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Difference in acetylcholine-induced nitric oxide release of swine lingual and pulmonary artery

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ABSTRACT

Nitric oxide plays an important role in the regulation of vascular tone, however the mechanism(s) of endothelium-dependent vasodilator-induced relaxation in oral and maxillofacial arteries have not yet been well characterized. This study compared the lingual artery with the pulmonary artery. Changes in the isometric tension and the nitric oxide concentration were measured with a nitric oxide-specific microelectrode in swine lingual arteries and swine pulmonary arteries that were contracted via perfusion with 5 μM noradrenaline. When a stable constricted plateau was reached with 5 μM noradrenaline, 5 μM noradrenaline containing acetylcholine was perfused, or when a stable constricted plateau was reached with 5 μM noradrenaline containing 100 nM Nw-Nitro-L-arginine methyl ester hydrochloride, 5 μM noradrenaline containing 100 nM Nw-Nitro-L-arginine methyl ester hydrochloride and acetylcholine was perfused. 30 μM acetylcholine inhibited the contractions induced by noradrenaline; maximal relaxations were 50.8 ± 5.6 % in the lingual artery, and 52.6 ± 8.2 % in the pulmonary artery. 30 μM acetylcholine induced endothelium-dependent simultaneous increases in nitric oxide concentration of 114.4 ± 17.7 nM in the lingual artery, and 37.3 ± 6.1 nM in the pulmonary artery. However, in the presence of L-NAME (100 nM) and contracted with 5 μM NA, ACh-induced relaxation and increase in NO concentration were almost abolished. While the NO continued to be produced by ACh, a sustained decrease occurred in isometric tension and L-NAME inhibited the ACh-induced relaxation, thus suggesting that NO might play an important role in relaxation.

KEYWORDS : relaxation; nitric oxide concentration; acetylcholine; smooth muscle artery

INTRODUCTION

The vascular endothelium plays an important role in the regulation of vascular tone via endothelium-derived relaxing factors (EDRFs) such as nitric oxide (NO) (1). A direct relationship between endothelium-derived NO and endothelium-dependent vasodilator-induced relaxation can be investigated using NO-selective electrodes (2-4).

NO is a powerful endothelium-dependent pulmonary vasodilator that is used clinically to attenuate severe pulmonary hypertension (5). The pulmonary circulation is markedly different from the systemic circulation, as the pulmonary artery is subject to low pressure. Under hypoxic conditions, the pulmonary circulation matches its perfusion to ventilation by increasing the tone of resistant vessels of hypoxic alveoli (6,7). In addition, pulmonary vasoconstriction plays a central role in pulmonary arterial hypertension (8).

Among the craniofacial arteries, the lingual artery is relatively unexplored, even though it is a prominent artery that nourishes the tongue, a pivotal organ for oral functions such as tasting, mastication, swallowing, and speech. The lingual artery is a part of systemic circulation. It should be more like a systemic artery with high blood pressure and high oxygen-contained blood in the artery. Characterization of the contraction of lingual artery will reveal its features both common to and distinct from those of other vascular beds (9).

Hence the relaxation and the NO release between the lingual artery and pulmonary were compared, as the lingual artery is a part of systemic circulation and the pulmonary artery is different from the systemic artery as to the pressure and the oxygen.

In the present study, we investigated a direct relationship between endothelium-derived NO and acetylcholine (ACh)-induced relaxation by the use of a NO-selective electrode introduced into the lumen of mounted swine lingual and pulmonary arterial rings. Simultaneous measurements of relaxation and NO concentration were performed in swine lingual and pulmonary artery.

MJETHODS

This study was approved by the Institutional Review Committee on the Ethics of Animal Experiments of Iwate Medical University (approval number 26-010). All experiments were conducted in accordance with the Institutional Animals Care and Use Committee guidelines.

Reagents and solutions

$_{L}$-NA bitartrate monohydrate (NA) was purchased from Alexis Corporation (Lausen, Switzerland), acetylcholine chloride (ACh) from Wako Pure Chemical Industries (Osaka, Japan), N_w-Nitro-L-arginine methyl ester hydrochloride minimum 98 % TLC (L-NAME) from Sigma-Aldrich (St Louis, USA). The other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). In all experiments, air-equilibrated Hanks' balanced salt solution (HBSS; kawaguchi) was used to keep arteries under resting conditions. HBSS consisted of 137 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 1.26 mM $CaCl_2$, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, and 5.55 mM glucose (pH 7.34). All other salt solutions (SS) used as perfusate were formulated by modifying HBSS. SS containing 100 mM KCl (100KSS) was prepared by substituting the respective concentration of KCl for the equivalent concentration NaCl in HBSS. Each SS containing NA, ACh, and L-NAME (alone or in combination) was prepared by adding the component agents into the respective SS base immediately before use.

