1 mM PMSF using a Potter-Elvehjem tissue grinder (Wheaton Science Products, Millville, NJ). Differential centrifugation was used to obtain a crude membrane fraction (Chow et al., 2010). In brief, the brain homogenates were centrifuged at 9000 g for 60 min at 4°C, and the resulting pellet was resuspended in phosphate-buffered saline containing 0.1 mM PMSF. Samples were then sonicated on ice for 10 s to ensure homogeneity. Protein concentration was determined using the method of Lowry et al. (1951), using bovine serum albumin as a reference protein. Aliquots were stored at −80°C up to Western blot analysis.

Western Blot Analysis. Major brain drug transporters were assessed by Western blot analysis following a previously described protocol (Leblond et

Materials and Methods

Experimental Model. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI), weighing 176 to 225 g, were housed in the Research Center animal care facility and maintained on Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, QC, Canada) and water ad libitum. Animals were allowed an acclimatization period of at least 7 days before the

Materials and Methods

Experimental Model. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI), weighing 176 to 225 g, were housed in the Research Center animal care facility and maintained on Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, QC, Canada) and water ad libitum. Animals were allowed an acclimatization period of at least 7 days before the

Materials and Methods

Experimental Model. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI), weighing 176 to 225 g, were housed in the Research Center animal care facility and maintained on Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, QC, Canada) and water ad libitum. Animals were allowed an acclimatization period of at least 7 days before the
Effect of Uremic Serum on Brain Drug Transporters. To determine whether uremic serum could affect the expression and function of blood-brain barrier drug transporters, we incubated RBECs and astrocytes with serum from nephrectomized rats and evaluated the drug transporters' protein expression.

Isolation and Culture of Rat Astrocytes. Rat astrocytes were isolated and cultured as described previously by Perrière et al. (2005) and Booher and Sensenbrenner (1972). When cells reached confluency, the standard culture medium was changed to culture medium containing 10% serum from either control or nephrectomized rats (one serum per flask). After 48 h of culture, the culture medium was recovered under sterile conditions to be used for RBECC conditioning, and cells were trypsinized, pelleted, and homogenized in phosphate-buffered saline containing 0.1 mM PMSF. Protein concentrations were determined using the Micro BCA Protein Assay Kit (Thermo Fisher Scientific). One hundred micrograms was loaded for Western blot analysis of each studied transporter.

Isolation and Culture of RBECs. RBECs were isolated and purified for 72 h as published previously (Perrière et al., 2005). Culture medium was then changed, for a 24-h period, to endothelial cell basal medium (ECBM) containing 20% bovine plasma-derived serum, 2 ng/ml basic fibroblast growth factor, 500 ng/ml hydrocortisone, and 1% Penstrep (a mixture of 20,000 U/mg penicillin and streptomycin) (Perrière et al., 2005). Four days after seeding, the culture medium was again changed to a 50:50 mix of predescribed ECM culture medium and culture medium recovered from astrocyte cultures containing 10% fetal bovine serum to simulate coculture with astrocytes. Finally, the culture medium was changed 48 h later to a new 50:50 mix of predescribed ECM containing 20% serum from CTL or nephrectomized rats and astrocyte preconditioned medium with sera from CTL of CRF rats. 8-(4-Chlorophenylthio)-cAMP (250 μM) and the cAMP phosphodiesterase-4-specific inhibitor RO 20 1724 (17.5 μM) were added to the final mix. After 48 h of culture, cells were trypsinized, pelleted, and homogenized in phosphate-buffered saline containing 0.1 mM PMSF. Protein concentrations were determined using the Micro BCA Protein Assay Kit (Thermo Fisher Scientific). One hundred micrograms was loaded for Western blot analysis of each studied transporter.

Other Assays. Blood and urine chemistries were determined with an Architect C16000 clinical analyzer (Abbott Laboratories, Saint-Laurent, QC, Canada).

