Comparison of Development of Tolerance between Nicorandil and Nitroglycerin in Anesthetized, Open-Chest Dogs

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Abstract—Development of tolerance to nicorandil (NCR), N-(2-hydroxyethyl)nicotinamide nitrate (ester), was compared with that to nitroglycerin (NTG) in dogs. An intra-coronary arterial (i.a.) injection of NCR (30 μg) or NTG (3 μg) produced coronary vasodilation. Development of tolerance (including cross tolerance) was determined by examining whether the coronary vasodilating effect of i.a. injection of these drugs was attenuated by a 2 hr-infusion of NCR or NTG. The effect of i.a. injection of NCR was not affected by either NCR infusion (10 μg/kg/min, i.v.) or NTG infusion (1 or 3 μg/kg/min, i.v.). The effect of i.a. injection of NTG, however, was attenuated by the NTG infusion, while it was not affected by the NCR infusion. Additionally, the coronary vasodilating effect of NCR infusion (30 μg/kg/min, i.v.) was not attenuated by NTG infusion (3 μg/kg/min, i.v.). These results suggest that NCR does not produce tolerance, whereas NTG does, and that there is no cross-tolerance between NCR and NTG in terms of the coronary vasodilating effect.

Nicorandil, N-(2-hydroxyethyl)nicotinamide nitrate (ester), has been newly developed as an orally efficacious antianginal drug (1) with strong vasodilating (2-5) and vasospasmolytic effects (5-7). A considerable number of experimental and clinical investigations have contributed to clarify its pharmacological profile (3, 4, 8) and to prove its therapeutic benefit in patients with angina (1). For the past years, the efficacy of orally administered nicorandil has been well-established in patients not only with vasospastic angina but also with effort angina. At the present time, its use is being further extended to a number of cardiovascular syndromes in addition to angina pectoris, including congestive heart failure and acute myocardial infarction. Thus, for the possibility of greater utility in therapy, the i.v. route application of nicorandil has been explored.

The aim of this experiment was to investigate some aspects of the development of acute self- and cross-tolerance to nitroglycerin during i.v. infusion of nicorandil in anesthetized dogs. Nitroglycerin was included in the study as a reference drug.

Materials and Methods
Preparation of the animal: Experiments were carried out on 33 adult beagle dogs (10-12 kg) of both sexes. The animals were anesthetized initially with 35 mg/kg sodium pentobarbital, i.v., and 12 mg/kg pentobarbital, dissolved in 20 ml of 0.9% saline solution was infused i.v. at a rate of 0.11 ml/min by means of an infusion pump (Perfusor V, B. Braun, Melsungen AG, West Germany). The animals were respired with room air with a tidal volume of 20 ml/kg at a rate of 20 breaths/min by the use of a Harvard dog respirator (model 607). Systemic blood pressure (SBP) was measured with a Nihon Kohden pressure transducer (MPU-0.5) by inserting a polyethylene tube filled with 5 ml of heparin solution (1000 units) into the right brachial artery. The heart rate (HR) was monitored continuously with a Nihon Kohden heart
rate counter (AT-600G). The chest was opened by removing a portion of the left fourth and fifth ribs, and the phrenic nerves were severed. The pericardium was cut, and a pericardial cradle was prepared to keep the heart in the proper position. The left circumflex coronary artery was isolated near the origin and cleaned of connective tissue. A precalibrated noncannulating electromagnetic flow probe (i.d., 1.5–2.5 mm; Nihon Kohden, MFV-2100) was positioned around the exposed vessel. Zero flow references were obtained periodically by briefly occluding the vessel distal to the probe. A fine polyethylene tube (o.d., 0.8 mm) was cannulated into a distal branch of the vessel for intra-arterial injections of drugs. The coronary vascular resistance (mmHg/ml/min) determined from the mean SBP and coronary blood flow was calculated with a Nihon Kohden analog multiplier (EO-600G). To measure left ventricular (LV) pressure, a microtip pressure manometer (model PC-350; Millar Instruments, Houston, Texas) was introduced into the left ventricle via the left atrium from the left femoral artery. The first derivative (LVdP/dt max) of the LV pressure was obtained using a Nihon Kohden resistance-capacitance differentiating circuit (EQ-600G). The pressure-rate product, systolic SBP×HR, was calculated as a crude indicator of myocardial oxygen consumption (9). All recordings were made on a chart by using a Watanabe Linearorder (WR-3101). Arterial blood PO2, PCO2 and pH were kept at approximately 80 mmHg, 20 mmHg and 7.40, respectively (measured with a blood gas analyser, ABL2 Acid-Base Laboratory, Radiometer, Copenhagen). The animal’s rectal temperature was kept at 37–38°C by a heating device (Aquamatic K, Hamilton Industries).

