

Short Communication

Biphasic Tracheal Relaxation Induced by Higenamine and Nantenine From *Nandina domestica* THUNBERGTakuro Ueki^{1,2}, Tatsuhiro Akaishi¹, Hidenobu Okumura², Tsuneo Morioka², and Kazuho Abe^{1,*}¹Laboratory of Pharmacology, Faculty of Pharmacy and Research Institute of Pharmaceutical Sciences, Musashino University, Tokyo 202-8585, Japan²TOKIWA Pharmaceutical Co., Ltd., 3-5-12 Azuchimachi, Chuo-ku, Osaka 541-0052, Japan

Received October 1, 2010; Accepted December 22, 2010

Abstract. We compared the effects of the extract from fruits of *Nandina domestica* THUNBERG (NDE) and its constituents, higenamine and nantenine, on contractile responses in isolated guinea-pig trachea. NDE (1 mg/ml) caused biphasic relaxation of the trachea precontracted with high-K⁺ stimulation: the fast component was blocked by propranolol and mimicked by higenamine; and the slow was resistant to propranolol and mimicked by nantenine. Ca²⁺-induced contraction under high-K⁺ stimulation was antagonized by nantenine or NDE + propranolol. These results suggest that NDE relaxes the trachea quickly through β -adrenoceptor stimulation by higenamine and slowly through Ca²⁺ antagonism by nantenine.

Keywords: *Nandina domestica* THUNBERG, tracheal relaxation, higenamine

The fruits of *Nandina domestica* THUNBERG (ND, Berberidaceae), called “nantenjitsu” in Japan, or the crude extract of ND (NDE) has been used for the treatment of respiratory diseases such as asthma, whooping cough, and pharynx tumor for many years in Japan (1). However, very little is known about the mechanism underlying the beneficial effects of NDE.

We have recently found that NDE inhibits histamine- and serotonin-induced contraction of isolated guinea-pig trachea (2, 3). The NDE-induced tracheal relaxation may lead to an improvement of breathing difficulty. Furthermore, we have identified higenamine as a major active constituent of NDE in relaxing guinea-pig trachea precontracted with histamine (3). Higenamine is a tetrahydroisoquinoline alkaloid originally isolated from the roots of *Aconitum japonicum* THUNBERG (4), and has been reported to show several pharmacological activities, including positive chronotropic and inotropic effects in the heart (5, 6), inhibitory effect on nitric oxide production (7), and anti-thrombotic effect (8). Another major alkaloid isolated from NDE is nantenine (1), which has been reported to inhibit serotonin-induced contraction in rabbit aorta or rat stomach (9), inhibit phenylephrine-induced

contraction in rat aorta (10), and exert Ca²⁺-antagonistic effects in several rat tissues (11 – 13).

In the present study, in order to elucidate how the two major constituents of NDE, higenamine and nantenine, contribute to the tracheal relaxing effect of NDE, we compared the effects of NDE, higenamine, and nantenine on smooth muscle contraction in isolated guinea-pig tracheal ring preparations. High-K⁺ stimulation was used to ask if NDE directly relaxes tracheal smooth muscles irrespective of neurotransmitter receptors.

Fruits of ND were collected in Japan and China, in December 2003, and identified by Hiroshi Okumura, the head researcher, Department of Chemical Analysis, Tokiwa Pharmaceutical Co., Ltd. (Osaka). A voucher specimen (ND129) has been deposited at the same company. NDE was manufactured through a conventional process by Tokiwa Pharmaceutical Co., Ltd., Noevir Group (Osaka) as described in our previous paper (2, 3). Briefly, the dried fruits of ND were extracted with 10 parts of hot water (90°C – 100°C) for 1 h, and the solution was spray-dried to give NDE. The yield of NDE was 25.0%. The contents of higenamine and nantenine in NDE were determined with a high performance liquid chromatograph (HPLC)–mass spectrometer (MS) and a gas chromatograph–MS, respectively, as in our previous paper (3). Synthetic higenamine hydrochloride was purchased from Sequioa Research Products, Ltd.

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Published online in J-STAGE on January 26, 2011 (in advance)
doi: 10.1254/jphs.10251SC

(Pangbourne, UK). Nantenine was purchased from Matsuura Yakugyo Co., Ltd. (Nagoya). Indomethacin was from Sigma Chemical (St. Louis, MO, USA). Other chemicals were from Wako Pure Chemical Industries, Ltd. (Osaka).

