Superoxide Generation and Reversal of Acetylcholine-Induced Cerebral Arteriolar Dilation after Acute Hypertension

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SUMMARY. The appearance of superoxide anion radical in cerebral extracellular space during and after acute hypertension induced by intravenous norepinephrine was investigated in anesthetized cats equipped with cranial windows. Superoxide was detected by demonstrating the presence of superoxide dismutase-inhibitable reduction of nitroblue tetrazolium. The superoxide dismutase-inhibitable rate of reduction of nitroblue tetrazolium was 4.1 ± 1.61 nM/min per cm² during hypertension and 4.55 ± 0.62 nM/min per cm² one hour after hypertension had subsided. During norepinephrine administration in the absence of hypertension, the superoxide dismutase-inhibitable rate of reduction of nitroblue tetrazolium was 0.44 ± 0.17 nM/min per cm². The reduction of nitroblue tetrazolium during hypertension was also inhibited by prior treatment of the brain surface with phenylglyoxal at pH 10, to induce irreversible inhibition of the anion channel. The results show that acute hypertension is associated with the generation of superoxide which enters the extracellular space of the brain via the anion channel. Following hypertension, the sustained vasodilation caused by acute hypertension was inhibited significantly by topical application of superoxide dismutase and catalase, showing that it was due in part to superoxide and other radicals derived from it. The vasodilator response of cerebral arterioles to topical acetylcholine was converted to vasoconstriction following acute hypertension, and restored to vasodilation following topical application of superoxide dismutase and catalase. The results show that superoxide and other radicals generated after acute hypertension interfere with acetylcholine-induced endothelium-dependent vasodilation, probably because they destroy the endothelium-derived relaxant factor. (Circ Res 57: 781-787, 1985)

ACUTE severe hypertension causes cerebral arteriolar abnormalities manifested by dilation, which is sustained well beyond the duration of hypertension, by reduced responsiveness of the dilated vessels to the vasoconstrictor effects of arterial hypocapnia, and to the vasodilator effects to arterial hypercapnia and arterial hypotension, by morphological lesions of the endothelium and vascular smooth muscle, and by reduced oxygen consumption of the vessel wall (Kontos et al., 1981). These abnormalities are inhibited by pretreatment with cyclooxygenase inhibitors or with oxygen radical scavengers (Kontos et al., 1981). For this reason, they were ascribed to the generation of oxygen radicals in association with accelerated endogenous arachidonate metabolism via cyclooxygenase.

These cerebral vascular effects of acute hypertension are mimicked by topical application of arachidonate in high concentration (200 μg/ml) (Kontos et al., 1980). The effects of arachidonate are also inhibited by pretreatment with cyclooxygenase inhibitors or with oxygen radical scavengers, suggesting that they are also due to the production of oxygen radicals in association with metabolism of arachidonate via cyclooxygenase (Kontos et al., 1980). Consistent with this view is our recent observation that during topical application on the brain surface of arachidonate or bradykinin, a polypeptide which releases endogenous arachidonate via activation of phospholipases, superoxide anion radical appeared in the extracellular space of the brain (Kontos et al., 1985).

Since acute hypertension seemed to produce the cerebral vascular abnormalities noted above by a mechanism similar to that of arachidonate, we examined the possibility that superoxide anion radical appears in extracellular cerebral space during and after acute hypertension. Since acute hypertension causes endothelial injury (Kontos et al., 1981), we also examined endothelium-dependent vasodilation due to acetylcholine before and after acute hypertension.

Methods

Experiments were carried out in cats anesthetized with sodium pentobarbital (30 mg/kg, iv). After tracheostomy, each animal was ventilated with a positive pressure respirator and received 5 mg/kg of gallamine triethiodide for skeletal muscle paralysis. The end-expiratory CO₂ of the animal was monitored continuously with a Beckman infrared CO₂ analyzer and was maintained at a constant
level of about 30 mm Hg, by adjusting the respirator rate and volume. Arterial blood pressure was measured with a Statham transducer connected to a cannula introduced into the aorta via the femoral artery. Arterial blood samples were collected periodically for the determination of $P_{CO_2}$, $P_{O_2}$, pH, and hematocrit. Blood gases and pH were measured with Radiometer electrodes; hematocrit was measured by a micro method.

