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Treatment with Tin Prevents the Development of Hypertension in Spontaneously Hypertensive Rats

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Cytochrome P-450–dependent metabolites of arachidonic acid (AA) increased in the kidneys of young, spontaneously hypertensive rats (SHRs) during the period of rapid elevation of blood pressure (BP) but not in adult SHRs or in Wistar Kyoto rats (WKYs) with normal BP. Treatment of SHRs and WKYs with stannous chloride ($SnCl_2$), which selectively depletes renal cytochrome P-450, restored BP to normal, coincident with a natriuresis, in young but not in adult SHRs and did not affect either BP or sodium excretion in WKYs. Depletion of renal cytochrome P-450 was associated with decreased generation of these AA metabolites only in young SHRs. The antihypertensive effect of $SnCl_2$ in young SHRs was greatly reduced by prevention of its cytochrome P-450–depleting action.

THE MECHANISMS RESPONSIBLE FOR the elevation of BP in essential hypertension are not understood (*1*). The animal model most frequently used to study essential hypertension is the SHR (*2*). In the young SHR, between ages 5 and 13 weeks, BP increases rapidly: for example, systolic BP rises from 100 to 170 mmHg, whereas in the age-matched WKY systolic BP rarely exceeds 130 mmHg (*3*). Hypertension in the SHR can be corrected by renal transplant from a normotensive donor (*4*), suggesting that abnormalities in renal function contribute to elevation of BP. Alterations in salt and water excretion, renal blood flow, and glomerular filtration rate have been described in the SHR at 4 to 8 weeks of age (*3, 5*) but are compensated for in the older SHR. It has been suggested that these alterations in renal function in the young SHR are the functional expression of abnormal renal AA metabolism through the cyclooxygenase pathway (*6, 7*). Thus, increased production of thromboxane (Tx) A_2 has been described in the SHR (*8*) and may contribute to the aforementioned abnormalities in renal hemodynamics and excretory function (*9*).

Metabolites of AA generated by cytochrome P-450 monooxygenases also have the potential to alter BP by affecting vascular tone and Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase) activity (*10, 11*). We have recently reported that renal cytochrome P-450 levels and metabolism of AA by cytochrome P-450–dependent monooxygenases are elevated in the SHR during the developmental phase of hypertension, ages 5 to 13 weeks, when compared to those in the WKY (*12*). When hypertension becomes

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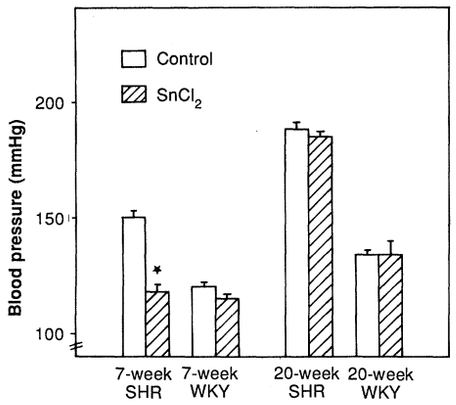

Fig. 1. The effect of Sn^{2+} treatment on BP in 7- and 20-week-old SHRs and WKYs. We measured BP from the tail without anesthesia, using a plethysmograph, every day for four consecutive days. Rats were treated for 4 days with $SnCl_2$, 10 mg per 100 g of body weight, subcutaneously. Results are expressed as the mean ± SEM ($n = 6$ for each of the four groups). Values obtained before and after treatment were compared by the unpaired Student's t test; * indicates $P < 0.05$ for control versus $SnCl_2$ treatment in 7-week-old SHRs. The range of BP in 7-week-old SHRs, treated and untreated, was 115 to 140 and 150 to 160 mmHg, respectively. For corresponding groups of 7-week-old WKYs, BP ranged between 105 to 120 and 110 to 130 mmHg. The BPs in 20-week-old SHRs and WKYs ranged between 175 to 190 and 110 to 140 mmHg, respectively, and were unaffected by $SnCl_2$ treatment.

established in the adult SHR, abnormal AA metabolism is not apparent.

