Oxygen Radicals in Cerebral Vascular Injury

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SUMMARY. Acute, severe increases in arterial blood pressure cause sustained cerebral arteriolar dilation, abnormal reactivity to carbon dioxide and to changes in blood pressure, abolition of endothelium-dependent dilation from acetylcholine, discrete morphological lesions of the endothelium and vascular smooth muscle, and breakdown of the blood-brain barrier to plasma proteins. The dilation, abnormal reactivity, and morphological abnormalities are inhibited by pretreatment with cyclooxygenase inhibitors or with free radical scavengers. Superoxide dismutase-inhibitable reduction of nitroblue tetrazolium applied to the brain surface was detectable both during hypertension and one hour after hypertension subsided. Nitroblue tetrazolium reduction is also reduced by inhibitors of the anion channel. The abnormalities seen after hypertension are reproduced by topical application of arachidonate. The results are consistent with the view that acute hypertension induces generation of superoxide anion radical in association with accelerated arachidonate metabolism via cyclooxygenase. This radical enters cerebral extracellular space via the anion channel and gives rise to hydrogen peroxide and hydroxyl radical. All three radicals are capable of causing vasodilation by relaxation of cerebral vascular smooth muscle. The hydroxyl radical is the most likely candidate for vascular wall damage. The significance of this mechanism in chronic experimental hypertension or its relevance to human disease is not known. (Circ Res 57: 508–516, 1985)

THE sequential univalent reduction of oxygen produces a number of reactive intermediate species. These include superoxide anion radical, hydrogen peroxide, and the hydroxyl radical. Superoxide and hydrogen peroxide are normal products of several enzymatic reactions. Superoxide is produced by mitochondria (Boveris, 1977) and by certain oxidative enzymes, such as xanthine oxidase (McCord and Fridovich, 1968) and cytochrome P-450 reductase (Kuthan and Ulrich, 1982). It is also produced by leukocytes via a NADPH-dependent oxidase (Babior, 1978). Hydrogen peroxide can be produced directly by oxidative enzymes such as xanthine oxidase, or it can be generated by the spontaneous or catalyzed dismutation of superoxide. The hydroxyl radical can be produced directly in biological fluids, under certain circumstances, such as under the influence of radiation. A more likely source for this radical is the interaction of superoxide and hydrogen peroxide, via the Haber-Weiss reaction (Haber and Weiss, 1934). The uncatalyzed Haber-Weiss reaction proceeds at a very slow rate under the conditions prevalent in biological materials. However, in the presence of catalytic iron, the reaction can proceed at a sufficiently fast rate to generate significant amounts of hydroxyl radical (McCord and Day, 1978).

Oxygen radicals are capable of producing cellular damage. Superoxide or its derivatives have been shown to damage or destroy cells in a variety of experiments (Fridovich, 1978). Because of their high reactivity and capacity to produce cellular damage, they have been implicated as mediators for such damage in a number of pathological conditions including ischemia (Demopoulos et al., 1980), inflammation (McCord, 1974), acute hypertension (Kontos, et al., 1981), and traumatic brain injuries (Wei et al., 1981).

The purpose of this paper is to consider the evidence implicating oxygen radicals in the cerebral vascular abnormalities seen in experimental acute hypertension.

Effects of Acute Hypertension on Cerebral Vessels

Acute severe elevations of the arterial blood pressure induce severe abnormalities in the cerebral circulation. These abnormalities are independent of the means used for raising the blood pressure. They are seen in association with hypertension due to administration of exogenous vasoconstrictor agents (MacKenzie et al., 1976; Auer, 1978; Kontos et al., 1981), as well as in association with hypertension from increased sympathetic activity secondary to...
fluid-percussion brain injury (Wei et al., 1980a) or to seizures (Cutler et al., 1968; Johansson et al., 1970; Mueller et al., 1979). The cerebral arterioles undergo marked dilation which may outlast the duration of the hypertensive episode by several hours (Kontos et al., 1981) (Fig. 1). This dilation may be preceded by a short constriction. If the increase in pressure is sufficiently pronounced, the vessels become maximally dilated. This dilation affects both small and large arterioles and is accompanied by marked hyperemia of the brain (MacKenzie et al., 1976). In most cases, the dilation involves the arterioles uniformly. Sometimes the dilation is non-uniform. Localized dilations resembling microaneurysms, or markedly dilated segments alternating with less dilated or constricted segments (sausage-string phenomenon), have been described (MacKenzie et al., 1976; Auer, 1977; Kontos et al., 1981).