The detection of superoxide anion radical in cerebral extracellular space relied on the demonstration of superoxide dismutase (SOD)-inhibitable reduction of nitroblue tetrazolium (NBT). The technique has been described previously in detail (Kontos et al., 1985). NBT is a water-soluble dye, which when reduced by superoxide anion radical or by other reducing agents is converted to an insoluble blue form which precipitates. We used a double-window technique. Each window was installed in symmetrical fashion to overlies the parietal cortex of the animal. The cranial window technique was otherwise identical to the single-window technique described in detail before (Levasseur et al., 1975). The space under the cranial window was filled with artificial cerebrospinal fluid (CSF) having the same composition as endogenous CSF of cats (Davson, 1967). Both window spaces were filled with CSF containing 2.4 mM solution of NBT. One window contained, in addition to NBT, 20 $\mu$g/mL SOD (from bovine blood 2800 U/mg protein). These solutions were allowed to stay in contact with the brain surface for 15 minutes. Then they were washed away with fresh CSF containing no additives. Subsequently, the brain was fixated by perfusion. For this purpose, a catheter was placed into the left ventricle via a thoracotomy, and the brain was perfused first with 0.9% sodium chloride solution and then with a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer. Fixation by perfusion was used to eliminate red cells from the field, because hemoglobin absorbs light in the same wavelength region as reduced NBT. After fixation was completed, the brain was removed from the cranial cavity and the amounts of NBT deposited were determined spectrophotometrically. This was done with a modified Perkin-Elmer double-beam model 124 spectrophotometer equipped with a 3-mm (diameter) fiberoptics light guide which transmitted the incident and reflected light to and from the brain surface. The area of the brain corresponding to the cranial window, which has a diameter of 12 mm, was divided into four quadrants. Absorbance readings were taken at the appropriate wavelengths from each of these four quadrants. We used a two-wavelength technique, taking measurements at 570 and 450 nm. Calibration of the surface spectrophotometric measurements was done by comparing the results to direct chemical determination of the reduced NBT following extraction with pyridine. Correlation between the two methods was excellent up to a concentration of 200 nM/cm$^2$ brain surface (Kontos et al., 1985). The amounts of NBT deposited were converted to the rate of deposition per minute, assuming that the latter was constant. By subtraction of the rates of deposition in the two windows, the rate of SOD-inhibitable NBT reduction was calculated. This was used as a measure of superoxide anion radical generation.

The experimental design was as follows: In the first group, 15 cats were divided into three subgroups. In one subgroup, norepinephrine (3–6 $\mu$g/min) was administered intravenously to induce severe hypertension for 15 minutes. The dose of norepinephrine was designed to increase mean arterial blood pressure to approximately 190–200 mm Hg. Measurements of superoxide generation were made during the 15-minute period of hypertension. In the second subgroup, norepinephrine was again administered in the same dose as before to raise the blood pressure for 15 minutes. The blood pressure then was allowed to return to the baseline, and the measurement of superoxide was made for 15 minutes beginning 1 hour after hypertension had subsided. In the third subgroup, norepinephrine was administered intravenously in the same doses as before, but the arterial blood pressure was maintained at the resting level by connecting the descending aorta to a reservoir which was placed at a fixed level to keep a pressure equal to the animal’s own resting mean arterial blood pressure. The measurement of superoxide was made for 15 minutes during the administration of norepinephrine.

In a second series of seven cats, we examined whether superoxide generated during acute hypertension entered the extracellular space by traversing the membrane of the cells in which it was generated via the anion channel. We had found previously that this was the case with superoxide generated from topical arachidonate or bradykinin (Kontos et al., 1985). We used phenylglyoxal, which is known to inhibit irreversibly the anion channel in red cells following only a very brief exposure at high pH, presumably by modifying arginine residues in transport protein (Wieth et al., 1982). We first treated one window site with a solution of 20 mM phenylglyoxal for 2 minutes at pH 10, and treated the other window site with CSF at pH 10 for 2 minutes. After washout with fresh CSF, the two windows were filled with CSF containing NBT, and the animal was subjected to an acute hypertensive episode with intravenous norepinephrine for 15 minutes.

