Comparative Effects of Nicorandil and Nitroglycerin on Tracheal and Vascular Smooth Muscle in the Dog, in Vivo and in Vitro

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Abstract—The effects of nicorandil (NCR) on tracheal and vascular smooth muscle in the dog were compared with those of nitroglycerin (NTG) in in vitro and in vivo preparations. In the isolated tracheal strip and coronary artery preparations contracted with KCI (30 mM), the ability of NCR to relax these muscles and arteries by 50% was 1/10–1/15 as potent as NTG. In blood-perfused tracheal preparations, single doses of NCR and NTG injected into the tracheal artery produced dose-related decreases in the intraluminal pressure (ILP) of the trachea (relaxation) and increases in the tracheal blood flow (TBF). When the potency of NCR relative to that of NTG was compared on the basis of doses decreasing the ILP and increasing the TBF by 50%, NCR was 822 times less potent than NTG in producing tracheal relaxation and 572 times less potent in producing tracheal vasodilation. The effects of NCR on the ILP and TBF were not antagonized by propranolol. In non-perfused tracheal preparations, the two drugs administered i.v. elicited the effects in a similar dose-dependent manner: decreases in systemic blood pressure (SBP), left ventricular systolic pressure (LVP), pressure-rate product (PRP), femoral vascular resistance (FVR), and ILP and increases in heart rate (HR) and LVdP/dt max. The results show that NCR has a potent bronchodilating action and that its pharmacological profile is somewhat similar to NTG.

Nicorandil (NCR) is a newly developed, orally active antianginal agent (1). It has been reported that this compound significantly increases coronary blood flow, virtually without affecting cardiac contraction, myocardial oxygen consumption and atrioventricular conduction in anesthetized open-chest dogs (2, 3) and in canine heart-lung preparations (4). Thus, it seems that NCR has a relatively selective action on the vasculature. This compound has a nitrate moiety in its chemical structure, and this site undoubtedly plays an important role in the development of its pharmacological activity (2, 5). Recently, the effects of locally applied nitroglycerin (NTG) (6) and NCR (7) on the tracheal musculature and vasculature were investigated in the arterially blood-perfused preparation of the dog trachea (8). However, there have been no reports concerning the tracheodilator effects of NCR administered systemically, particularly in relation to its cardiohemodynamics.

The purpose of this investigation was to elucidate more systematically the tracheodilator effects of NCR, compared with NTG, in in vitro and in vivo tracheal preparations of the dog.

Materials and Methods

Twenty-seven adult beagle dogs of either sex, weighing 10 to 12 kg, were anesthetized with sodium pentobarbital (35 mg/kg i.v.).

In vitro experiments: Under anesthesia, dogs were killed by bleeding from the common carotid artery. Rectangular pieces of smooth muscle with attached epithelium were taken from the median part of the

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trachea and placed in a modified Krebs-Henseleit solution. The strips measured 10–12 mm long, 1–1.5 mm wide, and 1.5 mm thick. The circumflex branch of the left coronary artery was dissected free from the heart, cleaned of adhering connective tissue and cut into 4 mm wide rings. These strips were mounted vertically in 10 ml tissue baths containing the Krebs-Henseleit solution. An initial tension of 1 g was applied to the tracheal strips and 2 g to the coronary artery strips. The medium contained (in mM): 

- NaCl, 119;
- KCl, 4.8;
- CaCl₂, 2.5;
- MgSO₄, 1.2;
- KH₂PO₄, 1.2;
- NaHCO₃, 24.8;
- glucose, 10.0.

The medium was aerated with a gas phase mixture containing 95% O₂, 5% CO₂ and was kept at a pH of 7.4 and a temperature of 37±0.2°C. The preparation was equilibrated for at least 2 hr, with washes every 10–15 min, before exposure to the drugs. The drugs were added to the 10 ml bath in a volume less than 0.1 ml. A series of sequential doses was administered, each subsequent one being introduced when the effect of the preceding one had reached a steady value. Isometric muscle contractions and relaxations were recorded on a pen recorder (Yokogawa 3066) using a transducer (Nihon Kohden TB-611T). Cumulative concentration-percentage maximal response curves for muscle relaxation of drugs were generated after contraction with 30 mM KCl. The value of ED₅₀ was defined as the concentration of drugs which induced a relaxation of 50% of the KCl-induced muscle contraction and which showed a confidence interval of 95%.

