Superoxide Anion Radical Does Not Mediate Vasodilation of Cerebral Arterioles by Vasoactive Intestinal Polypeptide

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SUMMARY We examined the hypothesis that oxygen radicals may mediate the vasodilator effect of VIP on cerebral arterioles in cats equipped with cranial windows. The appearance of superoxide anion radical in cerebral extracellular space during VIP application was examined by measuring the rate of superoxide dismutase (SOD)-inhibitable reduction of nitroblue tetrazolium (NBT). Although VIP (1 and 10 ng/ml) caused substantial reduction of NBT, the rate of the SOD-inhibitable portion was not significantly different from zero. We also examined the effect of scavenging of superoxide and hydrogen peroxide by topical application of SOD plus catalase on the vasodilator effect of VIP (0.05-1.0 μg/ml). The dilation in response to VIP was not significantly affected in either large or small arterioles by scavenging of superoxide and hydrogen peroxide. We conclude that VIP does not cause generation of superoxide and that superoxide or other reactive oxygen species derived from it, such as hydrogen peroxide and hydroxyl radical, are not mediators of the cerebral vasodilation caused by VIP.

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THE SEQUENTIAL UNIVALENT REDUCTION of oxygen gives rise to reactive products, including superoxide, hydrogen peroxide, and the free hydroxyl radical, which relax cerebral vascular smooth muscle and dilate cerebral arterioles. These agents are also capable of causing vascular damage upon prolonged exposure. Several experimental interventions, including acute severe hypertension, fluid-percussion brain injury, and topical application of arachidonate or bradykinin, have recently been shown to induce the generation of superoxide which then appears in cerebral extracellular space. Superoxide and/or its derivatives, such as hydrogen peroxide or the hydroxyl radical, by subsequent reactions have been shown to be responsible for the vasodilation which is seen in cerebral extracellular space. Since inhibition of cyclooxygenase or scavenging of oxygen radicals inhibit the vasodilation seen in these conditions, it was concluded that the vasodilation was mediated by oxygen radicals generated in association with accelerated arachidonate metabolism via cyclooxygenase.

To date, there has been no evidence linking oxygen radicals to any physiological vasodilator responses in the cerebral circulation. Vasoactive intestinal polypeptide (VIP) is normally present as a putative neurotransmitter in nerve endings of cerebral arterioles. Since the dilation of cerebral arterioles of cats from topical application of VIP on the brain surface is blocked by pretreatment with cyclooxygenase inhibitors, the possibility exists that the effects may be mediated via oxygen radicals in a manner analogous to what is seen with bradykinin. Since VIP is a normal constituent of the vessel wall, its release might provide the link between such abnormal interventions as acute severe hypertension and brain injury and the initiation of accelerated arachidonate metabolism which leads to the production of superoxide and other oxygen radicals.

Accordingly, we tested the possibility that VIP induces cerebral arteriolar vasodilation via generation of superoxide and other radicals derived from it.

Methods

Experiments were carried out in 22 cats weighing 2.5 to 3.5 kg. The animals were anesthetized with sodium pentobarbital (30 mg/kg iv). After completion of tracheostomy each animal was ventilated with a
positive pressure respirator and received 5 mg/kg of gallamine triethiodide for skeletal muscle paralysis. 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 PO$_2$, PCO$_2$, pH and hematocrit. Blood gases and pH were measured with Radiometer electrodes. Hematocrit was measured by a micromethod. The end-expiratory CO$_2$ of the animals was monitored continuously with a Hewlett-Packard infrared CO$_2$ analyzer and was maintained at a constant level of about 30 mm Hg throughout the experiment by adjusting the respirator rate and volume.

We used two approaches: 1) Detection of superoxide anion radical in cerebral extracellular space and 2) examination of the effect of scavenging of superoxide and hydrogen peroxide on the vasodilation induced by VIP.

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. 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 cranial window technique. Each window was installed in symmetrical fashion to overlay the parietal cortex of the animal. The space under the cranial window was filled with artificial CSF (having the same composition as endogenous CSF of cats). Both window spaces were filled with CSF containing 2.4 mM solution of NBT and 1 or 10 μg/ml VIP. One window contained, in addition to VIP and NBT, 20 μg/ml SOD. These solutions were allowed to stay in contact with the brain surface for 15 min. They were then washed away with fresh CSF containing no additives. Subsequently, the brain was fixed 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. 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 fiber optics 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 and absorbance readings were taken at the appropriate wave-lengths 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 by pyridine. Correlation between the two methods was excellent up to a concentration of 200 nM/cm$^2$ brain surface. 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.