In a third series of nine cats, we determined cerebral arteriolar caliber changes in response to topical application of SOD and catalase after an acute hypertensive episode to see whether the radicals released after hypertension were mediating the sustained dilation seen under these conditions. In seven of the same animals, we also studied responses to topical application of $10^{-7}$ m acetylcholine. Acetylcholine hydrochloride was dissolved in artificial CSF and was administered by superfusion via the cranial window at a rate of 1 ml/min, with a constant infusion pump. This infusion rate gives a turnover rate of the fluid under the cranial window equal to about 5 times/min. Vessel caliber measurements are made 2–4 minutes after onset of the infusion, when a steady state was established. The animals were equipped with a single acutely implanted cranial window to observe the surface microcirculation in the parietal cortex. Arteriolar diameters were measured with a Vickers image-splitting device attached to a Leitz Ortholux microscope, as described previously (Levasseur et al., 1975). One opening of the window was connected to a plastic tubing whose other end was set at a fixed level to give a constant intracranial pressure of 5–7 mm Hg. After control diameter measurements were made and control responses to acetylcholine were examined, the animals were subjected to an acute hypertensive episode for 15 minutes with intravenous norepinephrine. Subsequently, the arterial blood pressure was allowed to return to the baseline, and repeat measurements of resting vessel caliber and of the responses to acetylcholine were made 30 minutes later. Then the space under the window was filled with CSF containing 20 $\mu$g/ml of SOD and 20 $\mu$g/ml of catalase (from bovine liver, 2000 U/mg protein). Measurements of vessel caliber were repeated after 10 minutes. Then we reexamine the responses to acetylcho-
line, using a solution which contained, in addition to acetylcholine, SOD and catalase in the concentrations given above.

Since, after acute hypertension, cerebral arterioles are dilated (Kontos et al., 1981), we performed two additional tests to find out if this dilation influenced the effects of acetylcholine. First, we examined the vasodilator responses to arterial hypertension induced by controlled bleeding; this was done in the group of seven cats in which the response to acetylcholine was tested after application of SOD and catalase. For this purpose, the abdominal aorta was connected via a catheter to a reservoir set at a level to keep a mean arterial blood pressure equal to 60 mm Hg. Vessel caliber measurements were repeated 4–6 minutes later, when a steady state had been established. Second, in a separate group of four cats, we examined the effect of dilation by arterial hypercapnia, induced by inhalation of 5% CO₂, and by topical application of 1 μg/ml histamine, on the responses to acetylcholine.

After completion of the functional studies, morphological examination of the pial vessels via scanning electron-microscopy was conducted. The animal’s head was fixed by perfusion as described before. After fixation, the cranial window was removed and the portion of the parietal cortex containing the vessels whose caliber was previously measured was taken out and processed for electron microscopy. The method for processing the tissue and identifying the same vessels which were previously studied from the functional standpoint has been described before in detail (Dietrich et al., 1980). Scanning electron microscopy was used to determine quantitatively the number of lesions in the endothelium. This was achieved by counting the number of lesions in each of five randomly selected microscopic fields, measuring 2100 μm² each. The average number of lesions per microscopic field was used as a measure of the density of these lesions. Counting was done by an electron microscopist who was not aware of the physiological results. SOD, catalase, NBT, phenylglyoxal, and acetylcholine were obtained from Sigma.

Results

In the five animals in which superoxide was measured during hypertension, mean arterial blood pressure was raised from 130 ± 5.0 to 199 ± 3.0 mm Hg. In the five cats in which superoxide was measured 1 hour after hypertension, mean arterial blood pressure was raised from 120 ± 3.0 to 197 ± 4.0 mm Hg and returned to 109 ± 2.0 mm Hg at the time of measurement of superoxide. In four of the cats in the control group, mean arterial pressure was 128 ± 3.0 mm Hg before norepinephrine was infused and was kept at 122 ± 4.0 mm Hg during the infusion of norepinephrine by bleeding. One animal was eliminated from this group, because its mean arterial blood pressure could not be maintained at the resting level during the administration of norepinephrine.

The rates of NBT reduction during and after hypertension are shown in Table 1. It is seen that the administration of norepinephrine without change in blood pressure induced virtually no SOD-inhibitable reduction of NBT. This finding is consistent with the earlier observation that there was no SOD-inhibitable reduction of NBT on the brain surface under normal resting conditions in anesthetized cats (Kontos et al., 1985). The rate of SOD-inhibitable NBT reduction during hypertension was approximately 10 times higher. The same was true 1 hour after hypertension.

In the seven cats in which the effect of phenylglyoxal was studied, mean arterial blood pressure was raised from 123 ± 5.0 to 190 ± 7.0 mm Hg. The rate of reduction of NBT was 5.23 ± 1.46 and 1.13 ± 0.42 nm/min per cm² in the absence and presence of phenylglyoxal, respectively. Thus, phenylglyoxal inhibited NBT reduction by 78%.