We evaluated the possible contribution of increased renal cytochrome P-450 levels and attendant enhanced synthesis of AA metabolites by the cytochrome P-450 monooxygenases of the kidney to the elevation of BP in the SHR. We studied the effects of decreasing renal cytochrome P-450 content on BP and renal cytochrome P-450–dependent AA metabolism. We depleted renal cytochrome P-450 by inducing heme oxygenase, which is the rate-controlling enzyme in heme catabolism (*13*) and thereby affects the availability of heme for hemoproteins such as cytochrome P-450 monooxygenases. As induction of heme oxygenase with cobalt (Co^{2+}) has been reported to reduce renal AA metabolism by cytochrome P-450 monooxygenases (*14*), we elected to treat the SHRs with Co^{2+}. Treatment with cobaltous chloride ($CoCl_2$) (12 mg per kilogram of body weight, subcutaneously, for 4 days) reduced BP in the 7-week-old SHRs from 140 ± 5 to 80 ± 5 mmHg ($n = 4$, mean ± SEM, $P < 0.05$) and reduced cytochrome P-450–dependent AA metabolism from 788 ± 139 to 617 ± 132 ng of AA converted per milligram of protein per 30 min ($n = 4$). However, the BP-lowering effect of Co^{2+} was associated with unacceptable toxicity and high mortality even in the short term. Among the metal ions, which are inducers of heme oxygenase, tin (Sn^{2+}) is unique as it is selective for the kidney, having little effect on hepatic heme oxygenase (*15*). Moreover, the potency of Sn^{2+} surpasses that of any of the known inducers of renal heme oxygenase. Further, stannous chloride ($SnCl_2$) is not associated with acute toxicity in the rat and renal function is unimpaired during the 4-day period of treatment (*16*).

We treated SHRs and WKYs with $SnCl_2$ and related the changes in heme oxygenase activity, cytochrome P-450 content, and AA metabolite formation to the effects on BP. SHRs and WKYs, ages 6 and 20 weeks, were injected with either $SnCl_2$ (10 mg per 100 g of body weight, subcutaneously) or vehicle control for 4 days and then killed to measure heme oxygenase activity and cytochrome P-450 levels and the formation of cytochrome P-450–dependent AA metabolites within the kidneys. Treatment with Sn^{2+} for 4 days reduced BP in 7-week-old SHRs, whereas it was essentially without effect in 20-week-old SHRs (Fig. 1). A significant effect of $SnCl_2$ on BP of young SHRs was evident by day 1 of treatment: BP was reduced by 12 ± 3 mmHg on day 1 and by 25 ± 3, 31 ± 3, and 32 ± 4 mmHg on days 2, 3, and 4, respectively, from a control level of 155 ± 4 mmHg ($P < 0.01$ for each day versus control; multiple comparisons were done by the Newman-Keuls' multiple range test). Treatment with Sn^{2+} did not affect the BP of 7- and 20-week-old WKYs. Renal heme oxygenase in both young and adult SHRs and WKYs was stimulated five- to eightfold by treatment with Sn^{2+} and was associated with an approximately 50% reduction in renal cytochrome P-450 content (Table 1). In contrast to the effect on the kidney, hepatic heme oxygenase activity and hepatic cytochrome P-450 content were unaffected by treatment with $SnCl_2$ in the SHR and the WKY, confirming the reported selective stimulation of renal heme oxygenase by tin (*15*).

Renal cytochrome P-450–dependent metabolites of AA, defined as those metabolites whose formation is dependent on the addition of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) (1 m*M*) and inhibited by SKF-525A (100 μ*M*) (*14*), were formed in greater quantities by renal cortical microsomes obtained from 7-week-old SHRs as compared to age-matched WKYs (Table 1). Treatment with $SnCl_2$ for 4 days caused a significant reduction in cortical and medullary synthesis of these AA metabolites only in the young SHR. A dose-response relation was demonstrated in the young SHR for the effects of $SnCl_2$ on BP and renal cytochrome P-450–related AA metabolism. Thus, increasing $SnCl_2$ doses of 1, 5, and 15 mg per 100 g of body weight resulted in corresponding

