Age-Related Changes in Release of Endothelium-Derived Relaxing Factor From the Carotid Artery

Roberto Paternò, MD; Frank M. Faraci, PhD; Donald D. Heistad, MD

Background and Purpose  The goal of this study was to determine whether release of endothelium-derived relaxing factor (EDRF) from carotid artery in response to acetylcholine is altered by aging.

Methods  Responses were examined in arteries from Wistar rats 6 to 8 months old (young rats), 24 to 26 months old (old rats), and 30 to 32 months old (very old rats). We used a bioassay technique to measure release of EDRF from the carotid artery (donor vessel) and, in the same experiment, measured the diameter of pressurized donor arteries.

Results  Responses of the donor arteries and the detector vessels (aortas) to sodium nitroprusside were not altered in old (24 to 26 months) and very old (30 to 32 months) rats compared with responses in the young rats. Dilator responses in the carotid artery to acetylcholine tended to be lower in the old rats and were significantly lower in very old rats compared with the young rats. Relaxation of the detector vessel during administration of acetylcholine to the donor artery was not significantly different in young, old, and very old rats.

Conclusions  Endothelium-dependent dilatation in carotid arteries of old rats is impaired, but release of EDRF and responsiveness of vascular muscle to nitroprusside are normal. Thus, impairment of endothelium-dependent relaxation in the carotid artery during aging is not due to impaired release of EDRF. (Stroke. 1994;25:2459-2462.)

Key Words  • acetylcholine  • aging  • carotid arteries  • endothelium-derived relaxing factor

Several but not all in vitro studies of large arteries from experimental animals and humans suggest that agonist-induced endothelium-dependent relaxation is impaired by aging. Many mechanisms potentially contribute to impaired endothelium-dependent relaxation: impaired synthesis or release of endothelium-derived relaxing factor (EDRF), impaired responsiveness of vascular smooth muscle, formation of an endothelium-derived contracting factor (EDCF), formation of an endogenous inhibitor of nitric oxide synthase, or generation of oxygen-derived free radicals that inactivate EDRF.

The goal of this study was to determine whether release of EDRF in response to acetylcholine and responsiveness of vascular muscle to EDRF are impaired by aging. To do this, we studied young, old, and very old rats. Most previous studies of the effects of aging on endothelium-dependent relaxation were performed with rings or strips of blood vessels in an organ chamber. In this study we used a perfusion-cascade bioassay system, in which a carotid artery was perfused. Endothelial cells were exposed to pressure and flow, and release of EDRF could be assayed. We also developed a new method that allowed measurement of responses to acetylcholine in the carotid artery.
vasodilatation is impaired in old rats. We developed a young, old, and very old rats were not different (see "Results"). After making these findings, which demonstrated that release of EDRF is normal in old rats, it was important that we confirm our previous finding that endothelium-dependent relaxation in response to acetylcholine (Figs 1 and 2) exposed to sodium nitroprusside (10^{-9} to 10^{-4} mol/L) added to phenylephrine in Krebs' solution.

### Drugs
Phenytoin, acetylcholine, and sodium nitroprusside were purchased from Sigma Chemical Co. (St. Louis, Mo).

### Calculations and Statistical Analysis
Relaxation in response to acetylcholine and sodium nitroprusside is expressed as the percentage of phenylephrine-induced force of the detector vessel and as the percentage of the phenylephrine-induced tone of the donor. Responses in the detector vessel were calculated using the formula (isometric force during phenylephrine infusion−isometric force during acetylcholine infusion)×100%/(isometric force during phenylephrine infusion). Responses in the donor were calculated using the formula ([diameter during acetylcholine infusion−diameter during phenylephrine infusion]×100%)/(baseline diameter−diameter during phenylephrine infusion).

Constriction of the donor artery to phenylephrine is expressed as percentage of the isometric force baseline of stretched bioassay detector vessel. Responses were calculated from the formula (isometric force during phenylephrine infusion−isometric force baseline)×100%/(isometric force during phenylephrine infusion).

Constriction of the donor artery to phenylephrine is expressed as percentage of the quiescent tone of pressurized donor artery. Responses were calculated from the formula ([diameter during phenylephrine infusion−diameter baseline]×100%)/(diameter during phenylephrine infusion).

Several experiments in which the bioassay detector did not respond to high concentrations of acetylcholine (10^{-4} mol/L) infused in the donor artery were not included in the data. We did not include data from five animals (1 young, 2 old, 2 very old). Results were expressed as mean±SEM. Comparisons between three groups were evaluated by ANOVA and Bonferroni test. Values of P<.05 for a one-tailed Student's t-test were considered to be significant.

