THE HYPOTENSIVE MECHANISMS OF THE NEW ANTI-ANGINAL DRUG, N-(2-HYDROXYETHYL)NICOTINAMIDE NITRATE (SG-75) IN BEAGLE DOGS

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Abstract—The hypotensive mechanisms of N-(2-hydroxyethyl) nicotinamide nitrate (SG-75, Nicorandil) were studied in anesthetized dogs. Intravenous injections of SG-75 (0.03–1 mg/kg) decreased systemic blood pressure (SBP) and increased peripheral (coronary, renal, mesenteric and femoral) blood flow (PBF) dose-dependently. The duration of the PBF increase, however, was much shorter than that of the SBP decrease. When peripheral vascular beds were perfused by means of a pump under a constant perfusion pressure near the SBP, the duration and magnitude of the SBP decrease and the PBF increase were equal. In doses of 0.03–0.3 mg/kg i.v., SG-75 did not significantly affect pulse pressure, heart rate, aortic blood flow, left ventricular pressure (LVP) and LVdP/dt max. Intra-arterial injections of SG-75 (0.003–1 mg) increased coronary, renal, mesenteric and femoral blood flow dose-dependently, without affecting SBP and cardiac function. In heart-lung preparations the drug (0.1–2 mg) did not cause cardio-depression. No hypotensive effect was observed following the administration of SG-75 (3 mg) into the cisterna magna. The results indicate that the hypotensive effect of SG-75 may be due mainly to its peripheral mechanisms, relating to vasodilation.

For many years much effort has been devoted to the development of new anti-anginal drugs. As a result about a dozen new coronary vasodilating remedies have appeared on the market, none of which however fully satisfies all the criteria as an ideal anti-anginal drug. N-(2-Hydroxyethyl) nicotinamide nitrate (SG-75, Nicorandil), a newly synthesized nicotinamide derivative, is a novel coronary vasodilator under development as a remedy for angina pectoris. At a dose doubling the coronary blood flow this compound decreases the systemic blood

pressure, whereas it has little effect on myocardial oxygen consumption and atrio-ventricular conduction in anesthetized dogs (1–4). SG-75 has a nitrate moiety in the chemical structure, which seems to play an essential role in the development of pharmacological activity of this compound (3, 5). According to our previous study (4), however, it seems that there are some differences in the pharmacological profile between SG-75 and nitroglycerin. Although a series of studies has contributed to the clarification of the pharmacological properties of SG-75 (1–13), the mechanisms of its hypotensive effects remain to be in-

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vestigated.

In the present study, extensive pharmacological experiments with SG-75 were carried out to analyze the hypotensive responses to this compound in beagle dogs.

MATERIALS AND METHODS

Experiments were performed exclusively on adult beagle dogs (8–12 kg) of both sexes. The animals were initially anesthetized with sodium pentobarbital, 35 mg/kg, i.v., and pentobarbital, 12 mg/kg, dissolved in 20 ml of 0.9% saline solution was infused i.v. at a rate of 0.11 ml/min by means of a Harvard infusion/withdrawal pump (model 944), except in those animals used for heart-lung (H-L) preparations. Systemic blood pressure was measured with a pressure transducer (Nihon Kohden, MPU-0.5) by inserting a polyethylene tube into the femoral artery. Heart rate was continuously monitored with a heart rate counter (Nihon Kohden, AT-600G). For a cisternal application of drug solutions, a polyethylene catheter of 1 mm ID was inserted 2–3 cm into the cisterna magna. In this case, 0.1 ml of drug solutions was introduced into the cisterna magna by a tube and flushed in with 0.2 ml of 0.9% saline.