In vivo experiments: The animals were anesthetized initially with sodium pentobarbital (35 mg/kg i.v.) supplemented with a continuous i.v. infusion, 5 mg/kg/hr, throughout the experiments. The blood-perfused tracheal preparation was made according to a slight modification (9) of the method of Himori and Taira (8). Briefly, the upper cervical region was incised at the midline. The left and right cranial thyroid arteries and their branches were exposed and carefully freed. After heparinization (500 U/kg i.v.), the tracheal branches of both cranial thyroid arteries were cannulated, and the tracheal vascular bed was perfused through these arteries with blood from the femoral artery via a peristaltic pump (Harvard Apparatus, Model 1215). Constant pressure perfusion was achieved by shunting a portion of blood through a Starling pneumatic resistance to the femoral vein, and tracheal perfusion pressure (TPP) was set at a value slightly higher than the mean systemic blood pressure (SBP) at the beginning of perfusion and kept constant throughout the experiment. The SBP in the left femoral artery and the TPP were measured with pressure transducers (Nihon Kohden, MPU-0.5). Heart rate (HR) was obtained from the arterial pulse wave with a linear cardiograph (Nihon Kohden, AT-600G). Tracheal blood flow (TBF) was measured with a square wave electromagnetic flowmeter (Nihon Kohden, MF-27). A tracheal tube with a water-filled cuff attached was introduced into the trachea. Responses of the tracheal smooth muscle were measured as changes in the intraluminal pressure (ILP) of this cuff which was measured with a pressure transducer (Nihon Kohden, LPU-0.1). The volume of water in the cuff was adjusted initially to give a resting ILP of 21–41 cm H₂O. By this procedure, constant tracheal tone was maintained through the experiment. Drug solutions were injected locally (i.a.) into the rubber tube connecting the right femoral artery to the tracheal artery cannula, over a period of 10 sec in a volume of 0.1 ml.

The effects of drugs administered i.v. were examined in non-perfused tracheal preparations; although the water-filled cuff attached to a tracheal tube was introduced into the trachea, the tracheal vascular bed was not perfused. The femoral blood flow (FBF) was measured by placing a precalibrated noncannulating electromagnetic flow probe (Nihon Kohden, MF-27) around the left femoral artery. The femoral vascular resistance (FVR) was determined from the mean SBP and FBF by means of an analog multiplier (Nihon Kohden, EO-600G). To measure the left ventricular systolic pressure (LVP), a microtip PC-350 pressure manometer (Millar Instruments, Houston, Texas) was introduced through the right femoral artery into the left ventricle.
first derivative \((LVdP/dt \text{ max})\) of the LVP was derived using a resistance-capacitance differentiation circuit (Nihon Kohden, EQ-600G). The pressure rate product (PRP), systolic SBP\(\times\)HR, was calculated as a crude indicator of myocardial oxygen consumption (10). The cephalic vein was cannulated, and drug solutions were injected i.v. for 10 sec in a volume of 0.1 ml/kg, which was subsequently flushed in with 0.9% saline. All recordings were made on a chart using a Watanabe Linearrecorder (Model WR-3001). The peak responses to drugs were expressed as the percentage change in each parameter from preadministration levels.

**Drugs:** The drugs used were: N-(2-hydroxyethyl) nicotinamide nitrate (ester) (SG-75, Nicorandil) (synthesized in the Chugai Institute), nitroglycerin (kindly provided by Nihon Kayaku Co., Tokyo; dissolved in 99.8% ethylalcohol at a concentration of 10 mg/ml), \((-\)-isoproterenol hydrochloride (Nikken Kagaku) and \((-\)-propranolol hydrochloride (ICI). All drugs were dissolved in and diluted with 0.9% saline solution.

**Statistics:** Values in the text are represented as means±S.E. Dose-response curves were treated as linear regressions and parallelism of the curves was analyzed.