Preparation of artery rings

Fresh swine tongues and lungs were obtained at a local abattoir. A segment of the lingual artery in the proximal region of the tongue, and a third of the pulmonary artery were dissected out. After adventitia was removed, lingual artery segments about 2 mm in diameter, and pulmonary artery segments about 2–3 mm in diameter, were cut into 3-mm long rings. The artery rings were kept in HBSS at 5 °C until used for measurements.

Calibration of NO-sensitive electrodes

The microelectrode (ISONOP30, World Precision Instruments) used was constructed from carbon fiber with a polymer coating. Calibration of the coated carbon electrode was carried out with S-nitroso-N-acetyl-D, L-penicillamine (SNAP) solution. When the background current became stable, three aliquots of the SNAP solution, 5 μl (50 nM), 10 μl (100 nM), and 20 μl (200 nM), were injected into the vial containing cupric chloride solution with 100 % oxygen bubbled. The calibration curve was constructed simply by plotting the signal output (in pA) vs. the concentration of SNAP added at that time. The ISO-NO meter could be calibrated to display data in either concentration

mode (nM) or redox current (pA).

Simultaneous measurements of isometric tension and NO concentration

An artery ring was held by two tungsten needles in a perfusion chamber (volume of perfusate was 3 ml). One needle was fastened to a displacement transducer (Type UL-2GR, Minebea Co., Fujisawa, Japan), and the other to a micromanipulator. The solution was bubbled with a mixture of 95 % O_2 and 5 % CO_2, held at a temperature of 37 °C, and flowed at a rate of 1.6 ml/min using a peristaltic pump (SMP-23, Tokyo Rikakikai Co., Tokyo, Japan). Because the strength of contraction did not change when the resting tone was 3–7 mN, artery rings were extended to give a resting tone of about 4 mN and immediately tested for contractility with two 2.5-min perfusions with 100 KSS separated by a 10-min HBSS perfusion.

The NO-sensitive microelectrode (L-type ISONOP30) was inserted after calibration into the arterial lumen and placed close to the endothelial surface by touching the vessel lumen until the force increased 0.1 mN, and then retouching the electrode until the force decreased 0 mN by means of the micromanipulator. The NO electrode was connected to an amplifier (APPOLO 4000 free radical analyzer, World Precision Instruments), and the amplified signal was registered on a recorder permitting simultaneous measurement of contraction and NO concentration. Then after a 30-min HBSS perfusion, artery rings were perfused with 5 μM NA. When a stable constricted plateau was reached with 5 μM NA, 5 μM NA containing ACh (30 μM) was perfused, or when a stable constricted plateau was reached with 5 μM NA containing L-NAME (100 μM), 5 μM NA containing L-NAME (100 μM) and ACh (30 μM) was perfused. Each solution was perfused for 12 min, and HBSS perfusion was always done after all drug perfusion was completed. Isometric tension was measured with the displacement transducer, and signals detected were amplified with a carrier amplifier (CSD-815 Digital indicator, Minebea Co., Fujisawa, Japan) and recorded with a Powerlab 16/30T data acquisition system (ADInstruments, Bella Vista, Australia). The isometric tension during an experiment was normalized to the isometric tension immediately before ACh perfusion, and expressed as a percentage.

Statistical analysis

Relaxation was determined by measuring the cumulative reduction in induced tone in the arterial segments, and was expressed as the percentage of the contraction induced by 5 μM NA. A value of 0 % indicated the initial resting tension (5 mN), and a value of 100 % indicated the isometric tension generated by exposure to 5 μM NA. Values smaller than 100 % indicated that vasodilation had occurred in response to ACh. Values are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS), version 11.0 (SPSS, Chicago, Illinois, USA). The Shapiro–Wilk test was used for normality, and Bartlett's test was used for homogeneity of variance. Repeat measure analysis of variance (ANOVA) was performed, followed by Bonferroni's post-test. Differences were considered significant at $p < .05$.

Results

Effects of ACh on contractile force in the lingual and pulmonary artery with NA

ACh relaxed both lingual and pulmonary arteries in a concentration-dependent manner (Fig. 1).