Cardiohemodynamic studies and the measurement of peripheral (coronary, renal, superior mesenteric and femoral) blood flow followed procedures as described previously (4). Aortic and peripheral blood flows were measured by placing precalibrated non-cannulating electromagnetic flow probes (Nihon Kohden, MF-27) around the respective vessels. The peripheral vascular resistance (mmHg/ml/min) was calculated from the mean systemic blood pressure and each blood flow. In some femoral circulation experiments, the perfusion pressure was maintained constantly near the systemic blood pressure by means of a pneumatic artificial resistance (14). To measure the left ven-

tricular systolic pressure (LVP), a microtip pressure manometer (model PC-350; Millar Instruments, Houston, Texas) was introduced into the left ventricle *via* the left atrium. The first derivative (LVdP/dt max) of the LVP was derived using a resistance-capacitance differentiating circuit (Nihon Kohden, EQ-600G). To inject drugs intra-arterially, a polyethylene tube (0.8 mm OD) was introduced into a distal circumflex or anterior descending branch of the left coronary artery, and a 30 mm, 25 gauge needle with a polyethylene tube (PE 50) was inserted through a direct puncture of the renal, superior mesenteric or femoral vessel wall and fixed there with biotissue adhesive (Aron Alpha®, Sankyo).

The H-L preparations were prepared according to the Krayer-Mendez modifications of the original Starling method (15) with arrangement and experimental conditions as previously described (16, 17). Artificial respiration was performed with a gas mixture of 95% O₂:5% CO₂ in a tidal volume of 20 ml/kg at a rate of 16 breaths/min using a dog respirator (Harvard Apparatus, model 607). Right atrial pressure was recorded with a pressure transducer (Nihon Kohden, LPU-0.1). Arterial pressure was recorded with a pressure transducer (Nihon Kohden, MPU-0.5) connected to a side branch of the arterial cannula. Systemic output (cardiac output minus coronary blood flow) and coronary sinus outflow led out by a Morawitz cannula were measured by means of electromagnetic flowmeters (Nihon Kohden, MF-27). The pneumatic artificial resistance was set at about 100 mmHg and the height of the blood level of the venous reservoir was kept constant at 70 mm above the right atrium. All recordings were made on a chart by using a Nihon Kohden WI-680G recorder. Standard limb II was used for the ECG with a Nihon Kohden ECG-3002. Measurements of PO₂, PCO₂ and pH

of blood samples were made using a blood gas analyzing system (Radiometer, BMS3-MK2).

The drugs used were: N-(2-hydroxyethyl) nicotinamide nitrate ester (SG-75, Nicorandil) (synthesized in the Chugai Institute), ephedrine hydrochloride (Dainippon) and clonidine hydrochloride (Boehringer Ingelheim). The drugs were dissolved in or diluted with 0.9% saline. For intra-arterial administration, 0.1 ml of drug solutions were injected over a 10 sec interval and flushed in with 0.2 ml of 0.9% saline. For intravenous administration, the cephalic vein was cannulated, and less than 2 ml of drug solutions were injected over a 20 sec interval and flushed in with 0.9% saline. Peak responses to drugs were expressed as the percentage changes in each parameter from preadministration levels. Values given in the text are means $\pm$ SE. The difference of mean values were analyzed by the Student's *t*-test and judged to be significant when *p* values were less than 0.05.

RESULTS

Hypotensive effects of SG-75 in the closed-chest dog

SG-75 in doses of 0.03 to 3 mg/kg was administered intravenously. At 0.03 mg/kg, this drug had no effects on the systemic blood pressure (SBP) and heart rate (HR). In doses between 0.1 mg/kg and 3 mg/kg SG-75 caused a sustained decrease in SBP and a moderate increase in HR in a dose-related fashion (Fig. 1). The increase in HR induced by SG-75 was significantly reduced by cutting the vagus nerves bilaterally at the midcervical region: 100 μ g/kg, before $7.7\pm0.7\%$; after cutting $2.7\pm1.2\%$, $P<0.05$, $N=4$; 300 μ g/kg, before $15.0\pm4.0\%$; after cutting $5.2\pm2.9\%$, $P<0.05$, $N=4$. The time course of the depressor response to SG-75 (300 μ g/kg) followed over 1 hr is shown in Fig. 2A and B (show the results from the

