0026-895X/04/6603-413–419
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 66:413–419, 2004

Vol. 66, No. 3
3275/1171408
Printed in U.S.A.

Pregnane X Receptor Up-Regulation of P-Glycoprotein Expression and Transport Function at the Blood-Brain Barrier

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Received February 12, 2004; accepted June 8, 2004

This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

P-glycoprotein, an ATP-driven drug export pump, is a critical, selective component of the blood-brain barrier responsible for the poor penetration of many therapeutic drugs. In liver, ligand-activated, nuclear receptors are transcriptional regulators of drug metabolizing enzymes and drug export pumps, but only one, the pregnane X receptor (PXR in rodents, SXR in humans), regulates p-glycoprotein expression. We report for the first time that PXR is expressed in rat brain capillaries. Moreover, exposing isolated capillaries to the PXR ligands pregnenolone-16α-carbonitrile (PCN) and dexamethasone increased p-glycoprotein expression and p-glycoprotein–specific transport of a fluorescent cyclosporine A derivative into capillary lumens. Dosing rats with PCN and dexamethasone increased p-glycoprotein expression in liver plasma membranes and in brain capillaries and up-regulated specific transport in capillaries. This is the first evidence for PXR expression in brain and for regulation by nuclear receptors of a xenobiotic export pump at the blood-brain barrier. These results imply selective tightening of the barrier in patients exposed to the wide range of xenobiotics that are PXR/SXR ligands, including drugs, dietary constituents, and toxicants.

Our ability to treat disorders of the CNS is greatly impaired by poor transport from blood to brain of a large number of therapeutic drugs. The structure responsible for this low penetration is the nonfenestrated endothelium of brain capillaries that constitutes the blood-brain barrier. Although originally thought to present a passive, anatomical barrier to xenobiotics, it is now clear that the capillary endothelium is a complex, dynamic structure with selective, active components. The molecular basis for the active barrier is a number of multispecific efflux transporters that remove metabolic wastes and xenobiotics from the CNS and block xenobiotic entry from the blood. Recent studies have identified the ATP-driven drug export pump p-glycoprotein as a critical factor in blood-brain barrier function (Schinkel et al., 1994). For example, compared with wild-type controls, p-glycoprotein–null mice show substantially increased penetration of a number of drugs into the brain, including chemotherapeutic agents, HIV protease inhibitors, anticonvulsant agents, and antipsychotic agents (Schinkel et al., 1996), and inhibition of p-glycoprotein in an animal model greatly increases both blood to brain transport of the chemotherapeutic agent paclitaxel (Taxol) and its effectiveness against an intracerebrally implanted human glioblastoma (Fellner et al., 2002). At present, the physiological mechanisms that regulate p-glycoprotein expression and function in brain capillaries are unknown.

A number of ligand-activated nuclear receptors are key transcriptional regulators of hepatic drug metabolizing enzymes and drug export pumps (Muller, 2000; Francis et al., 2003; Staudinger et al., 2003). Although several nuclear receptors have been implicated in regulation of xenobiotic metabolism and excretion, only one, the pregnane X receptor [PXR; steroid and xenobiotic receptor (SXR) in humans], has been shown to regulate p-glycoprotein expression (Geick et al., 2001; Synold et al., 2001; Dussault and Forman, 2002). Like p-glycoprotein, PXR is multispecific, recognizing a number of endogenous metabolites and xenobiotics as ligands, including steroids, chemotherapeutic agents, HIV protease inhibitors, glucocorticoids, and anticonvulsive agents (Jones et al., 2000). Herein we present the first evidence for regulation by nuclear receptors of a xenobiotic export pump at the blood-brain barrier. We show that PXR is expressed in rat

This work was supported in part by grant DFG FR1211/8-1 (to G.F.) from the German Research Foundation.

ABBREVIATIONS: CNS, central nervous system; PXR, pregnane X receptor; SXR, steroid and xenobiotic receptor; PCN, pregnenolone-16α-carbonitrile; DPBS, Dulbecco's phosphate-buffered saline; BSA, bovine serum albumin; NBD-CSA, [N-ϵ(4-nitrobenzofurazan-7-yl)-D-Lys^8]-cyclosporine A; PSC-833, valspodar; DMSO, dimethyl sulfoxide; Mrp2, multidrug resistance-associated protein isoform 2; AED, antiepileptic drug; PCR, polymerase chain reaction; RT, reverse transcription.

brain capillaries and that in vitro or in vivo exposure to PXR ligands leads to increased p-glycoprotein expression and transport function. These results argue for selective tightening of the blood-brain barrier in patients exposed to the wide range of xenobiotics that are PXR/SXR ligands. They imply increased protection against neurotoxicants but at the cost of reduced effectiveness of therapeutic drugs.

Materials and Methods

Chemicals. Pregnenolone-16α-carbonitrile (PCN), dexamethasone, corn oil, Dulbecco's phosphate-buffered saline (DPBS), and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO). [N-ε(4-nitrobenzofurazan-7-yl)-D-Lys^8]-cyclosporine A (NBD-CSA) was custom-synthesized by Novartis (Basel, Switzerland) (Schramm et al., 1995). PSC833 was a kind gift from Novartis. All other chemicals were of analytical grade and were obtained from commercial sources.

