Xanthine oxidase activity associated with arterial blood pressure in spontaneously hypertensive rats

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ABSTRACT Recent evidence in vivo indicates that spontaneously hypertensive rats (SHR) exhibit an increase in oxyradical production in and around microvascular endothelium. This study is aimed to examine whether xanthine oxidase plays a role in overproduction of oxidants and thereby may contribute to hypertensive states as a consequence of the increasing microvascular tone. The xanthine oxidase activity in SHR was inhibited by dietary supplement of tungsten (0.7 g/kg) that depletes molybdenum as a cofactor for the enzyme activity as well as by administration of the xanthine oxidase inhibitor (−)-8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo(1,5-α)-1,3,5-triazine-4-monohydrate, a synthetic inhibitor of the enzyme. The characteristic elevation of mean arterial pressure in SHR was normalized by the tungsten diet, whereas Wistar Koto (WKY) rats displayed no significant alteration in the pressure. Multifocal intravital videomicroscopy in mesenteric microvessels with hydroethidine, an oxidant-sensitive fluoroprobe, showed that SHR endothelium exhibited overproduction of oxyradicals that coincided with the elevated arteriolar tone as compared with WKY rats. The tungsten diet significantly repressed these changes toward the levels observed in WKY rats. The activity of oxyradical-producing form of xanthine oxidase in the mesenteric tissue of SHR was ~3-fold greater than that of WKY rats, and pretreatment with the tungsten diet eliminated detectable levels of the enzyme activity. The inhibitory effects of the tungsten diet on the increasing blood pressure and arteriolar tone in SHR were also reproducible by administration of (−)-BOF4272. These results suggest that xanthine oxidase accounts for a putative source of oxyradical generation that is associated with an increasing arteriolar tone in this form of hypertension.

Several hypothesis have been advanced to account for the high blood pressure in SHR. Among the factors that have been proposed to account for the increase in arteriolar resistance are smooth muscle medial hypertrophy (9), vessel rarefaction (2), an elevation of tone in resistance arteries (10), an increased sympathetic activity (11), differences in catecholamine transmitter disposition and sensitivity of smooth muscle cells (12,13), and differences in central vascular regulation (14). Previously reported that the level of microvascular oxidative stress in SHR was significantly above that displayed in its Wistar Kyoto (WKY) normotensive control strain. The enhanced level in SHR showed a linear correlation with the increase in arteriolar tone (15). Superoxide of a synthetic xanthine oxidase (XO) inhibitor (−)-BOF4272, (−)-8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo(1,5-α)-1,3,5-triazine-4-monohydrate led to a normalization of the enhanced oxidative conditions in SHR (15). Furthermore, although the increase in mesenteric arteriolar blood flow following topical histamine application was significantly attenuated in SHR as compared with WKY rats, the response to this agent could be normalized by topical application of a synthetic XO inhibitor (16). The use of animals deprived of molybdenum and fed a tungsten supplement has been used as an effective method for XO inhibition in which molybdenum is replaced by tungsten in the active site of this enzyme (17).

Accordingly, the present study was designed to explore the effect that a blockade of XO has on the elevated arterial blood pressure, as well as on the enhanced level of oxidative stress observed along the arteriolar walls in SHR.

MATERIALS AND METHODS

Animals. All animal procedures were reviewed previously and approved by the University of California, San Diego, Animal Subject Committee. SHR (n = 60) and WKY rats (n = 57) (14–16 weeks of age, Charles River Breeding Laboratories) were used for the study. A group of SHR (n = 17) and WKY rats (n = 14) were fed with a tungsten-enriched (sodium tungstate, 0.7 g/kg), molybdenum-depleted diet (ICN Nutritional Biochemicals, Cleveland) and water ad libitum and were maintained in a light-controlled holding facility for 2 weeks.

Abbreviations: SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto rat; XO, xanthine oxidase; XD, xanthine dehydrogenase; HE, hydroethidine; EB, ethidium bromide; (−)BOF4272, (−)-8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo(1,5-α)-1,3,5-triazine-4-monohydrate; (+)BOF4272, (−)-8-(3-methoxy-4-phenylsulfinylphenyl)pyrazolo(1,5-α)-1,3,5-triazine-4-olate monohydrate.

