EFFECTS OF N-(2-HYDROXYETHYL)NICOTINAMIDE NITRATE (SG-75) ON METHACHOLINE-INDUCED ECG CHANGES IN INTACT ANESTHETIZED RATS

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Abstract—The effects of a newly developed nicotinamide derivative, N-(2-hydroxyethyl)nicotinamide nitrate (SG-75, Nicorandil), were examined in an experimental model of angina pectoris, utilizing methacholine-induced ECG changes as main parameter in intact anesthetized rats. The right carotid artery was exposed and through it a special cannula was inserted to a point near the right and left coronary ostium. Such a device made it possible to inject drugs more selectively into the coronary artery. Single intra-aortic injections of 4 to 8 μg of methacholine caused a transient elevation of the ST segment and T wave of the electrocardiogram (ECG). SG-75 (3 mg/kg i.v. or 10 mg/kg p.o.) prevented these changes in the ECG, while a potent vasodilator, papaverine, failed to do so. In the isolated, donor-perfused rat heart, SG-75 (1–30 μg) injected into the coronary perfusion system caused dose-dependent vasodilation, while 0.1–0.8 μg acetylcholine as well as methacholine produced marked vasoconstriction. SG-75 (10 mg/kg) administered orally to the donor rat inhibited the coronary vasoconstriction produced by the cholinomimetic drugs, whereas papaverine (30 μg i.a.) failed to prevent it. The inhibitory effects of SG-75 on methacholine-induced ECG changes in intact rats seemed to be due to its spasmyolytic action.

N-(2-Hydroxyethyl)nicotinamide nitrate (SG-75, Nicorandil), a nicotinamide derivative, produces a potent coronary vasodilating effect (1), virtually without affecting cardiac contraction, heart rate and myocardial oxygen consumption in anesthetized dogs (2–4). Thus, SG-75 increases the oxygen supply without increasing myocardial oxygen demand. Since angina pectoris is a clinical syndrome caused by transient myocardial ischemia resulting from an imbalance between myocardial oxygen supply and demand (5, 6), it is suggested that SG-75 possesses favorable properties as a remedy for angina pectoris. Among the several types of anginal syndromes there is a variant form at rest, caused by coronary vasospasm (7–11). In this syndrome, pain and ST segment elevation occur without hemodynamic indication of increased myocardial oxygen demand.

The present experiment was undertaken to investigate inhibitory effects of SG-75 on ECG changes due to coronary vasospasm induced by methacholine in the intact anesthetized rat (12).
MATERIALS AND METHODS

Male Sprague-Dawley rats were anesthetized with sodium pentobarbital, 65 mg/kg given intraperitoneally. An experimental model of angina pectoris in the intact anesthetized rat: In rats weighing 500 to 550 g, the trachea, esophagus, femoral artery and vein were cannulated with polyethylene tubing. For selective bolus injections of methacholine into the ostia of the coronary arteries, a double walled cannula was introduced through the exposed right carotid artery to a point near the aortic valve. A schematic diagram of this experimental preparation has been published (12). Systemic blood pressure was measured with a pressure transducer (Nihon Kohden, MPU-0.5). Heart rate was measured by means of a heart rate counter (Nihon Kohden, AT-600G). All recordings were made on a Nihon Kohden WI-680G recorder. The standard limb lead II of the electrocardiogram (ECG) was recorded by means of an electrocardiograph (Nihon Kohden, ECG-3002). Single doses of 4 to 8 μg of methacholine in a volume of 0.01 ml were injected into the aorta over a 1 sec interval using microsyringes (Jintan Terumo Co.). For i.v. administration, 0.01 ml of drug solutions were injected into the femoral vein during 10 sec intervals and flushed in with 0.05 ml of 0.9% saline. For i.a. administration, less than 1 ml of drug solutions were introduced into the stomach of the donor over 20 sec and flushed in with 0.9% saline. Increases (vasoconstriction) or decreases (vasodilation) in mean coronary perfusion pressure caused by drugs were taken as the drug responses, since the perfusion flow rate was constant. Maximal drug effects were expressed as changes from the preadministration control levels.