Animals and Tissue Isolation. Male Sprague-Dawley retired breeder rats (Taconic, Germantown, NY), weighing approximately 500 to 750 g, were used. Animal housing and dosing protocols were in accordance with National Institute of Environmental Health Sciences guidelines. For in vitro dosing experiments, rats were euthanized by CO_2 inhalation and decapitated. Brains were taken immediately for capillary isolation as described previously (Miller et al., 2000). Freshly isolated capillaries in DPBS (with 5 mM glucose and 1 mM pyruvate) were exposed to PXR ligands (6 h at room temperature) added to the medium in DMSO stock solutions; control medium contained DMSO. We previously found that the concentration of DMSO used (<0.1%) had no effect on drug transport in isolated capillaries (Miller et al., 2000). For in vivo induction experiments, five animals per group were dosed daily by i.p. injection for 3 d with PCN (10–50 mg/kg in corn oil) or dexamethasone (1–50 mg/kg in corn oil); control animals received corn oil alone. Twenty-four hours after the last injection, rats were euthanized, and brains were taken immediately for capillary isolation. Livers were removed, snap-frozen in liquid nitrogen, and stored at −80°C until use.

Western Blotting. Tissue was homogenized in mammalian tissue lysis buffer (Sigma, St. Louis, MO) containing Complete protease inhibitor cocktail (Roche, Mannheim, Germany). After 1 h on ice with occasional vortexing, samples were centrifuged at 10,000*g* for 30 min. Denucleated supernatants were centrifuged at 100,000*g* for 90 min. Pellets (crude plasma membranes) were resuspended in DPBS containing protease inhibitor cocktail and protein concentrations were determined. Western blots were performed using the Invitrogen (Carlsbad, CA) NuPage Bis-Tris electrophoresis system. In brief, brain capillary plasma membrane proteins (1 μg/lane) and liver plasma membrane proteins (20 μg/lane) were electrophoresed on 4 to 12% Bis-Tris NuPage gradient gels and subsequently blotted on Invitrolon polyvinylidene difluoride membranes (Invitrogen). After blocking with SuperBlock buffer (Pierce, Rockford, IL) for 8 h, membranes were incubated overnight with a 1:100 dilution (1 μg/ml) of primary monoclonal mouse antibody C219 (Signet, Dedham, MA) for p-glycoprotein or a monoclonal antibody to MRP2/cMOAT (M2 III-6; Alexis Biochemicals, San Diego, CA). Membranes were then washed and incubated with horseradish peroxidase-conjugated ImmunoPure secondary goat anti-mouse IgG (1:10,000; Pierce) for 1 h. After washing, antibody was detected using SuperSignal West Pico Chemoluminescent Substrate (Pierce). Bands were visualized with a Gel Doc 2000 gel documentation system (Bio-Rad, Hercules, CA).

Immunohistochemistry. Isolated rat brain capillaries adhering to glass cover slips were fixed for 15 min with 3% paraformaldehyde/0.2% glutaraldehyde at room temperature. After washing with DPBS, capillaries were permeabilized for 30 min with 0.1% (v/v) Triton X-100 in DPBS, and washed with DPBS containing 1% BSA for blocking. Then, capillaries were incubated for 1 h at 37°C with a 1:100 dilution (1 μg/ml) of polyclonal rabbit antibody mdr ab-1 (Oncogene Research Products, Cambridge, MA) to detect p-glycoprotein or a 1:100 dilution (2 μg/ml) of polyclonal goat anti-PXR antibody (Santa Cruz Biotechnology, Santa Cruz, CA) to detect PXR. After washing (DPBS and 1% BSA), capillaries were incubated with the corresponding Alexa Fluor 488-conjugated secondary IgG (all 1:1000; Molecular Probes, OR) for 1 h at 37°C. Nuclei were counterstained for 15 min with 5 μg/ml propidium iodide. Negative controls for each treatment were processed without primary antibody, and these showed only background fluorescence. Immunofluorescence was visualized by confocal microscopy (model 510 laser scanning confocal microscope; Zeiss, Welwyn Garden City, UK). For quantitation of p-glycoprotein immunofluorescence, confocal images of 10 to 20 capillaries per treatment were acquired and saved to disk. Luminal membrane immunofluorescence for each capillary was measured using ImageJ software (version 1.29). A 10 × 10 grid was superimposed on each image, and measurements were taken at every intersection of a grid line with a capillary luminal plasma membrane. The fluorescence intensity for each capillary was the mean of all measurements.

Transport. Capillaries were transferred to incubation chambers with cover slip bottoms (Miller et al., 2000) and incubated for 1 h at room temperature in DPBS with 2 μM NBD-CSA or NBD-CSA plus 5 μM PSC833, a specific inhibitor of p-glycoprotein, or with 1 mM NaCN. For each treatment group, confocal images of 10 to 15 capillaries were acquired, and luminal fluorescence intensity was measured using Scion Image software (Scion Corp., Frederick, MD) as described previously (Miller et al., 2000). P-glycoprotein–mediated transport was taken as the difference between total luminal NBD-CSA accumulation and accumulation in the presence of PSC833.

