Nitroarginine Inhibits Endothelium-Derived Relaxation

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Abstract—Effects of N\textsuperscript{G}nitro-L-arginine (NO\textsubscript{2}Arg), a
guanidinonitro arginine derivative, on acetylcholine-
duced relaxation of rabbit thoracic aorta ring
preparation were studied. Relaxation by acetylcholine
was inhibited by NO\textsubscript{2}Arg dose-dependently and the maximum
relaxation with 10\textsuperscript{-6} M NO\textsubscript{2}Arg was reduced to 10.1±4.3%
(n=5). L-arginine (10\textsuperscript{-6} M) did not affect the
acetylcholine-induced relaxation. Relaxation by
glyceryl trinitrate or papaverine was not affected by
NO\textsubscript{2}Arg. These results indicate that NO\textsubscript{2}Arg is a novel
inhibitor of endothelium-derived relaxation.

Acetylcholine (ACh)-induced relaxation of arteries is endo-
thelium-dependent and it is mediated through the release of
endothelium-derived relaxing factor(s) (EDRFs)(1). Subsequent
studies suggested that at least one of EDRFs was nitric oxide
(NO) (2, 3) and it was formed from the terminal guanidino
nitrogen atom of L-arginine (4, 5). Schmidt et al. (4) reported
that EDRF dependent relaxation was decreased by L-canavanine, a
guanidinooxy structural analogue of L-arginine. Palmer et al.
(5) reported a guanidinomethyl arginine derivative, N\textsuperscript{\alpha}monomethyl-
L-arginine, reduced ACh-induced relaxation. In the present
study, effects of N\textsuperscript{G}nitro-L-arginine (NO\textsubscript{2}Arg), a guanidinonitro
derivative, on ACh-induced relaxation of rabbit thoracic aorta
were investigated.

Rabbits of either sex (2.0-2.3 kg) were exsanguinated under
pentobarbital sodium anesthesia (30 mg/kg, i.v.). Ring strips
of thoracic aorta (2-3 mm width) were fixed vertically under a
resting tension of 2 g in a 20 ml organ-bath containing a Krebs-
Henseleit buffer solution of the following composition (mM): NaCl
118.4; KCl 4.7; CaCl\textsubscript{2} 2.2; KH\textsubscript{2}PO\textsubscript{4} 1.2; MgSO\textsubscript{4} 1.2; NaHCO\textsubscript{3} 25.0;
and Dextrose 5.6. The solution was maintained at 37°C and
aerated with a gas mixture of 95% O\textsubscript{2} and 5% CO\textsubscript{2}. The strips
were allowed to equilibrate for 90 min before the start of
experiments. Tension change was measured isometrically using a
force-displacement transducer (Nihonkoden Kogyo Co. Ltd., Tokyo).
The preparations were contracted with 10 \textmu M prostaglandin F\textsubscript{2}\alpha and
ACh or glyceryl trinitrate (GTN) was added cumulatively. The
maximum relaxations by ACh or GTN in these first responses were
taken as a 100%. Then, the strips were incubated with or
without NO\textsubscript{2}Arg (Peptide Institute Inc., Minoo, Japan) or
L-arginine for one hour and the second series of vasodilatory
responses by ACh or GTN were obtained. Papaverine (100 \textmu M) was
added finally to obtain the maximum relaxation.

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Vasodilatory responses of rabbit thoracic aorta to acetylcholine (ACh; a, b and c) and glyceryl trinitrate (GTN; d). The ring preparation had been contracted with 10 μM prostaglandin F$_2$α (arrow). Concentrations of ACh and GTN were designated in terms of negative log M. 10 μM N$^2$-nitro-L-arginine (NO$_2$Arg; b and d) or 10 μM L-arginine (L-Arg; c) was added one hour before the experiment. 100 μM papaverine was added to obtain the maximum relaxation (arrow head).

The relative relaxation by ACh or GTN was compared by Student's $t$-test.

Dose-dependent relaxation by ACh (1 nM–3 μM) was shown and the maximum relaxation was obtained at 1 to 3 μM (Fig. 1a). The responses were not different between the first and the second cumulative addition of ACh and the maximum relaxation by ACh at the second responses without NO$_2$Arg or L-arginine were 87.4±10.4% (n=5). NO$_2$Arg (1 μM–10 μM) inhibited ACh-induced relaxation dose-dependently and the maximum relaxation with 10 μM NO$_2$Arg was 10.1±4.3% (n=5, Fig. 1b). Lower doses of NO$_2$Arg inhibited ACh-induced relaxation in non-competitive manner. The inhibition was irreversible after several washings in one hour. L-arginine (10 μM) showed no significant effect on ACh-induced relaxation (Fig. 1c). Dose-dependent relaxation by GTN (1 nM–1 μM) was shown and NO$_2$Arg (10 μM) did not affect the relaxation by GTN (Fig. 1d) or papaverine.

NO$_2$Arg (10 μM) did not inhibit negative inotropic effects of ACh in rat atrium preparation (Y. Kobayashi et al., unpublished observation). This result suggests that NO$_2$Arg does not affect on muscarinic receptors. The present results indicated that NO$_2$Arg was a novel inhibitor of EDRF-dependent relaxation, but not of EDRF-independent relaxation by GTN. Schmidt et al. (4) reported that the ATP-induced relaxation of rat thoracic aorta was decreased by 2 mM of L-canavanine. Palmer et al. (5) observed that the ACh-induced relaxation of rabbit thoracic aorta was reduced to half by 30 μM of N$^2$-monomethyl-L-arginine. These results support the suggestion that NO is the EDRF and suggest
that such arginine derivatives, which guanidino-group is modified, may inhibit the formation of NO from the terminal guanidino nitrogen atom of L-arginine. The inhibitory effect of NO$_2$Arg seemed to be more potent than those of L-canavanine and N$^\text{N}$-monomethyl-L-arginine.

ACh-induced relaxation in rabbit thoracic aorta was reduced to 10% by 10 μM NO$_2$Arg, suggesting that EDRF(s) released by ACh in this preparation was mainly NO$_2$Arg sensitive. Kelm et al. (6) reported that NO is solely responsible for EDRF using cultured endothelial cells. The present results seem to agree with their results.

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References