Table 2 shows the cerebral arteriolar diameter for 32 small (<100 μm) and 23 large (>100 μm) arterioles in nine cats before, during, and 30 minutes after hypertension, as well as after application of SOD and catalase. It is seen that acute hypertension caused pronounced dilation of both small and large arterioles which was sustained 30 minutes after hypertension had subsided. The application of SOD and catalase reduced this residual dilation by 52% and 42% in small and large arterioles, respectively. It should be noted that topical application of SOD and catalase in cats not subjected to hypertension has no significant effect on arteriolar caliber (Kontos et al., 1984).

During topical application of acetylcholine in seven cats prior to the induction of hypertension, all 19 large arterioles studied and 23 of 24 small arterioles dilated; one small arteriole showed no change in diameter. After acute hypertension, 13 large and

![Image](http://circres.ahajournals.org/isses/pdf/mic ח związki z przemysłem, w tym w formie handlu. Przeciwko temu dochodzi jednak coraz więcej protestów. Ostatnich najnowszych badań w tej dziedzinie wskazuje, że...)

**Table 1**

Reduction of NBT during and after Acute Hypertension

<table>
<thead>
<tr>
<th>n</th>
<th>NBT reduction rate (nm/min per cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBT</td>
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<tr>
<td>----</td>
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<tr>
<td></td>
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<tr>
<td><strong>During hypertension</strong></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8.17 ± 1.7</td>
</tr>
<tr>
<td>5</td>
<td>5.43 ± 0.46</td>
</tr>
<tr>
<td><strong>Norepinephrine infusion</strong></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.24 ± 0.92</td>
</tr>
</tbody>
</table>

Values are mean ± SE. n = number of cats.

*Significantly (P < 0.05) different from corresponding value in group without hypertension, based on analysis of variance and Dunnett's t-tests.
TABLE 2

Effect of SOD and Catalase on Cerebral Arteriolar Dilation Due to Acute Hypertension

<table>
<thead>
<tr>
<th>Change in arteriolar diameter (% of control)</th>
<th>Hypertension</th>
<th>30 Min after hypertension</th>
<th>After SOD and catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small arterioles</td>
<td>57 ± 6.5*</td>
<td>31 ± 3.5*</td>
<td>15 ± 4.1*</td>
</tr>
<tr>
<td>Large arterioles</td>
<td>38 ± 3.7*</td>
<td>24 ± 3.6*</td>
<td>14 ± 4.0*</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>202 ± 3.3*</td>
<td>105 ± 9.3</td>
<td>103 ± 7.7</td>
</tr>
</tbody>
</table>

Values are mean ± se from 32 small and 23 large arterioles in nine cats. MABP: mean arterial blood pressure. In the control period, vessel diameters were 69 ± 2.5 and 154 ± 7.0 μm for small and large arterioles, respectively, and MABP was 122 ± 4.8 mm Hg.

* Significantly (P < 0.05) different from other two means in group, based on analysis of variance and t-tests modified by Bonferroni’s method.

20 small arterioles constricted, while six large and four small arterioles dilated during acetylcholine application. After topical application of SOD and catalase, 11 large and 16 small arterioles dilated in response to acetylcholine, while eight arterioles in each group constricted. The average changes in arteriolar diameter caused by acetylcholine in small and large arterioles are shown in Figure 1. Before the induction of acute hypertension, acetylcholine caused significant dilation of both small and large arterioles. After hypertension, acetylcholine constricted small arterioles significantly, but caused no significant change in the diameter of large arterioles. After application of SOD and catalase, acetylcholine significantly dilated both small and large arterioles, but in both groups of arterioles the response was less pronounced than before the occurrence of hypertension.

To evaluate the effect of dilation of cerebral arterioles after hypertension on the response to acetylcholine, we compared arterioles which constricted in response to acetylcholine after application of SOD and catalase to those which dilated in response to acetylcholine with respect to two variables. The degree of residual dilation after application of SOD and catalase using the prehypertension diameters as baseline and the response to arterial hypotension. The analysis was done separately for small (<100 μm) and large (>100 μm) arterioles, since the responses of these two classes of arterioles may differ quantitatively (Kontos et al., 1978). The results in Table 3 show that the residual dilation and the response to arterial hypotension of small arterioles which constricted in response to acetylcholine did not differ from those which dilated in response to acetylcholine. On the other hand, the residual dilation of large arterioles which constricted in response to acetylcholine was significantly greater than that of those that dilated in response to acetylcholine. Also, the additional dilation in response to arterial hypotension was greater in large arterioles which dilated in response to acetylcholine. Arterial hypotension dilated all arterioles studied except one small arteriole which had dilated in response to acetylcholine; this arteriole showed 3% reduction in diameter.