PXR Cloning and Sequencing. Total capillary RNA was isolated using TRIzol reagent (Invitrogen) and purified using the RNeasy Mini kit (QIAGEN, Valencia, CA). A 353-bp amplicon of rat PXR (GenBank accession no. AF151377) was generated by RT-PCR (PowerScript Reverse Transcriptase and Titanium *Taq*PCR, both from BD Biosciences Clontech, Palo Altom CA) with the following primers: 5′-GATGATCATGTCTGATGCCGCTG-3′ (forward, bases 538–560) and 5′-GAGGTTGGTAGTTCCAGATGCTG-3′ (reverse, bases 890–868), both custom synthesized by QIAGEN. The PCR product was cloned into the pcDNA3.1/V5-His-TOPO vector (Invitrogen), followed by transformation of the construct in One Shot TOP10 chemically competent *Escherichia coli* cells (Invitrogen). Plasmid DNA was isolated using the UltraClean Mini Plasmid Prep Kit (MoBio Laboratories, Solana Beach, CA). Positive clones were selected by double-restriction digest (HindIII and XhoI; New England BioLabs, Beverly, MA) and agarose gel electrophoresis. Sequencing reactions of plasmid DNA were performed using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and universal T7 and BGH primers (Invitrogen). PCR reaction products were spin-column purified using the DyeEx 2.0 Spin Kit (QIAGEN), vacuum dried, and sequenced on a Prism 377 DNA sequencer (Applied Biosystems).

Results

Although PXR is highly expressed in liver, kidney, and certain regions of the gut, previous experiments detected no evidence of expression in whole brain homogenates (Kliewer et al., 1998; Zhang et al., 1999; Jones et al., 2000). However, capillaries comprise less than 1% of brain volume. If PXR expression were restricted primarily to those structures, mRNA levels in whole brain might be below the detection limits of the technique used (Northern blotting). Using RT-PCR, we detected PXR mRNA in whole-brain homogenates and in isolated brain capillaries (Fig. 1A). The PCR product was confirmed by direct sequencing to be identical to the rat PXR gene (GenBank accession no. AF151377). Consistent with PXR expression at the blood-brain barrier, immuno-

staining of isolated brain capillaries with an antibody to PXR showed immunofluorescence extending over endothelial cell cytoplasm and nucleus (Fig. 1B).

PXR is a member of the NR1I subfamily of nuclear receptors, which exhibit species differences in specificity. Thus, PCN is the prototypical ligand for rodent PXR but a poor ligand for the human ortholog (SXR). In contrast, paclitaxel, rifampicin, and hyperforin are high-affinity ligands for SXR but not PXR (Jones et al., 2000; Moore et al., 2000b; Watkins et al., 2001). To determine whether PXR ligands altered p-glycoprotein expression at the blood-brain barrier, we exposed freshly isolated rat brain capillaries to PCN and measured immunoreactive p-glycoprotein in isolated capillary plasma membranes (Western blots) and in the luminal membrane of intact capillaries in situ (quantitative immunostaining). In initial time course experiments, p-glycoprotein immunostaining increased significantly after 3-h exposure to 5 μM PCN and increased further after 6-h exposure (Fig. 2). Over this period, luminal membrane immunofluorescence in control capillaries did not change. We used a 6-h exposure period for subsequent in vitro experiments. Exposing isolated capillaries to PCN caused concentration-dependent increases in immunoreactive p-glycoprotein in Western blots of capillary plasma membranes (Fig. 3A) and in the luminal plasma membrane of immunostained, intact capillaries (Fig. 3B). In individual experiments, PCN concentrations as low as 1 μM significantly increased immunofluorescence. In five experiments, 5 μM PCN increased luminal membrane immunofluorescence on average by 86 $\pm$ 17% (Fig. 3B).

To determine whether increased immunoreactive p-glycoprotein in PCN-treated capillaries was accompanied by a parallel increase in transport function, we measured steady-state accumulation of a fluorescent cyclosporin A derivative (NBD-CSA) in capillary lumens using confocal microscopy and quantitative image analysis. Figure 4A shows luminal accumulation of NBD-CSA in a control capillary after 60-min