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The remainder of the animals, were fed with normal chow and water ad libitum. All animals appeared alert and in good health.

**Blood Pressure Measurement.** About 1 h before general anesthesia and vital microscopy of the mesentery, a femoral arterial catheter (PE 50 tubing, Clay Adams) was inserted under local anesthesia (4% lidocaine s.c.; Abbott). Mean and pulsatile components of the arterial pressure were measured for about 30 min in animals that were allowed to roam freely in the cage (18).

**Intravital Fluorescence Microscopy.** Under general pentobarbital sodium anesthesia (40 mg/kg), the mesenteric microcirculation was visualized through an intravital microscope (×55 water immersion objective lens; Leitz, Wetzlar, Germany) by using a digital color charge-coupled device (CCD) camera (DEI-470, Optronics Engineering, Goleta, CA). The tissue was continuously superfused at 36.5°C and pH 7.4 with a Krebs–Henseleit bicarbonated-buffered solution saturated with a 95% N2 and 5% CO2 gas mixture to minimize the presence of vascular smooth muscle, and divergent bifurcations, whereas venules are classified on the basis of their position on the in-flow side of the microvascular network, the arterioles or venules (NEB) was determined at 60 min after the onset of HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion. At the end of the experiments, the tissue was superfused with absolute ethanol for 10 min following HE superfusion.
state lumen diameter \( (d_{ss}) \) for each vessel at 30 min after topical mesenteric application of 10 nM (–)BOF4272 and the dilated diameter \( (d_{max}) \) during papaverine superfusion (without BOF4272).

**Statistical Analysis.** Statistical differences between the two animal strains and treatment modalities were determined by one-way layout ANOVA and Bonferroni-type multiple comparison test. All values are expressed as means ± SD. Statistical significance was set at \( P < 0.05 \).

**RESULTS**

After tungsten treatment, WKY rats exhibited a mild tendency to gain weight after tungsten supplementation but without statistically significant differences when compared with the control WKY rats \( (P = 0.091) \) or the SHR strain on tungsten supplement \( (P = 0.217) \) (Table 1).

The mean arterial blood pressure in SHR under local anesthesia was significantly above normal as compared with that of WKY rats (Fig. 1a). This elevation in blood pressure was significantly lowered by the tungsten diet (Fig. 1a). In WKY rats, mean arterial blood pressure was in no case decreased but tended to show a marginal and insignificant increase after the tungsten diet (Fig. 1a).

The systolic and diastolic blood pressures followed the same trend as the mean blood pressure (Table 2). There were no significant differences in heart rate among the four groups (Table 2). There were no significant differences in average arteriolar flow rates of the mesentery after the tungsten diet in either rat strain (measured in about five animals per group with five to six arterioles per rat; results not shown).

The level of HE oxidation, as demonstrated by the number of EB-positive nuclei (%) along mesenteric arteriolar walls, increased in SHR compared with WKY rats (Fig. 1b, Left). The tungsten diet served to significantly decrease the number of EB-positive nuclei along arterioles of SHR (Fig. 1b, Left), whereas in WKY rats, the tungsten diet had no effect on the number of EB-positive nuclei.

In the venules, the level of HE oxidation was significantly higher in SHR than in WKY rats (Fig. 1b, Right). The trend toward an elevated number of EB-positive nuclei along SHR venules was significantly attenuated after tungsten intake (Fig. 1b, Right).

**Table 1. Body weight with chow or tungsten diet**

<table>
<thead>
<tr>
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<th>Body weight, g</th>
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<tr>
<td></td>
<td>WKY</td>
</tr>
<tr>
<td>Chow diet</td>
<td>328.0 ± 26.9</td>
</tr>
<tr>
<td>( n = 13 )</td>
<td>( n = 13 )</td>
</tr>
<tr>
<td>Tungsten-enriched diet</td>
<td>344.5 ± 22.5</td>
</tr>
<tr>
<td>( n = 14 )</td>
<td>( n = 17 )</td>
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</table>

Data are the mean value ± SD. The number of animals is specified by \( n \).