Effects of ACh and L-NAME on contractile force in the lingual and pulmonary artery with NA

In NA-contracted preparation, the NO sensor in swine lingual and pulmonary arterial rings detected that ACh-induced relaxation was associated with NO release. However, in the presence of L-NAME (100 nM) and contracted with 5 μM NA, ACh-induced relaxation and increase in NO concentration were almost abolished (Fig. 2, 3).

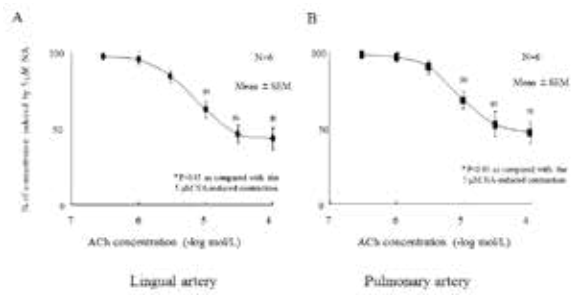

Fig.1 Acetylcholine dose effect on lingual and pulmonary vasorelaxation

(a) Lingual artery (n = 6)
(b) Pulmonary artery (n = 6)

The arteries are constricted with noradrenaline and relaxations are expressed as percentage of initial constriction level. Data are expressed as mean ± SEM.

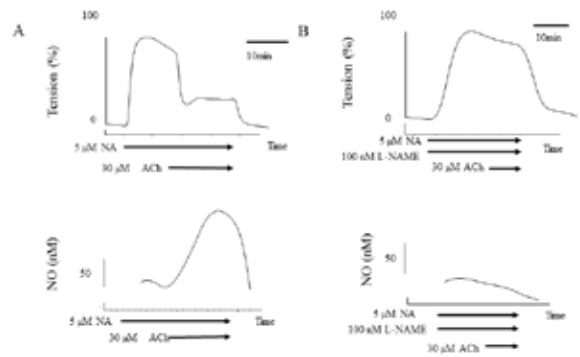

Fig. 2 Changes in tension and nitric oxide (NO) in lingual arteries

Simultaneous measurements of isometric tension (upper traces) and NO concentration (lower traces) in endothelium-intact rings of swine lingual arteries contracted with 5 μM NA and relaxed with either 30 μM acetylcholine (Ach) (A), or 30 μM ACh containing 100 μM L-NAME. The traces are representative of eight experiments.

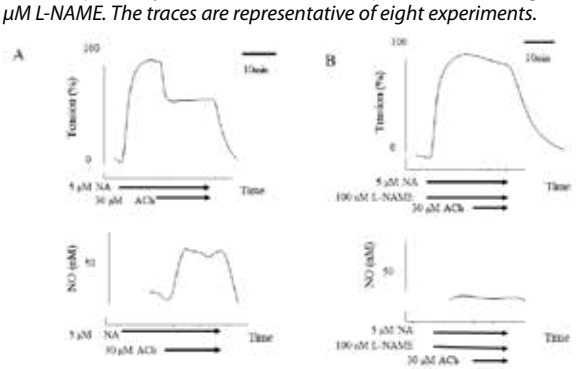

Fig. 3 Changes in tension and nitric oxide (NO) in pulmonary arteries

Simultaneous measurements of isometric tension (upper traces) and NO concentration (lower traces) in endothelium-intact rings of swine pulmonary arteries contracted with 5 μM NA and relaxed with either 30 μM acetylcholine (Ach) (A), or 30 μM ACh containing 100 μM L-NAME. The traces are representative of eight experiments.

Time relationship for simultaneously measured relaxation

In NA-contracted preparation, stimulation of the lingual arterial rings with ACh induced a sustained decrease in isometric tension; the minimum isometric tension measured was 50.8 ± 5.6 % (Fig. 4A and Table 1). Stimulation of the pulmonary arterial rings with ACh also induced a sustained decrease in isometric tension; the minimum isometric tension measured was 52.6 ± 8.2 % (Fig. 5A and Table 1).