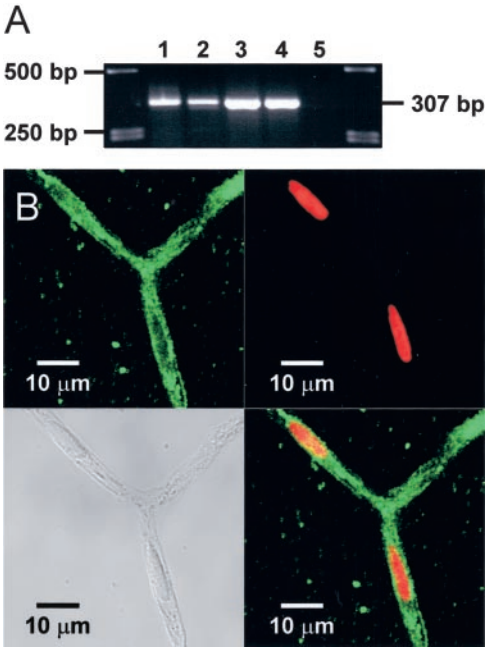

Fig. 1. Expression of PXR in rat brain capillaries. A, RT-PCR of a 307-bp sequence from PXR mRNA. Lane 1, brain capillaries; lane 2, whole-brain homogenate; lane 3, kidney; lane 4, liver; lane 5, negative control. B, representative confocal image of isolated brain capillaries immunostained for PXR (green) and counterstained for nuclei with propidium iodide (red). Top left, PXR immunostaining; top right, propidium iodide; bottom left, transmitted light; bottom right, overlaid green and red channels.

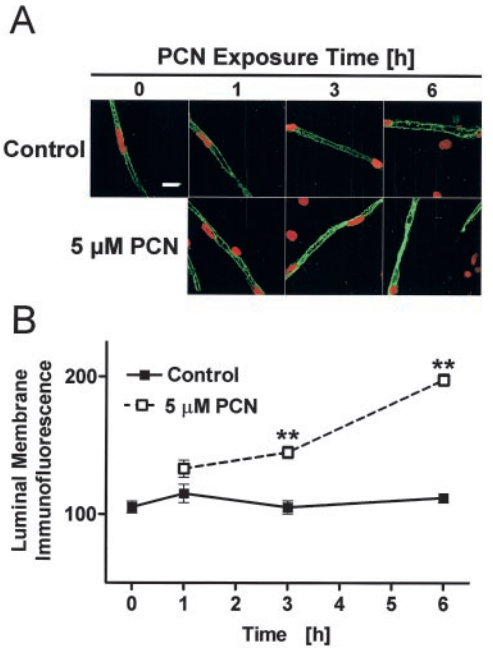

Fig. 2. Time course of increased p-glycoprotein expression in rat brain capillaries exposed to 5 μM PCN in vitro. A, representative confocal images of immunostained capillaries (green, p-glycoprotein; red, propidium iodide). The scale bar indicates 10 μm. B, luminal membrane immunofluorescence as a function of time of exposure to PCN. Each point represents the mean value (arbitrary fluorescence units, scale 0–255) for 10 to 20 capillaries from a single preparation (pooled tissue from 10 rats); variability is given by S.E. bars. **, significantly greater than control, $p < 0.01$.

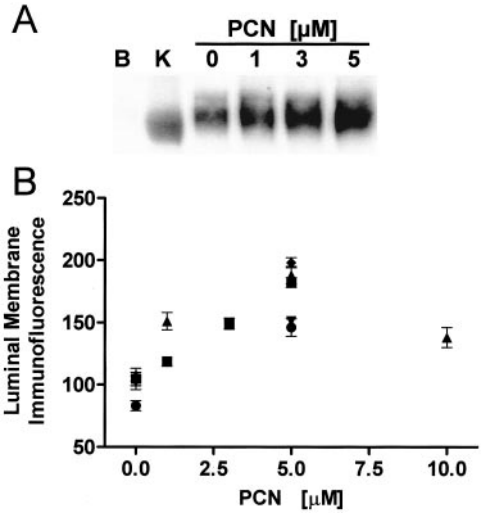

Fig. 3. Increased expression of p-glycoprotein after 6-h exposure of rat brain capillaries to PCN in vitro. A, Western blot of brain capillary plasma membranes after exposure to the indicated concentrations of PCN. B indicates rat brain homogenate; K indicates brush border membranes from rat kidney cortex, a fraction rich in p-glycoprotein. B, luminal plasma membrane immunofluorescence as a function of PCN concentration. Each point represents the mean value (arbitrary fluorescence units, scale 0–255) for 10 to 20 capillaries from a single preparation (pooled tissue from 10 rats); variability is given by S.E. bars. In individual experiments, all PCN concentrations tested significantly increased membrane immunofluorescence over controls.

incubation in medium with 2 μM NBD-CSA. In agreement with previous studies with NBD-CSA and other p-glycoprotein substrates (Miller et al., 2000; Fellner et al., 2002), approximately 50% of NBD-CSA luminal accumulation in control capillaries was blocked by the metabolic inhibitor NaCN or by the p-glycoprotein–specific inhibitor PSC833 (Fig. 4A).

Note that brain capillaries also express multidrug resistance-associated protein isoform 2 (Mrp2) on the luminal plasma membrane (Miller et al., 2000). Mrp2 is another ATP-driven xenobiotic efflux pump that primarily handles anionic xenobiotics. Our experiments show that inhibitors of Mrps had no significant effects on NBD-CSA transport. For example, when capillaries were incubated with 2 μM NBD-CSA, luminal fluorescence averaged 92 ± 7 (SE for 10 capillaries per group), 84 ± 4 and 85 ± 4 fluorescence units for controls and capillaries exposed to 10 μM MK571 or 0.3 μM leukotriene C_4, respectively. These results are consistent with previous experiments in which neither of the Mrp inhibitors affected transport of fluorescent p-glycoprotein substrates in brain capillaries (Miller et al., 2000; Fellner et al., 2002). Thus, PSC833-sensitive, luminal NBD-CSA accumulation seems to measure p-glycoprotein function, and the uninhibited component of accumulation seems to reflect nonspecific processes.

