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 μg/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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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. Hemato-
crit was measured by a micromethod. The end-expira-
atory 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 superox-
ide 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 su-
peroxide dismutase (SOD)-inhibitable reduction of ni-
troblue tetrazolium (NBT). The technique has been
described previously in detail.$^4$ NBT is a water soluble
dye which, when reduced by superoxide anion radical
or by other reducing agents, is converted to an insol-
uble blue form which precipitates. We used a double
cranial window technique.$^4$ Each window was in-
 stalled in symmetrical fashion to overlie the parietal
cortex of the animal. The space under the cranial win-
dow was filled with artificial CSF having the same composi-
tion as endogenous CSF of cats.$^11$ Both window spaces were filled with
CSF containing 2.4 mM solution of NBT and 1 or 10
$\mu$g/ml VIP. One window contained, in addition to VIP
and NBT, 20 $\mu$g/ml SOD. These solutions were al-
lowed 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. After fixa-
tion was completed, the brain was removed from the
cranial cavity and the amounts of NBT deposited were
examined to verify that the vessels responded normal-
ly. 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 $\mu$g/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 win-
dow 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 estab-
ishment 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 $\mu$g/ml) in the
absence and presence of SOD. It is seen that with
either concentration VIP there was substantial reduc-
tion 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 dilution of
small (< 100 $\mu$m in diameter) or large (> 100 $\mu$m 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-
Ulcer ± SE of control diameters are given at the top left. Number of vessels studied is given in parentheses after appropriate control diameter values. Vasodilator response to VIP in the presence of SOD and catalase was not significantly different from VIP alone (analysis of variance). Mean arterial blood pressure in control period before VIP was 116 ± 5.4 mm Hg and did not change significantly during application of VIP. Arterial CO₂ tension was 30.6 ± 0.9 mm Hg and did not change during application of VIP.

Table 1: NBT Reduction Induced by Topical VIP with and without SOD

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>Number of cats</th>
<th>NBT</th>
<th>NBT plus SOD</th>
<th>SOD-Inhibitable Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>4.7±1.1</td>
<td>5.0±0.9</td>
<td>-0.4±1.1</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>5.1±1.4</td>
<td>7.5±1.4</td>
<td>-2.4±1.5</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Value for each cranial window site is the average of all readings. The SOD-inhibitable reduction rate was not significantly different from zero (paired t test).

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 kininogen. 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. These results show that the effect of bradykinin on these vessels is mediated by oxygen radicals generated in the course of arachidonate metabolism. The oxygen radical generating capability of bradykinin therefore contrasts with the absence of such an effect from VIP. 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 G₂ 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 endothe-
lum derived relaxant factor. It would appear that in the cerebral microcirculation at least, the endothelial 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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