Deliberate increase in arteriolar caliber induced by hypercapnia or by topical application of histamine in four cats reduced the vasodilator effect of acetylcholine significantly (Fig. 2), but in no case was the response converted to vasoconstriction.

The density of endothelial lesions was determined in nine small and in 17 large arterioles. In the small arterioles, the density averaged 12.6 ± 1.5 and in the large arterioles 11.5 ± 1.4 lesions per 2100 μm². We compared the density of endothelial lesions in arterioles which dilated in response to acetylcholine following application of SOD and catalase and in arterioles in which acetylcholine induced constriction, to find out whether a different degree of morphological damage accounted for this difference. In the 15 arterioles which showed dilation in response to acetylcholine, the density of endothelial lesions was 11.1 ± 1.2, whereas in the 11 arterioles which...
**Residual Dilation and Response to Arterial Hypotension of Cerebral Arterioles after Acute Hypertension**

<table>
<thead>
<tr>
<th>Change in vessel diameter (% of baseline)</th>
<th>Arterioles with constriction from acetylcholine</th>
<th>Arterioles with dilation from acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (n = 8)</td>
<td>21.1 ± 8.1</td>
<td>19.3 ± 5.7</td>
</tr>
<tr>
<td>L (n = 8)</td>
<td>44.6 ± 6.7*</td>
<td>15.3 ± 2.7*</td>
</tr>
<tr>
<td>Residual dilation</td>
<td>21.1 ± 8.1</td>
<td>44.6 ± 6.7*</td>
</tr>
<tr>
<td>Response to arterial hypotension</td>
<td>17.6 ± 5.4</td>
<td>10.0 ± 5.7 ± 5.7</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se. S = small; L = large. Residual dilation is difference in diameter seen after SOD plus catalase application and prehypertension baseline value, expressed as percent of latter value. Response to arterial hypotension is increment in diameter caused by hypotension as percent of diameter after application of SOD and catalase.

* Significantly (P < 0.05) different from corresponding value in vessels showing dilation with acetylcholine based on t-test.

showed constriction, it was 12.6 ± 2.2 lesions per 2100 μm². There was no significant difference between these two.

**Discussion**

The occurrence of SOD-inhibitable reduction of NBT supports the conclusion that during and after acute hypertension, superoxide anion radical is generated and appears in cerebral extracellular space. The substantial inhibition of NBT reduction by phenylglyoxal shows that superoxide anion radical enters extracellular fluid space by traversing the anion channel of the membranes of the cells in which it is generated. The present and earlier experiments support the conclusion that the mechanisms of the superoxide anion generation during and after hypertension is similar to that seen during application of arachidonate, 15-hydroxy-eicosatetraenoic acid (15-HPETE), or bradykinin. As noted previously (Kontos et al., 1985), the most likely mechanism for superoxide anion production from arachidonate metabolism is an indirect one. It is known that the prostaglandin hydroperoxidase oxidizes a large number of compounds (Egan et al., 1979, 1980, 1981; Marnett et al., 1975, 1979; Marnett and Bienkowski, 1980; Marnett, et al., 1982; Zenser et al., 1980). It has been shown that these oxidations follow chain reactions involving free radical generation (Egan, et al., 1979; Egan et al., 1981; Marnett, et al., 1979). These oxidations provide a possible mechanism for the indirect generation of superoxide anion radical from the reaction with oxygen of one of the radicals produced by the oxidations of substrate. Such a mechanism finds precedent in the action of peroxidases. The initial reaction of peroxidases with hydroperoxy acids is known to generate enzyme-centered radical species (Yokota and Yamazaki, 1965, 1977; Dunford, 1979). These intermediates react with NADH to form the radical-NAD. This radical, in turn, is known to react with oxygen to produce superoxide (Yokota and Yamazaki, 1965, 1977; Land and Swallow, 1971).

The similarity of action of prostaglandin hydroperoxidase to the action of peroxidases prompted us to study the production of superoxide by this enzyme in vitro. In unpublished experiments, Kukreja, Hess, Ellis, and Kontos measured superoxide production during the action of cyclooxygenase on arachidonate in vitro, as the SOD-inhibitable portion of the reduction of ferricytochrome c. In the absence of NADH, no superoxide production was detected, confirming the findings of Marnett et al. (1975). In the presence of NADH there was substantial production of superoxide. Maximum rates of production between 3000 and 3600 nm/min per 100 units of enzyme were observed in the presence of 80–120 μM NADH. With arachidonate as substrate, the production of superoxide was inhibited in a
dose-dependent fashion by indomethacin, aspirin, or AHR-5850, all of which inhibit cyclooxygenase. Superoxide production was also seen when PGF₂ was used as substrate rather than arachidonate, but not when PGH₂ was used as substrate, showing that superoxide generation depended on the action of hydroperoxidase.

