CIRCUMSTANTIAL EVIDENCE FOR INCREASED POTASSIUM CONDUCTANCE OF MEMBRANE OF CARDIAC MUSCLE BY 2-NICOTINAMIDOETHYL NITRATE (SG-75)

Teruyuki YANAGISAWA, Keisuke SATOH and Norio TAIRA
Department of Pharmacology, Tohoku University School of Medicine, Sendai 980, Japan
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Abstract—The mechanism of action of 2-nicotinamidoethyl nitrate (SG-75), was investigated by the use of arterially blood-perfused papillary muscle preparations of the dog. All drugs were administered intra-arterially. SG-75 shortened the effective refractory period (ERP) and decreased the rate of automaticity and developed tension of the papillary muscle, whereas verapamil failed to change the ERP despite a decrease in the developed tension. SG-75 in extremely high doses induced ventricular fibrillation. Methacholine produced decreases in the rate of automaticity and developed tension, and the actions were abolished by atropine. The SG-75-induced decreases in two parameters were not modified by atropine. These results indicate that the cardiac action of SG-75 differs from that of calcium-antagonistic vasodilators and it is suggested that the basic mechanism of action of SG-75 involves an increase in potassium conductance in the membrane of cardiac muscle, without mediation through muscarinic receptors.

2-Nicotinamidoethyl nitrate (SG-75) is a new compound found by Uchida et al. (1) to have a coronary vasodilator action comparable in potency to that of papaverine. Subsequently Taira et al. (2) confirmed the results obtained by Uchida et al. (1) and further found that unlike calcium-antagonistic coronary vasodilators SG-75 scarcely impaired the atioventricular (AV) conduction in the canine blood-perfused AV node preparation and scarcely affected the developed tension of the canine blood-perfused papillary muscle in doses which increased markedly blood flow through these preparations. Calcium-antagonistic coronary vasodilators are well-known to impair AV conduction and to depress force of contraction of the myocardium (3). Taira et al. (2) also found that unlike nitroglycerin SG-75 given into the ascending aorta increased venous return in anesthetized dogs. Reduction of venous return is characteristic of vascular action of nitroglycerin (4). Based on these observations, it was suggested that the mechanism of cardiovascular action of SG-75 may differ from either that of calcium-antagonistic coronary vasodilators or that of nitrates (2).

In the present experiments, we investigated the effects of SG-75 on the effective refractory period and automaticity of the ventricular myocardium using the canine blood-perfused papillary muscle preparation, because such an investigation would give some insight into the mechanism of action of this drug.

MATERIALS AND METHODS

Mongrel dogs of either sex, weighing 8–12 kg were anesthetized with sodium pentobar-
bital (30 mg/kg, i.v.), given sodium heparin (500 units/kg, i.v.) and exanguinated. The heart was quickly removed and the preparation was dissected in cooled Tyrode solution (about 4°C). The anterior papillary muscle of the right ventricle was excised together with the ventricular septum and a polyethylene cannula was placed in the anterior septal artery. The preparation was placed in a glass water-jacket maintained at about 38°C, and perfused via the cannulated anterior septal artery with arterial blood led from a donor dog. Constant pressure perfusion at about 100 mm Hg was achieved by the use of a peristaltic pump (Harvard Apparatus, Model 500-1200) and by the placement of a Starling pneumatic resistance in parallel with the preparation. Donor dogs were 15–22 kg in body weight, and were anesthetized with sodium pentobarbital initially at a dose of 30 mg/kg, i.v. and hourly at a supplemental dose of 4–5 mg/kg, i.v. Sodium heparin was given initially at a dose of 500 units/kg, i.v. and hourly at a supplemental dose of 100 units/kg, i.v. Details of the preparation have been described by Endoh and Hashimoto (5). The developed tension of the papillary muscle was measured with a strain-gauge transducer (Grass, FT03B). The muscle was preloaded so as to develop maximal tension, the load being about 3 g. Bipolar stimulating electrodes were placed diagonally at the base of the papillary muscle and bipolar recording electrodes were placed in a position perpendicular to the stimulating electrodes at the base of the papillary muscle. Blood flow through the anterior septal artery was measured by an electromagnetic flowmeter (Nihon Kohden, MF-26). All recordings were made on a chart by the use of a rectilinear recorder (San-ei Instrument, 8S).