The dilated vessels display abnormal reactivity. In response to arterial hypocapnia, normally a strong vasoconstrictor stimulus for these vessels, they do not constrict at all or show reduced responsiveness (Wei et al., 1980a; Kontos et al., 1981). Such vessels also show reduced ability to dilate in response to arterial hypercapnia, or in response to arterial hypertension (Kontos et al., 1981); in some cases they display passive reduction in vascular caliber when blood pressure is reduced (Wei et al., 1980a). The blood-brain barrier, which normally does not allow the transfer of plasma proteins from the intravascular compartment into the vessel wall and into the brain parenchyma, becomes abnormally permeable after this type of acute hypertension. The extravasation of plasma proteins under these conditions has been demonstrated by a number of investigators (Johansson et al., 1970; MacKenzie et al., 1976; Auer, 1977).

Arterioles that have been subjected to acute hypertension display morphological lesions of the endothelium and of the vascular smooth muscle (Wei et al., 1980a; Kontos et al., 1981). The endothelium displays focal destructive lesions which are usually localized at the junctions between endothelial cells. These lesions have the appearance of craters or balloon-like protrusions into the lumen of the vessel when viewed by scanning electron microscopy. By transmission electron microscopy, the craters correspond to localized destructions of the cell membrane, while the balloon-like lesions appear to be blebs, possibly due to swelling of the endoplasmic reticulum. The endothelium also displays increased pinocytic activity and an increase in the number of microvilli. The vascular smooth muscle of arterioles which have remained dilated after acute hypertension also displays morphological abnormalities. These consist occasionally of outright necrosis of vascular smooth muscle cells, but more commonly of inclusion bodies and vacuolization. The extent of these lesions seems to be limited. In one detailed study (Kontos et al., 1981), only 5% of the vascular smooth muscle cells were affected. The severe functional derangement with the marked dilation and abnormal responsiveness contrasts with the limited extent of the morphological abnormalities of the vascular smooth muscle. This raises the question of whether these morphological abnormalities could fully explain the functional derangement. It was thought that this would be possible, if the abnormal vascular smooth muscle cells were incapable of developing tension and were stretched by adjacent cells connected to them in series, and still capable of generating tension (Kontos et al., 1981). This mechanism has been invoked to explain the reduced ability of vascular and skeletal muscle to develop tension when induced to contract at long lengths (Dobrin, 1978).

The arterioles dilated by acute hypertension also display biochemical abnormalities. Their oxygen consumption measured in vitro in a microrespirometer is 15–20% lower than the oxygen consumption of vessels of comparable size from normal control animals (Wei et al., 1980a; Kontos et al., 1981).
Role of Arachidonate Metabolism in the Cerebral Vascular Abnormalities from Acute Hypertension

The cerebral vascular abnormalities in acute severe hypertension usually are ascribed to direct mechanical effects of the high transmural pressure prevailing under these conditions. Although the mechanical effects of the increased pressure seem to be a logical initiator of the abnormalities, the precise sequence of events leading to the vascular abnormalities remains unclear. Since the dilation outlasts the duration of hypertension considerably, passive stretching of the vessel wall is not a sufficient explanation. Dilation due to vascular smooth muscle relaxation must therefore play a role.

We discovered that pretreatment with cyclooxygenase inhibitors prevented the cerebral vascular abnormalities induced by acute hypertension (Wei et al., 1981). After pretreatment with indomethacin or sodium amfenac (AHR-5850), two cyclooxygenase inhibitors, acute hypertension induced by either intravenous administration of vasoactive agents or by experimental fluid-perfusion brain injury did not cause the expected abnormalities. Although the vessels dilated during the phase of acute hypertension, after hypotesis the vessels retained their normal caliber and responsiveness to vasoactive agents and did not show morphological abnormalities of the endothelium or vascular smooth muscle. Concurrent controls treated with the appropriate vehicles showed the usual abnormalities described above. These experiments provided pharmacological evidence implicating metabolites of arachidonic acid via the cyclooxygenase pathway in the production of the vascular abnormalities from hypertension. Indomethacin and sodium amfenac did not alter the responsiveness of normal vessels to hypercapnia, hypocapnia, or hypoxia (Wei et al., 1980).