Statistical Analysis. Results are expressed as mean ± S.D. Differences between groups were assessed using an unpaired Student’s t-test or an analysis of variance test. Significant analysis of variance was followed by a Scheffe’s post hoc comparison of groups. The threshold of significance was p < 0.05.

Results

Biochemical Parameters and Body Weight in Control and CRF Rats. Table 2 presents the biochemical parameters and body weights of both groups of rats. Compared with control animals, CRF rats had higher levels of serum creatinine and urea and lower creatinine clearance (reduced by 80%; p < 0.001). There was no difference in body weight between control and CRF rats.

Protein Expression of Brain Drug Transporters in CRF Rats. Figure 2 presents the measured protein expression of various drug transporters in crude brain membranes of control and CRF rats. The expression level of Bcrp, Mrp2, Mrp4, Oat3, Oatp2, and Oatp3 was significantly decreased from 20 to 50% in CRF rat crude brain membranes as shown by Western blot. The only measured protein that remained stable was Mrp5. We found significant correlations between protein expression and the rat clearance of creatinine for Bcrp (r = 0.64, n = 32, p < 0.001).

TABLE 2

<table>
<thead>
<tr>
<th>Control (n = 32)</th>
<th>CRF (n = 32)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>352.8 ± 29.5</td>
<td>333.0 ± 54.5</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>55.4 ± 10.5</td>
<td>205.0 ± 90.3</td>
</tr>
<tr>
<td>Creatinine clearance (μL/100 g b.w.t/min)</td>
<td>309.1 ± 82.4</td>
<td>70.1 ± 55.5</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>4.5 ± 1.0</td>
<td>38.4 ± 17.9</td>
</tr>
</tbody>
</table>

Data are the mean ± S.D. Measurements were made at the time of sacrifice. Twenty-four-hour urinary collection was begun the day before. Blood urea and creatinine and urine creatinine were determined with an Architect C16000 clinical analyzer (Abbott Laboratories).
mRNA Expression of Brain Drug Transporters in CRF Rats. Figure 3 presents results from the analysis of mRNA coding for brain drug transporters. There were significant decreases in the expression of Bcrp (35%), Mrp2 (40%), Mrp4 (30%), Oat3 (40%), Oatp2 (35%), Oatp3 (70%), and P-gp (35%) mRNA in CRF rat brain compared with controls. Again, only Mrp5 mRNA remained stable between the groups.

In Vivo Accumulation of Radiolabeled Drugs in the Brain of Rats with Surgically Induced Chronic Renal Failure. Two hours after administration, digoxin plasma radioactivity was similar in control and CRF rats, whereas the plasma radioactivity of benzylpenicillin was slightly elevated by 17% in CRF rats compared with control animals (Fig. 4). There was 33% less [14C]benzylpenicillin in this tissue compared with controls. This would suggest a change in the uptake of benzylpenicillin across the blood-brain barrier due to changes in the expression of drug transporters in the brain of CRF rats.
brain of CRF rats. On the other hand, no significant differences in \[\text{[H]}\text{digoxin}, \text{[14C]}\text{doxorubicin}, \text{or [H]}\text{verapamil brain accumulation}\] were observed between CTL and CRF rats. These data were obtained via a ratio of radioactivity for 1 g of brain tissue/1 ml plasma.

**Protein Expression of Drug Transporters in RBECs Incubated with Serum from CRF Rats.** Protein expression of drug transporters was measured in rat brain endothelial cells incubated for 48 h with serum from control or CRF animals (Fig. 5). There was a significant decrease in the expression of Bcrp (40%), Mrp2 (25%), Mrp4 (45%), Oat3 (35%), Oatp2 (35%), Oatp3 (25%), and P-gp (30%) protein when incubated with CRF serum compared with controls. On the other hand, the expression of Mrp5 was unaltered. These results were similar to those obtained in vivo in CRF rats.