Experimental protocols: The dogs were divided into five groups (each n=5) as follows: I. Group treated with 0.9% saline solution. II and III. Groups treated with nicorandil. IV and V. Groups treated with nitroglycerin. According to a previous experiment in anesthetized dogs (4) it was found that to elicit a similar degree of coronary vasodilation in magnitude and duration, the dose of intra-coronary nicorandil was approximately ten times larger than that of nitroglycerin, and so in the present experiment, nicorandil was infused continuously into the cannulated cephalic vein at a rate of 10 μg/kg/min (group II) or 30 μg/kg/min (group III), and nitroglycerin was infused i.v. at a rate of 1 μg/kg/min (group IV) or 3 μg/kg/min (group V). In each group (except for group III), just before starting the infusion of either 0.9% saline or the drugs, bolus injections of nitroglycerin (3 μg) and nicorandil (30 μg) were made locally into the coronary artery, and then 0.9% saline or the drug solution was infused for 2 hr at a rate of 0.11 ml/min by using a glass syringe with a Braun Perfusor V pump (Melsungen, West Germany). At 30, 60, 90 and 120 min following the onset of the infusion and at 60 min after its cessation, injections of intracoronary nitroglycerin (3 μg) and then nicorandil (30 μg) were made and the effects were tested each time. Thus, cardiohemodynamic and acute tolerance studies were carried out in the same preparation.

For additional studies, two groups (each n=4) were prepared. In one (group VI) of these groups, 0.9% saline was infused i.v. for 1 hr at a rate of 0.11 ml/min, after which a combination of 0.9% saline and nicorandil (30 μg/kg/min) solutions was infused for a further 1 hr. In the other group (VII), nitroglycerin (3 μg/kg/min) alone was first infused i.v. for 1 hr at a rate of 0.11 ml/min, after which the simultaneous infusion of nitroglycerin (3 μg/kg/min) and nicorandil (30 μg/kg/min) solutions was continued for a further 1 hr. In both the groups, bolus injections of nitroglycerin (3 μg) and nicorandil (30 μg) were made locally into the coronary artery just before infusion and 1 hr after the onset of the infusion of 0.9% saline or nitroglycerin (3 μg/kg/min) solution.

Drugs used: nicorandil, N-(2-hydroxyethyl)nicotinamide nitrate (ester) (MW 211.18, C₈H₉N₃O₄), was synthesized in our Research Laboratories. Nitroglycerin from commercially available ampoules was used (Nippon Kayaku Co.). Just before use, the drugs were dissolved in or diluted with 0.9% saline solution.

Statistical analysis: Data are presented as means and standard errors. Responses to drugs were expressed as the percentage
|                           | I              | II             | III            | IV             | V              |
|---------------------------|----------------|----------------|----------------|----------------|----------------|
| **Systemic blood pressure (mmHg)** |                |                |                |                |                |
| Systolic                  | 143.2±2.3      | 129.8±9.1      | 141.6±9.5      | 135.6±4.7      | 144.6±7.4      |
| Mean                      | 116.4±3.3      | 106.8±7.7      | 114.4±9.6      | 113.2±5.2      | 119.0±7.6      |
| Diastolic                 | 94.6±2.7       | 90.0±6.5       | 98.2±8.1       | 98.2±4.7       | 101.2±7.0      |
| Heart rate (beats/min)    | 168.8±8.9      | 144.4±8.4      | 158.4±11.3     | 159.8±7.5      | 167.8±4.6      |
| Pressure-rate product     | 24123.0±1102.3 | 18877.8±2136.7 | 22590.8±2713.7 | 21728.4±1411.5 | 24320.4±1650.1 |
| Left ventricular (LV) systolic pressure (mmHg) | 125.6±2.9 | 117.6±7.0 | 127.4±8.4 | 125.0±6.4 | 131.6±5.8 |
| LV end-diastolic pressure (mmHg) | 7.05±1.37 | 5.14±0.39 | 4.93±0.99 | 6.74±1.35 | 4.27±0.61 |
| LVDp/dt max (mmHg/sec)    | 3152.0±174.2   | 2832.0±241.1   | 2824.0±292.5   | 2680.0±258.6   | 3196.0±261.0   |
| Coronary (left circumflex branch) blood flow (mean, ml/min) | 20.8±1.2 | 18.2±1.8 | 16.2±2.0 | 17.2±2.1 | 17.4±1.4 |
| Coronary vascular resistance (mmHg/ml/min) | 5.68±0.36 | 6.33±1.26 | 7.34±0.76 | 6.90±0.68 | 7.02±0.69 |