### Results
#### Nonpressurized Donor Artery
Administration of acetylcholine to the nonpressurized donor artery produced relaxation of the bioassay vessel. In the bioassay detector vessel paired with the unpressurized carotid artery, relaxation in response to acetylcholine and sodium nitroprusside (Figs 1 and 2) and contraction in response to phenylephrine (not shown) were not significantly different in young, old, and very old rats.
TABLE 2. Response of Bioassay Detector to Effluent From Pressurized, Perfused Carotid Arteries

<table>
<thead>
<tr>
<th></th>
<th>Acetylcholine</th>
<th></th>
<th>Nitroprusside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^{-8}$ M</td>
<td>$3\times10^{-7}$ M</td>
<td>$10^{-7}$ M</td>
</tr>
<tr>
<td>Young (n=8)</td>
<td>8±2</td>
<td>24±5</td>
<td>35±4</td>
</tr>
<tr>
<td>Old (n=6)</td>
<td>9±3</td>
<td>18±6</td>
<td>32±10</td>
</tr>
<tr>
<td>Very old (n=4)</td>
<td>3±2</td>
<td>18±8</td>
<td>31±10</td>
</tr>
</tbody>
</table>

|                | $10^{-6}$ M   | $10^{-7}$ M   | $10^{-8}$ M   |
| Young (n=9)    | 23±7          | 76±8          | 104±6         |
| Old (n=9)      | 18±4          | 58±9          | 85±9          |
| Very old (n=6) | 19±6          | 75±8          | 100±5         |

Values are percent change in isometric force (mean±SEM). No values were significantly different in young, old, and very old rats.

Pressurized Donor Artery

In pressurized arteries preconstricted with phenylephrine, acetylcholine produced dilatation. Dilation in response to acetylcholine by the donor carotid artery tended to be lower in old rats and was significantly lower in very old rats (Table 1). Dilation in response to sodium nitroprusside was not significantly different in young, old, and very old rats (Table 1). Constriction in response to phenylephrine was not significantly different in young, old, and very old rats (not shown).

In the bioassay detector vessel paired with the pressurized carotid artery, responses to acetylcholine in the young, old, and very old rats were not significantly different (Table 2). Relaxation of the bioassay detector in response to sodium nitroprusside (Table 2) and contraction in response to phenylephrine (not shown) were not significantly different in the three groups of rats.

Discussion

The major finding of this study is that agonist-induced release of EDRF in a cascade bioassay model is similar in young, old, and very old rats. Responsiveness of vascular smooth muscle to nitroprusside was not altered by aging. In these experiments we found, as in earlier ones, that endothelium-dependent dilatation is impaired in the donor artery by aging. The findings suggest that impairment of endothelium-dependent dilatation by aging is not due to impaired release of EDRF.
measure the effects of pressure in canine donor and bioassay detector vessels; responses in the perfused donor artery were measured with stainless-steel stirrups inserted into the vessel wall to measure isometric force. In our system, diameter, not force, was measured in the pressurized perfused vessel.

Impaired endothelium-dependent relaxation in response to acetylcholine may be due to several mechanisms, including impaired synthesis or release of EDRF, impaired responsiveness of vascular smooth muscle to EDRF, or generation of oxygen-derived free radicals that inactivate EDRF. Using the cascade bioassay model, it is possible to study release of EDRF.

Release of EDRF

Several studies suggest that agonist-induced endothelium-dependent relaxation is impaired by aging. In this study, there was no difference between young, old, and very old rats in release of EDRF, as detected with a perfusion-cascade bioassay system. Sodium nitroprusside was administered to test responsiveness of vascular smooth muscle. Responsiveness of vascular muscle was not altered by aging. Thus, in the bioassay rings that detect release of EDRF, relaxation is not the result of impaired responsiveness of vascular muscle.

Mechanism of Impaired Vasodilatation

Responses of the donor artery in the present study confirmed previous findings that vasodilator responses to acetylcholine are impaired by aging. The important finding of this study is that the decrease in dilator responses in old rats is due to a decrease in release of EDRF, indicating that other mechanisms are involved.

In a recent study, we found that L-arginine, the precursor of EDRF, partially restores dilatation of the carotid artery in response to platelets, ADP, or acetylcholine in vessels from old animals. Improvement in dilatation may or may not indicate that there is a reversible deficiency in L-arginine, because responses may improve with excess generation of EDRF.

Impairment of endothelium-dependent vasodilatation with aging could also be due to production of EDCF, as has been found in large arteries and cerebral arterioles in chronically hypertensive rats, or to the presence of oxygen-derived free radicals. Because the concentration of superoxide dismutase appears to decrease in several different tissues with aging, susceptibility to superoxide anion may increase. Oxygen radicals can influence endothelium-dependent relaxation, and superoxide anion and hydrogen peroxide in particular can inactivate EDRF. Superoxide anion may also act as a direct vasoconstrictor in vascular smooth muscle under some conditions.

In a previous study we found that in old rats, superoxide dismutase and indomethacin increase dilator responses of the carotid artery to platelets, ADP, and acetylcholine. This finding suggests that a cyclooxygenase-dependent constrictor factor, either superoxide or a prostanoid or both, may contribute to inactivation of EDRF or may produce direct vasoconstriction in vascular muscle.

In conclusion, the major findings of this study are that, although in old rats endothelium-dependent dilatation is impaired in carotid arteries, intraluminal release of EDRF (as detected by bioassay) and responsiveness of vascular muscle are normal. We speculate, on the basis of findings in a previous study, that impairment of responses to acetylcholine may be related to release of EDCF or increased destruction of endothelium-derived relaxing factor that is released abuminally.

References

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