Table 1. Effect of 4-day treatment with $SnCl_2$ on cortical heme oxygenase activity, cortical cytochrome P-450 content, and cytochrome P-450–dependent AA metabolism. The microsomal fraction was obtained from the renal cortex and outer medulla of control and Sn^{2+}-treated rats by homogenization of the tissue in tris-HCl sucrose buffer, *p*H 7.6, and centrifugation at 2,000*g* for 10 min and at 40,000*g* for 60 min. We measured heme oxygenase activity by following bilirubin formation as described by Abraham *et al.* (*24*). We determined the cytochrome P-450 content by measuring the carbon monoxide difference spectra as described by Omura and Sato (*25*). Cytochrome P-450–dependent AA metabolites were determined by incubating the microsomes (0.3 mg of protein) with [^{14}C]AA (0.2 to 0.4 μCi) in the presence of an NADPH-generating system (glucose C-6 phosphate, 0.1 m*M*; NADP, 0.4 m*M*; glucose C-6 phosphate dehydrogenase, 1 unit) in a total volume of 1 ml, for 30 min at 37°C ($n = 5$). The reaction was terminated and AA metabolites were extracted and separated by thin-layer chromatography as described by Sacerdoti *et al.* (*12*). Results are expressed as mean ± SEM. Results obtained were compared between SHRs and WKYs (*) of the same age or after treatment with Sn^{2+} with controls (†) of corresponding age within a group by analysis of variance with Newman-Keuls' test. The difference was considered to be significant for $P < 0.05$.

Treatment	Rat	Heme oxygenase activity per hour (pmol per mg of protein)	Cytochrome P-450 content (pmol per mg of protein)	Cytochrome P-450 metabolism per 30 min (ng of AA converted per mg of protein)	
				Cortex	Medulla
		At 7 weeks			
Control	SHR	771 ± 182	185 ± 11	954 ± 58	754 ± 25
	WKY	776 ± 193	141 ± 13*	762 ± 40*	600 ± 48
Tin	SHR	4858 ± 422†	87 ± 22†	746 ± 38†	534 ± 69†
	WKY	3576 ± 282†	77 ± 13†	702 ± 56	530 ± 73
		At 20 weeks			
Control	SHR	874 ± 115	200 ± 36	744 ± 37	484 ± 64
	WKY	820 ± 61	155 ± 17	674 ± 34	512 ± 29
Tin	SHR	5774 ± 1126†	100 ± 36†	628 ± 45	386 ± 59
	WKY	6420 ± 315†	72 ± 18†	566 ± 76	438 ± 70

greater reductions in BP of 16 ± 3, 24 ± 2, and 36 ± 2 mmHg (control, 151 ± 2 mmHg) and in renal cortical microsomal AA metabolism; namely, 665 ± 98, 618 ± 100, and 570 ± 10 ng of AA converted per milligram of protein per 30 min (control, 835 ± 57 ng/mg per 30 min). The magnitude of the decrease in BP produced by $SnCl_2$ in the young SHR was linearly related to the degree of reduction in renal cytochrome P-450 AA metabolism (slope ± SE = 7.6 ± 1.1).

We measured the 24-hour urinary sodium (Na^+) excretion on the day before treatment with Sn^{2+} (day −1) and on the fourth day of treatment (day 4) before the rat was killed. Excretion of Na^+ increased from 1059 ± 193 to 1682 ± 158 microequivalents per day ($P < 0.025$) in 7-week-old SHRs treated with $SnCl_2$. Corresponding values (day −1 versus day 4) for untreated 7-week-old SHRs were 1342 ± 220 versus 984 ± 160 μeq/day and for $SnCl_2$-treated and untreated, 7-week-old WKYs were 1700 ± 107 versus 1383 ± 57 μeq/day and 1491 ± 181 versus 1487 ± 114 μeq/day, respectively ($n = 5$). Urinary volume and potassium (K^+) excretion also increased by the 4-day treatment with $SnCl_2$ in 7-week-old SHRs as compared to control SHRs of the same age; values at day 4 were as follows: volume, 15.1 ± 1.6 versus 10.6 ± 1.0 ml/day ($P < 0.05$); K^+ excretion, 3430 ± 328 versus 1931 ± 283 μeq/day ($P < 0.01$).