Isolated donor-perfused spontaneously beating rat heart: The procedures for the cross-circulation of hearts were as already (13, 14) reported. Briefly, the isolated heart (left in situ) of the recipient rat (300–350 g) was perfused at a fixed flow rate through the coronary arteries via the aorta with heparinized blood from the carotid artery of a donor (500–600 g) using a precalibrated peristaltic pump (Mitsumi Science, SJ-1210). The systemic blood pressure of the donor (BP) and the mean coronary perfusion pressure (PP) were measured with pressure transducers (Nihon Kohden, MPU-0.5). Left ventricular pressure (LVP) was measured by means of a concentric double walled cannula, as described elsewhere (15), attached to a pressure transducer (Nihon Kohden, MPU-0.5). Heart rate was monitored by means of a heart rate counter (Nihon Kohden, AT-600G). For p.o. administration, less than 1 ml of drug solutions were introduced into the stomach of the donor over 20 sec and flushed in with 0.9% saline. Values in the text are represented as means±S.E. The difference of paired mean values was analysed by the Student’s t-test and judged to be significant when P values were less than 0.05.

RESULTS

An experimental model of angina pectoris in the intact anesthetized rat

Control observations: The basal values of
systemic blood pressure (BP) and heart rate (HR) from 24 different preparations were as follows: BP, 115.7±7.0 mmHg; HR, 350.0±15.3 beats/min.

ECG changes induced by methacholine administered intra-arterially: Intra-aortic bolus injections of methacholine (4 to 8 μg) caused decreases in BP and transient cardiac arrest, followed thereafter by a transient elevation of the ST segment and T wave. The methacholine-induced ECG changes lasted about 10 to 20 sec and were reproducible.

Effects of SG-75 administered intravenously or orally on the ECG changes due to intra-aortic methacholine: The effects of the SG-75 on methacholine-induced ECG changes were examined in different animals. The SG-75 produced a dose-dependent decrease in BP, but caused no significant changes in HR. Summarized data are shown in Table 1.

SG-75 administered intravenously (3 mg/kg) or introduced into the stomach (10 mg/kg) via a tube into the esophagus significantly protected against the methacholine-induced elevation of the ST segment and T wave, whereas a potent vasodilator papaverine (1 mg/kg) administered i.v. was unable to prevent it. An i.v. administration of papaverine (1 mg/kg) produced peak decreases in BP as follows: 108.3±3.3 mmHg to 66.0±7.6 mmHg; (N=5). The effects of SG-75 on the ECG changes due to intra-aortic methacholine are shown in the original record (Fig. 1) and the summarized data (Table 2).

Influence of SG-75 on systemic blood pressure responses to methacholine: Methacholine (1–30 μg/kg) administered intravenously produced dose-dependent decreases in BP. After dose-response curves to methacholine for BP changes had been obtained, SG-75 (10 mg/kg) was given p.o.

![Graph showing the effects of SG-75 on the ECG changes caused by methacholine](image)

**Table 1. Influence of SG-75 administered intravenously or orally on systemic blood pressure (BP) in intact anesthetized rats**

|            | Before SG-75 | After SG-75 |
|------------|--------------|-------------|
| SG-75      |              |             |
| 3 mg/kg p.o. | 117.5±9.5   | 83.3±6.5    |
| 10 mg/kg p.o. | 125.8±6.1  | 67.5±7.4    |
| 1 mg/kg i.v.  | 125.0±9.1   | 65.0±10.4   |
| 3 mg/kg i.v.  | 115.8±4.7   | 53.5±5.4    |

Values are represented as means±S.E. from observations on five different rats.
to the rat. As shown in Fig. 1 and Table 2, the preventive effects of SG-75 (10 mg/kg) on methacholine-induced ECG changes appeared about 10 min after p.o. administration and lasted 40 min. The depressor responses to SG-75 recovered near the initial level, 20–25 min after the oral administration, and then the dose-response curves to methacholine for BP changes were re-examined. The depressor responses to methacholine remained unchanged before and after the administration of SG-75 (Fig. 2).