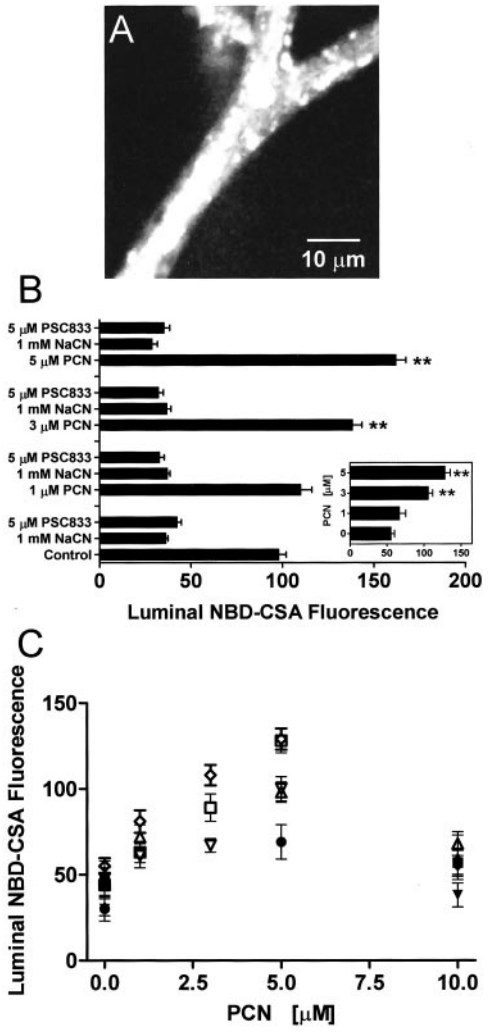

Fig. 4. Stimulation of NBD-CSA accumulation in capillary lumens after 6-h exposure to PCN in vitro. A, confocal image of a control capillary after 60-min incubation in medium with 2 μM NBD-CSA. Note luminal accumulation of the p-glycoprotein substrate. Scale bar, 10 μm. B, a representative dose-response experiment. Each point represents the mean value (arbitrary fluorescence units, scale 0–255) for 20 capillaries from a single preparation (pooled tissue from 10 rats); variability is given by S.E. bars. The inset shows p-glycoprotein-mediated transport (PSC833-sensitive component). **, significantly greater than control, $P < 0.01$. C, P-glycoprotein-mediated (PSC833-sensitive) luminal accumulation of NBD-CSA as a function of PCN concentration. Each point represents the mean value (arbitrary fluorescence units, scale 0–255) for 20 capillaries from a single preparation (pooled tissue from three to ten rats); variability is given by S.E. bars. For individual experiments, 1 to 5 μM PCN significantly increased luminal NBD-CSA accumulation over controls.

Exposing isolated capillaries to PCN increased total luminal NBD-CSA accumulation without changing the component of transport insensitive to NaCN or PSC833. Thus, the p-glycoprotein–specific component of transport increased with increasing PCN concentration (Fig. 4B, inset); with 5 μM PCN, specific transport had more than doubled. Together, results from eight in vitro exposure experiments show increased specific NBD-CSA transport with 1 to 5 μM PCN followed by a decline with 10 μM PCN (Fig. 4C). With 5 μM PCN, transport had increased on average by 106 ± 15% (data from five experiments). The apparent fall in transport with the high concentration of PCN may represent toxicity.

The widely prescribed anti-inflammatory glucocorticoid dexamethasone is a ligand for both PXR and SXR (Kliewer et al., 2002). Exposing capillaries to this drug increased capillary luminal membrane immunofluorescence (Fig. 5A), but at much lower concentrations than PCN. With 0.1 to 0.5 μM dexamethasone, luminal membrane immunofluorescence increased on average by 76 ± 9% (data from 5 experiments, $p <$ 0.01). We found a similar doubling of p-glycoprotein–mediated transport in capillaries exposed to dexamethasone (Fig. 5B). As with immunostaining, increases in transport were evident with dexamethasone concentrations (0.01 to 0.5 μM) that were at least an order of magnitude lower than for PCN.

In contrast to the results with PCN and dexamethasone, we found no increase in p-glycoprotein expression in rat brain capillaries exposed to the chemotherapeutic agent paclitaxel or the St. John's Wort component hyperforin (not shown). Both are potent activators of human SXR but not rodent PXR (Moore et al., 2000a; Schuetz and Strom, 2001; Synold et al., 2001; Kliewer et al., 2002). Finally, initial quantitative real-time PCR experiments indicated a doubling of p-glycoprotein mRNA in brain capillaries exposed to 5 μM PCN or 0.5 μM dexamethasone (B. Bauer, A. Hartz, D. Miller, unpublished data). Together, these in vitro experiments demonstrate that PXR ligands increased transcription, translation, and transport function of p-glycoprotein in brain capillaries.