All pharmacological experiments were performed in accordance with the "Guiding Principles for the Care and Use of Laboratory Animals" approved by The Japanese Pharmacological Society. Contractile responses of isolated guinea-pig trachea were measured as described in our previous paper (2). Briefly, the trachea was isolated from 6–9-week-old male Hartley guinea pigs (Tokyo Laboratory Animals Science, Tokyo), weighing 350–550 g, and immersed in Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 10 mM glucose) gassed with 95% O₂ / 5% CO₂ (pH 7.4). Each tracheal ring preparation (2–3-mm-wide) was mounted at a resting tension of 5 mN in a 5-ml organ bath (UC-5TD; UFER Medical Instrument, Kyoto), which contained Krebs-Henseleit solution maintained at 32°C and gassed with 95% O₂ / 5% CO₂. The isometric tension was measured with a force displacement transducer. The trachea was contracted by changing the bathing medium to high-K⁺ Krebs-Henseleit solution containing 62.7 mM NaCl,

60 mM KCl, atropine, and indomethacin. Atropine and indomethacin were added in order to abolish the basal, tonic contraction possibly produced by endogenous acetylcholine or arachidonate metabolites. After high-K⁺-induced contraction reached a steady state, NDE, higenamine, or nantenine was added in a cumulative manner to obtain its concentration–effect curve or at a single concentration to analyze the time-course of relaxation. To determine the maximal relaxation in each tissue, papaverine (3×10^{-4} M) as a positive control was added at the end of all experiments.

Cumulative addition of NDE (0.1–1 mg/ml) caused a concentration-dependent relaxation of guinea-pig trachea precontracted with high-K⁺ stimulation (Fig. 1A). Chromatographic analysis showed that 1 g NDE contained 216 nmol (0.0585 mg, 0.00585%) higenamine (Fig. 2) and 20 μ mol (6.7 mg, 0.67%) nantenine. Therefore, the effect of NDE at 0.01–1 mg/ml was compared with the effects of higenamine at 2.16–216 nM and nantenine at 0.2–20 μ M. Cumulative addition of higenamine (21.6–216 nM) caused a concentration-dependent relaxation of high-K⁺-precontracted trachea (Fig. 1B). Nantenine at 2 μ M had no effect, but at 20 μ M caused a relaxation of high-K⁺-precontracted trachea (Fig. 1C).

