**INTRODUCTION**

In the US, chronic renal failure (CRF) affects an estimated 8 million people as of 2006. Knowledge about what causes CRF, especially genetic factors, is limited. The Brachyrrhine (Br) mutant mouse with heritable renal hypoplasia could be a novel mouse model of CRF. The Br mutation came about as a result of irradiation experiments in the 3H1 strain in 1966. This mutation has been mapped to the distal chromosome 17 and seems to silence gene expression of the homeobox transcription factor Six2. This mutation is inherited in a semidominant manner in which both the Br homozygote (Br/Br) and Br heterozygote (Br/+), have similar phenotypes characterized by midfacial retrognathia and renal hypoplasia, although the Br/+ is less severe. Renal hypoplasia of the Br/+ adult kidney has been accurately defined as 85% fewer glomeruli than a normal wild type (+/+) kidney, based on stereological tests. This is significant because renal hypoplasia is often associated with CRF. Investigating other markers of CRF is important in characterizing the renal physiological state of our Br/+ mice strain.

To determine whether the adult Br/+ mouse exhibits markers of CRF, cardiorenal physiological analyses have been previously performed. It has been determined that the Br/+ exhibits increased cardiovascular and renal physiological complications including increased plasma osmolality and creatinine, increased urine output, decreased urine osmolality and creatinine, and hypertension. These values are highly suggestive of CRF. We hypothesized that it is possible to determine if CRF is present in the adult Br/+ mutant mouse by accurately measuring changes of mRNA expression of genes whose upregulation is associated with CRF.

The genes investigated in this study include endothelin-1 (ET-1), endothelin receptors A and B (ET_A and ET_B, respectively), and Na^+K^+ ATPase. ET-1 is a potent vasoconstrictor that regulates renal hemodynamics by binding to antagonist receptors, ET_A and ET_B. ET-1 also acts directly on Na^+K^+ ATPase to regulate sodium reabsorption in the tubules. In patients with CRF, an increased production of these four genes has been noted and complications including hypertension, cardiovascular hypertrophy, and overall decline in renal function have occurred. We expect an increased production of these genes based on the cardiorenal physiological analyses and observations of immuno-histochemical stainings previously conducted that revealed a greater intensity of these four genes in the Br/+ adult kidney. By looking deeper into the significance of these results, we may optimize our findings of markers of CRF in the Br/+ kidney. In this study we accurately measured the gene expression of ET-1, ET receptors, and Na^+K^+ ATPase using quantitative real-time polymerase chain reaction.

**METHODS**

Ten (4 +/-, 6 Br/+) male, 30-day-old, 3H1 × Balb F2 mice were sacrificed for kidney extraction. Mice
were identified based on morphological phenotype. Each kidney was weighed, diced and immediately stored in RNA-later (Sigma) at 4°C until RNA extraction the following day. RNA extraction was performed using the RNeasy kit (Qiagen) following the total RNA purification for animal tissues protocol. From each kidney sample, 20 mg of the cortex tissue was used in order to obtain optimal RNA yield and purity. After elution of the purified RNA, a spectrophotometer (Shimadzu) was used to measure the RNA concentration by measuring the UV light absorbency at 260 nm. RNA samples were diluted to 1 μg/μL and stored at −70°C until cDNA synthesis. cDNA was generated from 1 μg of each RNA sample using the Superscript III First Strand Synthesis System for RT-PCR reverse transcription reactions (Invitrogen). After first strand cDNA synthesis was completed, cDNA concentrations were determined by spectrophotometer, and each sample was then diluted to equal 100 ng/μL. cDNA samples were stored at −20°C until they were used for polymerase chain reaction (PCR). Primers for Na⁺,K⁺ ATPase, ET-1, ETₐ, ETₐ, and beta-actin genes were chosen based on published primers previously used for PCR. Each primer pair was optimized to achieve single amplified bands, as analyzed by agarose gel electrophoresis. This initial PCR was performed using Accuprime polymerase under conditions recommended by the manufacturer (Invitrogen). Once primer pairs were optimized, we conducted quantitative real-time PCR (qPCR). qPCR was used to measure individual gene expression levels in the extracted kidney samples with the use of BioRad’s iQ Sybrgreen Supermix and MyiQ thermal cycler. For each primer pair, control samples were analyzed by agarose gel electrophoresis after pilot qPCR runs to verify that only single amplified bands were present. Subsequently, quantitative mRNA expression of beta-actin, Na⁺,K⁺ ATPase, ET-1, ETₐ, and ETₐ in the 10 samples were measured, with each sample in triplicate to minimize experimental error. The expression levels of the individual samples were compared as a function of their threshold cycles, and results were normalized with beta-actin gene expression using a relative comparison formula. For each gene tested, the Student t test was used to compare the mean expression levels between +/+ and Br/+ kidneys using Prism statistical software (Graphpad Software). Mean expression values of the +/+ samples were set to 100%. A P value of less than 0.05 was considered statistically significant.

RESULTS

The adult Br/+ mutant mouse displays a significant upregulation of Na⁺,K⁺ ATPase and ET-1 genes in the kidney. ETₐ and ETₐ receptor gene mRNA expression differences between +/+ and Br/+ kidneys were not statistically significant.

DISCUSSION

The purpose of this project was to measure and compare gene expression of ET-1, ET receptors, and Na⁺,K⁺ ATPase in Br/+ vs +/+ kidneys. Based on these results, we were able to confirm the observed increased gene expression in the Br/+ in immunohistochemical stainings for the genes Na⁺,K⁺ ATPase and ET-1. The ET receptors, however, showed no significant upregulation in the Br/+ kidney. Because there was a significantly upregulated mRNA expression of ET-1 and Na⁺,K⁺-ATPase in the Br/+ kidney in comparison to the +/+ kidney, and despite the results of the ET receptors gene expression levels, these results can be used to show characteristics of CRF that contribute to cardio-renal complications such as hypertension. In future experiments, older mice can be tested for expression levels of these genes to demonstrate progressive worsening of renal function.

REFERENCES