In the experiments designed to measure the effective refractory period (ERP), to prevent frequency-induced changes in this parameter, the preparation was stimulated at a basic driving rate of 120 beats/min (stimulus interval: 500 msec) with rectangular pulses of twice the threshold voltage (2–4 V) and 5 msec in duration. The ERP was measured by delivering a test stimulus (S') after every 7th basic stimulus (S) (a conditioning stimulus). Test stimuli had the same voltage and duration as conditioning stimuli. Intervals between successive conditioning and test stimuli were automatically decreased until responses to test stimuli disappeared. In this study we defined the ERP as the shortest S-S' interval for S' to elicit the propagated response. In the present experiments the intensity of stimuli was low enough and the distance between the stimulating and recording electrodes was long enough for the recording electrodes to record propagated cardiac impulses. Details of the apparatus which measures the ERP automatically have been described elsewhere (6).

In the experiments designed to measure ventricular automaticity the preparation was not driven. Bipolar electrograms obtained by the recording electrodes were fed to a cardiotachometer (Nihon Kohden, RT-5).

The drugs used were 2-nicotinamidoethyl nitrate (SG-75) (Chugai Pharmaceutical Co.), verapamil hydrochloride (Knoll AG), methacholine chloride (Merck) and atropine sulfate (Hoei Pharmaceutical Co.). All drugs were dissolved in 0.9% saline. Except in the ERP experiments drug solutions in a volume 10–30 μl were injected into rubber tubing connected to the arterial cannula of the preparation over a period of 4 sec with individual micro-syringes. In the ERP experiments solutions of SG-75 or verapamil were infused intra-
arterially at a rate of 0.1 ml/min by means of an infusion pump (Harvard Apparatus, Model 600-900). Doses of all drugs refer to their bases.

Experimental values are expressed by means±S.E. The significance of difference between means was evaluated by Student's t-test and expressed in terms of p values. A p value smaller than 0.05 was considered to be significant.

RESULTS

Blood flow through the anterior septal artery of 29 blood-perfused papillary muscle preparations under a constant perfusion pressure of about 100 mm Hg was 5.5±0.6 ml/min.

Effects of SG-75 on the effective refractory period and developed tension of the blood-perfused papillary muscle: Control values of the effective refractory period (ERP) and developed tension of 6 blood-perfused papillary muscles were 205±8 msec and 5.8±1.4 g, respectively. Measurements of the ERP under drug action were made at least 10 min after the start of infusion of SG-75, as by this time, the reduced developed tension had reached a steady level. As summarized in Fig. 1, infusion of SG-75 (0.1–3 mg/min) produced dose-dependent decreases in ERP and developed tension. At the highest dose of SG-75 (3 mg/min) the ERP was shortened to 138±11 msec (about 67% of control) and developed tension was reduced to 1.3±0.7 g (about 22% of control), and fibrillation appeared in 2 of the 6 preparations. Because of occurrence of fibrillation the experimental number with 3 mg/min of SG-75 was 4 (Fig. 1). Fibrillation disappeared in 10–30 min after the discontinuation of infusion. The changes in the ERP and developed tension were also reversible.

Effects of verapamil on the effective refractory period and developed tension of the blood-perfused papillary muscle: Control values of the ERP and developed tension of 8 blood-

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**FIG. 1.** Dose-response curves to intra-arterial infusion of SG-75 and verapamil for changes in effective refractory period and developed tension of canine blood-perfused papillary muscles. Each symbol is the mean value and vertical bars are S.E. of the mean. The experimental numbers are indicated in parentheses. *: p<0.05; **: p<0.01.
perfused papillary muscles were 217±6 msec and 6.4±1.0 g, respectively. Measurements of the ERP under drug action were made about 10 min after the start of infusion of verapamil. Infusion of verapamil (0.01–0.3 mg/min) produced a dose-dependent decrease in developed tension but no significant change in ERP (Fig. 1). A decrease in developed tension produced by verapamil was not readily reversible, that is, the developed tension remained still reduced even 3 hr after the discontinuation of the infusion. Unlike SG-75, verapamil elicited no fibrillation.

