Superoxide Dismutase Decreases Mortality, Blood Pressure, and Cerebral Blood Flow Responses Induced by Acute Hypertension in Rats

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Oxygen radicals are known to be produced by the cerebral vasculature during acute, pressor-induced hypertension and are also known to inactivate endothelium-derived relaxing factor. The objective of our present study was to determine if the oxygen radical scavenger superoxide dismutase (24,000 units/kg plus 1,600 units/kg/min) alters the pressor, cerebral blood flow, and mortality responses to systemic norepinephrine in rats. Increasing doses (0.01–30 μg/kg i.v.) of norepinephrine were given by bolus injection to eight rats, and changes in the cortical microcirculatory blood flow were measured by laser-Doppler flowmetry. Superoxide dismutase shifted the norepinephrine-blood pressure and -cerebral blood flow dose-response curves moderately, but significantly, to the right such that it took more norepinephrine to reach a given blood pressure. However, superoxide dismutase had no effect on the autoregulation of cerebral blood flow. Additionally, whereas five (63%) of the eight control rats died after the 10 μg/kg norepinephrine dose, all eight rats treated with superoxide dismutase survived this dose. The mechanism by which superoxide dismutase reduced mortality is uncertain. The blood pressure and cerebral blood flow results suggest that superoxide dismutase prevents oxygen radicals from destroying endothelium-derived relaxing factors, which reduce the pressor effects of norepinephrine. (Stroke 1991;22:489–494)

Acute, drug-induced hypertension is known to cause abnormalities in the cerebral circulation. These abnormalities include increased cerebral vascular permeability to dye and proteins, a loss or "breakthrough" of autoregulation, and forced dilation of the arterial vasculature, which imparts a "sausage-string" appearance. Additionally, we and our colleagues have shown that following acute hypertension induced by pressor agents or experimental traumatic brain injury, abnormalities of the cerebral arterioles are caused by oxygen radicals formed during increased arachidonic acid metabolism. The abnormalities include sustained dilation, decreased reactivity to hypocapnia, endothelial lesions, and altered reactivity to vasoactive substances. These abnormalities are known to be caused by cyclooxygenase-derived free radicals since they can be prevented by cyclooxygenase inhibitors and oxygen radical scavengers. Additionally, we have very recently shown that the increases in cerebrovascular permeability and brain water content caused by norepinephrine-induced hypertension can be prevented by the systemic administration of superoxide dismutase.

In recent years, investigators have become interested in the endothelium and its generation of relaxing factors. These endothelium-derived relaxing factors (EDRFs) are formed by the endothelium in response to various vasoactive agents and are short-lived. Experimental evidence also shows that EDRFs are destroyed by oxygen radicals. This implies that a condition that increases the formation of oxygen radicals may alter cardiovascular reactivity by decreasing or eliminating the action of EDRFs. The purpose of our present investigation was to determine whether superoxide dismutase alters the blood pressure and cerebral blood flow (CBF) responses to intravenous bolus injections of norepinephrine, which cause hypertension and induce the production of cyclooxygenase-dependent oxygen rad-

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the probe to be held stable and fixed over the cortical plug, which was inserted into the needle hub, allowed This Teflon plug also contained a hole that was equal to the diameter of the needle hub was then inserted into it. Following tracheotomy, the rats were ventilated with room air and the rate and volume of the ventilator were adjusted such that the end-expiratory CO2 was controlled at approximately 30 mm Hg. The temperature of all animals was maintained at 37°C. Mean arterial blood pressure was measured with a pressure transducer connected to a cannula introduced into the aorta through the left femoral artery. The right femoral artery and vein were cannulated for the withdrawal of arterial blood samples and the infusion of superoxide dismutase, respectively. Blood gases and pH were analyzed to ensure adequate and consistent ventilation.

The CBF was continuously measured using laser-Doppler flowmetry, a technique on which we have recently reported in detail.16,17 We previously validated laser-Doppler flowmetry as a technique for measuring alterations in the cerebral microcirculation by comparing it with CBF changes measured with the hydrogen clearance technique or by correlating it with changes in pial arteriolar diameter. Using laser-Doppler flowmetry, changes in CBF are measured in a 1 mm³ volume of tissue on the cortical surface of the brain. Infrared light of 760 nm wavelength is generated by the laser in the central laser-Doppler flowmeter unit and transmitted through a fiber-optic cable to a 0.84-mm-diameter probe tip. The laser light shines on the brain surface and is Doppler-shifted by moving cells in the cerebral microcirculation. Doppler-shifted light is returned by other optical fibers to the central laser-Doppler flowmeter unit and processed to yield voltage and frequency information, which is processed to yield blood velocity and blood volume, the product of which yields a CBF signal.

