Renal Phosphodiesterase 4B Is Activated in the Dahl Salt-Sensitive Rat

Urmila Tawar, Kumar Kotlo, Shilpa Jain, Sagar Shukla, Suman Setty, Robert S. Danziger

Abstract—Reduced β-adrenergic signaling is associated with increased sympathoadrenal activity in hypertensive patients and animal models of hypertension. However, the mechanism that accounts for this characteristic decline in β-adrenergic signaling is unclear. In the present study, we investigated renal phosphodiesterase 4B, which metabolizes cAMP. Immunoblot analysis detected only the phosphodiesterase 4B isoform present in kidney tissue from spontaneously hypertensive rats, hypertensive Dahl salt-sensitive (SS) rats, and Dahl salt-resistant rats. The phosphorylated (activated) form of the protein was present at 2-fold greater levels in Dahl SS rats than in spontaneously hypertensive rats and Dahl salt-resistant rats, whereas the unphosphorylated form of the protein was reduced by approximately one half in SS animals. In accord with immunoblot data, rolipram-inhibitable cAMP hydrolyzing activity, a measure of PDE4 activity, was ≈3-fold greater in kidney cytosolic extracts from SS rats than in extracts from spontaneously hypertensive rats and salt-resistant rats. Phosphodiesterase 4B expression was detected by immunohistochemistry in the renal vasculature, proximal tubules, and distal tubules. These results raise the possibility that increased PDE4 activity, specifically phosphodiesterase 4B, reduces β-adrenergic signaling in the kidney and contributes to salt-sensitive hypertension in the Dahl SS rat. (Hypertension. 2008;51:762–766.)

Key Words: phosphodiesterase 4B ■ cAMP ■ Dahl rat ■ β-adrenergic signaling

Activation of β-adrenergic receptors triggers the formation of cAMP through G protein–coupled adenylate cyclase, which subsequently stimulates cAMP-dependent protein kinase A (PKA). cAMP phosphodiesterase (PDE) negatively regulates β-adrenergoreceptor signaling by hydrolyzing cAMP and reducing PKA activity. Reduced vascular β-adrenergic responsiveness has been demonstrated in both hypertensive patients and rat models of genetic and acquired hypertension (see review8). This reduced responsiveness has been hypothesized to play an important role in the pathogenesis of hypertension. However, the mechanism(s) underlying reduced β-adrenergic signaling remain unclear. β-Adrenergic receptor number has been measured in different animal hypertension models, and the results have varied, with receptor number increasing, decreasing, or remaining unchanged in the heart and vasculature (see review9). Other evidence suggests that, in certain models of hypertension, expression of the stimulatory G protein Gs is reduced, and/or the expression of the inhibitory G protein Gi is increased.4–7 Another possibility, which we have investigated here, is that PDE activity may be increased in hypertension.

The PDE 4 family of PDEs, formerly known as cAMP-PDE and rolipram-sensitive PDE, is expressed in most cell types and is generally considered to account for most of the cAMP-hydrolyzing activity of cells (see review9). PDE4s are generated from 4 distinct genes (PDE4A through D), and each gene generates long and short splice variants using alternate splicing and promoters (see review9). Long PDE4 variants contain 2 conserved regions at the amino terminus known as upstream conserved regions 1 and 2, whereas short variants lack upstream conserved region 1 and may have a truncated upstream conserved region 2. PKA phosphorylates and activates many long PDE4 isoforms, whereas long and short forms are differentially regulated by extracellular signal regulated kinase–mediated phosphorylation of a conserved serine residue.8,10

The PDE4B family consists of 5 different isoforms (PDE4B1 through 5). PDE4B2 and PDE4B3 isoforms are strongly conserved among species, with the human and rat proteins being of identical length and sharing >90% of the amino acid sequence identity.11,12 PDE4B4, a 659-amino acid protein, is unique among the other long forms of PDE4B because of the presence of a 17-amino acid NH₂-terminal region that regulates its catalytic activity. PDE4B4 is also unique, because, unlike the other forms, it is found exclusively in the cytosol of transfected COS7 cells.13 Recombinant PDE4B4 is activated through PKA-mediated phosphorylation at Ser-56.13 Recently, PDE4B5, a novel, super-short,
brain-specific cAMP PDE4 variant of which the isoform-specifying NH$_2$-terminal region is identical to that of cAMP PDE4D6, has been reported. In the present study, we investigated the potential role of PDE4B in 2 rodent models of renal hypertension.