**Effects of SG-75 on the automaticity and developed tension of the blood-perfused papillary muscle:** Control values of the rate of automaticity and the developed tension of 10 spontaneously beating blood-perfused papillary muscles were 43±4 beats/min and 2.9±0.4 g, respectively. By a single intra-arterial injection of SG-75 (0.01–1 mg) the rate of automaticity and the developed tension were reduced. A typical experiment is shown in Fig. 2 and summarized data obtained from 10 preparations are shown in Fig. 3. With 1 mg of SG-75 fibrillation appeared in 1 of the 10 preparations. Fibrillation subsided in about 1 min, and automaticity reappeared in the papillary muscle. These changes in the rate of automaticity and the developed tension were reversible.

**Effects of methacholine on the automaticity and developed tension of the blood-perfused papillary muscle:** The control values of the rate of automaticity and the developed tension of 5 spontaneously beating blood-perfused papillary muscles were 37±3 beats/min and 3.7±0.5 g, respectively. A single injection of methacholine (0.1–3 μg) into the anterior septal artery reduced the rate of automaticity and the developed tension. The reduction of the developed tension by methacholine, however, was less pronounced than that by SG-75 (Fig. 4). These changes in the rate of automaticity and the developed tension were reversible.

**Effects of atropine on changes in automaticity and developed tension of the blood-perfused papillary muscle in response to SG-75 and methacholine:** A single injection of 100 μg of atropine into the anterior septal artery of 5 spontaneously beating blood-perfused papillary muscle preparations produced no significant changes in rate of automaticity and in developed

![Fig. 2. Effects of SG-75 injected into the anterior septal artery on the developed tension and on the rate of automaticity of a canine blood-perfused papillary muscle.](image-url)
tension (42 ± 5 beats/min and 4.1 ± 0.6 g against 40 ± 6 beats/min and 4.0 ± 0.7 g in control). This dose of atropine abolished the reduction of both rate of automaticity and developed tension produced by methacholine (0.1-3 μg) for about 30 min. The effects of SG-75 on the rate of automaticity and the developed tension observed during this 30-min period were not significantly different from those obtained before atropine (Fig. 4).

**FIG. 3.** Dose-response curves to intra-arterial injection of SG-75 for decreases in rate of automaticity and developed tension of 10 blood-perfused papillary muscles. Each symbol is the mean of 10 muscles. Vertical bars are S.E. of the mean. *: p<0.05; **: p<0.01.

**FIG. 4.** Effects of atropine on decreases by methacholine (MeCh) and SG-75 in rate of automaticity and developed tension of 5 blood-perfused papillary muscles. Each symbol is the mean of 5 muscles. Vertical bars are S.E. of the mean. *: p<0.05; **: p<0.01.

**DISCUSSION**

In the present experiments SG-75 injected or infused intra-arterially shortened the ERP and decreased the rate of automaticity and the developed tension of the blood-perfused papillary muscle preparation. The SG-75-induced reduction of the developed tension in the papillary muscle suggests that this drug has the calcium-antagonistic action (3, 7) or the local anesthetic or quinidine-like action as the fundamental mode of action, although the doses of SG-75 required to produce the decrease in developed tension in the present experiments were greater than those producing coronary vasodilation in the same preparation in the previous experiments (2). However, the previous study (2) has demonstrated that SG-75 differs from either calcium-antagonistic vasodilators or local anesthetics in several aspects. One of the characteristics which distinguish SG-75 from calcium-antagonistic vasodilators is that unlike calcium-antagonistic vasodilators SG-75 injected into the AV node artery produced neither marked prolongation of AV conduction time nor AV block (2). Calcium-antagonistic vasodilators injected into the AV node artery readily impair AV conduction (8). Occurrence of ventricular fibrillation with extremely large doses of SG-75
administered into the anterior septal artery as observed in the previous (2) and also in the present experiments is another characteristic of SG-75. Ventricular fibrillation was not elicited with calcium-antagonistic vasodilators administered into the anterior septal artery of the blood-perfused papillary muscle (9) or AV node (8) preparation. In the present experiments SG-75 shortened the ERP of ventricular muscle, whereas with verapamil, a prototype of calcium-antagonistic vasodilator, little change was seen. In this respect also, SG-75 differs from calcium-antagonistic vasodilators. Thus, the question arises what is responsible for the basic mechanism of action of SG-75 on cardiac tissues. The shortening of the ERP and occurrence of fibrillation by SG-75 do provide clues. Rosenblueth (10) has suggested that substances like acetylcholine which decrease the ERP of myocardial tissues accelerate the rate of fibrillation and sensitize the heart to fibrillation. In recent experiments Farges et al. (11) confirmed this, and suggested that the facilitation of the repolarizing potassium outward current may be a fundamental mechanism of action of such substances. Our results strongly suggest that SG-75 belongs to such a class of substances.