To hold the fiber-optic probe rigidly over the surface of the rat's brain, a probe holder device was fashioned as previously reported.18 Briefly, a 4-mm-diameter craniotomy was made using a pediatric trephine. Next, the metal needle portion of a syringe needle was removed, and the plastic hub of the needle was positioned over the craniotomy such that the diameter of the needle hub was equal to the diameter of the craniotomy. The needle hub was then fixed in place over the craniotomy using first cyanoacrylate glue and then dental acrylic. A piece of Teflon with an outside diameter equal to the inside diameter of the needle hub was then inserted into it. This Teflon plug also contained a hole that was equal to the 0.84 mm diameter of the laser-Doppler probe. Inserting the laser-Doppler probe into the Teflon plug, which was inserted into the needle hub, allowed the probe to be held stable and fixed over the cortical surface. At the end of the experiment, the implanted needle hub was discarded with the animal, whereas the Teflon probe holder was removed and reused.

The laser-Doppler probe is inserted into the probe holder device such that the tip of the probe is 1–2 mm from the surface of the cortex. Our previous studies and those of others show that this technique in rats can measure changes in CBF produced by hemorrhage, norepinephrine-induced hypertension, indomethacin-induced vasodilation, bacterial infection, and arterial occlusion.18–20 Changes in CBF, blood velocity, and blood volume can be recorded directly from the laser-Doppler flowmeter and can also be displayed on a polygraph.

In this rat preparation the dura is left intact, which allows the cortical surface to be bathed by normal cerebrospinal fluid and reduces any potential drying of the cortical surface. Additionally, intracranial pressure is more normally maintained. The rat dura mater is only a few microns thick while the laser light penetrates to approximately 1 mm and measures blood flow in surface as well as subsurface vessels. Since the dura in rats is translucent and extremely thin, blood flow in it makes a relatively insignificant contribution to laser-Doppler–measured CBF. Additionally, the laser-Doppler probe is positioned such that it is not over any large dural vessels. In our previous studies we have shown that laser-Doppler flowmetry measures predominantly subsurface flow since dramatic alterations in pial arteriole diameters in the absence of changes in subsurface vessels produce little effect on laser-Doppler–measured CBF.16 This was particularly striking in one of our previous studies wherein washout of the topically applied vasodilator 2-chloroadenosine caused a return to control diameter in pial arterioles but did not significantly decrease laser-Doppler–measured CBF since subsurface vessels were still dilated.18 Additionally, successful laser-Doppler examination of rat cortical blood flow through an intact dura has been reported by other laboratories.19,20

The eight superoxide dismutase–treated rats were given a 0.5–ml bolus dose of 24,000 units/kg followed by a constant infusion of 1,600 units/kg/min in a volume of 33 µl/min. Superoxide dismutase (3,400 units/mg) was from bovine erythrocytes and was obtained from Sigma Chemical Co., St. Louis, Mo., in saline vehicle. Therefore, the rats received an initial loading dose plus a continuous supplement throughout the period of the norepinephrine injection. The eight control rats received saline administered in a similar manner.

Hypertension was produced by the intravenous administration of increasing doses of norepinephrine (0.01, 0.03, 0.1, 0.3, 0.6, 1, 3, 10, and 30 µg/kg). Five minutes of a stable blood pressure was allowed before each successive bolus injection of norepinephrine. The control and superoxide dismutase–treated rats were examined in a random order. Blood pressure, CBF, and cerebral blood volume and velocity were recorded during the maximum response to
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SOD and Acute Hypertension

Table 1. Baseline Physiologic Parameters and Mortality Response to 10 μg/kg Norepinephrine for Control and Superoxide Dismutase-Treated Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=8)</th>
<th>Superoxide dismutase (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>94±3</td>
<td>89±3</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>30±1</td>
<td>29±1</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>96±3</td>
<td>98±2</td>
</tr>
<tr>
<td>pH</td>
<td>7.52±0.01</td>
<td>7.50±0.01</td>
</tr>
<tr>
<td>Survival</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>37</td>
</tr>
</tbody>
</table>

Values are mean±SEM unless noted. *p<0.03 different from control by Fisher’s exact test.

norepinephrine and 1, 2, and 5 minutes after each dose of norepinephrine. The protocol was as follows: after implantation of the cannula and the laser-Doppler probe, CBF as well as blood pressure were recorded over a 10-minute baseline period. Next, the superoxide dismutase or saline bolus was given and the infusion started. Ten minutes later the norepinephrine doses were started, and each dose was given at least 5-minute intervals or when blood pressure following the preceding dose had stabilized. The same parameters were observed after each successive dose of norepinephrine. The dose–response curves were analyzed by repeated-measures analysis of variance; p<0.05 was considered to be significant.