**Results**

**Effects of NCR and NTG on isolated tracheal muscle and coronary artery preparations:** With tissues in the contractile state induced by KCl (30 mM), NCR and NTG added cumulatively to the organ bath caused a concentration-dependent relaxation of the contraction in both the tracheal muscle and coronary artery preparations, as shown in Figs. 1 and 2. The ED50 values of NCR and NTG in the two kinds of preparations were as follows: tracheal smooth muscle, NCR: 2.09 (1.46–3.06)\(\times\)10\(^{-4}\) M (n=17) and NTG: 1.38 (0.61–3.11)\(\times\)10\(^{-5}\) M (n=12); coronary vessel, NCR: 3.89 (2.07–7.32)\(\times\)10\(^{-6}\) M (n=7) and NTG: 3.89 (2.84–5.34)\(\times\)10\(^{-7}\) M (n=9). When doses relaxing tracheal smooth muscle by 50% (ED50) were compared, NCR was 1/15 as potent as NTG. The ED50 of NCR for relaxing the coronary artery preparation was 1/10 as
potent as NTG.

Effects of intra-arterial NCR and NTG on tracheal intraluminal pressure (ILP) and blood flow (TBF) in blood-perfused tracheal preparations: Basal values of mean SBP, TBF, perfusion pressure (TPP) and ILP from four different preparations were as follows: SBP, 176.5±14.6 mmHg; TBF, 12.1±2.5 ml/min; TPP, 185.0±14.4 mmHg; ILP, 34.0±2.2 cm H,O.

The two drugs were administered to each preparation in a randomized protocol. Each dose of drugs was given after the effect of the preceding dose had disappeared; and the intervals between drug injections thus ranged from 5 to 10 min, depending on the duration of the effect. Single injections of NCR (3–300 μg) and NTG (0.03–3 μg) into the tracheal artery produced dose-dependent decreases in the ILP of the trachea (tracheal relaxation) and increases in the TBF. Original tracings are illustrated in Fig. 3, and the dose-response curves for peak changes in the ILP and TBF are shown in Fig. 4. The curves for NCR for tracheal relaxation and vasodilation were almost parallel to those for NTG. Within the dose range tested, these drugs had virtually no effects on SBP. The ED50 values of NCR and NTG in the ILP and TBF were as follows: ILP, NCR: 51.8 (32.3–83.3) μg (n=4) and NTG: 0.063 (0.026–0.149) μg (n=4); TBF, NCR: 30.3 (23.6–38.8) μg (n=4) and NTG: 0.053 (0.002–1.745) μg (n=4). When the potency of NCR relative to that of NTG was compared on the basis of doses decreasing the ILP and increasing the TBF by 50% (ED50), NCR was 822 times less potent than NTG in producing tracheal relaxation and 572 times less potent in producing tracheal vasodilation. Unlike isoproterenol (0.1 μg), the effects of NCR (300 μg) on the ILP and TBF were not antagonized by propranolol (100 μg i.a.) (data not shown).

Tracheal dilator and cardiovascular effects of intravenous NCR and NTG in non-perfused tracheal preparations: The basal values of the main parameters from 11 different preparations were as follows: ILP, 30.4±2.2 cm H,O; mean SBP, 186.2±4.9 mmHg; HR, 182.1±3.1 beats/min; LVP, 195.4±9.5 mmHg; LVP/dt max, 3150±310 mmHg/sec; FBF, 105.5±15.6 ml/min and FVR, 1.8±0.1 mmHg/ml/min. Typical effects of the two drugs studied with regards to
Fig. 5. Responses of systemic blood pressure (SBP), heart rate (HR), tracheal intraluminal pressure (ILP), femoral blood flow (FBF), femoral vascular resistance (FVR), left ventricular systolic pressure (LVP), LVdP/dt max and pressure rate product (PRP) (systolic SBP×HR) to nicorandil and nitroglycerin administered i.v. in non-perfused tracheal preparations. A) Original tracings B) Dose-response curves for peak changes in each parameter from the preadministration level. Each value represents the mean±S.E. of 11 experiments. The two drugs caused a decrease (-----) in FBF, preceded by an increase (------).
these parameters are shown in Fig. 5A, and the data are summarized in Fig. 5B.