To determine whether in vivo exposure to PXR ligands altered p-glycoprotein expression at the blood-brain barrier, we dosed rats daily with PCN or dexamethasone by i.p. injection for 3 days, isolated brain capillaries on day 4, and measured p-glycoprotein content and p-glycoprotein–mediated transport. Livers were also removed and crude membrane fractions were isolated and analyzed by Western blotting. In agreement with previously published studies (Salphati and Benet, 1998; Demeule et al., 1999), dosing with PCN and dexamethasone increased immunoreactive p-glycoprotein in liver plasma membranes (Fig. 6, A and B). It is noteworthy that transporter expression in Western blots of brain capillary plasma membranes also increased. Immuno-

staining of capillaries showed that luminal membrane p-glycoprotein increased as dose levels of PCN and dexamethasone were raised (Fig. 6C); at the highest doses used, immunofluorescence about doubled. When p-glycoprotein–specific transport in capillaries from control and PXR ligand-dosed rats was measured, significant dose-dependent increases were found (Fig. 6D). As with the in vitro dosing experiments (above), dexamethasone was a more potent inducer of p-glycoprotein than PCN. Specific transport in isolated capillaries increased by 120% with PCN doses of 25 mg/kg/day and by 160% with dexamethasone doses of 5 mg/kg/day (Fig. 6D).

Results of five in vivo dosing experiments with PCN and dexamethasone are summarized in Fig. 7, where p-glycoprotein immunostaining is plotted against specific transport. Control values cluster at the bottom left of the plot, values for animals given low doses of inducers cluster in the middle, and values for animals given higher doses (up to 50 mg/kg PCN) cluster in the upper right. It is noteworthy that both immunofluorescence and specific transport increased in parallel as PXR was activated by increasing dose of ligand.

In addition to p-glycoprotein, PXR also regulates Mrp2 expression in excretory epithelia (Kast et al., 2002; Francis et al., 2003). We previously demonstrated expression and function of Mrp2 in brain capillaries from rat and pig (Miller et al., 2000). As with p-glycoprotein, this transporter was immunolocalized to the luminal plasma membrane of capillary endothelial cells. To determine whether Mrp2 expression was also increased by PXR ligands, we dosed rats with PCN and dexamethasone and assayed Mrp2 protein in Western blots of plasma membranes from liver and brain capillaries. Figure 8 shows that both drugs increased Mrp2 expression in membranes from both tissues.

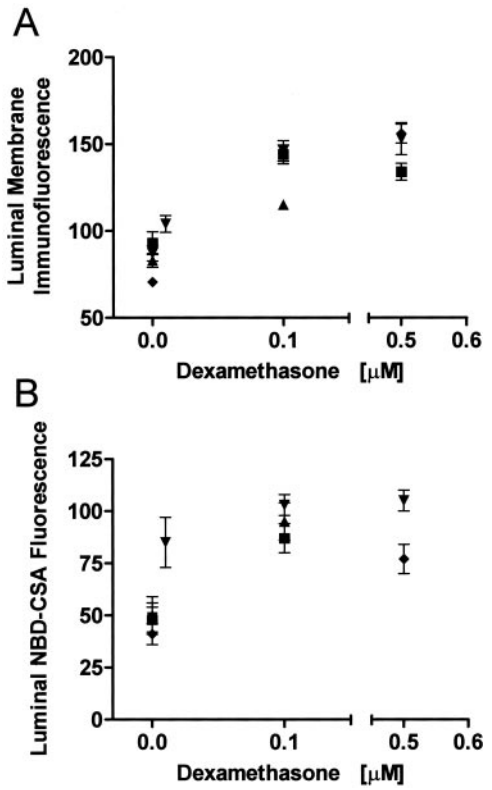

Fig. 5. Increased p-glycoprotein expression in brain capillaries exposed for 6 h to dexamethasone in vitro. Luminal plasma membrane immunofluorescence (A) and p-glycoprotein-mediated (PSC833-sensitive) luminal accumulation of NBD-CSA (B) are shown. Each point represents the mean value (arbitrary fluorescence units, scale 0–255) for 10 to 20 capillaries from a single preparation (pooled tissue from three to ten rats); variability is given by S.E. bars. For individual experiments, all dexamethasone concentrations tested significantly increased membrane immunofluorescence and NBD-CSA transport over paired controls.