Additional biochemical evidence that arachidonate metabolism is accelerated following acute hypertension was obtained in animals subjected to fluid-perfusion brain injury, which is associated with this type of hypertension. In such animals, phospholipase C activity in the brain was increased 2- to 3-fold (Wei et al., 1982). Also, there was a transient increase in the concentration of the stable prostaglandins PGE$_2$ and PGF$_2$ in the brain following this type of injury (Ellis et al., 1981). On the other hand, in acute hypertension induced by vasoactive agents, the evidence for phospholipase activation and increased prostaglandin formation is negative. Preliminary unpublished experiments by Lamb, Ballon, Ellis, and Wei in this laboratory failed to show an increase in prostaglandin concentration or in phospholipase C activity in the brain of cats subjected to acute hypertension due to iv norepinephrine. It is possible that in acute hypertension induced by vasoactive agents, the increased phospholipase activation and increased prostaglandin synthesis occur in the vessel wall only, whereas, in experimental brain injury, they occur in the brain parenchyma as well as in the cerebral vessels.

If accelerated arachidonate metabolism produces injurious agents which are responsible for the vascular damage from hypertension, it should be possible to reproduce the abnormalities seen in hypertension by arachidonate or by some of its products. This prediction proved to be correct. Topical application of arachidonate in high concentration reproduced all the abnormalities seen after acute hypertension (Kontos et al., 1980). In such experiments, the topical application on the brain surface of cats of arachidonate in a concentration of 200 ng/ml induced sustained arteriolar dilation and reduced responsiveness to arterial hypoxemia. The dilated vessels displayed morphological abnormalities of the vascular smooth muscle and of the endothelium identical to those seen in acute hypertension. Recent unpublished experiments by Wei and myself also showed that arachidonate caused increased permeability of the blood-brain barrier to plasma albumin. The dilated arterioles after topical application of arachidonate showed reduced oxygen consumption of the vessel wall (Levasseur et al., 1985). The concentration of arachidonate used in these experiments was very high compared to the concentrations usually used in in vitro experiments. The high concentration required in the in vivo experiments probably because arachidonate in these experiments is limited in its access to the vessels by the meninges and also because part of it undoubtedly is washed away or taken up by cells. Comparisons between in vivo and in vitro experiments in which the effects of arachidonate on oxygen consumption of the vessel wall were measured show that the concentration of arachidonate in in vitro experiments which produces the same effect is one-tenth of the high in vivo concentrations used in these experiments (Levasseur et al., 1985).

Bradykinin, a polypeptide which activates phospholipase and produces vasodilation, in part due to activation of arachidonate metabolism in vessels, including isolated cerebral vessels (Toda, 1977), also produced abnormalities similar to those due to arachidonate. The same was true with the endoperoxide PGG$_2$ (Kontos et al., 1980) and 15-hydroperoxyeicosatetraenoic acid (15-HPETE) (Christian et al., 1984), a product of arachidonate metabolism via lipoxigenase. On the other hand, the endoperoxide PGH$_2$ and the prostaglandins PGE$_2$ and PGF$_2$ did not lead to sustained cerebral vascular abnormalities or to morphological alterations, despite the fact that during their application they produced dilation as pronounced as that produced by arachidonate (Ellis et al., 1979; Kontos et al., 1980).

It should be noted that 15-HPETE is as good a substrate of the prostaglandin hydroperoxidase as the natural substrate of this enzyme, PGG$_2$ (Egan et al., 1979; Marnett et al., 1979). Thus, arachidonate, or agents which release arachidonate from endoge-
Role of Oxygen Radicals in the Vascular Abnormalities from Acute Hypertension

The metabolism of arachidonate generates radicals (Porter, 1980). Because of the known capacity of radicals to cause tissue damage, we tested the possibility that a radical might be responsible for the abnormalities seen in the brain vessels following acute hypertension. Pretreatment with a variety of radical scavengers, including superoxide dismutase (SOD), an enzyme which eliminates superoxide anion radical, mannitol, a scavenger of the hydroxyl radical, and nitroblue tetrazolium (NBT), a dye which is reduced by superoxide anion radical and, in the process, becomes converted to an insoluble form which precipitates, prevented the vascular abnormalities from acute hypertension induced by administration of vasoactive agents or induced by fluid-perfusion type brain injury (Wei et al., 1981; Kontos et al., 1981). The radical scavengers did not alter resting vessel caliber or vessel reactivity.