**Fig. 1.** (a) Mean arterial blood pressure under local anesthesia. All values are reported as mean ± SD. In SHR, a significant elevation of mean arterial blood pressure was observed relative to that in WKY rats \( (*, P < 0.05) \). After intake of the tungsten-enriched diet, the elevated mean arterial blood pressure characteristic of SHR was significantly blunted \( (†, P < 0.05) \). The number at the bottom of each column indicates the number of animals. (b) The number of EB-positive nuclei (%) along arteriolar and venular wall. All values are reported as mean ± SD. \( *, P < 0.05 \) as compared with those of WKY rats arterioles; \( †, P < 0.05 \) as compared with those of SHR arterioles; \( **, P < 0.05 \) as compared with those of WKY rats venules; \( ††, P < 0.05 \) as compared with those of control SHR venules. The number at the bottom of each column indicates the number of microvessels observed in the study. (c) The steady-state arteriolar tone after 60 min mesenteric superfusion. All values are reported as mean ± SD. \( *, P < 0.05 \) as compared with those of WKY rats; \( †, P < 0.05 \) as compared with those of SHR. The number at the bottom of each column indicates the number of arterioles observed in the study. (d) The level of total xanthine oxidoreductase (XD and XO) activity and XO activity in the mesentery. All values are reported as mean ± SD. \( *, P < 0.05 \) as compared with those of control WKY rats. \( **, P < 0.05 \) as compared with control SHR. Six rats were used in each group.
1b, Right). In WKY rats, the tungsten diet had no significant effect on the number of EB-positive nuclei along the venules.

The level of tone displayed by the SHR arterioles was significantly higher than that of WKY arterioles (Fig. 1c). In SHR, tungsten intake serves to significantly lower the level of arteriolar tone (Fig. 1c), whereas in WKY rats, the arteriolar tone showed a slight and statistically insignificant increase (Fig. 1c).

XO and XD activity in the mesentery of SHR (1.94 ± 0.77 mU/mg) was 2.8 times higher than that of WKY rats (0.70 ± 0.15 mU/mg) (Fig. 1d, Upper). The treatment with tungsten enriched diet lowered this activity to undetectable levels in both strains (Fig. 1d, Upper). XO activity in the mesentery of WKY rats (0.65 ± 0.16 mU/mg) encompasses 93% of the total (XO and XD) activity (1.75 ± 0.64 mU/mg); similarly, almost 90% of the total (XO and XD) activity in SHR is caused by XO (Fig. 1d, Lower). The level of XO activity in the mesentery of SHR was 2.7 times higher than that of WKY rats (Fig. 1d, Lower). Treatment with a tungsten-enriched diet reduced this activity in both strains to undetectable levels.

The mean arterial blood pressure in SHR (188.4 ± 8.4 mmHg), was significantly reduced to (140.7 ± 12.2 mmHg) after 10 mg/kg (-)BOF4272 administration (Fig. 2a). In contrast, 10 mg/kg (+)BOF4272 administration did not significantly shift the arterial blood pressure in SHR. In WKY rats, the mean arterial blood pressure was not affected, neither did (+)BOF4272 administration reduce the arterial blood pressure in these rats. With 30 mg/kg of (-)BOF4272, the mean arterial blood pressure was not only further reduced in SHR, but a significant reduction was observed also in WKY rats (Fig. 2a).

Arteriolar tone at the initial level in SHR was significantly higher than that in WKY rats (Fig. 2b). The tone values in SHR were significantly reduced after (-)BOF4272 application, whereas no such effect could be detected after (+)BOF4272 application. In WKY rats, the intervention with (-)BOF4272 or (+)BOF4272 did not bring about a significant change in the arteriolar tone (Fig. 2b).

### DISCUSSION

Previous studies reported that, in hypertensive animals such as SHR, the increase in arteriolar tone occurs in a variety of tissues (i.e., skeletal muscle, mesentery, and skin) and thus serves as a major mechanism that determines elevation of systemic blood pressure (5, 25). The detailed mechanisms for the increasing tone, however, have been unknown. The present study provides evidence that the increasing activity of XO, an oxidant-producing enzyme, plays an important role in the increasing arteriolar tone in SHR.