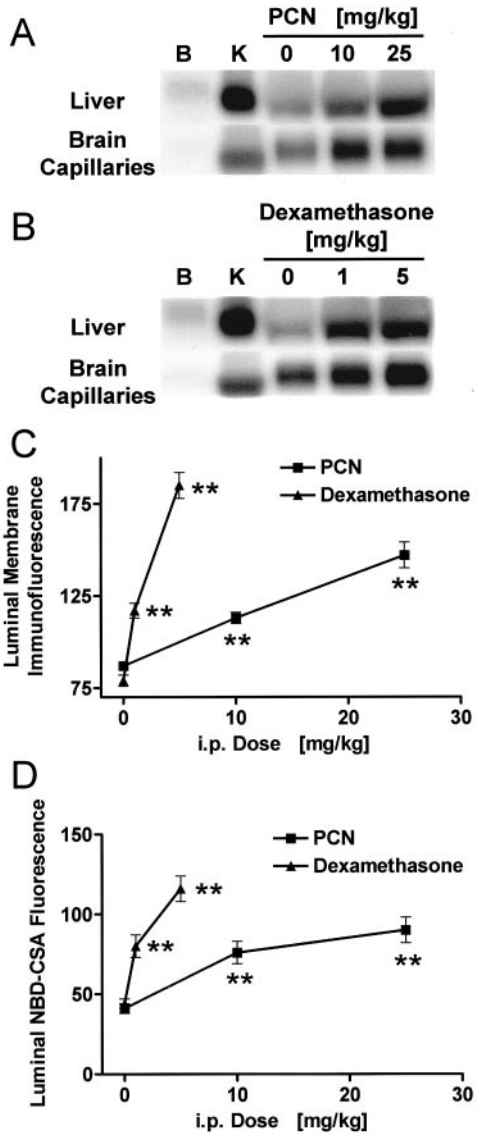

Fig. 6. Increased expression of p-glycoprotein after dosing of rats with PXR ligands. Over 3 days, rats were given daily i.p. injections of the indicated doses of PCN or dexamethasone; tissues were collected on day 4. A and B, Western blots of plasma membranes from liver and brain capillaries of animals given the indicated daily doses of PCN (A) or dexamethasone (B). B indicates brain homogenate; K indicates brush border membranes from rat kidney cortex. C, p-glycoprotein immunostaining in the luminal plasma membrane of brain capillaries. D, stimulation of p-glycoprotein-mediated (PSC833-sensitive) luminal accumulation of NBD-CSA. Each point represents the mean value (arbitrary fluorescence units, scale 0–255) for 20 capillaries from a single preparation (pooled tissue from five rats); variability is given by S.E. bars. All PCN and dexamethasone doses tested significantly increased luminal membrane immunofluorescence and NBD-CSA transport over control values. **, significantly greater than control, $p < 0.01$.

Discussion

PXR/SXR has been called "a master xenobiotic sensor" because of the large number of drugs, components of herbal remedies, dietary constituents, and environmental pollutants that act as ligands and because of the importance of its target genes (enzymes and transporters) to xenobiotic uptake, distribution and excretion (Dussault and Forman, 2002; Kliewer et al., 2002). P-glycoprotein, which is uniquely regulated by PXR/SXR, is expressed at the blood-brain barrier and is a primary "gatekeeper" for drug entry into the CNS (Schinkel, 1999; Geick et al., 2001; Synold et al., 2001; Dussault and Forman, 2002). The present experiments identify one underlying mechanism by which drug export pump activity at the blood-brain barrier is modulated. They show that PXR is indeed expressed in brain capillaries and that p-glycoprotein expression and transport function at the blood-brain barrier increase after in vitro or in vivo exposure to the PXR ligands PCN and dexamethasone. Thus, one consequence of exposure to PXR ligands is selective tightening of the blood-brain barrier to those chemicals that are p-glycoprotein substrates.

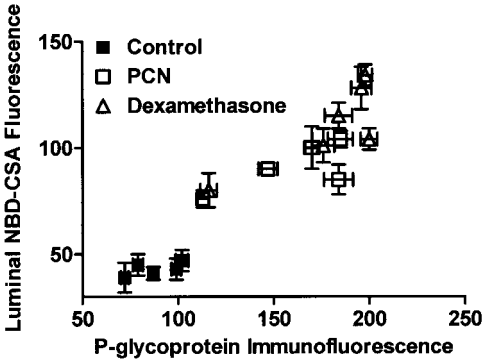

Fig. 7. Correlation between p-glycoprotein–specific (PSC833-sensitive) NBD-CSA transport and luminal membrane immunofluorescence in brain capillaries from control and PCN- or dexamethasone-dosed rats. Each point represents the mean values for 20 capillaries from a single preparation (pooled tissue from five rats); S.E. bars give variability. Linear regression analysis showed a significant positive correlation between transport and expression ($r = 0.97$; $p < 0.0001$).

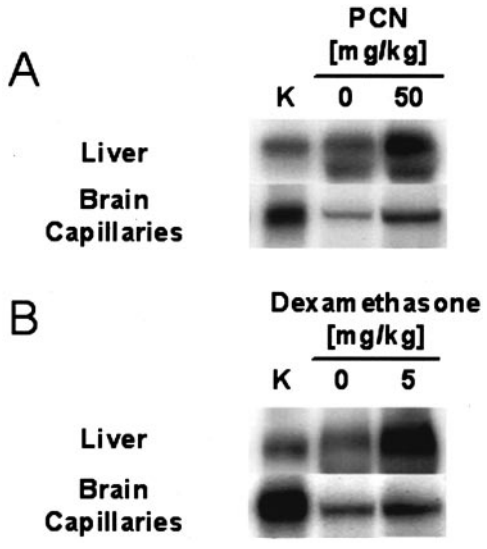

Fig. 8. Increased expression of Mrp2 in liver and brain capillaries of rats dosed with PXR ligands of PCN (A) or dexamethasone (B). Shown are Western blots of plasma membranes from liver and brain capillaries of animals given the indicated daily doses of PCN (A) or dexamethasone (B). Dosing was over 3 days; each rat was given a single daily i.p. injection; tissues were collected on day 4. K indicates brush border membranes from rat kidney cortex (positive control).