Since these experiments implicated oxygen radicals as mediators of the vascular abnormalities from hypertension, it was necessary to determine what effect exogenous oxygen radicals have on cerebral vessels. We used the xanthine oxidase reaction as a source of oxygen radicals (Wei et al., 1985). This reaction is known to produce superoxide anion radical as well as hydrogen peroxide in varying proportions. If these two radicals are not eliminated, they interact and produce hydroxyl radical via the catalyzed Haber-Weiss reaction. We studied the effects of xanthine oxidase together with xanthine as substrate by topical application on the brain surface of cats during a 15-minute application, as well as after washout. By using SOD, we selectively eliminated superoxide anion radical, and by using catalase, we selectively eliminated hydrogen peroxide. In this manner, the effects of these two agents were gauged separately. Xanthine oxidase plus xanthine in the presence of both SOD and catalase produced effects that were indistinguishable from those seen when heat-inactivated xanthine oxidase plus xanthine were applied. It appeared, therefore, that any additional effects of the active enzyme could be ascribed entirely to superoxide anion radical and hydrogen peroxide. Xanthine oxidase plus xanthine with either SOD or catalase produced dilation during application, but no sustained effects on vascular caliber after washout were produced, nor were any morphological abnormalities. On the other hand, the active enzyme, in the absence of scavengers, caused dilation during application as well as after washout, with reduced responsiveness and with morphological lesions of the endothelium. Short-lasting applications caused only transient effects. It is clear from these experiments that both superoxide anion radical and hydrogen peroxide are capable of producing relaxation of cerebral vascular smooth muscle. Consistent vascular damage is produced only in the presence of both radicals. It is likely, therefore, that the vascular damage is caused by the hydroxyl radical generated by the interaction of these two agents. In experiments of this type, there is usually sufficient free iron contaminating the various reagents to permit the operation of the catalyzed Haber-Weiss reaction. It is also known that endogenous cerebrospinal fluid contains enough free iron to catalyze the Haber-Weiss reaction (Halliwell and Gutteridge, 1984).

Scavengers of oxygen radicals were also effective in preventing the cerebral vascular effects of arachidonate and of some of its products. For example, the combination of SOD and catalase prevented the dilation from topical arachidonate or topical bradykinin (Kontos et al., 1984). Similarly, these enzymes prevented the morphological abnormalities in the endothelium from topical arachidonate. They also prevented the increased permeability of the blood-brain barrier to plasma proteins during topical application of arachidonate (unpublished data). SOD by itself or catalase by itself reduced the dilation during topical application of arachidonate or 15-HPETE and prevented completely the residual dilation and reduced responsiveness to hypocapnia (Kontos et al., 1980; Christman et al., 1984). Mannitol reduced the residual dilation from arachidonate and prevented the morphological abnormalities of the endothelium, while SOD by itself had similar effects on the action of topical PGG2 (Kontos et al., 1980).

These results provided evidence supporting the hypothesis that oxygen radicals are generated in the course of acute hypertension in association with increased arachidonate metabolism via cyclooxygenase, and that these radicals are responsible for the vascular consequences of acute hypertension. The evidence supported the view that superoxide anion radical was the first oxygen radical produced, and that it led to hydrogen peroxide production by dismutation and to hydroxyl radical by interaction of superoxide and hydrogen peroxide. The hydroxyl radical was considered the radical most likely to be immediately responsible for vascular injury.

