Increased superoxide levels in ganglia and sympathoexcitation are involved in sarafotoxin 6c-induced hypertension

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Li M, Dai X, Watts S, Kreulen D, Fink G. Increased superoxide levels in ganglia and sympathoexcitation are involved in sarafotoxin 6c-induced hypertension. Am J Physiol Regul Integr Comp Physiol 295: R1546–R1554, 2008. First published September 3, 2008; doi:10.1152/ajpregu.00783.2007.—Endothelin (ET) type B receptors (ETB) are expressed in multiple tissues and perform different functions depending on their location. ETB mediate endothelium-dependent vasodilation, clearance of circulating ET, and diuretic effects; all of these should produce a fall in arterial blood pressure. However, we recently showed that chronic activation of ETB in rats with the selective agonist sarafotoxin 6c (S6c) causes sustained hypertension. We have proposed that one mechanism of this effect is constriction of capacitance vessels. The current study was performed to determine whether S6c hypertension is caused by increased generation of reactive oxygen species (ROS) and/or activation of the sympathetic nervous system. The model used was continuous 5-day infusion of S6c into male Sprague-Dawley rats. No changes in superoxide anion levels in arteries and veins were found in hypertensive S6c-treated rats. However, superoxide levels were increased in sympathetic ganglia from S6c-treated rats. In addition, superoxide levels in ganglia increased progressively the longer the animals received S6c. Treatment with the antioxidant tempol impaired S6c-induced hypertension and decreased superoxide levels in ganglia. Acute ganglion blockade lowered blood pressure more in S6c-treated rats than in vehicle-treated rats. Although plasma norepinephrine levels were not increased in S6c hypertension, surgical ablation of the celiac ganglion plexus, which provides most of the sympathetic innervation to the splanchnic organs, significantly attenuated hypertension development. The results suggest that S6c-induced hypertension is partially mediated by sympathoexcitation to the splanchnic organs driven by increased oxidative stress in prevertebral sympathetic ganglia.

WE RECENTLY REPORTED that chronic activation of endothelin (ET) type B receptors (ETB) using intravenous infusion of the ETB-selective agonist sarafotoxin 6c (S6c) in rats causes an increase in arterial pressure (S6c-induced hypertension) (16). This was surprising in light of the fact that the two best described physiological responses to ETB activation, i.e., release of vasodilators from endothelial cells, and increased renal sodium and water excretion, should lead to a fall in arterial pressure (42, 48, 50). We believe venoconstriction contributes to S6c-induced hypertension, because in vitro S6c has been shown to constrict veins from most vascular regions but to have little effect on most arteries (15, 27, 41, 49).

It is likely, however, that other mechanisms contribute to S6c-induced hypertension. Reactive oxygen species (ROS) are a variety of oxygen-containing molecules that are by-products of cellular metabolic processes under physiological condition, including superoxide anion (O2−), hydrogen peroxide (H2O2), and hydroxyl ion (OH−). Recently, increasing evidence has shown that ROS can function as cellular signaling molecules (5). Increased ROS levels have been found in blood vessels, kidneys, and other tissues in many experimental models of hypertension, including renovascular (32), DOCA-salt (53), Dahl salt-sensitive (56), and lead-induced hypertension (62). ROS generation also is elevated in hypertensive patients (60). Increased tissue ROS levels can cause hypertension, because blocking ROS generation or increasing their removal lowers blood pressure (4, 30, 55). Recent reports indicate that ETB activation increases oxidative stress in sympathetic ganglia in vitro (12) and in vivo (31). Although the precise effects of increased ROS levels in sympathetic ganglia are not known, it is possible that ROS signaling could enhance ganglionic neurotransmission and thereby increase sympathetic activity. Therefore, we tested the hypothesis that increased superoxide generation in vascular tissues or sympathetic ganglia is involved in S6c-induced hypertension. We focused our attention on sympathetic ganglia supplying the splanchnic region because changes in arterial and venous tone in this region have a major role in both vascular resistance and capacitance responses of the systemic circulation as a whole (19, 46, 47).