Higenamine has been reported to relax tracheal smooth

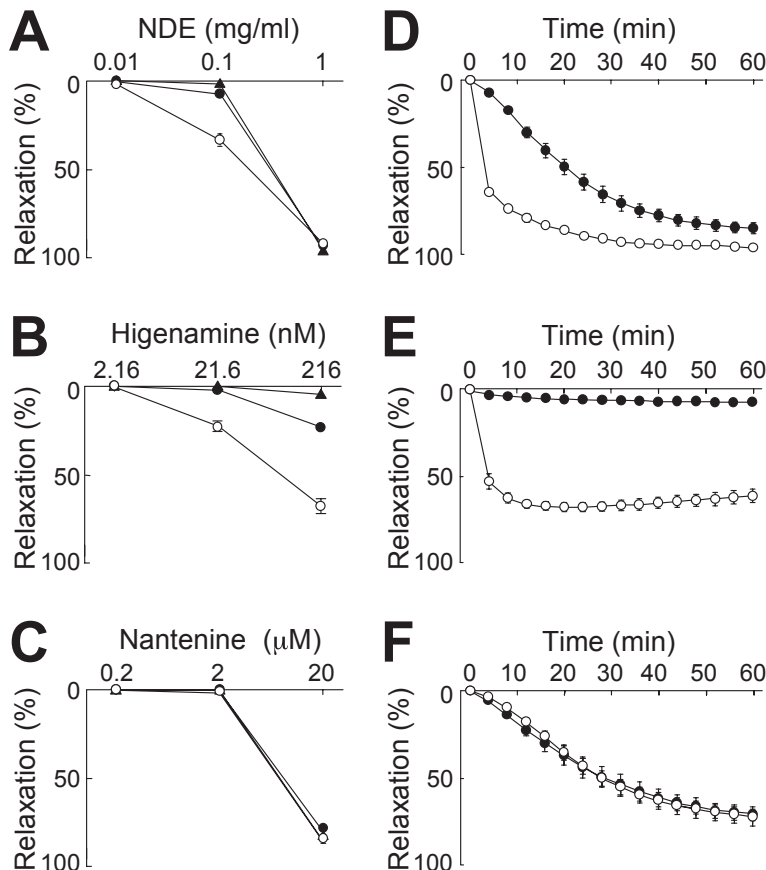


Fig. 1. Comparison of relaxing effects of NDE, higenamine, and nantenine in guinea-pig trachea precontracted with high-K⁺ stimulation. A–C) Concentration–effect curves for NDE (A), higenamine (B), and nantenine (C) in relaxing guinea-pig trachea precontracted with high-K⁺ stimulation in the absence (white circles) or presence of the β -adrenoceptor antagonist propranolol (10^{-8} M, black circles; 10^{-7} M, black triangles). Propranolol was added at the same time as high-K⁺ solution. D–F) Time courses of tracheal relaxations induced by 1 mg/ml NDE (D), 216 nM higenamine (E), and 20 μ M nantenine (F) in the absence (white circles) or presence of 10^{-7} M propranolol (black circles). The relaxation was expressed as a percentage of the maximal relaxation induced by papaverine in each preparation. All data are the mean $\pm$ S.E.M. of 5 observations.

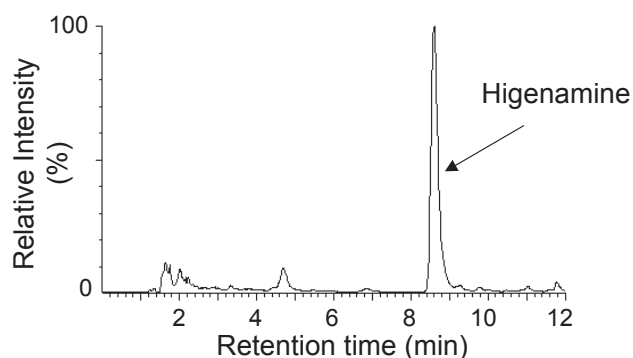


Fig. 2. HPLC-MS chromatogram of NDE. The content of higenamine in NDE was determined by HPLC-MS analysis as described in our previous paper (3).

muscles by stimulating β_2 -adrenoceptors (3). In fact, the concentration–effect curve for higenamine in relaxing high- K^+ -precontracted trachea was inhibited by the presence of the β -adrenoceptor antagonist propranolol (10^{-8} – 10^{-7} M, Fig. 1B). On the other hand, the concentration–effect curve for nantenine was not at all affected by propranolol (Fig. 1C). The effect of NDE at 0.1 mg/ml was inhibited by propranolol, but the effect of NDE at 1 mg/ml was not significantly affected by propranolol (Fig. 1A).

To explore the possibility that different mechanisms underlie the effects of NDE at 0.1 and 1 mg/ml, the time course of tracheal relaxation was analyzed in the absence or presence of propranolol. The time course of relaxation induced by 0.1 mg/ml NDE was very similar to that by 21.6 nM higenamine, and the effects of 0.1 mg/ml NDE and 21.6 nM higenamine were both completely abolished by the presence of 10^{-7} M propranolol (data not shown, $n = 5$). The relaxation induced by 1 mg/ml NDE appeared to consist of two components with different time-course and mechanism. The fast component was inhibited by 10^{-7} M propranolol, while the slow one remained in the presence of propranolol (Fig. 1D). The time course of the propranolol-sensitive, fast relaxation induced by 1 mg/ml NDE (Fig. 1D) was similar to that by 216 nM higenamine, which was almost completely blocked by 10^{-7} M propranolol (Fig. 1E). The time-course of the propranolol-resistant, slow relaxation induced by 1 mg/ml NDE (Fig. 1D) was similar to that by 20 μ M nantenine, which was not at all affected by 10^{-7} M propranolol (Fig. 1F).

To explore the possibility that nantenine causes the slow tracheal relaxation through Ca^{2+} antagonism, its effect on Ca^{2+} -induced contraction was investigated. The trachea was preincubated with Ca^{2+} -free, high- K^+ Krebs-Henseleit solution containing 62.7 mM NaCl, 60 mM KCl, 0 mM $CaCl_2$, atropine, and indomethacin and then exposed to 0.01–10 mM $CaCl_2$ in a cumulative manner.