In a second series of cats, the animals were implanted with a single cranial window overlying the parietal cortex. Pial arterioles were visualized via a Leitz Ultraphot microscope equipped with a Vickers imagesplitting device for measuring vessel diameter. Usually 4–6 arterioles covering a wide range of vessel caliber were studied in each animal. Initially, the space under the cranial window was filled with artificial CSF without additives. The responses of the arterioles to arterial hypocapnia induced by hyperventilation was examined to verify that the vessels responded normally. After restoration of the arterial PCO$_2$ to normal and return of the vessel caliber to the control level, dose-response curves were obtained to topical application of VIP 0.05 to 1.0 μg/ml. VIP was dissolved in artificial CSF and the resultant solution was used to fill the space under the cranial window. Intracranial pressure was kept constant by connecting one outlet of the window to a coiled tube whose open end was placed at a predetermined height to give a constant intracranial pressure of 5 mm Hg. Dose-response curves to VIP were repeated in the presence of SOD, (60 units/ml) plus catalase (40 units/ml). Measurements of vessel diameter were made 2–4 min after the application of each solution. This allowed ample time for the establishment of a new steady state.

Porcine VIP, SOD (from bovine blood 3,000 units/mg protein), catalase (from bovine liver 2,000 units/mg protein) and NBT were obtained from Sigma.

Results

Table 1 shows the reduction rates of NBT from topical application of VIP (1 and 10 μg/ml) in the absence and presence of SOD. It is seen that with either concentration VIP there was substantial reduction of NBT. However, the SOD-inhibitable reduction rate of NBT was not significantly different from zero with either dose of VIP.

Figures 1 and 2 show that the application of SOD plus catalase had no significant effect on the dilation of small (< 100 μm in diameter) or large (> 100 μm in diameter) arterioles induced by VIP.

Application of VIP to the brain surface produced no significant changes in mean arterial blood pressure or in arterial blood CO$_2$ tension.

Discussion

The main findings of these experiments are that VIP does not generate superoxide when applied on the brain surface and that the combination of SOD and catalase do not influence the cerebral arteriolar vasodi-
We are faced therefore with the seemingly paradoxical finding that the two polypeptides accelerate prostaglandin synthesis and yet one leads to generation of superoxide and the other does not. This difference may be reconciled if one considers the mechanism by which accelerated prostaglandin synthesis generates superoxide. We recently found that cyclooxygenase or lipooxygenase in vitro produce superoxide in the presence of NADH or NADPH but not in their absence. The generation of superoxide is an indirect one, analogous to that described for peroxidases in general. Briefly, the mechanism involves the generation of enzyme-centered radicals which interact with NADH or NADPH to produce the radical -NAD or -NADP. These radicals, in turn, react readily with oxygen to produce superoxide. In the case of cyclooxygenase, the generation of superoxide is dependent on the hydroperoxidase action of the enzyme which is responsible for the conversion of prostaglandin G3 to prostaglandin H2. In order to produce superoxide, cyclooxygenase must be coupled with NADH or NADPH or a similarly acting co-substrate. The possibility exists that such a coupling might occur in some tissues and not in others. Hence, accelerated arachidonate metabolism via cyclooxygenase might produce superoxide in some cells in which the enzyme is coupled with NADH or NADPH but not in others. It is known that bradykinin induces smooth muscle relaxation via action on the endothelium and subsequent release of an endothelium and subsequent release of an unidentified reducing agent. It is not known whether this action of VIP has anything to do with its ability to cause cerebral arteriolar vasodilation.

It is interesting to contrast the effects of VIP and bradykinin. Both polypeptides are present in normal brain. VIP is located in nerve fibers innervating vessels where it presumably acts as a neurotransmitter. Bradykinin is also present and mammalian brain where it can be formed by the action of kallikrein on its precursor kinogen. Both polypeptides cause cerebral arteriolar dilation due to relaxation of the vascular smooth muscle. This vasodilation is blocked by cyclooxygenase inhibitors. The effect of bradykinin on cerebral arterioles is also blocked by the combination of SOD and catalase. In addition, during the application of bradykinin on the brain surface, superoxide appears in the cerebral extracellular space. It is interesting to contrast the effects of VIP and bradykinin. Both polypeptides are present in normal brain. VIP is located in nerve fibers innervating vessels where it presumably acts as a neurotransmitter. Bradykinin is also present and mammalian brain where it can be formed by the action of kallikrein on its precursor kinogen. Both polypeptides cause cerebral arteriolar dilation due to relaxation of the vascular smooth muscle. This vasodilation is blocked by cyclooxygenase inhibitors. The effect of bradykinin on cerebral arterioles is also blocked by the combination of SOD and catalase. In addition, during the application of bradykinin on the brain surface, superoxide appears in the cerebral extracellular space. It is known whether this action of VIP has anything to do with its ability to cause cerebral arteriolar vasodilation.

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ium derived relaxant factor. It would appear that in the cerebral microcirculation at least, the endothelium relaxant factor released by bradykinin is an oxygen radical. The dependence of the vasodilation of bradykinin in cerebral arterioles on the endothelium was demonstrated by Rosenblum who found that injury to the endothelium eliminated the vasodilation from bradykinin. On the other hand, the endothelium is not needed for the vasodilation induced by VIP in cerebral vessels. Evidently, this polypeptide acts directly on vascular smooth muscle. The present findings may therefore indicate that cyclooxygenase in the endothelium may be coupled with co-substrates, allowing it to generate superoxide and other oxygen radicals, while in smooth muscle, this is not the case.

References
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