**Materials and Methods**

**Animals and Treatments**

Dahl salt-sensitive (SS) rats and spontaneously hypertensive rats (SHRs) were used as models of renal hypertension. Male SHRs, Dahl hypertensive SS/Jr rats, and Dahl salt-resistant (SR/Jr) rats (200 to 250 g) were purchased from Harlan (Indianapolis, Ind). Only males were studied to eliminate the confounding effects of the estrus cycle. Animals were housed at controlled temperatures (22°C to 25°C) and lighting (12-hour light/12-hour dark) with free access to food and water before experimentation. All of the animals used in this work received humane care in compliance with institutional animal care guidelines. All of the animals were maintained on high-salt diets (Purina 5501 chow with 8% NaCl) for 10 days before sacrifice.

**Immunoblotting**

On removal, brain and kidneys were fast-frozen in liquid nitrogen and dounce homogenized at 4°C in 50 mmol/L of Tris-HCl (pH 7.4), 150 mmol/L of NaCl, 1 mmol/L of EDTA, 0.25% sodium deoxycholate, 1% Nonidet P-40, and protease inhibitor mixture. The homogenate was centrifuged at 13 000 rpm for 15 minutes at 4°C, and the supernatant was collected as the total protein fraction. The samples were boiled for 3 minutes in Laemmli buffer and subjected to electrophoresis in a 10% SDS-PAGE. The proteins were transferred to a nitrocellulose membrane and then blocked in a Tris-buffered saline solution (20 mmol/L of Tris, 140 mmol/L of NaCl, and 0.05% Tween-20 [pH 7.6]). After 2 washes, the membrane was incubated with rabbit polyclonal PDE4B antisera. Against the PDE4B carboxyl-terminus (LEETDIDIEDAKSLIDT, Fabgennix International, Inc), the membrane was then washed 3 times in Tris-buffered saline-Tween 20 (TBST) and incubated for 1 hour with peroxidase-linked anti-rabbit secondary antibody (Santa Cruz Biotech) at a dilution of 1:2000 in TBST. After 3 additional washes in TBST, the membrane was incubated for 1 minute with ECL detection reagents (Amersham) and exposed to x-ray film (Kodak) for varying times to obtain desirable band intensity within a linear detection range. The sections were examined using a Nikon E600 microscope with Axioplan objectives. Immunohistochemistry was performed with 2 negative controls as follows. In the first condition, the tissue was incubated with a blocking solution (5% normal goat serum) for 30 minutes in the absence of the primary antibody. In the second condition, the tissue was incubated with the blocking solution for 30 minutes, followed by a 1-hour incubation with a combination of PDE4B peptide (×4, Fabgennix International, Inc), which corresponds with the linear epitope against which the PDE4B antibody has been raised (1:100, Fabgennix International, Inc).

Images were acquired at ×400 and ×1000 using a Microlumina Ultra Resolution Scanning Digital Camera (3380×2700 pixels, Leaf Systems). In all of the cases, files were saved in tagged-image file format.

**Statistics**

Comparisons were by ANOVA and Student’s $t$ test. Data are expressed as means±SDs. $P<0.05$ was significant.

**Results**

**Phosphorylated PDE4B4 Is Increased in Kidneys From Dahl SS Rats**

Immunoblot analysis using an antibody to the “common” carboxyl terminus of the PDE4B family revealed the presence of PDE4B1, PDE4B2, PDE4B3, and PDE4B in brain extracts from Dahl SS rats, Dahl SR rats, and SHRs (Figure 1). The levels of these 4 PDE4 isoforms did not differ among strains. In contrast, only the PDE4B4 isoform could be detected in kidney extracts from these animals. Unphosphorylated PDE4B4 appeared as a 62-kDa species and was associated with a slightly heavier species (66 kDa), which likely represented phosphorylated PDE4B4.
ylated PDE4B4 was more abundant in the SHR and Dahl SR groups than in the Dahl SS group. Conversely, levels of phosphorylated PDE4B4 in the Dahl SS group were 2-fold greater than those in the Dahl SR and SHR groups. Similar results were obtained after immunoblot analysis of anti-PDE4 immunoprecipitates using an anti-phospho serine-threonine antibody (Figure 2). Interestingly, only the phosphorylated form of PDE4B4 was detected in the brain (Figure 1).