Values represented are means±S.E. of 5 observations on 5 preparations from each group. The values from each group demonstrated no significant differences relative to the corresponding values from the 0.9% saline infusion group. I. Group infused with 0.9% saline; II and III. Groups infused with low (10 μg/kg/min) and high dose (30 μg/kg/min) of nicorandil, respectively; IV and V. Groups infused with low (1 μg/kg/min) and high dose (3 μg/kg/min) of nitroglycerin, respectively. (See Materials and Methods in details.)
changes in each parameter from the preadministration level, unless otherwise stated. Inter-
group differences were analyzed by Student's t-test for unpaired data. A P value less than
0.05 was considered statistically significant.

Results
After completion of the surgical procedure, a minimum of 30–60 min was allowed for
stabilization of the preparation.

Cardiohemodynamics
The basal values of the cardiovascular parameters from 5 individual groups are
shown in Table 1. In the control group (I), 0.9% saline solution was infused i.v. for 2 hr
at a rate of 0.11 ml/min. The cardiovascular changes were examined during the infusion
and for 1 hr after cessation of the infusion. Parameters remained relatively stable
throughout the total 3 hr-experimental period, except for slight reductions in LVdP/dt max.

Nicorandil: Typical effects of nicorandil on cardiovascular parameters are illustrated in
Fig. 1A, and the data are summarized in Fig. 1B (left panel). Nicorandil was infused i.v.
for 2 hr at a rate of 10 (group II) or 30 µg/kg/min (group III), and the changes in the cardiovascular parameters were examined, compared with those in the control group (I).
Following the start of the infusion, nicorandil caused dose-dependent gradual decreases in
systemic (systolic, mean, diastolic) blood pressure and LV systolic pressure, and it
causued a decrease in LV end-diastolic pressure. Slight but significant increases in
LVdP/dt max and heart rate were also observed. The pressure-rate product was
significantly reduced, due primarily to a decrease in systolic blood pressure. The
coronary blood flow was progressively increased in a dose-dependent manner,
accompanied by a concomitant decrease in the coronary vascular resistance. Approximately 120 min after the i.v. infusion at a rate of 30 µg/kg/min, the increase in the coronary blood flow reached a level which was twice as high as the control one. After cessation of the infusion, the cardiovascular parameters tended to return to the preinfusion level.

Nitroglycerin: Nitroglycerin was infused i.v. for 2 hr at a rate of 1 (group IV) or 3 µg/
kg/min (group V), and the changes in the cardiovascular parameters were examined, compared with those in the control group (I). Summarized data are shown in Fig. 1B (right panel). Following the onset of the infusion, nitroglycerin produced a dose-dependent decrease in systemic (systolic, mean, diastolic) blood pressure, LV systolic and end-diastolic pressure, and pressure-rate product. No significant changes in LVdP/dt max and heart rate were observed. Within 60 min following the start of the nitroglycerin (3 µg/kg/min) infusion, coronary blood flow decreased slightly but significantly. However, coronary vascular resistance remained virtually unaltered throughout the infusion period. Within 60 min after the cessation of the infusion, all of the cardiovascular parameters were restored to the preinfusion level.