XO is an enzyme that is known to distribute widely in mammalian tissues (26). Among the tissue components, endothelial cells in microvascular systems constitute the most abundant source of the enzyme as compared with those in larger vessels (27). Because the enzyme reaction that transfers electrons from hypoxanthine to uric acid is coupled with a reduction of molecular oxygen into superoxide anions, XO has been considered to play a crucial role in the pathogenesis of oxidant-induced microvascular changes and tissue injury (26, 28–30). However, few reports have addressed the role of oxidants in hypertension, except for previous observations by Nakazono et al. (31), showing that a selective delivery of superoxide dismutase to the vascular endothelial cells causes a decrease in systemic blood pressure in SHR but not in WKY rats.

The present observation that chronic tungsten diet as well as administration of a selective inhibitor of XO lowers microvascular tone (Fig. 1c) and systemic blood pressure (Figs. 1a and 2a) lead us to hypothesize that XO-mediated superoxide generation contributes to the mechanisms for the increasing arteriolar tone in SHR. The results indicate that there is a greater oxyradical-producing form of XO in vessels of SHR than in those from WKY rats. Taken together with the observation that both the tungsten diet and (-)BOF4272 significantly attenuated the increasing levels of HE oxidation in SHR (Fig. 1b) (15), these results suggest that the elevation of XO activities accounts for overproduction of oxyradicals in and around microvessels in this type of hypertension.

Overproduction of oxygen radicals in and around endothelial cells may cause pathologic responses of microvascular tone through a variety of mechanisms. Among such mechanisms, most important is the overproduction of superoxide anions is likely to be an event responsible for the increasing arteriolar tone in SHR. Aisaka et al. (32) reported that NO-scavenging systems around the microvasculature (e.g., superoxide dismutase) leads to the development of NO oxidation in the SHR (31), showing that a selective delivery of N-acetyl-L-arginine, an inhibitor of NO synthase, increases mean arterial pressure not only in WKY rats but also in SHR. Furthermore, our previous observation showed that NO-dependent arteriolar relaxation in SHR can be restored by topical application of (-)BOF4272 but not (+)BOF4272 (16). These findings suggest that, in SHR, NO is well synthesized but is canceled out by unidentified superoxide dismutase. Although NADH/NADPH oxidase induced by way of renin-angiotensin system (33) is a candidate for the oxyradical-producing systems that can lower the vasorelaxing action of NO, the current results firmly support the concept that XO serves as such a putative oxidant-generating system in the hypertensives.

Studies on vascular endothelial cells show that they have a highly enriched XO/XD system (34). Our present results indicate that both XO and XD + XO activities in the mesentery of SHR were about 3-fold higher than those of WKY rats. Nakazono et al. (31) have reported that in the aorta there were no significant differences in XO activities between WKY rats and SHR. However, because the mass of endothelial cells within the aorta is less than that of other cells, such as smooth muscle cells, and the nonendothelial cell content of the aorta is higher in SHR than those in WKY rats because of medial hypertrophy, XO activity within vascular endothelial cells may in fact be even higher than that estimated by Nakazono et al. (31). Because, in the vessels of the mesentery, the ratio of endothelial to nonendothelial cells is higher than in the aorta, the XO activity recorded in the mesentery may represent a closer estimate of the actual value of endothelial XO activity.

### Table 2. Systolic blood pressures (SBP), diastolic blood pressures (DBP), and heart rate (HR) with and without dietary tungsten supplement

<table>
<thead>
<tr>
<th></th>
<th>WKY (n = 13)</th>
<th>Tungsten (n = 14)</th>
<th>SHR (n = 13)</th>
<th>Tungsten (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>170.3 ± 26.6</td>
<td>185.1 ± 26.2</td>
<td>242.7 ± 45.3*</td>
<td>191.0 ± 20.0*</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>112.2 ± 11.6</td>
<td>121.9 ± 17.7</td>
<td>163.0 ± 10.4*</td>
<td>120.8 ± 14.0*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>409.3 ± 67.7</td>
<td>380.0 ± 46.3</td>
<td>396.6 ± 50.8</td>
<td>395.3 ± 65.3</td>
</tr>
</tbody>
</table>

Data are the mean value ± SD. n = number of animals; bpm = beats per min.