METHODS

Animals

All protocols were approved by the Michigan State University All University Committee on Animal Use and Care. Normal male Sprague-Dawley rats (300–325 g, Charles River, Portage, MI) were used in these experiments. All rats were housed two or three per cage in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle. Pelleted rat chow (8640 Rodent Diet; HarlanTeklad) and water were given ad libitum.

Vascular Catheterization

Pentobarbital sodium (30–50 mg/kg plus 0.4 mg atropine sulfate ip) was used for anesthesia. One polyvinyl catheter with a silicone rubber tip was inserted into the abdominal aorta via a femoral artery for drawing blood samples and hemodynamic measurements. A similar catheter was placed into a femoral vein for drug administration. Free ends of the catheters were passed through a stainless steel spring tether attached to a plastic harness fixed around the chest of the rat. Enrofloxacin (5 mg/kg iv) was given for bacterial prophylaxis. Rats were allowed to recover consciousness on a heated pad under constant observation. When they became conscious, they were placed in individual cages allowing continuous access to both catheters without handling or otherwise disturbing the rats. Acetaminophen was given...
for 3 days after the surgery to relieve surgical pain. Arterial lines were filled with a heparin-saline solution and occluded when not in use. Rats were allowed at least 3 days for recovery after the surgery before measurements were begun.

**Hemodynamic Measurements**

Arterial pressure was determined by connecting the arterial catheter to a low-volume displacement pressure transducer linked to a digital pressure monitor, as previously described (3). Briefly, hemodynamic recordings, including systolic, mean, and diastolic pressures, and heart rate, were recorded for 30 min each morning between 10:00 AM and 12:00 PM without handling or otherwise disturbing the rat sitting quietly in its home cage. All data were averaged minute-by-minute and saved using a computerized data acquisition system (DMSI-400 System Integrator, Micro-Med).

**Celiac Ganglionectomy**

The surgery was conducted through a midline laparotomy. The small intestine was gently moved aside from the midline and covered with saline-soaked gauze. Cotton swabs were used to rub off the fat covering the celiac plexus and main vessels (e.g., aorta, celiac artery, and mesenteric artery) in the vicinity. Visible nerves around the three arteries were removed as completely as possible by stripping. For sham rats, the small intestine was displaced and overlying fat was removed, but the nerves stayed intact. After the rats awakened, they were fed with normal food and tap water for 10 days during recovery from surgery. Acetaminophen was given for 3 days after the surgery to relieve surgical pain.

**Dihydroethidium Fluorescence**

Dihydroethidium (DHE), an oxidative fluorescent dye, was used to measure superoxide ($O_2^-$) levels in mesenteric arteries, mesenteric veins, and inferior mesenteric ganglion (IMG) (35) (36). Rats were killed with an intraperitoneal (ip) injection of pentobarbital sodium. Mesenteric vessels and IMG were removed from each rat. Blood vessels were placed into oxygenated Krebs buffer at 4°C, dissected free of loosely adhering tissue, and cut into 3- to 4-mm-wide ring segments. Unfixed frozen ring segments were cut into 25-μm-thick sections using a cryostat and were placed on a glass slide. DHE (5 × 10^{-6} mol/l) was topically applied to each tissue section. Slides were incubated in a light-protected humidified chamber at 37°C for 60 min for vessels and 45 min for IMG. Fluorescent images were observed immediately after incubation, tubes were incubated for 10 min at 37°C in the dark. After incubation, tubes were put into a luminometer (TD-20e; Turner Designs, Sunnyvale, CA). Fluorescence was quantified by ImageJ software (U.S. National Institutes of Health, Bethesda, MD).