Results

Table 1 shows that the baseline physiologic parameters were the same for the control and superoxide dismutase–treated groups. The administration of superoxide dismutase had no effect on blood pressure or any other physiologic parameter (data not shown).

Figure 1 shows the effect of increasing doses of norepinephrine on the maximum blood pressure response. Rats treated with superoxide dismutase responded with a lower blood pressure than the control rats to most doses of norepinephrine. Repeated-measures analysis of variance showed that the two dose–response curves differed at p<0.04. Multiple range tests showed that the responses to 0.1–0.6 μg/kg norepinephrine to differ at p<0.02 and responses to 1 and 3 μg/kg had p values of 0.08 and 0.06, respectively. Response to 30 μg/kg norepinephrine for controls is not shown since most control animals died after receiving 10 μg/kg norepinephrine, whereas all superoxide dismutase–treated animals survived this dose (see Table 1).

norepinephrine on the CBF response as measured by laser-Doppler flowmetry. Repeated-measures analysis of variance showed that the two curves displayed significant dose×group interactions (p<0.0001). Closer examination of the curves with a multiple range test showed that the responses to 1, 3, and 10 μg/kg norepinephrine differed at p<0.03. In essence, the two curves are divergent, with the maximum CBF.
response occurring at a lower norepinephrine dose in the control group. Interestingly, in the control group the 10 μg/kg dose caused a smaller CBF increase than the 3 μg/kg dose even though the maximum blood pressure response to the former dose was higher than that to the latter. Similarly, there was a less pronounced increase in CBF with the 30 μg/kg dose than with the 10 μg/kg dose in the superoxide dismutase-treated group compared with control group.

While a given dose of norepinephrine produced larger effects on blood pressure and CBF in the control group, Figure 3 shows that the blood pressure–CBF responses, irrespective of norepinephrine dose, were the same in the two groups. It can be seen that with the bolus injection of norepinephrine the CBF increase per mm Hg blood pressure increase was comparatively less between baseline blood pressure and approximately 150 mm Hg. It should be noted that bolus injections do not allow for a steady-state response to the blood pressure increase induced by norepinephrine. However, above 150 mm Hg a comparative loss or breakthrough of autoregulation occurred, resulting in much larger increases in CBF. This breakthrough of autoregulation is similar to that previously and extensively described by others and our own laboratory.18

The bolus injection of high doses of norepinephrine is a stressful challenge to rats. While all rats survived the 3 μg/kg dose, five of eight control rats died when challenged with 10 μg/kg norepinephrine (Table 1). In these control rats blood pressure and CBF peaked quickly and then declined rapidly to low levels, and subsequently death occurred. There was often a bloody or watery exudate in the respiratory tract that appeared in the ventilatory tubing of the control rats. Following the 10 μg/kg norepinephrine dose in the superoxide dismutase–treated rats, the blood pressure peaked, fell below baseline levels, and then gradually returned to near normal. Unlike the control animals, all rats treated with superoxide dismutase survived the 10 μg/kg dose of norepinephrine, and many survived the 30 μg/kg dose. The mortality responses in the control and superoxide dismutase–treated groups differed at p<0.03. Subjectively, there appeared to be less exudate in the respiratory tract of the superoxide dismutase–treated rats. In summary, most control rats died in response to 10 μg/kg norepinephrine whereas all superoxide dismutase–treated animals survived this challenge. This occurred despite the fact that the maximum blood pressure responses to 10 μg/kg norepinephrine were the same in the two groups (Figure 1).

Discussion

Our studies show that superoxide dismutase decreases the blood pressure and CBF responses to submaximal pressor doses of norepinephrine in rats without affecting CBF autoregulation. Additionally, superoxide dismutase reduced mortality in response to 10 μg/kg norepinephrine despite the fact that the blood pressure increases in response to this high dose of norepinephrine were similar in the control and superoxide dismutase–treated groups. Our data also indicate that superoxide dismutase does not affect the perfusion pressure at which breakthrough and loss of autoregulation occurs. Superoxide dismutase therefore increases the dose of norepinephrine needed to reach the blood pressure at which breakthrough occurs.

The mechanism(s) by which superoxide dismutase reduces the pressor response to norepinephrine is uncertain, but we hypothesize that it may involve an interaction between EDRFs and oxygen radicals. If a tonic EDRF release or a release of EDRF in response to norepinephrine-induced hypertension occurs, it would tend to counteract the pressor effects of norepinephrine. However, acute hypertension is known to stimulate free radical generation in the cerebral microcirculation5–7 and quite possibly in other vascular beds as well. This is important since oxygen radicals have been shown to inhibit EDRF in other vascular beds as well. This is important since oxygen radicals have been shown to inhibit EDRF in other vascular beds as well. It is important to note that EDRF acting, vasodilator EDRF to act, thus reducing the tonic EDRF release or a release of EDRF in response to norepinephrine. However, acute hypertension is known to stimulate free radical generation in the cerebral microcirculation5–7 and quite possibly in other vascular beds as well. This is important since oxygen radicals have been shown to inhibit EDRF in other vascular beds as well.