Of considerable interest is the fact that the production of superoxide continues for at least an hour after hypertension subsided. This has practical significance because it allows one to intervene after the occurrence of hypertension and reverse at least some of its effects. As we showed here, the sustained dilation after acute hypertension is at least in part dependent on the continued production of superoxide anion radical. The residual dilation seen after application of SOD and catalase may be due to the vascular smooth muscle damage which the radicals caused during and in the 30 minutes following hypertension before the application of the scavengers. We have previously demonstrated that acute hypertension causes morphologically demonstrable lesions in a small percentage of the smooth muscle cells of cerebral arterioles (Kontos et al., 1981).

Acetylcholine causes vasodilation via an indirect mechanism which involves the production of an endothelium-derived relaxant factor, which presumably reaches the vascular smooth muscle by diffusion (Furchgott, 1983). This mechanism has been studied almost exclusively in large vessels, usually under in vitro conditions. After removal of the endothelium, acetylcholine usually causes a small constriction which probably represents the direct effect of the drug on vascular smooth muscle. Our findings show that acute hypertension eliminates vasodilation from acetylcholine. In view of the endothelial damage caused by hypertension, it seems reasonable to attribute the inhibition of the vasodilator response to acetylcholine following hypertension to the consequences of endothelial injury. Therefore, this finding is consistent with the view that the vasodilation in response to acetylcholine in small cerebral arterioles is endothelium-dependent, as is the case in large cerebral arteries (Lee, 1982).

Several mechanisms for the altered response to acetylcholine after acute hypertension may be considered. It is clear from the findings that dilation due to relaxation of the vascular smooth muscle may have contributed to a reduction in the response to acetylcholine, particularly in large arterioles. However, this dilation could not account fully for the changes seen. It could not be responsible for the reversal of the response to acetylcholine in small arterioles. Also, the cerebral arterioles studied were still capable of dilating in response to arterial hypertension, and comparable dilation of normal vessels with CO₂ and histamine did not reverse or eliminate the dilator response to acetylcholine. Finally, the residual dilation of small arterioles in which the response to acetylcholine was converted to vasoconstriction was not different from that seen in vessels which retained a dilator response to this agent. In in vitro experiments, removal of 20-30% of the endothelium from large vessels is usually insufficient to eliminate the vasodilation from acetylcholine (Furchgott, 1983). Since the extent to which the endothelium was affected by destructive lesions in our experiments is much less pronounced, it seems unlikely that the reversal of the effect was due to elimination of the endothelium. We rejected the possibility that oxygen radicals generated by hypertension destroyed acetylcholine itself, because the presence of significant vasoconstriction in response to acetylcholine following hypertension shows that the drug was still present in sufficient concentration to have significant vascular effects. It is more likely that superoxide and other oxygen radicals, generated in response to hypertension, either interfere with the production of the endothelium-derived relaxant factor or actually destroy it. This interpretation is consistent with the restoration of the vasodilator effect of acetylcholine following elimination of the radicals by SOD and catalase. Recent studies have shown that the endothelium-relaxant factor in response to acetylcholine is an unstable, short-lived compound of uncertain identity (Griffith et al., 1984). Therefore, it is probable that it is easily susceptible either to reduction by superoxide or to oxidation by hydrogen peroxide, or by the hydroxyl radical which are likely to be generated from superoxide. It is worth noting that methylene blue and hemoglobin eliminate endothelium-dependent vasodilation from acetylcholine in large arteries studied in vitro (Furchgott et al., 1984). Both compounds have been shown to generate superoxide (McCord and Fridovich, 1970; Misra and Fridovich, 1972). Recent studies have shown that oxygen radicals generated by electrical stimulation are capable of attacking vasoactive agents, such as noradrenaline, thereby affecting vascular responses (Lamb and Webb, 1984).

We do not yet know fully the consequences of interference with the endothelium-dependent mechanisms from oxygen radicals generated by hypertension. Since, in some vessels, the elimination of the endothelium-dependent responses appears to be irreversible after scavenging of the radicals, this may set the stage for subsequent vasoconstriction and possibly ischemia.

References


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