The present results, which show the consequences of receptor activation in rats dosed with PXR ligands, provide a "proof of principle" with regard to PXR expression and action in the blood-brain barrier. The extent to which other PXR ligands will alter blood-brain barrier function in vivo in rats or in other species will depend on several interrelated factors. For example, the nature of the ligand, its pharmacokinetics, and the exposure (dosing) protocol will determine whether ligand accumulates in brain capillary endothelial cells at sufficient levels to affect gene expression. Indeed, for PXR ligands that are also p-glycoprotein substrates, accumulation may be limited at the barrier itself or more distally at the intestine or liver. In addition, substantial species differences in PXR ligand profiles (Jones et al., 2000; Moore et al., 2000b; Watkins et al., 2001) indicate that effective activators in rodents will not necessarily be effective in humans (e.g., PCN is a potent activator of rodent PXR but not human PXR, whereas the reverse is true for rifampicin).

Up-regulation of p-glycoprotein has important clinical implications. On the one hand, p-glycoprotein is responsible for the efflux of neurotoxic chemicals and metabolites from the CNS, and increased pump expression should provide increased neuroprotection. On the other hand, many p-glycoprotein substrates are used therapeutically (e.g., to treat brain tumors, viral and bacterial infections, and epileptic seizures), and increased pump expression also implies reduced access of those drugs to sites of action within the CNS. Consider epilepsy, where multidrug resistance is encountered in more than 30% of patients. Commonly prescribed antiepileptic drugs (AEDs) are substrates for the drug efflux pumps p-glycoprotein and Mrp2, a second ATP-driven efflux pump that handles primarily steroid and xenobiotic conjugates but also some unmetabolized drugs (Rizzi et al., 2002; Potschka et al., 2003; Sisodiya, 2003). The present results indicate that PCN and dexamethasone up-regulate expression of both p-glycoprotein and Mrp2 (present study) at the blood-brain barrier. It is clear that activation of PXR, increased expression of drug efflux pumps, and the resulting decrease in AED entry into the CNS may be one mechanism contributing to drug resistance in epilepsy. Furthermore, recent studies with patients and with animal models of epilepsy show focal up-regulation of p-glycoprotein and Mrp2 expression in brain tissue (Loscher and Potschka, 2002; Sisodiya, 2003). Mechanisms responsible for this have not been identified, but tonic activation of PXR by AEDs or by endogenous ligands produced during seizures are certainly possibilities.

In liver and gut, ligand-activated nuclear receptors regulate a network of genes that encode enzymes responsible for xenobiotic oxidation and conjugation as well as excretory transport of xenobiotics and products of xenobiotic metabolism (Rosenfeld et al., 2003). Although PXR/SXR is the only ligand-activated nuclear receptor that regulates p-glycoprotein expression, the blood-brain barrier does express other multispecific drug-metabolizing enzymes and transporters that in other tissues can be regulated by PXR/SXR, (e.g., cytochrome P450s, glutathione transferases, Mrps, and organic anion transport polypeptides) (Guo et al., 2002; Kast et al., 2002; Chen et al., 2003). Consistent with this, Fig. 8

shows that exposure to PCN and dexamethasone increased Mrp2 expression in rat brain capillaries. Thus, it is likely that multiple blood-brain barrier enzymes and transporters are transcriptionally regulated by PXR.

PXR is only one member of a family of ligand-activated nuclear receptors that regulates expression of drug metabolizing enzymes and transporters (constitutive androstane receptor and farnesoid X receptor are others) (Muller, 2000; Kast et al., 2002; Francis et al., 2003). It is currently not clear whether any of these other receptors are expressed at the blood-brain barrier. Those nuclear receptors that are actually expressed in brain capillaries will importantly determine 1) the range of chemicals that alter the selective blood-brain barrier, 2) the range of drugs for which barrier function can be modified, and 3) the nature and extent of the change in barrier properties for each specific drug.

Finally, the present results provide for the first time a molecular basis for regulation of specific xenobiotic transport at the blood-brain barrier. A better understanding of the mechanisms that regulate the selective barrier to drug entry also holds the promise of treatments (e.g., drugs or dietary modifications) that could down-regulate pump expression. For example, transient p-glycoprotein down-regulation could provide a window in time during which normally impermeant drugs would be able to access sites within the CNS. It remains to be demonstrated whether this can be done effectively through manipulation of PXR or through other faster acting regulatory mechanisms.

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