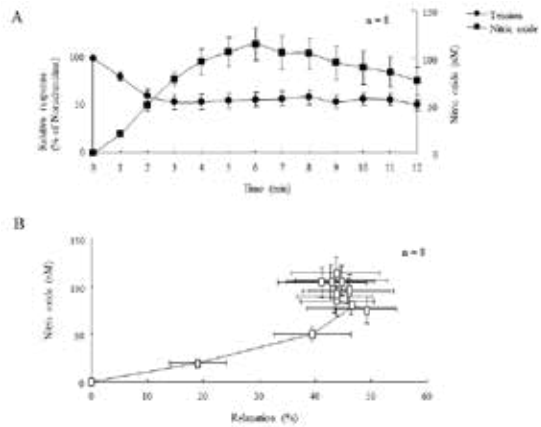

Fig. 4 Time relationship for simultaneously measured artery relaxations and nitric oxide (NO) concentration in the lingual artery

A: average of simultaneously obtained tension (filled circle) and NO concentration (filled square) curves for acetylcholine (30 μM) in the segment of the lingual artery contracted with noradrenaline (5 μM).

B: the same results as A with relaxation plotted against NO concentration.

The results are means of eight experiments, with horizontal and vertical bars representing SEM.

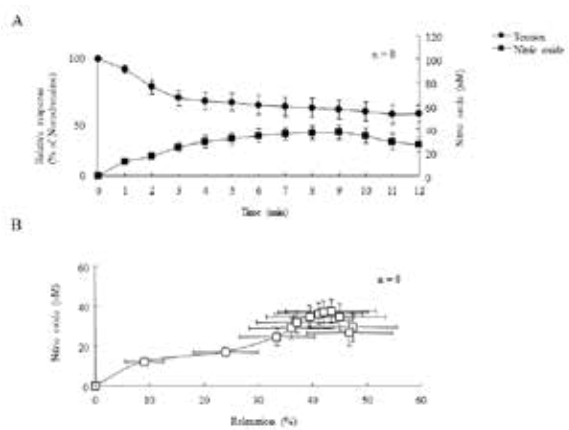

Fig. 5 Time relationship for simultaneously measured relaxations and nitric oxide (NO) concentration in pulmonary arteries

A: average of simultaneously obtained tension (filled circle) and NO concentration (filled square) curves for acetylcholine (30 μM) in the segment of the pulmonary artery contracted with noradrenaline (5 μM).

B: the same results as A with relaxation plotted against NO concentration.

The results are means of eight experiments, with horizontal and vertical bars representing SEM.

Time-concentration relationship of NO

Stimulation of the lingual arterial rings with ACh induced an increase in NO concentration at 6 min, followed by a sustained decrease that lasted for 6 min; the maximum concentration of NO was 114.4 ± 17.7 nM (Fig. 4A, B and Table 2). Stimulation of the pulmonary arterial rings with ACh induced an increase in NO concentration at 9 min, followed by a sustained decrease that lasted for 3 min; the maximum concentration of NO was 37.3 ± 6.1 nM (Fig. 5A, B and Table 2).

Discussion

The present study made three important discoveries: the decrease in isometric tension induced by ACh is most closely linked to the production of NO, there is a time-associated increase in NO concentration and relaxation, and L-NAME inhibits the relaxations and increases in NO concentration induced by ACh.

Decrease in isometric tension induced by ACh is most closely linked to NO production

The NO concentration in the vessel lumen was measured with the ISONOP30. This microelectrode is constructed from a carbon fiber with a polymer coating, giving selectivity and reference electrodes that have been combined within a Faraday shield designed to minimize susceptibility to environmental noise. The electrode has high selectivity for NO and a detection sensitivity of 1 nM (10). This present study revealed that the maximum concentration of NO was 114.4 ± 17.7 nM in the lingual arterial rings and 37.3 ± 6.1 nM in the pulmonary arterial rings, and so the NO concentrations were in the nanomolar (nM) range. A previous study measured NO concentration with a microelectrode (ISONP30), and reported that ACh (10 μM) induced an endothelium-dependent increased in lumen NO concentration of 21 ± 6 nM in isolated rat superior mesenteric artery contracted with 1 μM NA (2). In isolated rabbit carotid artery contracted with phenylephrine, the NO concentration rose with increasing concentrations of ACh, and reached a maximum (above 240 nM) at an ACh concentration of 3×10^{-5} M (11). In the presence of indomethacin, the peak concentration of NO usually appeared within 2.0–2.5 min after the addition of bradykinin (BK) (10^{-7} M), and reached 105.0 ± 19.6 nM (3). In isolated porcine coronary artery and cardiac vein, the maximum concentration of NO after the addition of BK (10^{-7} M) was 237.1 ± 27.2 nM in artery and 135.6 ± 14.5 nM in vein (4). Pulmonary artery is different from the systemic artery as to the pressure and the oxygen content, and therefore it is possibility that the NO release may be different from systemic artery, lingual artery. In these previous reports and our study, the NO concentrations measured in response to the endothelium-dependent vasodilators were in the low nanomolar ranges (12-14). However, it has also been reported that endothelium-dependent vasodilators are associated with much higher NO concentrations (15,16). This may be due to differences with respect to preparation examined, species, and conditions (for example, high or low temperature compared with physiological temperature) under which the experiments were performed; the different structure of the porphyrinic microelectrode used and the ISONOP30 (with a polymer coating) microelectrode applied could also have influenced the measured NO concentration (2). There were differences between the concentration and the style of ACh-induced NO release, which was shown by the time-concentration curves of NO release in lingual arteries and those in pulmonary arteries; however, we do not have enough data to determine why the differences between lingual artery and pulmonary artery exist. Nevertheless, in our study as well as previous studies the nanomolar increases in NO concentration observed with ACh were sustained for a long period; that is, the nanomolar concentrations of endogenously released NO could initiate and maintain the ACh-induced relaxation of the swine lingual and pulmonary artery.