Isolated donor-perfused spontaneously beating rat hearts

Control observations: Experiments were performed on 19 preparations. The mean coronary perfusion pressure was set at a value slightly lower than the mean systemic blood pressure of the donor at the onset of perfusion. Within 30 min after the preparations became stable, the functional parameters were as follows: coronary perfusion pressure (PP), 96.5±2.1 mmHg; left ventricular pressure (LVP), 122.5±3.4 mmHg; coronary blood inflow, 3.0±0.1 ml/min; heart rate (HR), 287.1±16.3 beats/min; systemic blood pressure (BP) of the donor, 111.3±5.0 mmHg.

Vasodilator effects of intra-arterial SG-75: When injected into the coronary perfusion system, single doses of SG-75 (1–30 μg) decreased PP, in a dose-dependent manner. The data from 6 different preparations are summarized in Fig. 3.

Influences of SG-75 administered orally to the donor rat on the coronary vasoconstriction caused by intra-arterial acetylcholine:

Table 2. Preventive effects of SG-75 administered intravenously or orally on methacholine-induced ECG (lead II) changes in intact anesthetized rats

|          | Control | Methacholine 4 to 8 μg before SG-75 or papaverine | Methacholine 4 to 8 μg after SG-75 or papaverine | Duration of protection min |
|----------|---------|--------------------------------------------------|--------------------------------------------------|---------------------------|
|          | Changes in S waves (mV) |                                                  |                                                  |                           |
| SG-75    |         |                                                  |                                                  |                           |
| 3 mg/kg p.o. | -0.05±0.02 | +0.10±0.02                                      | +0.05±0.02                                      | 21±9                      |
| 10 mg/kg p.o. | -0.05±0.02 | -0.12±0.02                                      | 0 ±0.01                                         | 40±6                      |
| 1 mg/kg i.v. | -0.03±0.02 | +0.12±0.01                                      | +0.08±0.02                                      | 6±2                       |
| 3 mg/kg i.v. | -0.04±0.02 | +0.10±0.02                                      | -0.02±0.02*                                     | 9±2                       |
| Papaverine |         |                                                  |                                                  |                           |
| 1 mg/kg i.v. | -0.08±0.03 | +0.10±0.02                                      | +0.10±0.04                                      |                           |

Changes in the S waves from the baselines were measured and taken as an indicator of myocardial ischemia. Values are represented as means±S.E. from observations of five different rats. Significant differences at P<0.05, **P<0.01, as compared with the value obtained with methacholine (4 to 8 μg) before SG-75 was administered.

Fig. 2. Dose-response curves for peak decreases in systemic blood pressure (BP) induced by i.v. methacholine before and after SG-75 10 mg/kg was administered p.o. to intact anesthetized rats. Vertical bars represent means ± S.E. from 5 different preparations. There were no significant differences (P>0.05) between the corresponding values before and after SG-75 administered p.o. to intact anesthetized rats. Initial systemic blood pressure (BP): 111.7±6.2 mmHg.
Acetylcholine (ACh) (0.1–0.8 μg) produced dose-dependent decreases in LVP and HR, and prominent increases in PP (vasoconstriction) (Fig. 4). After the dose-response curves to ACh for PP changes had been obtained, 10 mg/kg SG-75 was administered into the stomach of the donor rat. The SG-75 slightly decreased the BP of the donor.

Even though it did not significantly affect the HR, the SG-75 also reduced the PP and the LVP of the isolated heart, which reached a steady-state level within 10 to 15 min after the administration: BP of the donor, −13.6 ±2.4%; PP, −14.8±2.0%; LVP, −8.6±3.5%; N=7. Then the same dose range of ACh was again injected into the coronary perfusion system. The coronary vasoconstrictor response to ACh was inhibited significantly by the oral administration to the donor rat of 10 mg/kg of SG-75. The original tracings and the summarized data are shown in Figs. 4 and 5.