To confirm this hypothesis, we measured in semiquantitative fashion superoxide anion radical generation on the brain surface of cats subjected to acute hypertension with vasoactive agents (Kontos et al., 1985). The method relied on the demonstration of SOD-inhibitable reduction of NBT. The cats were implanted with two cranial windows in sym-
metrical fashion. The space under one window was filled with a solution of NBT; the other with a solution of NBT and SOD. The animals were subjected to acute hypertension with iv norepinephrine which led to reduction of NBT and deposition of the reduced dye on the brain surface. Subsequently, the brain was perfused with fixatives to eliminate hemoglobin, and the amounts of NBT deposited on the brain surface were measured spectrophotometrically. By subtraction, the rate of SOD-inhibitable reduction of NBT, which is a measure of the rate of superoxide generation, was determined. A typical example of such an experiment is shown in Figure 2. Acute hypertension led to the generation of superoxide anion radical, both during the period of increased pressure, and 1 hour after blood pressure had returned to the baseline (Fig. 3). On the other hand, the administration of norepinephrine while the animal was bled to prevent the arterial blood pressure from rising caused no superoxide anion radical generation. Similar results were obtained with fluid-percussion type brain injury. Also, topical application of arachidonate and bradykinin caused the appearance of superoxide anion radical at the site of application (Kontos et al., 1985). From the rate of reduction of NBT, we calculated that the production of superoxide per cm² of brain surface in these experiments was approximately equivalent to superoxide produced by one million fully activated leukocytes.

![Figure 2. Typical appearance of the fixed brain illustrating the deposition of reduced nitroblue tetrazolium during acute hypertension. The left cranial window site was treated with nitroblue tetrazolium, and the right cranial window site was treated with nitroblue tetrazolium plus superoxide dismutase. Note the inhibition of reduction of nitroblue tetrazolium on the right side by superoxide dismutase.](image)

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![Figure 3. Rates of superoxide dismutase-inhibitable reduction of nitroblue tetrazolium during hypertension, 1 hour after hypertension, and during norepinephrine infusion without hypertension. Values are mean ± ss.](image)

**Figure 3.** Rates of superoxide dismutase-inhibitable reduction of nitroblue tetrazolium during hypertension, 1 hour after hypertension, and during norepinephrine infusion without hypertension. Values are mean ± ss.

**Source of Superoxide Anion Radical**

Since NBT is a polar, water-soluble compound and SOD has high molecular weight, neither is likely to penetrate into the interior of intact cells very easily. The fact that they were capable of reacting with superoxide suggested that this radical must be present in the extracellular space of the brain. Three possible sources for the presence of superoxide in extracellular space may be considered: 1. Cells which normally secrete superoxide into the extracellular environment, such as leukocytes. This possibility was rejected, because no unusual accumulation of leukocytes could be found in the first few hours following acute hypertension. The same was true with topical application of arachidonate or 15-HPETE (Christman et al., 1984; Kontos et al., 1985). However, a few hours after application of these agents, leukocytes began adhering to the endothelium of microvessels and were seen to penetrate the vessel wall and accumulate in the extravascular space. Twenty-four hours after topical application of arachidonate, severe accumulation of leukocytes took place (Kontos et al., 1985). Therefore, leukocytes may be a source of superoxide and other oxygen radicals in later stages after topical application of arachidonate or 15-HPETE, but not in the first few hours.
2. Superoxide and other radicals may destroy the cell membrane, thereby removing any barriers to the movement of radicals and scavengers. This possibility seemed unlikely, because no parenchymal cellular damage could be detected following acute hypertension, brain injury, or topical application of arachidonate or bradykinin. Vascular injury to the endothelium was, of course, evident in these situations. In the presence of NBT, however, vascular injury was absent.

3. Superoxide is produced by intact cells and escapes into the extracellular space via normal membrane channels. Lynch and Fridovich (1978a, 1978b) showed that superoxide could easily penetrate the cell membrane of red cells in either direction. Furthermore, they found that this penetration of the red cell membrane by superoxide occurred via the anion channel, and that it could be blocked by anion channel inhibitors. This seemed to be the most likely explanation for the appearance of superoxide in extracellular cerebral space in our experiments. We tested this hypothesis by the use of anion channel inhibitors. Phenylglyoxal, an irreversible inhibitor of the anion channel (Wieth et al., 1982), prevented the reduction of NBT induced by acute hypertension (Fig. 4) or by topical application of bradykinin or arachidonate (Kontos et al., 1985).

The brain parenchymal cells, the vessel wall itself, the meninges, and the formed elements of the blood all are potential sources of superoxide. We tested whether the vessel wall could be a source of superoxide in sufficient amounts to cause vascular damage. We incubated cerebral arterioles with arachidonate and used the oxygen consumption rate of the vessel wall after washout as a marker of vascular injury (Levasseur et al., 1985). Arachidonate severely reduced oxygen consumption of the vessel wall. This effect was inhibited completely by a combination of SOD and catalase. SOD by itself was ineffective, and catalase by itself was partially effective. The same result was obtained after incubation with 15-HPETE. Xanthine oxidase, together with acetaldehyde as substrate, a known source of oxygen radicals, also reduced oxygen consumption, and this effect was partially inhibited by SOD and catalase (Levasseur et al., 1985). These findings showed that the vessel wall can by itself produce oxygen radicals from arachidonate metabolism.