Fig. 1. Dose-response curves of intravenous SG-75 for peak changes in systolic (SBP), mean (MBP), diastolic blood pressure (DBP) and heart rate (HR) in the closed-chest dog. Each point represents the mean of 4 experiments in anesthetized dogs and vertical bars indicate mean $\pm$ SE. Initial values were: SBP, 229.5 ± 12.4 mmHg; MBP, 162.3 ± 5.1 mmHg; DBP, 138.5 ± 5.0 mmHg; HR, 207.8 ± 9.8 beats/min.

open-chest dog). It can be seen that the depressor response reached a peak at about 1 min, thereafter it rose slowly, and blood pressure returned to normal after 1 hr.

Cardiohemodynamic studies of SG-75 in the open- or the closed-chest dog

Cardiovascular effects of intravenous SG-75: The basal values of the main parameters are presented in Table 1. Typical effects of SG-75 on these parameters are shown in Fig. 2A and the data are summarized in Fig. 2B and C. In the open-chest dog, SG-75 (0.03–1 mg/kg) caused a reduction in the SBP and coronary vascular resistance, and an increase in the left anterior descending artery flow (CBF) in a dose dependent manner. In doses of 0.03 to 0.3 mg/kg, SG-75 did not appreciably affect pulse pressure (PP), HR, right atrial pressure

Fig. 2A

Fig. 2. Cardiovascular effects of SG-75 administered intravenously. SBP, systemic blood pressure; PP, pulse pressure; HR, heart rate; RAP, right atrial pressure; ABF, aortic blood flow; LVP, left ventricular pressure; CBF, left anterior descending coronary artery flow; CVR, coronary vascular resistance; MBF, superior mesenteric blood flow; MVR, mesenteric vascular resistance; RBF, left renal blood flow; RVR, renal vascular resistance; FBF, left femoral blood flow; FVR, femoral vascular resistance. A) Original tracings, and B) Time course of the changes in SBP, peripheral blood flow and vascular resistance following intravenous administration of SG-75 (300 µg/kg). The vertical bars show means $\pm$ SE from 5 preparations. Experiments were performed in open- (A) or closed- (B) chest dogs. C) Dose-response curves for peak changes in cardiovascular parameters following intravenous administration of SG-75. $\blacktriangle$ — $\blacktriangle$, increase; $\bullet$ — $\bullet$, decrease. Values are the means of peak changes in each parameter from the preadministration level. Each point represents the mean from 5 preparations. The vertical bars show means $\pm$ SE. Renal, mesenteric and femoral blood flows and vascular resistance were calculated only at the doses of 100 and 300 µg/kg.

Fig. 2B

(RAP), aortic blood flow (ABF), LVP or LVdP/dt max. At 1 mg/kg, this drug decreased PP, HR, LVP and LVdP/dt max and increased RAP. A slight increase was observed in the ABF, often preceded by a transient decrease.

In the closed-chest dog, the effects of SG-75 on peripheral blood flow (PBF) and vascular resistance (PVR) were examined. When administered intravenously, SG-75

Fig. 2C

(0.1 and 0.3 mg/kg) increased the left renal (RBF), superior mesenteric (MBF) and left femoral blood flows (FBF) and decreased each PVR in a dose dependent fashion. Compared with the decrease in the SBP, the increases in the PBF had a very short duration: At 300 $\mu\text{g/kg}$, the effect on the SBP lasted over 1 hr, but that on the PBF wore off in about 5 min following the administration. The decrease in the PVR calculated from each PBF and the mean SBP, however, paralleled the decrease in the SBP in duration and magnitude (Fig. 2B).