A single dose of papaverine (30 μg) administered into the coronary perfusion system decreased PP (−10.6±1.2%, N=8). The effect of papaverine lasted about 5 min. Thirty sec after the administration of papaverine the decrease in PP reached a peak and then 0.3 μg ACh was injected into the perfusion system. The coronary vasoconstriction elicited by ACh was not significantly modified by 30 μg papaverine: before, +29.1±1.2 mmHg; after papaverine, +26.8±2.3 mmHg, P>0.05, N=8.
DISCUSSION

The present study shows that SG-75 has a preventive effect on the elevation of the ST segment and the T wave induced by methacholine in the intact anesthetized rat. The preventive effect of SG-75 on methacholine-induced ECG changes appeared about 10 min after dosing and lasted 40 min. It should be noted that maximal plasma concentrations of SG-75 administered orally to the rat were attained at 10–30 min after dosing (15). As in the previous studies (13, 14), when administered into the coronary perfusion system of the isolated, donor-perfused rat heart, the cholinomimetic drugs such as acetylcholine or methacholine caused a prominent vasoconstriction. This fact suggests that the ECG changes induced by intra-aortic methacholine in the intact rat can be ascribed to coronary vasoconstriction, indicating severe tissue anoxia or ischemia. It was interesting, therefore, that SG-75 administered orally to the donor rat inhibited the coronary vasoconstriction produced by acetylcholine.

It has been reported that cholinomimetic drugs such as acetylcholine administered intra-arterially to dogs produce coronary vasodilation but not vasoconstriction (16–21). However a recent study revealed that the cholinomimetic drugs administered intra-arterially could elicit coronary vasoconstriction in the monkey or pig heart (22). Thus, it seems that there are species differences in coronary vascular responses to cholinomimetic drugs.

The inhibitory effect of SG-75 against the cholinomimetic drugs in the intact rat or in the isolated donor-perfused rat heart cannot be attributed to an antagonistic action toward vascular muscarinic receptors, since SG-75 does not possess an atropine-like action. SG-75 is a potent coronary vasodilator (1–4). However, the afore-mentioned preventive effect of SG-75 on cholinomimetic drugs cannot be explained by only a dilator action of SG-75, since potent vasodilators such as papaverine and dipyridamole did not modify the increases in coronary perfusion pressure elicited by acetylcholine in the isolated, donor-perfused rat heart, and fur-
thermore had no preventive effect on the elevation of the ST segment and the T wave induced by methacholine in the intact rat (12).

According to the reports by Nakagawa et al. (2) and Sakanashi et al. (23), SG-75 causes a relaxation of the potassium-induced contraction and is antagonistic toward calcium in isolated smooth muscle preparations of canine coronary artery bathed in physiological saline solution. Similar results were obtained by us in dog coronary arterial strips contracted with potassium or prostaglandin E2 (Shiraki and Sakai, unpublished data). Furthermore, Uchida et al. (24) reported that SG-75 ameliorated the cyclic reduction of coronary blood flow and ST elevation in the ECG caused by partial occlusion of the left anterior descending coronary artery in dogs. Our present results taken together with the series of findings in the previous studies (2, 23, 24), indicate that SG-75 appears to have a non-specific vasospasmolytic action in vivo as well as in vitro. It is likely, therefore, that the elevation of the ST segment and T wave in the ECG patterns induced by methacholine in our angina model (12) was prevented mainly by the vasospasmolytic action of SG-75.

The methacholine-induced ECG changes in the intact rat resemble those seen during variant anginal attacks caused by severe vasospasm of a large coronary artery in patients (8–11). It has been reported, that cholinomimetic drugs such as methacholine or pilocarpine are capable of producing a coronary arterial spasm corresponding to the ST segment elevation in patients with variant angina (25).

In view of the pharmacological characteristics, SG-75 should elicit improvement in clinical sequelae in certain types of variant angina pectoris.

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