**Enzymatic Mechanism of Generation of Superoxide**

As noted above, it has been known for some time that the metabolism of arachidonate generates radicals. The initial conversion of arachidonate into PGG$_2$ is associated with the production of a radical which seems to be a carbon-centered radical associated with the enzyme (Mason et al., 1980). In the course of the action of hydroperoxidase in microsomal fractions on PGG$_2$ or 15-HPETE, an electron paramagnetic resonance (EPR) signal was obtained that was ascribed to the generation of a powerful oxidant, responsible for the inactivation of the cyclooxygenase enzyme (Egan et al., 1979). This radical was not fully identified, but it behaved in a manner similar to that expected of the hydroxyl radical. Subsequent evidence suggested that this may be an enzyme-centered radical due to the oxidation of an amino acid in close proximity to the iron of the cyclooxygenase (Kalyanaraman et al., 1982). However, the interpretation of this evidence is complicated further by the fact that the EPR signal attributed to this radical did not appear when the purified enzyme was used (Egan et al., 1981). This finding suggested that the EPR signal was due to a radical produced in the course of the oxidation of adventitious material isolated together with the cyclooxygenase enzyme.

The prostaglandin hydroperoxidase in the presence of suitable substrate causes the oxidation of a large number of reducing cosubstrates. It has been shown that oxidation of these cosubstrates frequently follows chain reactions involving the generation of radicals (Marnett et al., 1975, 1979; Egan et al., 1979, 1981). Superoxide could be produced by the interaction of one of these radicals with oxygen. The action of the prostaglandin hydroperoxidase is, in many ways, analogous to the action of peroxidases. The peroxidases are known to give rise to enzyme-centered intermediate radical species. These intermediates have been shown to interact with certain normal cellular constituents and produce radical species which are then capable of interacting with oxygen to produce superoxide (Yokota...
and Yamazaki, 1965, 1977; Dunford, 1979). For example, peroxidase can interact with NADH to produce the radical form of NAD$^+$ which then produces superoxide from molecular oxygen (Yokota and Yamazaki, 1965, 1977; Land and Swallow, 1971). It is of interest that during the action of microsomal cyclooxygenase and hydroperoxidase on PGG$_2$ or arachidonate, no superoxide anion radical is produced (Marnett et al., 1975). This does not exclude the production of superoxide from intact cells, however, since, in the in vitro preparations, both the availability of suitable substrates and the spatial relationships between the enzyme and such substrates are disturbed.

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 I 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 3,000 and 3,600 nM/min 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 also was seen when PGG$_2$ was used as substrate rather than arachidonate, but not when PGG$_2$ was used as substrate, showing that superoxide generation depended on the action of hydroperoxidase.

**Functional Consequences of Superoxide Generation during Hypertension**

As noted above, the discrepancy between the limited morphological damage to the vascular smooth muscle and the severe functional abnormalities observed in cerebral vessels following acute hypertension raises questions as to the mechanism of the functional abnormalities and the adequacy of the morphological damage to explain them fully. The finding that superoxide production continued for at least 1 hour after an acute hypertensive episode had subsided and the fact that superoxide and other radicals derived from it caused vascular smooth muscle relaxation provided an alternative explanation for the sustained dilation of cerebral arterioles following hypertension. If continued production of superoxide is in part responsible for this dilation, it should be possible to intervene with oxygen radical scavengers after hypertension has subsided and thereby inhibit the dilation. Topical application of SOD and catalase 30 minutes after an acute hypertensive episode had subsided was effective in significantly reducing the dilation of cerebral arterioles (unpublished data). Similar results were obtained after fluid-percussion brain injury. These results may have practical significance because they allow one to intervene and effectively inhibit vascular consequences of hypertension some time after the hypertensive episode subsides.