Studies on acute tolerance
Single infusion of nicorandil or nitro-
glycerin: Immediately after bolus injections of nitroglycerin (3 µg) and nicorandil (30 µg)
were made locally into the coronary artery, 0.9% saline solution was infused i.v. for 2 hr
at a rate of 0.11 ml/min in the control group (I). At 30, 60, 90 and 120 min following the
start of the infusion, and at 60 min after the cessation of the infusion, single doses of

Fig. 1. Cardiovascular effects of i.v. infused nicorandil and nitroglycerin. A) Original tracings. LVP, left ventricular pressure. Nicorandil (30 µg/kg/min) solution was infused i.v. for 2 hr at a rate of 0.11
ml/min. B) Time-response curves for changes in (systolic, mean, diastolic) SBP, heart rate (HR),
pressure-rate product (PRP, systolic SBP x HR), LV systolic pressure (LVSP), LV end-diastolic pressure
(LVEDP), LVdP/dt max, coronary blood flow (mean, CBF) and coronary vascular resistance (CVR).
Nicorandil (NCR, left panel) (●, 10 µg/kg/min, group II: ▲, 30 µg/kg/min, group III), nitroglycerin
(NTG, right panel) (●, 1 µg/kg/min, group IV: ▲, 3 µg/kg/min, group V) and 0.9% saline (O) solutions
(group I) were infused i.v. for 2 hr at a rate of 0.11 ml/min. Values are the means of changes from the
preinfusion level. The basal values before the infusion of each solution are shown in Table 1. The
vertical bars represent means±S.E. of 5 observations on 5 preparations. *P<0.05, **P<0.01, ***P<0.001
vs. the corresponding value from the 0.9% saline infusion group (I).
Fig. 2. Coronary vascular responses to intra-coronary (i.a.) injections of nitroglycerin (NTG) and nicorandil (NCR) before, during and after i.v. infusion of nicorandil. A) Original tracings. Bolus injections of nitroglycerin (3 μg, ■) and nicorandil (30 μg, ○) into the coronary artery caused a definite increase in coronary blood flow (CBF) and a decrease in coronary vascular resistance (CVR), but did not affect systemic blood pressure (SBP). B) Summarized data. During and after the i.v. infusion of nicorandil (NCR) (●, 10 μg/kg/min, group II), the vascular responses to i.a. nitroglycerin (3 μg) and nicorandil (30 μg) were not significantly attenuated, compared with the corresponding ones (○) from the 0.9% saline-treated group (I). Since CBF was significantly increased during nicorandil i.v. infusion, it was difficult to compare qualitatively the magnitude of the CBF increase elicited by i.a. nitroglycerin and nicorandil during nicorandil infusion (for the reason mentioned here, a tolerance study was not tried in group III). So, only in this series, each value obtained by i.a. nitroglycerin and nicorandil during and after nicorandil infusion was measured as the peak Δ CBF increase from the nicorandil preinfusion level. Peak Δ increase in CBF (control) induced by i.a. nitroglycerin and nicorandil prior to i.v. infusion of 0.9% saline or nicorandil solution was taken as 100%, and the peak Δ increase elicited by each drug during and after the infusion was expressed as % changes to the control. Vertical bars represent means ± S.E. of 5 observations on 5 preparations. There were no significant differences between the corresponding values of i.a. nitroglycerin (upper part) or nicorandil (lower part) obtained from 0.9% saline and nicorandil infusion groups. Peak Δ increases in CBF (control) just before the i.v. infusion of 0.9% saline and nicorandil solution: 31.2±2.0 ml/min for i.a. nicorandil (30 μg) and 32.6±2.3 ml/min for i.a. nitroglycerin (3 μg): each n=10, P>0.05.
nitroglycerin (3 μg) and nicorandil (30 μg) were injected into the coronary artery. During i.v. infusion of 0.9% saline solution, each of these drugs given locally elicited stable and reproducible coronary vasodilatation of similar magnitude each time.