In some experiments, femoral circulation was studied under constant perfusion pressure near the SBP. When 300 $\mu\text{g/kg}$ of SG-75 was administered intravenously, the decrease in the SBP and increase in the FBF were in parallel in duration and magnitude. A typical example from 3 experiments is shown in Fig. 3.

Effects of intra-arterial SG-75 on coronary, renal, mesenteric and femoral circulation: Basal values of the left circumflex coronary blood flow (CBF), RBF, MBF or FBF from 5 different preparations were as follows:

Table 1. Basal values of cardiohemodynamic parameters before intravenous administration of SG-75

Systemic blood pressure (mean, mm Hg)	134.0 $\pm$ 3.3
Pulse pressure (mm Hg)	76.0 $\pm$ 2.2
Heart rate (beats/min)	162.8 $\pm$ 7.6
Right atrial pressure (cm H ₂ O)	3.7 $\pm$ 0.7
Aortic blood flow (ml/min)	1776 $\pm$ 164
Left ventricular systolic pressure (mm Hg)	234.0 $\pm$ 23.1
Left ventricular dP/dt _{max} (mm Hg/sec)	4510 $\pm$ 674
Coronary (left anterior descending branch) blood flow (ml/min)	15.2 $\pm$ 2.1
Coronary vascular resistance (mm Hg/ml/min)	8.8 $\pm$ 1.3
Superior mesenteric blood flow (ml/min)	106.2 $\pm$ 23.2
Mesenteric vascular resistance (mm Hg/ml/min)	1.71 $\pm$ 0.28
Left renal blood flow (ml/min)	95.4 $\pm$ 12.1
Renal vascular resistance (mm Hg/ml/min)	1.71 $\pm$ 0.19
Left femoral blood flow (ml/min)	66.2 $\pm$ 11.2
Femoral vascular resistance (mm Hg/ml/min)	2.98 $\pm$ 0.49

Values represented are means $\pm$ SE of 5 different preparations.

CBF, 22.8 ± 6.6 ml/min; RBF, 95.6 ± 11.4 ml/min; MBF, 111.8 ± 19.4 ml/min; FBF, 68.4 ± 13.7 ml/min.

Each dose of SG-75 was given after the effect of the preceding dose had disappeared; the intervals between drug injections thus

Fig. 3. Changes in systemic blood pressure (SBP), heart rate (HR) and femoral blood flow (FBF) following an intravenous administration of SG-75 (300 μ g/kg). The hindlimb was perfused through the femoral artery by means of a pump under a constant pressure.

Fig. 4. Dose-response curves of intra-arterial SG-75 for peak increases of left circumflex coronary (CBF), left renal (RBF), superior mesenteric (MBF) and left femoral (FBF) blood flows. Each point represents the mean of 5 experiments in 5 dogs and vertical bars indicate means $\pm$ SE.

ranged from 5 to 10 min depending on the duration of the effect. Single injections of SG-75 (3–100 μ g) into the coronary artery of the open-chest dog produced dose-dependent increases in the CBF. Within the dose-range tested, this drug had virtually no effect on the SBP, HR, LVP or LVdP/dt max. When SG-75 was injected into the renal, superior mesenteric or femoral artery, a dose-dependent increase of blood flow was observed in each vascular bed. Figure 4

Fig. 5. Dose-response curves for peak changes in right atrial pressure (RAP), heart rate (HR), systemic output (SOP) and coronary sinus outflow (CBF) caused by single bolus injections of SG-75 to the venous reservoir in canine heart-lung preparations. Each point represents the mean of 5 preparations. The vertical bars show means $\pm$ SE. Each value ($\bullet$) of CBF was significantly different from control ($\blacktriangle$). The pO_2 , pCO_2 and pH in arterial blood were 433.5 ± 12.2 mmHg, 26.7 ± 0.6 mmHg and 7.37 ± 0.01 , respectively. The values of SOP and CBF were expressed as ml/min per 100 g of the left ventricular wet weight.

shows the dose-response curves for the peak increase of each blood flow.