**PDE4 Activity Is Increased in Kidneys From Dahl SS Rats**

Next, rolipram-inhibitable cAMP-hydrolyzing activity was measured to determine whether increased PDE4B4 phosphorylation was associated with an increase in protein activity. Because PDE4B4 is the sole PDE4B isoform localized to the cytoplasm, rolipram-inhibitable activity was measured in cytosolic extracts. Hydrolyzing activity was 2- to 3-fold greater in Dahl SS kidneys than in the Dahl SR and SHR Kidneys (Figure 3).

**PDE4B4 Is Expressed in the Renal Tubules and Vasculature of SS Dahl Rats**

To investigate the distribution of PDE4B4 in the kidney, we performed immunohistochemistry using anti-PDE4B antibody (Figure 4). Although this antibody cross-reacts with all of the isoforms, we inferred that labeling primarily is of the PDE4B4 isoform, because this was the only isoform detected by immunoblot analysis using the same antibody. Strong fine diffuse granular cytoplasmic and nuclear PDE4B staining were seen in a majority of the proximal convoluted tubules and distal convoluted tubules in SS Dahl rats (Figure 4A). PDE4B immunostaining was also observed in a subset of cells within the glomerulus, as well as in the arterioles (Figure 4B). At higher magnification, focal granular cytoplasmic and nuclear PDE4B immunostaining were detected in endothelial cells, as well as in the smooth muscle of the arterioles and smaller arteries in the kidney (Figure 4C). In the glomerulus, a fraction of cells showed nuclei and cytoplasmic immunoreactivity. In addition, parietal epithelial cells of the glomerulus were positive in the nucleus and cytoplasm (Figure 4D).

**Discussion**

PDE4B transcripts have been detected in a variety of tissues, including heart and lung tissue (PDE4B3), liver tissue...
Our finding that PDE4B is also expressed in proximal tubules which we have shown expresses PDE4B in the Dahl SS rat. Enhanced cAMP-mediated dilation of the renal vasculature, nephrons, and some glomerular cells. Rolipram has been shown to reduce renal vascular resistance and to increase glomerular filtration rate, urine flow rate, and urinary Na⁺ in dogs. These effects were attributed to enhanced cAMP-mediated dilation of the renal vasculature, which we have shown expresses PDE4B in the Dahl SS rat. Our finding that PDE4B is also expressed in proximal tubules and distal tubules suggests that cAMP signaling may also be augmented by rolipram in renal tubules. The effect of cAMP in tubules remains controversial. Evidence suggests that β-adrenergic signaling promotes Na⁺ excretion by facilitating a blood-lumen flux of Na⁺ whereas other studies have indicated that β-adrenergic signaling inhibits Na⁺ uptake in tubules by stimulating basolateral Na/K ATPase. Additional renal effects of rolipram that have been reported include regulation of the osmotic permeability response to antidiuretic hormone in neonatal tubules and renin release from juxtaglomerular cells.

Both the Dahl SS rats and SHRs are generally considered to be models of renal hypertension. Because the SHR is a model of salt-independent hypertension, these findings raise the possibility that PDE4 activity mediates renal adaptation to high salt and may play a pathogenic role specifically in SS hypertension. In accord with this possibility, rolipram is natriuretic in cirrhotic rats with ascites, a situation of enhanced sodium retention, but not in those without ascites.

Finally, our results also raise the possibility that increased cAMP PDE activity may account for β-adrenergic desensitization in arterial hypertension. Indeed, increased PDE4 activity has been shown to augment the primary vasconstrictive action of norepinephrine by reducing intracellular cAMP levels and PKA activity in rodent models of shock and multiple organ dysfunction syndrome.

**Perspectives**

These results raise the possibility that β-adrenergic signaling in general and cAMP-PDE specifically contribute to the pathogenesis of SS hypertension in the Dahl rat by showing that activated or phosphorylated form of the PDE4B4 is predominant in the kidney of the Dahl SS rat. On the other hand, renal PDE4B activity was less abundant and the unphosphorylated form of PDE4B4 more abundant in the SHR rat, thereby eliciting the possibility that PDE4B contributes to hypertension in the Dahl SS but not SHR rat. This raises the specter that polymorphisms of PDE4B with increased activity may explain or contribute to SS hypertension in some patients and cAMP-PDE may be a therapeutic target for certain hypertensive patients (see review).

This study further highlights the complexity of β-adrenergic signaling, specifically highlighting that a unique feedback loop may exist in which PKA-mediated phosphorylations may reduce cAMP signaling in cells by activating PDE4B.

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**Disclosures**

None.
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