**Protein Expression of Drug Transporters in Rat Astrocytes Incubated with Serum from CRF Rats.** Protein expression of drug transporters was measured in rat astrocytes incubated for 48 h with serum from control or CRF animals (Fig. 6). Significant decreases in the expression of Bcrp (25%), Mrp2 (25%), Mrp4 (30%), Oat3 (60%), Oatp2 (40%), Oatp3 (50%), and P-gp (30%) protein were observed when incubated with CRF serum compared with controls. The expression of Mrp5 remained unaltered. These results were similar to those obtained in vivo in CRF rats.

**Discussion**

This study demonstrated a significant (20–50%) decrease in protein and mRNA expression of Bcrp, Mrp2 and 4, Oat3, Oatp2 and 3, and P-gp in crude rat brain membranes from CRF rats compared with CTL rats, whereas Mrp5 was unchanged. Similar reductions were observed in RBECs and astrocytes incubated with serum from nephrectomized rat serum, compared with control. Influx and efflux drug transporter...
expression at the BBB and in the brain thus seem equally affected by CRF. For BBB permeability, we observed a 30% decrease in the BBB permeability to benzylpenicillin in nephrectomized rats and no change in the BBB permeability to digoxin, doxorubicin, and verapamil as measured by their brain/plasma radioactivity ratio.

We found significant correlations between the clearance of creatinine and the expression of all down-regulated transporters at the protein level, indicating that the changes in transporters are related to the severity of the renal failure. In addition, we have demonstrated that the modifications in drug transporter expression occurred in response to one or many factors present in the sera from uremic rats in at least two brain cell types, as demonstrated by the changes in protein expression of transporters in astrocytes and RBECs, which are similar to those observed in brain biopsies of CRF rats.

To our knowledge, no group has studied the impact of renal failure on the expression of brain drug transporters. However, many studies have reported a change in drug transporters in various organs in CRF (Huang et al., 2000; Laouari et al., 2001; Sun et al., 2004, 2006; Naud et al., 2007, 2008, 2011). In some of these studies, uremic circulating factors seemed involved in the expression and activity changes. Although no specific factor has been identified, many potential factors include uremic toxins such as CMPF, IS, or PAH and proinflammatory cytokines that are known to be elevated in CRF (Oettinger et al., 1994; Sunder-Plassmann et al., 1994; Ziesche et al., 1994; Akahoshi et al., 1995; Higuchi et al., 1997). Indeed, uremic toxins have been shown to inhibit drug uptake by Oat1, Oat3, and Oatp2 in the rat liver, kidney, and brain (Deguchi et al., 2003, 2006). They observed increased plasma and brain concentrations of doxorubicin in mice 6 h after the induction of inflammation compared with control. However, endotoxin-induced systemic inflammation did not significantly change the brain/plasma concentration ratio of doxorubicin (Zhao et al., 2002). The authors thus conclude that endotoxin-induced systemic inflammation has no effect on BBB integrity and doxorubicin transport across the blood-brain barrier in mice (Zhao et al., 2002). In addition, another study demonstrated that central nervous system inflammation produces a 50% decrease in P-gp mRNA expression and an almost 100% decrease in Oatp2 mRNA in the rat brain 6 h after induction (Torres et al., 2002). This group used [3H]digoxin as a probe for P-gp function in vivo and observed, 6 and 24 h after inflammation induction, an increase in brain and plasma [3H]-radioactivity (Torres et al., 2002). However, the brain/plasma radioactivity ratio was unchanged (Torres et al., 2002). Based on these results, and because CRF is a state of chronic inflammation, it could explain why, like Zhao et al. (2002) and Goralski et al. (2003), we observe decreased protein and mRNA expression of P-gp and Oatp2 in the brain of nephrectomized rats but no intracerebral accumulation of digoxin, doxorubicin, or verapamil.