Effects of SG-75 in canine heart (H)-lung (L) preparations

Experiments were performed in 5 different H-L preparations. Since SG-75 is not metabolized in blood (5), it was added cumulatively to the venous reservoir. Single bolus injections of SG-75 in doses smaller than 100 μ g had no effect on the RAP, HR, systemic output (SOP) or CBF. In larger doses, SG-75 increased the CBF dose-dependently, but did not significantly affect the RAP, HR or SOP (Fig. 5).

Effects of SG-75 administered into cisterna magna on SBP and HR

An intra-cisternal injection of 1 or 3 mg (corresponding to about 300 μ g/kg) of SG-75 produced no changes in the SBP and the HR, while intravenous administration of the same amount of SG-75 (3 mg) caused a sustained hypotension (Fig. 2A and B). When administered into the cisterna magna, ephedrine (4 mg) caused a rise in the SBP accompanying a decrease in the HR, and clonidine (1–10 μ g) produced a long-lasting depressor response preceded by a transient pressor one and a marked decrease in the HR (Figure is not presented).

DISCUSSION

The results of these experiments have shown that in the closed-chest dogs SG-75 administered intravenously produces a prolonged dose-dependent decrease in the systemic blood pressure and an increase in the heart rate. Since the increase in heart rate was markedly reduced by cutting the vagus nerves bilaterally at the midcervical region, the effects seem to be ascribable to a circulatory reflex induced by hypotensive effects. This view may be supported by the report of Taira et al. (3) that, when injected into the sinus node or the atrio-ventricular (A-V) node artery of the dog,

SG-75 at dose levels doubling the flows had virtually no effect on the sinus rate or the A-V conduction time.

In the present study, SG-75 administered intravenously increased the peripheral (coronary, renal, superior mesenteric and femoral) blood flow and decreased the peripheral vascular resistance, accompanied by a sustained decrease in systemic blood pressure. The increase in the peripheral blood flow, however, had an extremely short duration compared with the hypotensive effect of SG-75, although the decrease in the peripheral vascular resistance was in parallel in the duration and magnitude with that in the systemic blood pressure. The relation between systemic blood pressure and peripheral blood flow was examined by perfusing the hindlimb through the femoral artery of the dog under a constant pressure. The experiment suggested that the hypotensive response to SG-75 administered intravenously might be dependent on peripheral vasodilation.

It has also been shown that intra-arterial injections of SG-75 increases the coronary, renal, superior mesenteric and femoral blood flow in dogs. According to the previous results (4), the vasodilating effects of SG-75 are not mediated through cholinergic, adrenergic, histaminergic or adenosine potentiating mechanisms. Furthermore, the vasodilation caused by SG-75 may not be due to a papaverine-like action, since SG-75 was about 300-fold less potent than papaverine in inhibiting phosphodiesterase activity (9). Although the mechanism is still obscure, SG-75 clearly has a direct vasodilating action on the peripheral vascular beds.

The hypotensive response to SG-75 cannot be due to its cardiac action, since a dose which doubles the coronary blood flow in open-chest dogs, causes a long-lasting hypotension, but has virtually no effect on myocardial contraction, heart rate or

myocardial oxygen consumption (2–4). Similar results were obtained in the present experiment.

The hypotension due to SG-75 does not appear to be of central nervous system origin, since SG-75, unlike ephedrine and clonidine, elicited no response following administration into the dog's cisterna magna. In the present experiment, we used dogs weighing about 10 kg, and therefore the dose (3 mg) of SG-75 administered into the cisterna magna corresponds to the 300 μ g/kg intravenous dose which is sufficient to develop the hypotensive effect.

In summary, the present results indicate that the hypotensive response to SG-75 may not be mediated through the central nervous system or cardiac effects, but instead is mainly ascribable to the reduction of peripheral vascular resistance related to peripheral vasodilation.

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