**Lucigenin-Enhanced Chemiluminescence**

Vascular $O_2^-$ was quantified by lucigenin chemiluminescence. Isolated vessel segments were cleaned and incubated for 30 min in modified Jude Krebs Buffer at 37°C in the presence of 10 mM diethyldithiocarbamate. Vessels were transferred to small tubes that contained 5 μM lucigenin in modified Krebs-HEPES buffer and were incubated for 10 min at 37°C in the dark. After incubation, tubes were put into a luminometer (TD-20e; Turner Designs, Sunnyvale, CA). Luminescence measurements were integrated for 30-s periods. Ten repeated measurements were then averaged. After 10 cycles, the cell-permeant $O_2^-$ scavenger tiron (10 mM) was added, and 15 more cycles were read. The last eight values, which were maximally reduced, were averaged. Data were calculated as the change in the rate of luminescence per minute per milligram of tissue of values before and after tiron and then converted to $O_2^-$ (nmol·min^{-1}·mg tissue^{-1}) (10, 33).

**Plasma Sampling**

Blood samples (1 ml) were drawn from the arterial catheter over 25 μl of EGTA/glutathione solution into ice-chilled plastic syringes and transferred to ice-chilled plastic tubes. Blood samples were centrifuged at 10,000 g for 5 min at 4°C. Plasma were separated and stored in a −80°C freezer for later assays.

**Plasma Norepinephrine Assay**

Plasma norepinephrine was measured by a radioenzymatic assay based on the technique proposed by Peuler and Johnson (41a). The principle of this method is the conversion of norepinephrine and epinephrine to tritiated normetanephrine and metanephrine in the presence of catechol-O-methyltransferase and tritiated $S$-adenosylmethionine as a labeled methyl donor. After purification through a series of organic extractions, tritiated normetanephrine was separated from the derivatives of dopamine by oxidation with sodium periodate. Then tritiated vanillin was counted in a liquid scintillation spectrometer.

**Confirmation of Denervation**

Norepinephrine contents in splanchic organs were measured by high-performance liquid chromatography (HPLC), as previously described (29). Norepinephrine levels in mesenteric arteries and veins were measured by HPLC as well. Tyrosine hydroxylase staining of sympathetic nerves innervating mesenteric vessels and nerve functional studies were carried out to further validate celiac ganglionectomy (CGX; data are not shown here).

**Protocols**

**S6c infusion.** For S6c-treated rats, saline vehicle (3.47 μl/min) was infused intravenously for two days (control period). The animals were then treated with S6c (5 pmol·kg^{-1}·min^{-1}·iv) for 5 days (infusion period), followed by a 3-day-infusion of saline only (3.47 μl/min, recovery period). Sham rats received saline only (3.47 μl/min) for 10 consecutive days. Hemodynamic parameters were measured every day.

**Trimethaphan study.** In a separate study, trimethaphan (15 mg/kg iv) was given daily to S6c-treated and sham rats. The peak fall in blood pressure after trimethaphan injection (generally within 2–5 min) was recorded as an index of sympathetic pressor activity.

**Plasma norepinephrine measurement.** A separate group of S6c-infused and sham rats was used in this study. Blood samples were collected on the second control day, the third and the fifth days of infusion period, and the second recovery day.

**CGX study.** CGX and sham operation were performed 10 to 14 days before the start of the study. Rats were then subjected to S6c infusion as described above.

**Superoxide studies in S6c-induced hypertension.** For S6c-treated rats, saline (3.47 μl/min) was infused intravenously for two days (control period). The animals were then treated with S6c (5 pmol·kg^{-1}·min^{-1}·iv). Tissues were collected after S6c infusion for 1 day or 5 days. Sham rats received saline for 7 consecutive days. Animals were killed in pairs of S6c-treated and saline-treated rats.

**The effect of tempol treatment on S6c-induced hypertension.** After the recovery from catheterizations of femoral artery and vein, and a 2-day control period, one group of rats was infused with saline while drinking water containing tempol (1 mmol/l). Each water bottle was wrapped with aluminum foil to protect the drug from light exposure. The tempol solution was prepared fresh every day. The second group received S6c (5 pmol·kg^{-1}·min^{-1}·iv), and the third group received S6c while drinking tempol solution. S6c and tempol were removed after a 5-day-infusion in groups 2 and 3, followed by saline infusion (recovery period).