In doses smaller than 30 \( \mu g/kg \), NCR did not appreciably affect the tested parameters. In larger doses, it caused decreases in SBP, ILP, FVR, and LVP and increases in HR and LVDp/dt max in a dose-dependent fashion. The PRP was also decreased. An increase followed by a decrease was observed in FBF (left panel in Fig. 5B). Qualitatively, similar results were obtained with NTG (right panel in Fig. 5B). At 0.03 \( \mu g/kg \), NTG had little effect on the tested parameters. However, in larger doses, this drug produced decreases in SBP, PRP, ILP, FVR, and LVP and increases in HR and LVDp/dt max in a dose-dependent manner. Changes in FBF were biphasic: an initial increase followed by a decrease. The duration of the effects elicited by these drugs depended on the doses used.

Figure 6 shows the dose-response curves for the peak decreases in ILP and FVR caused by NCR and NTG applied i.v. The curves for tracheal relaxation and femoral vasodilation with NCR were almost parallel to those with NTG. The relative potency of NCR and NTG was compared on the basis of doses decreasing the ILP by 50% and the FVR by 25%, respectively. NCR was 347 times less potent than nitroglycerin in producing tracheal relaxation and 252 times less potent in producing femoral vasodilation. However, the effect of NCR on ILP was more long-lasting than that of NTG: the decrease in ILP by 300 \( \mu g/kg \) of NCR lasted more than 15 min, while the effect of 3 \( \mu g/kg \) of NTG disappeared within 5 min.

**Discussion**

This study shows that nicorandil causes dose-dependent relaxation of canine tracheal smooth muscle in both in vitro and in vivo conditions. This is consistent with the finding of Maruyama et al. (7) that nicorandil has a potent broncho-dilating activity. It has been reported that nicorandil has a nitrate moiety in its chemical structure which plays an important role in the pharmacological activity of this drug (2, 5). Recently, Maruyama et al. (7) also confirmed that in the arterially blood-perfused preparation of the dog trachea in situ, the nitrate site of nicorandil plays an important role in increasing its activity to produce both tracheal dilatation and tracheal vasodilation. In view of this, nicorandil may belong to the nitrates pharmacologically.

A series of experiments using a preparation for blood perfusion of arteries in the dog trachea have revealed that nitrates were unexpectedly more effective in producing tracheal relaxation than in producing tracheal vasodilation (6, 11, 12). This is in accordance with the present result: In the same sort of preparations, nitroglycerin and nicorandil were approximately 1.7 and 1.2 times, respectively, less potent in producing tracheal vasodilation than in causing tracheal relaxation. However, when compared with nitroglycerin, nicorandil seems to be more selective for the (tracheal, coronary and femoral) vasculature than for the tracheal musculature in both in vivo and in vitro preparations.

In the previous study using the open-chest preparation of the dog (3), nicorandil, at a dose (300 \( \mu g/kg \) i.v.) which doubles coronary blood flow, did not affect myocardial oxygen consumption, contractility and heart...
rate. However, in the present experiment using the closed-chest preparation, nicorandil in the same dose administered i.v. caused definite increases in cardioparameters such as heart rate and LVdP/dt max, although it elicited a marked tracheal relaxation. Similar results were obtained with nitroglycerin. The effects of nicorandil as well as nitroglycerin on cardioparameters seem to be ascribable to the more sensitive circulatory reflex induced by hypotensive effects (13) in the closed-chest condition.

Thus, the pharmacological profile of nicorandil resembles nitroglycerin, and yet differs somewhat from that drug: Nicorandil 1) has a relatively selective effect on the coronary vascular bed in the dog (3), 2) develops neither tolerance to the nicorandil nor cross-tolerance to nitroglycerin (14), 3) is not readily metabolized by a single passage of the liver (15), and 4) increases potassium conductance in cardiac muscle (16, 17) and vascular smooth muscle (18). These characteristics are not shared by conventional nitrates.

In conclusion, nicorandil, like nitroglycerin, has a potent bronchodilating activity in both in vitro and in vivo conditions. The properties of nicorandil may be beneficial in a wide range of applications as an antianginal agent, particularly in patients with bronchial hyper-reactivity.

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