Despite significant decreases of brain transporter expression in CRF rats, we did not observe in vivo brain accumulation of three different substrates, namely digoxin, doxorubicin, and verapamil. Such a discrepancy has also been observed in inflammation studies. Indeed, previously published data demonstrated that systemic inflammation, as seen in CRF, moderately down-regulated (~24%) the protein expression of P-gp at the BBB 6 h after the induction of inflammation by endotoxin (Zhao et al., 2002). In that study, P-gp expression returned to normal 24 h after inflammation (Zhao et al., 2002). They observed increased plasma and brain concentrations of doxorubicin in mice 6 h after the induction of inflammation compared with control. However, endotoxin-induced systemic inflammation did not significantly change the brain/plasma concentration ratio of doxorubicin (Zhao et al., 2002). The authors thus conclude that endotoxin-induced systemic inflammation has no effect on BBB integrity and doxorubicin transport across the blood-brain barrier in mice (Zhao et al., 2002). In addition, another study demonstrated that central nervous system inflammation produces a 50% decrease in P-gp mRNA expression and an almost 100% decrease in Oatp2 mRNA in the rat brain 6 h after induction (Torres et al., 2002). This group used [3H]digoxin as a probe for P-gp function in vivo and observed, 6 and 24 h after inflammation induction, an increase in brain and plasma [3H]-radioactivity (Torres et al., 2002). However, the brain/plasma radioactivity ratio was unchanged (Torres et al., 2002). Based on these results, and because CRF is a state of chronic inflammation, it could explain why, like Zhao et al. (2002) and Goralski et al. (2003), we observe decreased protein and mRNA expression of P-gp and Oatp2 in the brain of nephrectomized rats but no intracerebral accumulation of digoxin, doxorubicin, or verapamil.

We also observed a 30% decrease in the BBB permeability to benzylpenicillin in nephrectomized rats compared with controls as measured by the total brain/plasma radioactivity ratio. In vitro, studies showed that benzylpenicillin is a substrate of Mrp2 and Oat3, but no blood-to-brain transporter has been identified (Kikuchi et al., 2003; Choi et al., 2009). As mentioned previously, Oat3 is involved in the transport of uremic toxins from the brain (Ohtsuki et al., 2002; Kikuchi et al., 2003; Tahara et al., 2005; Deguchi et al., 2006). In fact, this transporter is typically thought to play a role in the efflux of drugs...
from the brain. However, a recent study hypothesized that it is also
involved in the uptake of 3\text{R},4\text{R},5\text{S}-4-acetamido-5-amino-3-(1-
ethyl-proproxy)-1-cyclohexene-1-carboxylate phosphate (Ro 64-0802), a
pharmacologically active form of oseltamivir, from the blood to the
brain (Ose et al., 2009). Thus, it could be responsible for the blood-
to-brain uptake of substrates, such as benzylpenicillin, or uremic
toxins and for their brain-to-blood transport. This hypothesis is sup-
ported by a study performed in the 1960s that demonstrated that
probenecid, a well known Oat3 inhibitor, limits the entry of ben-
zylpenicillin into rat brain as shown by a decrease in brain/plasma
concentration ratio of benzylpenicillin when coadministered with pro-
benecid (Fishman, 1966). The 30% decrease in the brain/plasma radio-
activity ratio of benzylpenicillin observed in the brain of nephrecto-
imized rats indicates a more efficient efflux of the drug compared with
its uptake. However, Mrp2 (efflux) and Oat3 (influx and efflux) were
equally reduced by 40% in the brain of nephrectomized rats compared with
controls. If the hypothesis of Oat3 involvement in the blood-to-
brain influx of benzylpenicillin and urmic toxins is correct, the
 greater functional reduction in benzylpenicillin brain uptake could be
attributed to competition with accumulating urmic toxins such as
CMPF, IS, and PAH for blood-to-brain transport by Oat3, preventing
benzylpenicillin entry into the brain. However, it is also possible that
Mrp2 plays a more important functional role in the BBB permeability
of benzylpenicillin, causing a greater efflux of the drug compared with
its influx.