**DHE staining in S6c-treated rats with tempol treatment.** One group of rats was given water containing tempol (4-hydroxy 2,2,6,6-tetramethyl piperidine 1-oxyl, a superoxide dismutase mimetic, 1 mmol/l)
immediately after catheterization surgery. After 6 days of tempol treatment, rats received S6c (5 pmol·kg⁻¹·min⁻¹ iv) infusion for 1 day while they continued to receive tempol. A second group of animals was infused with saline only (3.47 μl/min iv) for 7 days and did not receive tempol treatment. A third group of rats was treated with saline for 6 days, then received S6c (5 pmol·kg⁻¹·min⁻¹ iv) for 1 day. For DHE staining, one animal from each group was killed, and tissues were collected from all three rats and studied together.

Statistical Analyses

The effect of CGX on resting mean arterial pressure (MAP) was evaluated by Student’s t-test compared with the average of baseline MAP in intact rats. Within-group differences over time were assessed by a two-way ANOVA and post hoc multiple comparisons were assessed with Tukey’s test (GraphPad Instat 3). Between-group differences were evaluated using the protected least-significant difference test. Superoxide levels in lucigenin studies were reported (in nmol·min⁻¹·mg protein⁻¹). DHE staining was quantified by taking readings from three to five individual sympathetic neurons or vascular smooth muscle cells in each rat. The fluorescence density of the measured cells was the difference between the absolute number in arbitrary fluorescence units and the value of background. Control rats were used for comparison. The ratio of fluorescence density from treated rats to that from controls was used for statistical comparisons. When comparing two groups, the appropriate Student’s t-test was used. Analysis of variance followed by Tukey’s post hoc test was performed when comparing three or more groups. In all cases, *P < 0.05 was considered statistically significant. A *P < 0.05 was considered significant. All of the results are presented as means ± SE.

RESULTS

Acute Ganglion Blockade in S6c-Induced Hypertension

Figure 1A shows changes in MAP during 5-day S6c or vehicle infusion. MAP increased significantly during S6c infusion, whereas it did not change in vehicle-treated rats (average of 123.8 ± 1 vs. 106.7 ± 2 mmHg). Figure 1B shows acute depressor responses to ganglion blockade with trimethaphan. Infusion of S6c produced sustained hypertension, whereas no changes in blood pressure were observed in vehicle-treated rats. No differences in depressor responses to ganglion blockade were found in vehicle and S6c-treated rats during the control period or during the first 3 days of the infusion period. However, on days 4 and 5 of the infusion period, the response to trimethaphan was significantly larger in S6c-treated rats than in vehicle-treated control animals. Termination of S6c treatment was associated with a return of trimethaphan responses to control period values.

Plasma Norepinephrine Levels in S6c-Induced Hypertension

In a separate group of rats, MAP increased significantly during S6c infusion and the increase was sustained throughout the infusion period. MAP did not change in vehicle-treated animals (average of 127.4 ± 1 vs. 99.6 ± 2 mmHg, Fig. 2A). Plasma norepinephrine concentrations (Fig. 2B) did not change in either vehicle-treated rats or S6c-treated rats relative to their respective control period values, nor were differences between vehicle- and S6c-treated rats significant at any time during the study.

S6c-Induced Hypertension in Celiac Gangliectomyzed Rats

Figure 3 shows the effect of CGX on S6c-induced hypertension. Gangliectomy alone slightly decreased resting arterial pressure, although not significantly. The increase in pressure during S6c treatment, however, was markedly less in CGX than in sham-operated rats. In fact, during the last 3 days of S6c infusion, arterial pressure in CGX rats returned to values not significantly different from those measured during the control period.

