Extraluminally Applied Acetylcholine and Substance P on the Release of EDRF

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Abstract—Acetylcholine, substance P and nitroglycerin applied intra- and extraluminally to the perfused dog femoral artery segment with endothelium caused depressor responses. Endothelium denudation abolished the responses to acetylcholine and substance P. EC50 ratios of extra- versus intraluminal acetylcholine and substance P were 43 and 79, respectively, whereas those of nitroglycerin did not differ. Physostigmine potentiated the response to extraluminal acetylcholine. Acetylcholine seems to be degraded partly by cholinesterase in the arterial wall. Acetylcholine and substance P applied extraluminally are expected to reach the endothelium and release endothelium-derived relaxing factor.

Various agents, including acetylcholine and substance P, relax vascular smooth muscle by acting on the endothelium to liberate endothelium-derived relaxing factor (EDRF) (1). Vasodilatations via EDRF have been demonstrated in isolated blood vessels suspended in bathing media, in which the applied drugs act from both the intimal and adventitial sides, and also in in vivo experiments, in which drugs in the circulating blood are easily accessible to the endothelium. However, the data so far obtained as to whether perivascularly liberated substances, such as acetylcholine from cholinergic nerves innervating outside the adventitia-medial border (2), can cross the media and reach the endothelium are still controversial (3, 4). Therefore, the present study was undertaken to compare responses to intra- and extraluminally applied acetylcholine and substance P of the perfused dog femoral artery and to determine whether cholinesterase present in the arterial wall modifies the response to acetylcholine.

Mongrel dogs of either sex, weighing 7 to 14 kg, were anesthetized with sodium pentobarbital and killed by bleeding from the carotid arteries. Proximal portions of the femoral artery were isolated. The femoral artery segment with intact endothelium (approximately 20 mm long) was immersed in the modified Ringer-Locke solution (5) aerated with 95% O2 and 5% CO2 and maintained at 37±0.5°C. The segment was perfused with the aerated solution including 10−6 M indomethacin at a constant rate of 1 ml/min to maintain a perfusion pressure of 10–15 mmHg, which was raised by 30–40 mmHg following continuous infusions of norepinephrine. The perfusion pressure was measured by means of a pressure transducer placed in the upstream portion of the artery segments. Acetylcholine or other drugs were applied either to the perfusion fluid or the bathing medium. Drug-free solutions were perfused at the end of the experiment to restore the maximal vasodilatation.

The results shown in the text and figures are expressed as mean values±S.E.M. Statistical analyses were made using Student’s paired and unpaired t-test. Drugs used were acetylcholine chloride (Daichi Pharmaceutical Co., Tokyo), dl-norepinephrine hydrochloride (Sankyo Co., Tokyo), substance P (Peptide Research Foundation, Minoh, Japan), nitroglycerin (Nihon Kayaku Co., Tokyo) and physostigmine (Sigma, St. Louis, MO, U.S.A.).
Acetylcholine in concentrations ranging from $10^{-9}$ to $10^{-6}$ M applied to the femoral artery segment intra- and extraluminally caused a concentration-related depression of perfusion pressure (Fig. 1, left). The concentration of extraluminal acetylcholine equipotent to the median effective concentration (EC50) of intraluminal acetylcholine ($[2.35 \pm 0.47] \times 10^{-8}$ M) was estimated to be $1.0 \times 10^{-6}$ M on the basis of the concentration-response curves for acetylcholine. The responses to intra- and extraluminal acetylcholine were abolished by removal of the endothelium and by intra- and extraluminal application of $10^{-7}$ M atropine. Intra- and extraluminal treatment with $10^{-7}$ physostigmine moderately potentiated the response of the artery segment to extraluminal acetylcholine, but did not alter the response to intraluminal acetylcholine (Fig. 1, right). The equipotent concentration of intraluminal acetylcholine to $10^{-6}$ M extraluminal acetylcholine ($6.1 \times 10^{-8}$ M) was increased by physostigmine to $7.5 \times 10^{-8}$ M. Increase in the concentration of the enzyme inhibitor to $10^{-6}$ M did not cause an additional potentiation.