Free radical destruction of EDRF may therefore prevent a potential dilator mechanism from counteracting the direct vasoconstrictor effects of norepinephrine. By scavenging the injurious superoxide radical, superoxide dismutase will allow the counteracting, vasodilator EDRF to act, thus reducing the blood pressure response to norepinephrine. In fact, Beckman et al18 have provided evidence for such a mechanism, assuming that the EDRF acting systemically in our experiments is NO.23 These authors have shown that -NO and O2−, both of which contain unpaired electrons, react rapidly to form peroxynitrite (ONOO−). Peroxynitrite is stable but has a pK_a of 6.6 at 0°C and rapidly decomposes once protonated to form the hydroxyl radical (OH) plus nitro-
gen dioxide. Therefore, in our experiments superoxide dismutase may be preventing the inactivation of •NO by preventing the combination of O₂⁻ and •NO to form the peroxynitrite anion. It should be emphasized, however, that whether the proposed relations occur in our experiments is speculative.

Free radical generation has been shown to be induced by a side chain reaction associated with cyclooxygenase metabolism of arachidonic acid.²² In the pial microcirculation these cyclooxygenase-dependent free radicals cause endothelial lesions, arteriolar dilation, and loss of arteriolar reactivity to decreased Paco₂ or topically applied acetylcholine.⁶,⁷ The generation of free radicals and the subsequent arteriolar abnormalities can be prevented by cyclooxygenase inhibitors such as indomethacin. Alternatively, superoxide dismutase plus catalase can also prevent the cerebral abnormalities by preventing the action or interaction of superoxide anion and hydrogen peroxide. These previous findings, along with our current findings that superoxide dismutase does not prevent breakthrough of autoregulation at high blood pressures, imply that free radicals are produced subsequent to the damage produced by hypertension and breakthrough and that they are not a cause of the loss of autoregulation.

The blood pressure response in control rats to 10 µg/kg norepinephrine was slightly, but not significantly, greater than that to 3 µg/kg. However, the CBF response to the 10 µg/kg dose was significantly less than that to 3 µg/kg. A similar trend was noted in the superoxide dismutase–treated group when going from 10 to 30 µg/kg norepinephrine. The reason for CBF decreasing in light of sustained blood pressure is uncertain, but it appears to be a consistent and real finding. The decreased CBF might be due to a break in the blood–brain barrier and simultaneous edema, which might decrease blood flow. We have very recently shown that similar norepinephrine-induced hypertension does in fact increase cerebrovascular permeability and brain water content, which can be prevented by superoxide dismutase given systemically.⁶ However, the relatively small magnitudes of the brain water and permeability increases we observed argue against their causing the decreased CBF response. Therefore, the cause of the decreased CBF response at the highest norepinephrine dose is uncertain. Cardiac failure might be the cause in that the heart is unable to pump adequate output against such high peripheral vascular resistance. However, this explanation remains only speculative.

With respect to mortality, our studies clearly show that rats pretreated with superoxide dismutase better survive the hypertensive challenge. Again, we can only speculate as to the mechanism by which superoxide dismutase decreases mortality. As mentioned above, we have recently provided evidence that the superoxide dismutase–inhibitable brain permeability and edema responses to acute, norepinephrine-induced hypertension are too minor to cause death. Alternatively, since there is often a watery and bloody exudate in the respiratory tract following the administration of norepinephrine, the rats might die as a result of pulmonary edema and respiratory insufficiency. However, in other recent experiments we also examined the extravasation of protein in the lung and the lung percentage water following norepinephrine-induced hypertension.⁸ Immediately after hypertension we observed an increase in protein extravasation but were surprised to find no significant increase in the lung percentage water. This then argues against flooding of the respiratory passages as a cause of death. We are therefore uncertain of the mechanism by which superoxide dismutase decreases mortality. We note, however, that Levasseur et al²³ have recently found that the pretreatment of rats with superoxide dismutase greatly reduced mortality following fluid percussion–induced experimental brain injury. Interestingly, this model of brain trauma produces transient catecholamine hypertension reminiscent of that produced by the injection of norepinephrine.

In summary, superoxide dismutase reduces the blood pressure and CBF responses to submaximal pressor doses of norepinephrine and reduces mortality associated with acute hypertension in rats. While we can conclude that the superoxide anion induces these changes in reactivity and mortality, the mechanisms presented to explain these effects of superoxide anion are speculative and need further investigation.

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**KEY WORDS** • microcirculation • superoxide dismutase • rats
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