Superoxide Levels in Mesenteric Vessels of S6c-Treated Rats

DHE staining showed an apparent increase in superoxide level in smooth muscle cells from mesenteric arteries and veins from rats receiving S6c, compared with vessels from rats receiving saline only (Fig. 4A), but the difference was not statistically significant (Fig. 4B). Similarly, lucigenin-enhanced chemiluminescence measurements (Fig. 5) indicated that, although the mean values of superoxide levels were increased in blood vessels from the rats treated with S6c, the differences were not statistically significant. The
basal level of superoxide in veins is significantly higher than in arteries.

Superoxide Levels in Inferior Mesenteric Ganglion of S6c-Treated Rats

Figure 6A shows DHE staining of IMG from rats receiving saline for 7 days, or 1- or 5-day infusions of S6c. More intense fluorescence was observed in IMGs from rats treated with S6c for either 1 day or 5 days, compared with those treated with saline. In addition, more fluorescence was observed in IMG from rats receiving S6c for 5 days than in those receiving S6c for 1 day. Quantitative analyses revealed that these differences were statistically significant (Fig. 6B).

Effects of Tempol on Arterial Pressure and Ganglion Superoxide in S6c-Treated Rats

Figure 7 demonstrates that tempol treatment prevented a significant rise in blood pressure in rats receiving S6c but had no effect in control rats. Figure 8A shows DHE fluorescence in IMG of rats treated with saline, S6c, and S6c plus tempol. Quantification revealed that superoxide levels in IMG from rats treated with S6c and tempol were significantly lower than those in IMG from rats treated with S6c alone (Fig. 8B).

DISCUSSION

The data presented here suggest that the sympathetic nervous system contributes to S6c-induced hypertension. For example, depressor responses to the ganglion blocker trimethaphan were significantly larger during the final 2 days of the 5-day S6c infusion. This finding is generally accepted as evidence for elevated sympathetic activity to the cardiovascular system (6), although other explanations are possible (6, 13). Therefore, plasma norepinephrine levels were measured as another index of sympathetic nervous system activity. However, plasma norepinephrine concentrations were not elevated during S6c infusion. This could be due to the insensitivity of this method for detecting increased sympathetic nervous system activity (2, 40). Alternatively, it could indicate that sympathetic activity is increased in a regionally specific way in S6c-induced hypertension. Since the splanchnic veins and venules are the main capacitance segments of the circulation, we speculated that sympathetic nerves to the splanchnic vascular bed may be activated in S6c-induced hypertension. The celiac plexus contains the cell bodies of most sympathetic neurons innervating the splanchnic organs. Therefore, surgical CGX was performed to determine whether sympathetic nerves in the splanchnic region play a role in blood pressure responses to S6c infusion. The results showed that in celiac ganglionectomized rats, S6c increased blood pressure significantly for the first 2 days of the 5-day infusion period. However, subsequently, pressure returned to levels not significantly higher than preinfusion control period values, despite continued S6c administration.

Collectively, these findings support the idea that S6c-induced hypertension is partially dependent on increased activity of the sympathetic nerves (with cell bodies in the celiac ganglion plexus) supplying the splanchnic organs. These findings have implications for the treatment of hypertension.
nerves control smooth muscle activity in the arteries and veins of the intestine, stomach, pancreas, mesentery, liver, and other organs. They account for a substantial fraction of the norepinephrine released from the peripheral sympathetic nervous system (26, 54), and their activation leads to both increased systemic vascular resistance and decreased capacitance (22, 34, 39). As noted above, activation of the splanchnic sympathetic nerves can redistribute blood from compliant peripheral veins into the central circulation and thereby increase blood pressure (18, 28). A caveat to this overall conclusion is that other responses to chronic CGX besides reducing splanchnic sympathetic activity could contribute to the effects of this intervention on S6c-induced hypertension. Further study on this point is required.

