vascular reactivity either directly[2,3] or through intermediate pathways such as by reduction of nitric oxide (NO) availability[4,5] or by oxidation of arachidonic acid with the generation of vasoactive lipid mediators.[6,7] It has been demonstrated that virtually all types of cells produce ROS.[8] In addition to mitochondrial sources, ROS can be derived from nearly all oxidation reactions, including xanthine oxidase, cyclooxygenase, lipoxygenase, NO synthase, hemeoxygenases, peroxidases, hemoproteins such as heme and hematin, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. Normally levels of ROS are highly constrained in that there are many $\bullet O_2^-$-scavenging systems in cells and tissues that maintain tissue $\bullet O_2^-$ concentrations at very low levels. One of the important pathways is the reaction of superoxide dismutase (SOD), which produces the morestable ROS, H_2O_2, which in turn is converted to water by catalase and glutathione peroxidase.

In order to alleviate oxidative damage to living cells, 3 major scavenger enzymes, SOD, catalase, and glutathione peroxidase,[8] play protective and regulatory roles in the defense of cells against a variety of exogenous and endogenous oxidants.[9] Application of exogenous SOD to improve the disorders of acute hypertension has been documented,[10] but changes in endogenous defense enzymes in response to hypertensive stress remain obscure. The deoxycorticosterone acetate-salt (DOCA-salt-induced) hypertensive rat is an important model for studying salt-sensitive hypertension. In an attempt to determine whether changes in the SOD gene in this strain of rat heart are correlated with hypertension, the present study examined the changes in SOD, both in Mn-SOD and Cu, Zn-SOD subtypes, in hearts of 6, 9, and 12-week-old DOCA-salt-induced hypertensive rats (i.e., acquired hypertension) in comparison to age-matched normotensive Wistar-Kyoto (WKY) rats by using Northern blot analysis of mRNA levels and Western blot analysis of protein levels.

MATERIALS AND METHODS

Animals

All experiments were performed according to guidelines of the *Care and Use of Laboratory Animals* (NIH publication no. 92-23, revised 1985).

WKY rats, aged 6-12 weeks, were obtained from the Animal Center of National Cheng Kung University Medical College. They were housed 4 per cage at 23 ± 1 °C with a 12-hr light-dark cycle. The light-cycle lasted from 07:00 to 19:00. Purina rat chow and water were available ad libitum. Adult male WKY rats (weight, 0.20 to 0.25 kg) were given a subcutaneous silastic implant impregnated with DOCA (200 mg/kg) and were uninephrectomized (left side, flank incision) under isoflurane (Iso-Flo, Abbott Laboratories, USA) anesthesia. Control rats did not receive an implant but were uninephrectomized. Postoperatively, rats given DOCA received drinking water containing 1.0 NaCl and 0.2 KCl. Control rats received normal tap water. All animals were fed a diet of standard rat chow and received ad libitum access to food and water. After 4 weeks, the systolic blood pressure was measured by the standard tail-cuff method.

Determination of Blood Pressure

Systemic mean blood pressure was measured in conscious DOCA-salt and WKY rats at 6, 9, and 12 weeks old following a baseline stabilization period. An indirect tail-cuff method reading by a programmed autodetector (UR-5000; Ueda Seisaku, Tokyo, Japan) was performed, as described previously.[11] Recording on the laser computerized digital program was carried out at ambient temperature (28 ± 1 °C). The indicated blood pressure values were calculated from the means for each rat measured in triplicate before sacrifice.

Tissue Preparations

After recording the blood pressure, hearts of 6-, 9-, 12-week-old rats were obtained under ether anesthesia. Rat hearts were completly dissected clean of tissues other than the myocardium. Heart sections were then immediately homogenized (5:1, v/w) in 10 mmol/L Tris/HCI buffer (pH 7.0) containing 0.25 mol/L sucrose. Tissues for Western and/or Northern blotting were frozen in liquid nitrogen and stored at -80 °C until further analysis.