**Nicorandil infusion:** Immediately after coronary vascular responses to intra-coronary nitroglycerin (3 μg) and nicorandil (30 μg) were examined, nicorandil solution was infused i.v. for 2 hr at a rate of 10 μg/kg/min (group II). During the infusion of nicorandil, coronary blood flow was increased significantly, as shown in Figs. 1B (left panel) and 2A. It was therefore not straightforward to compare quantitatively the magnitude of the blood flow increase elicited by intra-coronary nitroglycerin and nicorandil during the nicorandil infusion, with those obtained from the 0.9% saline infusion group (I). However, it was noted that there were no significant differences between the cor-

![Diagram](image-url)
responding values of intra-coronary nitroglycerin (upper part in Fig. 2B) or nicorandil (lower part in Fig. 2B) obtained from the 0.9% saline and nicorandil infusion groups, when each value during the infusion was measured as the peak blood flow increase from the preinfusion level and expressed as percent changes against the preinfusion value.

**Nitroglycerin infusion:** Immediately after coronary vascular responses to intra-coronary nitroglycerin (3 μg) and nicorandil (30 μg) were examined, nitroglycerin solution was infused i.v. for 2 hr at a rate of 1 (group IV) or 3 μg/kg/min (group V). Only within the first 60 min following the start of the infusion, a slight decrease in coronary blood flow was observed (right panel in Fig. 1B). During the infusion and 1 hr after stopping the infusion, bolus injections of nitroglycerin (3 μg) and nicorandil (30 μg) were made repeatedly into the coronary artery. The vascular response to intra-coronary nitroglycerin was attenuated significantly during i.v. infusion of nitroglycerin (group IV and V) (upper part in Fig. 3), compared with the corresponding control (group I), while the response to intra-coronary nicorandil remained unchanged (lower portion of Fig. 3).

**Combined infusion of nicorandil and nitroglycerin:** When acute tolerance to intra-coronary nitroglycerin (3 μg) was provoked
at 1 hr after starting the infusion of nitroglycerin (3 μg/kg/min) alone, simultaneous i.v. infusion of nitroglycerin (3 μg/kg/min) and nicorandil (30 μg/kg/min) solutions was performed for a further 1 hr (group VII). As illustrated in Fig. 4A and B, the effects of the simultaneous infusion of nitroglycerin and nicorandil solutions on systemic blood pressure (except those at 30 min after the start of the infusion) and coronary vasculature were not essentially different, in the magnitude, from those observed during the combined infusion of nicorandil (30 μg/kg/min) and 0.9% saline solutions (group VI).

**Discussion**

The present experiment revealed in the anesthetized, open-chest dog that the essential cardiovascular effects of nicorandil, infused i.v., were a sustained, pronounced increase in coronary blood flow; a decrease in SBP, coronary vascular resistance, LV systolic and end-diastolic pressure; and a decrease in the pressure-rate product in spite of an increase in the LVdP/dt max and heart rate. Thus, in agreement with the previous result obtained by bolus i.v. injections in the anesthetized miniature pig (5), nicorandil seems to have the balanced hemodynamic effect of reducing both preload and afterload. Regarding SBP, LV systolic and end-diastolic pressure and pressure-rate product, similar results were obtained with nitroglycerin.

The problem of tolerance towards organic nitrates such as nitroglycerin has been given attention since the first clinical report of nitroglycerin therapy for hypertension (10).
Current studies have demonstrated that tolerance towards nitroglycerin is developed not only in animals, but also in humans. In addition, nitroglycerin induces cross-tolerance to other organic nitrates (11). In the present experiment, the coronary vascular response to nitroglycerin injected locally into the coronary artery was progressively attenuated during the i.v. infusion of nitroglycerin, whereas that to nicorandil remained virtually unchanged. Moreover, the vascular responses to bolus intra-coronary doses of the two drugs were not modified by the i.v. infusion of nicorandil. It was particularly important to note that simultaneous i.v. infusion of nicorandil and nitroglycerin, started just after the development of tolerance with nitroglycerin infusion, compared with the combined i.v. infusion of nicorandil and saline, elicited essentially no significant changes in coronary blood flow, coronary vascular resistance and systemic blood pressure. Thus, nicorandil did not provoke either self- or cross-tolerance to nitroglycerin. These findings and conclusion accord well with those obtained previously by us (12, 13). Despite the fact that nicorandil includes a nitrate moiety in the chemical structure, which plays an essential role in its pharmacological activity (3, 14), the site of action may be different from that of nitroglycerin.

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