It is not clear why an influence of the sympathetic nervous system on blood pressure (using trimethaphan) in S6c-induced hypertension was only demonstrable 4 days after starting S6c infusion. One possibility is that the early increase in blood pressure is nonneurogenic and causes a baroreflex-mediated reduction in sympathetic activity until significant resetting of the reflex occurs. This could be tested by performing S6c infusion in animals with sino-aortic denervation. Another question is, if splanchnic sympathetic nerve activity is increased during S6c infusion, why is this not reflected in elevated peripheral plasma concentrations of norepinephrine? The likely explanation is that norepinephrine released from splanchnic sympathetic nerves is efficiently cleared from the circulation by the liver (1, 14). This idea could be tested using regional norepinephrine spillover measurements in S6c-infused rats. Another means to test the role of sympathetic nervous activity in S6c-induced hypertension would be to examine the effects of α-adrenergic blockers or other sympatholytics drugs in the model.

Under physiological conditions, ROS are present at low concentrations and act as signaling molecules regulating the growth and function of vascular smooth muscle cells (11, 43). Under pathological conditions, ROS may impair endothelium-dependent vasorelaxation (53), induce vascular smooth muscle cell growth (65), elevate sympathetic nervous system activity (9), alter renal function (23), and cause increased deposition of extracellular matrix proteins (21). All of these effects may contribute to vascular and organ damage, and other
accumulating evidence indicates that ROS play physiological and pathophysiological roles in hypertension (61). Most studies indicate that endothelin-family peptides stimulate ROS generation in blood vessels and other tissues by acting on ETA subtype receptors (ETAR) (8, 38). However, endothelin-induced superoxide production in sympathetic neurons in vitro is mediated by ETBR (12). Furthermore, acute activation of ETBR in vivo in conscious rats produces increased superoxide concentrations in sympathetic ganglia (31). Therefore, we tested the hypothesis that chronic infusion of S6c also would increase superoxide in sympathetic ganglia. We made measurements in the IMG, a prevertebral ganglion providing innervation to splanchnic blood vessels, small intestine, colon, rectum, etc. (58), because fluorescent imaging is easier in this ganglion than in the celiac plexus. To assess a possible effect on blood vessels of chronic exposure to S6c in vivo, superoxide levels in mesenteric arteries and veins also were determined. Because evidence reported above suggested that the mechanisms of S6c-induced hypertension may differ during the early and later stages, we measured superoxide levels in rats treated with S6c for 1 or 5 days.

Both DHE staining and lucigenin-enhanced chemiluminescence measurements revealed that superoxide levels were not statistically different between vehicle-treated and S6c-treated rats. However, fluorescence intensity of IMG neurons was significantly greater in ganglia from rats receiving S6c for 1 or 5 days compared with sham rats. #Fluorescence of IMG neurons was significantly greater in ganglia from rats treated with S6c for 5 days compared with those treated for 1 day.

**Fig. 5.** Superoxide levels in SMA and SMV from control and S6c-infused rats, measured by lucigenin-enhanced chemiluminescence. Rats were treated with S6c either for 1 day or for 5 days. *Statistical difference (P < 0.05) from control rats.

**Fig. 6.** A–C: representative pictures showing superoxide levels in inferior mesenteric ganglion (IMG) neurons from control and S6c-treated rats. A: IMG from a sham rat. B: IMG from a rat receiving S6c for 1 day. C: IMG from a rat receiving S6c for 5 days. Scale bar in C applies to all panels. D: quantification of mean fluorescence intensity of sympathetic neurons from S6c-treated and saline-treated rats (n = 7 in each group). *Fluorescence of IMG neurons was significantly (P < 0.05) greater in ganglia from rats receiving S6c for both 1 day or 5 days compared with sham rats. #Fluorescence of IMG neurons was significantly (P < 0.05) greater in ganglia from rats treated with S6c for 5 days compared with those treated for 1 day.