The concentration–response curve for Ca^{2+} was shifted to the right by 6–20 μ M nantenine (Fig. 3A). The effect of nantenine was very similar to that of 0.3–1 mg/ml NDE in the presence of 10^{-7} M propranolol (Fig. 3B).

The majority of β -adrenoceptors in the guinea-pig trachea is β_2 -subtype (14, 15). The tracheal relaxing effect of higenamine was blocked by propranolol, and the relaxation induced by 0.1 mg/ml NDE was very similar to that by 21.6 nM higenamine, in terms of both time course and antagonism by propranolol. Therefore, the tracheal relaxing effect of NDE at a lower concentration can be accounted for by higenamine only.

The effect of 216 nM higenamine expected to be present in 1 mg/ml NDE was almost completely blocked by propranolol, while the effect of 1 mg/ml NDE was little affected by propranolol, suggesting that higenamine cannot account solely for the effect of higher concentration of NDE. Time-course analysis revealed that the relaxation induced by 1 mg/ml NDE consisted of two components different in terms of time-course and mechanism.

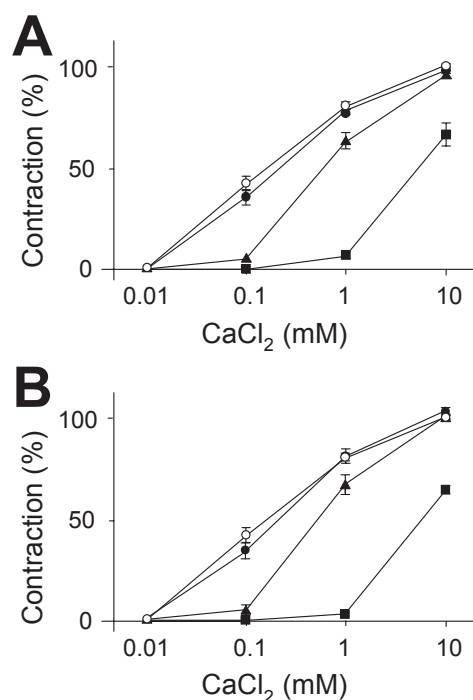


Fig. 3. Concentration–effect curves for Ca^{2+} -induced tracheal contraction in the absence (white circles) or presence of nantenine [2 μ M, black circles; 6 μ M, black triangles; 20 μ M, black squares (A)] or 10^{-7} M propranolol + NDE [0.1 mg/ml, black circles; 0.3 mg/ml, black triangles; 1 mg/ml, black squares (B)]. The trachea was preincubated with Ca^{2+} -free, high- K^+ Krebs-Henseleit solution and then exposed to 0.01–10 mM $CaCl_2$ in a cumulative manner. Drugs were added 10 min prior to cumulative addition of $CaCl_2$. The contraction was expressed as a percentage of the maximal contraction induced by 10 mM $CaCl_2$ in each preparation. All data are the mean $\pm$ S.E.M. of 5 observations.

The fast component was abolished by propranolol and mimicked by 216 nM higenamine, while the slow one was not affected by propranolol and mimicked by 20 μ M nantenine expected to be present in 1 mg/ml NDE. Therefore, both higenamine and nantenine are likely to contribute to the tracheal relaxing effect of 1 mg/ml NDE.

Nantenine at 20 μ M relaxed the trachea precontracted with high- K^+ stimulation. The result in this study was similar to the report by Orallo and Alzueta (13), in which 30 μ M nantenine completely inhibited high- K^+ -induced contraction in rat aorta. In addition, the concentration-response curve for Ca^{2+} in the guinea-pig trachea was shifted to the right by nantenine, suggesting that nantenine works as a Ca^{2+} antagonist. Furthermore, the Ca^{2+} -antagonistic effect of nantenine was mimicked by NDE + propranolol, supporting that nantenine contributes to the propranolol-resistant tracheal relaxation induced by NDE. Further investigations are underway in our laboratory to explore the possibility that nantenine directly or indirectly modulates tracheal Ca^{2+} channels.

In conclusion, we have demonstrated for the first time that NDE relaxes tracheal smooth muscles through a combination of β -adrenoceptor stimulation by higenamine and Ca^{2+} antagonism by nantenine. Patients who have not been helped by one drug often benefit from a combination of drugs. In this respect, NDE, a mixture of higenamine, nantenine, and others, may offer advantages over single drugs in treating breathing difficulty.

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