Prevention of $SnCl_2$ induction of heme oxygenase by concomitant treatment for 4 days with tin-protoporphyrin (50 μmol per kilogram of body weight, subcutaneously), a competitive inhibitor of heme oxygenase (*17*), reduced the BP-lowering effect of Sn^{2+} in 7-week-old SHRs. In these experiments, $SnCl_2$, when given alone, reduced BP from 152 ± 2 to 123 ± 3 mmHg ($n = 9$, $P < 0.01$), whereas cotreatment of 7-week-old SHRs with $SnCl_2$ and tin-protoporphyrin greatly attenuated the BP-lowering effect of Sn^{2+}, the BP falling only to 140 ± 3 mmHg from a control level of 153 ± 2 mmHg. Tin-protoporphyrin did not affect the BP of the young SHR when given alone. The coadministration of tin-protoporphyrin with $SnCl_2$ was also associated with a lesser reduction of renal cytochrome P-450 content. The latter fell to only 140 ± 25 pmol per milligram of protein from control cytochrome P-450 levels of 178 ± 11 pmol/mg when tin-protoporphyrin was coadministered with $SnCl_2$, as compared to the low levels of 60 ± 5 pmol/mg obtained when $SnCl_2$ was given without the moderating effect of tin-protoporphyrin.

It was critical to our hypothesis that Sn^{2+}-induced changes in BP could be linked to corresponding changes in renal cytochrome P-450 content. Thus, stimulation and inhibition of heme oxygenase was shown to produce corresponding effects on BP and on renal cytochrome P-450 content, lowering and raising the levels, respectively. Because renal mechanisms are critical to BP control, we gained considerable advantage in having a means to manipulate specifically the cytochrome P-450 system of the kidney independently without affecting that of the liver.

Depletion of cytochrome P-450 resulted in decreased formation of renal cytochrome P-450–dependent AA metabolites. A relation between the level of renal cytochrome P-450–dependent AA metabolites and changes in BP is supported by several observations. (i) Increased formation of these AA metabolites occurred only in the young SHR (*12*), and $SnCl_2$ treatment reduced their formation significantly only in this group (Table 1). (ii) Increasing doses of $SnCl_2$ caused corresponding greater reductions in BP in the young SHR, which were related to the magnitude of reduced formation of cytochrome P-450–dependent AA metabolites. (iii) Several of these AA metabolites have BP-elevating properties. Thus, the omega oxidation products of AA constitute a large component of the metabolites identified in the kidney of the young SHR (*12*) and have been shown to constrict blood vessels (*18*) and stimulate Na^+,K^+-ATPase (*19*).

Rapp and Dahl (*20*) have demonstrated in a salt-sensitive strain of genetically hypertensive rats enhanced activity of a cytochrome P-450–related hydroxylase, which gives rise to 18-hydroxycorticosterone, a potent mineralocorticoid. They presented evidence that the differences in steroidogenesis between salt-sensitive and salt-resistant strains of hypertensive rats are caused by mutation of a specific cytochrome P-450 controlled by a single genetic locus. More recently, Melby *et al.* (*21*) reported on excessive production of another potent mineralocorticoid, 19-nordeoxycorticosterone, in the young SHR. The natriuresis demonstrated in the young SHR coincident with the antihypertensive action of Sn^{2+} treatment may derive, then, from reduced formation of mineralocorticoids as well as reduced synthesis of Na^+-retaining AA metabolites. This natriuresis occurred despite a decrease in BP, indicating restoration toward normal of the relation between BP and Na^+ excretion. Thus, it has been proposed that hypertension acts as a compensatory mechanism to restore Na^+ homeostasis (*22*) in the face of enhanced activity of Na^+-retaining factors. Finally, Sn^{2+} treatment has long-term effects on BP of the SHR. In an on-going study in the SHR, normal BP persisted for the duration of the study, 7 weeks beyond the discontinuance of $SnCl_2$ treatment (*23*).

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