Time-associated increase in NO concentration and relaxation

The stimulation of the endothelium of the lingual artery with ACh evoked a rapid rise in NO concentration followed by a sustained elevation that lasted for 6 min. In contrast, stimulation of the pulmonary artery with ACh evoked a slow rise in NO concentration followed by a sustained elevation that lasted for 3 min. The concentration of NO reached a maximum of 114.4 ± 17.7 nM after 6 min and the relaxation reached a maximum of 50.8 ± 5.6 % after 12 min in the lingual artery, and in the pulmonary artery the NO concentration was 37.3 ± 6.1 nM after 9 min and the relaxation was 52.6 ± 8.2 % after 11 min. It was previously reported that the concentration of NO reached a maximum of 22 ± 9 nM and a simultaneous reduction of vascular tone averaging 87 ± 87.1% after 2 min, and the measured NO concentration after 10 min had decreased to 15 ± 4 nM and the relaxation was unchanged (88 ± 7 %) in the superior mesenteric artery of the rat (2). Stimulation of the endothelium of the coronary circumflex arteries and middle cardiac veins with BK evoked a rapid rise in NO concentration followed by a sustained elevation that lasted for 7 to 14 min; the concentration of NO reached a maximum after 3 min in the coronary artery and after 2 min in the cardiac vein, and the duration of BK-in-

duced NO release was 11.2 ± 0.5 min in the coronary artery and 7.3 ± 1.0 min in the cardiac vein (4). Stimulation of the endothelium of the pulmonary artery and pulmonary vein with BK evoked a rapid rise in NO concentration followed by a sustained elevation that lasted for 11 to 16 min; the concentration of NO reached a maximum after 4 min in the pulmonary artery and after 2 min in the pulmonary vein, and the duration of BK-induced NO release was 14.3 ± 1.3 min in the pulmonary artery and 12.1 ± 0.8 min in the pulmonary vein (6). In these previous studies, concentrations of NO release were measured within 12 to 14 min. Therefore, we determined that ACh was perfused to the organ chamber in this time, and the concentrations of NO were measured within 12 min in this study. The potential reason that time-concentration curves of NO release in the current study show a different pattern compared with these previous studies may be that each segment in the organ chamber was perfused with ACh in this study, but not added to the organ chamber with ACh.

L-NAME inhibited the relaxation and increase in NO concentration induced by ACh

When arterial rings reached a stable constricted plateau with 5 μM NA, 5 μM NA containing ACh (30 μM) was perfused. Alternatively, when reaching a stable constricted plateau with 5 μM NA containing L-NAME (100 nM), 5 μM NA containing L-NAME (100 nM) and ACh (30 μM) was perfused. An inhibitor of NO synthase, L-NAME inhibited the relaxation and increases in NO concentration induced by ACh. These results are in agreement in the swine lingual and pulmonary artery showing that inhibition of the NO-$_{L}$-arginine pathway almost abolishes the relaxations induced by Ach.

Conclusions

The present study demonstrated that the endogenous NO concentration correlates with relaxation of the swine lingual and pulmonary artery when these are stimulated with the endothelium-dependent vasodilator ACh. While the NO continued to be produced by ACh, a sustained decrease occurred in isometric tension and L-NAME inhibited the ACh-induced relaxation, thus suggesting that NO might play an important role in relaxation. The decrease in isometric tension induced by ACh is most closely linked to the production of NO.

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