**Fig. 7.** Average (±SE) MAP before (C1–C2), during (E1–E5), and after (R1–R3) continuous S6c infusion (5 pmol·kg⁻¹·min⁻¹ iv) or vehicle into rats, while tempol was given through drinking water (1 mmol/l). A third group of animals were on S6c, while drinking normal distilled water. Numbers in parentheses are number of rats in each group. MAP significantly increased in S6c-infused rats without tempol treatment. The rats with dual treatments of S6c and tempol did not show a significant increase in MAP. Tempol did not change MAP in vehicle-treated rats. *Statistical difference (P < 0.05) from control period.
significantly increased in either arteries or veins from rats treated with S6c for 1 or 5 days. This result is consistent with previous work (discussed above) indicating vascular superoxide production in response to endothelin peptides is mediated by ETAR. Interestingly, the basal level of superoxide in veins was significantly higher than in arteries, possibly due to a higher nitric oxide production in arteries that may interact with superoxide to lower the level, compared with veins (57).

Lucigenin was not used to determine ROS levels in ganglia because the method lacks sufficient sensitivity for very small amounts of tissue. However, DHE staining showed that superoxide levels were significantly increased in sympathetic ganglia from S6c-treated rats. Moreover, the increase was time-dependent. That is, superoxide levels were higher in sympathetic neurons from rats receiving S6c for 5 days versus 1 day.

With this evidence of increased ROS in sympathetic ganglia in S6c-induced hypertension, we designed an experiment to address the cause-and-effect relationship between increased oxidative ROS and elevated blood pressure. Tempol, a superoxide dismutase mimic (51, 52), was used to test whether increased blood pressure in S6c-infused rats required increased tissue levels of superoxide. Our results confirm previously published studies showing that tempol does not affect blood pressure in normotensive rats (55). However, tempol attenuated blood pressure elevations in S6c-infused rats, suggesting that increased tissue concentrations of superoxide contribute importantly to S6c-induced hypertension. To determine possible target tissues for the actions of tempol, we measured superoxide levels in sympathetic neurons from rats treated with S6c with and without tempol. These results showed that superoxide was lower in a prevertebral ganglion of rats treated with tempol compared with those treated with S6c alone, although levels were not completely normalized. These results are consistent with the idea that tempol ameliorated S6c-induced hypertension by reducing ROS levels in sympathetic ganglia. The residual pressor effect of S6c in the presence of tempol suggests, however, that S6c also engages pressor mechanisms not related to ROS.

How do ROS in sympathetic ganglia affect postganglionic sympathetic nerve activity? Although there is no direct evidence in sympathetic ganglia, in other neuron types, ROS have been shown to increase cell excitability by well-established signaling mechanisms (9). Therefore, we hypothesize that in S6c-induced hypertension, increases in superoxide content in the prevertebral sympathetic ganglia cause higher postganglionic nerve discharge and constriction of splanchnic blood vessels. Nevertheless, we cannot rule out the possibility that S6c increases sympathetic activity via alternative mechanisms, such as an action on the brain or sympathetic nerve terminals. Clearly, no matter how S6c may increase sympathetic nerve activity, the expression of this effect would be dampened by the actions of the baroreflex if S6c also caused an increase in blood pressure by nonneural mechanisms (as mentioned above in DISCUSSION). We further hypothesize that tempol decreases blood pressure in S6c-induced hypertension by reducing ROS levels in sympathetic ganglia and thereby postganglionic sympathetic nerve activity. Previous studies have shown that tempol is, in fact, sympathoinhibitory in hypertensive rats (17, 24). A caveat to this conclusion, however, is that tempol also has other pharmacological effects [e.g., opening large conductance, calcium activated potassium channels (64)] and sites of action (e.g., blood vessels, kidney or brain) that could attenuate sympathetic activity and/or hypertension.

In summary, the data presented here are consistent with the idea that hypertension produced by chronic activation of ET4R in rats is due to both neurogenic and nonneurogenic mechanisms. The neurogenic mechanism has a delayed onset and probably results from changes in sympathetic activity to the...
splanchnic vascular bed. Although it is not known whether chronic, selective activation of ET\(_B\)R occurs under normal circumstances, the model illuminates the possible importance the splanchnic sympathetic nerves in the development of hypertension.

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