In conclusion, although we reported significant reductions in the
expression of influx and efflux brain drug transporters, it seems that
the BBB integrity and permeability to drugs is preserved in renal failure
as shown by the unchanged intracerebral accumulation of
digoxin, doxorubicin, and verapamil and by the reduced accumulation
of benzylpenicillin, causing a greater efflux of the drug compared with

Authorship Contributions

Participated in research design: Naud, Laurin, and Pichette.
Conducted experiments: Naud, Laurin, Michaud, Beauchemin, and Leblond.
Performed data analysis: Naud, Leblond, and Pichette.
Wrote or contributed to the writing of the manuscript: Naud, Leblond, and Pichette.

References

induction of monocyte chemotactic and activating factor in patients with chronic renal failure.
Booher J and Sensenbrenner M (1972) Growth and cultivation of dissociated neurons and
Cefepime-induced neurotoxicity: an underestimated complication of antibiotics in patients
Choik MK, Kim H, Han YH, Song IS, and Shim CK (2009) Involvement of Mrp2/MRP2 in the
influx of benzylpenicillin, causing a greater efflux of the drug compared with its
influx.

In conclusion, although we reported significant reductions in the
expression of influx and efflux brain drug transporters, it seems that
the BBB integrity and permeability to drugs is preserved in renal failure
as shown by the unchanged intracerebral accumulation of
digoxin, doxorubicin, and verapamil and by the reduced accumulation
of benzylpenicillin.

from the brain. However, a recent study hypothesized that it is also
involved in the uptake of 3\text{R},4\text{R},5\text{S}-4-acetamido-5-amino-3-(1-
ethyl-proproxy)-1-cyclohexene-1-carboxylate phosphate (Ro 64-0802), a
pharmacologically active form of oseltamivir, from the blood to the
brain (Ose et al., 2009). Thus, it could be responsible for the blood-
to-brain uptake of substrates, such as benzylpenicillin, or uremic
toxins and for their brain-to-blood transport. This hypothesis is sup-
ported by a study performed in the 1960s that demonstrated that
probenecid, a well known Oat3 inhibitor, limits the entry of ben-
zylpenicillin into rat brain as shown by a decrease in brain/plasma
concentration ratio of benzylpenicillin when coadministered with pro-
benecid (Fishman, 1966). The 30% decrease in the brain/plasma radio-
activity ratio of benzylpenicillin observed in the brain of nephrecto-
imized rats indicates a more efficient efflux of the drug compared with
its uptake. However, Mrp2 (efflux) and Oat3 (influx and efflux) were
equally reduced by 40% in the brain of nephrectomized rats compared with
controls. If the hypothesis of Oat3 involvement in the blood-to-
brain influx of benzylpenicillin and urmic toxins is correct, the
 greater functional reduction in benzylpenicillin brain uptake could be
attributed to competition with accumulating urmic toxins such as
CMPF, IS, and PAH for blood-to-brain transport by Oat3, preventing
benzylpenicillin entry into the brain. However, it is also possible that
Mrp2 plays a more important functional role in the BBB permeability
of benzylpenicillin, causing a greater efflux of the drug compared with
its influx.

In conclusion, although we reported significant reductions in the
expression of influx and efflux brain drug transporters, it seems that
the BBB integrity and permeability to drugs is preserved in renal failure
as shown by the unchanged intracerebral accumulation of
digoxin, doxorubicin, and verapamil and by the reduced accumulation
of benzylpenicillin.


Address correspondence to: Dr. Vincent Pichette, Centre de Recherche de l’Hôpital Maisonneuve-Rosemont, 5415 boul. de l’Assomption, Montréal, Québec, Canada H1T 2M4. E-mail: vpichette.hmr@ssss.gouv.qc.ca