The fact that the combination of SOD and catalase effectively inhibited the dilation from the topical application of arachidonate and bradykinin drew attention to the so-called endothelium-derived relaxant factor (EDRF). A number of vasoactive agents including arachidonate, bradykinin, and acetylcholine are capable of relaxing vascular smooth muscle via an indirect mechanism which involves action on the endothelium and release of one or more endothelium-derived relaxant factors (Furchgott, 1983). Cerebral arterioles respond to topical application of acetylcholine with vasodilation. This vasodilation, unlike that due to topical arachidonate or bradykinin, was not affected by SOD and catalase (Kontos et al., 1984). This shows that oxygen radicals are not the endothelium-derived relaxant factor induced by acetylcholine. It is not known whether arachidonate and bradykinin induce relaxation of small cerebral vessels via an endothelium-derived factor. If this is the case, then this evidence suggests that we may be dealing with more than one endothelium-derived factor.

We tested the responses of cerebral vessels to topical acetylcholine before and after an acute hypertensive episode due to iv norepinephrine to find out if the endothelium-dependent dilation from acetylcholine was affected by the endothelial damage caused by hypertension (Kontos and Wei, 1985). We found that 30 minutes after hypertension, the response to acetylcholine was converted to vasoconstriction. This can be explained by one of the following mechanisms. (1) Since the vessels are dilated after acute hypertension, the response may be due to the fact that the vessels are incapable of responding with additional dilation. This explanation seemed unlikely, because normal vessels which had been dilated with hypercapnia or histamine responded with additional dilation to topical acetylcholine. (2) The oxygen radicals generated by acute hypertension may either prevent the production of the endothelium-derived relaxant factor or inactivate it. We tested this by determining the response to acetylcholine following treatment with SOD and catalase 60 minutes after the hypertensive episode had subsided. Vessels which had responded to acetylcholine with vasoconstriction, responded with vasodilation which was significant, but reduced, compared to responses seen before hypertension. This result is consistent with the interpretation that oxygen radicals either prevent the production of, or inactivate, the endothelium-derived relaxant factor. The latter seems more likely, in view of the recent report that oxygen radicals are capable of inactivating other vasoactive agents, such as norepinephrine (Lamb and Webb, 1984).
Kontos/Oxygen Radicals and Cerebral Vessels

It is of interest that acute hypertension causes cerebral arteriolar dilation in part by releasing oxygen radicals; at the same time these radicals interfere with endothelium-dependent vasodilation. We do not know whether, under different circumstances, the elimination of endothelium-dependent dilation may become irreversible. If this does happen, it may set the stage for subsequent vasoconstriction and, possibly, ischemia.

Significance

The evidence considered above supports the following sequence of events for the production of the cerebral vascular effects of acute hypertension. The initial event seems to be the activation of phospholipase in the vessel wall and release of free arachidonate. The mechanism by which hypertension accomplishes this is not known. In view of the fact that polypeptides, such as bradykinin, reproduce the effects of acute hypertension, I suggest the possibility of release of an endogenous polypeptide as the mediator for this step. The released free arachidonate causes accelerated metabolism via cyclooxygenase. The hydroperoxidase reaction leads to the production of superoxide which escapes into the extracellular space via the anion channel. In this space, by dismutation, it generates hydrogen peroxide, and the two together lead to the production of hydroxyl radical which then causes vascular injury. All three agents, superoxide anion radical, hydrogen peroxide, and hydroxyl radical, are capable of causing vascular relaxation.

It is pertinent to speculate whether this mechanism is relevant to chronic hypertension and whether it has any relationship to human hypertension. Endothelial lesions of the type seen in acute hypertension have also been found in large cerebral vessels in rats with genetic hypertension (Hazama et al., 1979). Byrom (1954) studied cerebral vascular effects of chronic renal hypertension in rats. He found extravasation of protein and edema. He also noted vascular caliber changes. These were of uneven caliber, with alternating segments of dilation and constriction. It is of interest that he thought that the primary problem was one of constriction. However, blood flow measurements in these animals showed hyperemia (Byrom, 1973).

The syndrome of human hypertension which resembles most the manifestations of acute experimental hypertension is hypertensive encephalopathy, where marked elevations of blood pressure are accompanied by marked hyperemia of the brain (Skinhoj and Strandgaard, 1973), edema, and disturbed cerebral function. The possibility that radical-mediated mechanisms are responsible for these manifestations is an attractive one.

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