Substance P and nitroglycerin applied intra- and extraluminally also produced a concentration-related decrease in perfusion pressure. The responses to the former drug were abolished by endothelium denudation, whereas those to the latter were unaffected. The responses are quantitatively compared in Fig. 2. From these curves, the equipotent concentration of extraluminal substance P to the EC50 of intraluminal substance P ($[1.51 \pm 0.40] \times 10^{-10}$ M) was estimated to be $1.2 \times 10^{-8}$ M. Responses to intra- and extraluminal

![Fig. 1. Concentration-depressor response curves for acetylcholine applied intra- and extraluminally in the isolated, perfused femoral artery segment with endothelium (left figure) and modification of the response by intra- and extraluminal treatment with $10^{-6}$ physostigmine (Physo., right). Perfusion pressure was raised by infusions of norepinephrine. Depressor responses were expressed as percent of the norepinephrine-induced pressor response. Mean values in 19 (left figure) and 8 and 3 (extra- and intraluminal, respectively, in the right figure) experiments are presented. aSignificantly different from values obtained by intraluminal application. P < 0.001. bSignificantly different from values obtained by extraluminal application without physostigmine. P < 0.01. Vertical bars represent S.E.M. Conc., concentration.](image-url)
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**DOG FEMORAL ARTERY**

![Concentration-depressor response curves for substance P (left figure) and nitroglycerin (right) applied intra- and extraluminally in the isolated, perfused femoral artery segment with endothelium.](image)

**Fig. 2.** Concentration-depressor response curves for substance P (left figure) and nitroglycerin (right) applied intra- and extraluminally in the isolated, perfused femoral artery segment with endothelium. Perfusion pressure was raised by infusions of norepinephrine. Depressor responses were expressed as percent of the norepinephrine-induced pressor response. Mean values in 7 (left) and 7 (right) experiments are presented. *a*Significantly different from values obtained by intraluminal application, *P*<0.001. Vertical bars represent S.E.M. Conc., concentration.

Nitroglycerin did not significantly differ; the EC50 values were [1.03±0.26] and [0.87±0.09]×10\(^{-7}\) M, respectively.

Acetylcholine applied intra- and extraluminally to isolated dog femoral artery segments produced a depression of perfusion pressure, which was abolished by endothelium denudation and by treatment with atropine. Substance P and nitroglycerin also produced the depressor response. Potency ratios of extraversus intraluminal acetylcholine and substance P were 43 and 79, respectively, whereas the potency of nitroglycerin applied intra- and extraluminally did not differ. Effects of nitroglycerin are not dependent on endothelium (6), and this drug is expected to act directly on smooth muscle from the adventitial and intimal sides. Similar results with acetylcholine were also observed by Toda et al. (4) with an EDRF bioassay method by the use of dog femoral artery segments with endothelium (donor tissue) and coronary artery strips without endothelium (assay tissue). These findings suggest that acetylcholine and substance P applied extraluminally are translocated to the endothelium and release EDRF, as do these substances applied intraluminally. Increase in blood flow in skeletal muscle caused by stimulation of sympathetic cholinergic nerves (7) may be explained by such an action of perivascularly-released acetylcholine, especially on resistance vessels.

The reason for the low susceptibility of artery segments to acetylcholine and substance P applied perivascularly may be associated with a rapid decline in their concentrations due to metabolizing enzymes present in the arterial wall. In our perfused preparations, potentiation by the cholinesterase inhibition with physostigmine of the response to extraluminal acetylcholine was significant but not so evident; therefore, a marked difference was still noted between
the potencies of intra- and extraluminal acetylcholine in the presence of cholinesterase blockade. It appears that cholinesterase in the arterial wall participates partly in the degradation of acetylcholine on the way to the endothelium; however, other mechanisms are also involved.

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