*P < 0.05 as compared with WKY rats (chow).

†P < 0.05 as compared with SHR (chow).
and has a higher affinity for aldehyde than purines, but produces O$_2$ in a manner similar to XO because both oxidases possess nearly identical internal electron transport systems (43).

Corticosterone 6β-hydroxylation is markedly higher in SHR than in WKY rats, and its reduction by the selective family 3A cytochrome P450 inhibitor is associated with a large blood pressure reduction (44). Recently we (7) demonstrated that the trend for the systemic blood pressure and arteriolar tone to be elevated during the SHR syndrome was blunted after bilateral adrenalectomy, and that the reduction in blood pressure after adrenalectomy could be circumvented by supplementation of dexamethasone, suggesting a dependence of the syndrome on glucocorticoids. The elevated levels of XO-mediated oxygen radical production in SHR could be reduced by adrenalectomy and again restored by dexamethasone supplementation (15). Although the relationship between elevated levels of glucocorticoid and XO activity in SHR remains to be clarified, a number of connections are conceivable. XO activity in the myocardium (45) as well as plasma uric acid (the major metabolite of xanthine) levels (31) are higher in SHR than in WKY rats. Gene expression of XO in epithelial cells is enhanced by glucocorticoid application (46). Although the level of XO activity is lower in man than in the rat, the potential shift in XO activity and effect on microvascular tone in essential hypertensive subjects remains to be examined.

In conclusion, the elevation of the arterial blood pressure in SHR, as well as the enhanced production of microvascular oxygen radicals, may be related and could be associated with an elevated activity of XO. The resulting free radical production may constitute a key mechanism that leads to organ injury and lesion formation in the hypertensives.

We thank Stephen Laroux (Louisiana State University) and Dan Katz (University of California at San Diego) for the excellent technical assistance. We thank Otsuka Pharmaceutical Factory (Naruto, Japan) for providing (−)BOF4272 and (+)BOF4272. This work was completed during the tenure of a Research Fellowship for H.S. from the American Heart Association, California Affiliate. The research was supported by Public Health Service Grants HL-10881, HL-48676, and DK-43785. A preliminary study was presented at the 6th World Congress for Microcirculation (August 1996, Munich, Germany) as well as the 3rd Asian Congress for Microcirculation (October 1997, Bangkok, Thailand).

Fig. 2. (a) The mean arterial blood pressure before and after the administration of (−)BOF4272 or (+)BOF4272 to WKY rats or to SHR. *P < 0.05 as compared with that of the control in each animal. (b) Arteriolar tone before and after the administration of (−)BOF4272 or (+)BOF4272 to WKY rats and SHR. *P < 0.05 as compared with that of control WKY rats; †P < 0.05 as compared with that of control SHR. The number at the bottom of each column indicates the number of animals.

The findings that blood pressure in SHR but not in WKY rats was markedly decreased by oxypurinol, a XO inhibitor (31), are also in line with the present results.

The requirement of molybdenum for the XO activity in rat intestine (35, 36) and the presence of this metal in the XO were recognized in 1953 (36). Higgins et al. (37) found that levels of molybdenum in the diet as low as 0.02 mg/kg were sufficient to maintain normal growth of rats and to sustain effective levels of XO in all tissues except the intestines. Based on the experiments of De Renzo (38), who first used tungsten as a competitive antagonist of molybdenum in animals, it is reported that high tungsten to molybdenum ratios in the diet were required for the expected antagonism to be achieved in rats (37). The concentration of dietary tungsten used in the present study should be sufficient to decrease the molybdenum content and XO activity (17).

There are two other enzymes identified as a molybdoproteins; sulfite oxidase (SO) (39–41) and aldehyde oxidase (AO). SO was purified from bovine, chicken, and rat livers and localized in the intermembrane space of mitochondria (41). XO is present in the cytosol (42). The rate of decay of SO was reported to be lower than that of XO (17). It is conceivable that mitochondrial localization bestows a longer half-life on SO activity. AO are predominantly present in liver and intestine...
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