As reported previously (12, 13), unless the blood-perfused papillary muscle preparation is stimulated, the papillary muscle contracts spontaneously at a rate of about 40 beats/min. This automaticity has been considered to derive from Purkinje fibers existing in the preparation (12, 13). In the present experiments, SG-75 decreased the rate of ventricular automaticity whereas verapamil failed to affect it as reported previously (13). Thus, in this respect also, SG-75 differs from calcium-antagonistic vasodilators. The virtual absence of the effect of verapamil on the ventricular automaticity as observed in the previous experiments (13) is consistent with the finding by Tsien (14) that verapamil and methoxyverapamil (D 600) do not affect the diastolic depolarization or the kinetics of $i_K$ of calf cardiac Purkinje fibers. The slope of the pacemaker potential of cardiac Purkinje fibers, a primary determinant of the rate of ventricular automaticity, is governed by the time- and voltage-dependent fall of $i_K$ (14, 15). The SG-75-induced reduction of the rate of ventricular automaticity, taken together with the shortening of the ERP of ventricular muscle and its liability to fibrillation, strongly suggests that the basic mechanism of action of this compound would be to increase potassium conductance in the membrane of myocardial fibers.

In the present experiments methacholine injected into the anterior septal artery decreased the rate of automaticity and the developed tension of the blood-perfused papillary muscle preparation. Since these effects of methacholine were blocked by atropine, such are thought to be mediated through muscarinic receptors in the preparation. Recently, it has been clearly demonstrated that acetylcholine decreases the rate of automaticity of canine Purkinje fibers by the action on muscarinic receptors (16, 17), and it was suggested that this decrease in automaticity is probably due to an increase in the membrane potassium conductance (16, 17). The effects of SG-75 on the rate of automaticity and developed tension, unlike those of methacholine, were not modified by a dose of atropine sufficient to block the effects of methacholine. This indicates that the effects of SG-75 are not mediated through muscarinic receptors. In view of the facts that muscarinic receptors are sparse in the ventricular myocardium and that acetylcholine scarcely changes the action potential (18) and the ERP
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of ventricular muscle (11), the effectiveness of SG-75 on the ventricular myocardium is of prime interest.

Finally, a plausible explanation should be given to the reduction of the developed tension produced by SG-75. The reduction of the developed tension as observed in the spontaneously beating preparations may partially be ascribed to decrease in the rate of automaticity. However, the reduction of developed tension occurred in the preparations driven at a fixed rate. Thus, not all of the reduction of the developed tension by SG-75 is attributable to a decrease in frequency of contraction. In the present experiments SG-75 shortened the ERP of ventricular muscle. The shortening of the ERP of cardiac muscle is generally accompanied by shortening of the action potential. Voltage clamp experiments have shown that in ventricular muscle there is a direct relationship between action potential duration and developed tension (19). Thus, it is tempting to speculate that in the present experiments, SG-75 decreased developed tension possibly by shortening the ventricular action potential. However, in canine ventricular muscle an inside-positive clamp pulse of 100–200 msec duration has been claimed to be sufficient to elicit the maximum developed tension (19). In the present experiments the ERP was decreased from about 205 msec only to about 140 msec even with the highest dose of SG-75 (3 mg/min). Thus, one may doubt if the ventricular action potential of about 140 msec duration might be sufficient to produce the developed tension much the same as that in control. Nevertheless, it is still tempting to assume that SG-75 decreased developed tension by shortening the ventricular action potential. Fig. 3 of the paper by Beeler and Reuter (19) which concerns one experiment clearly shows that a clamp pulse of 20 mV inside positive and of about 140 msec duration elicited developed tension by about a half of the maximum tension produced by a pulse of the same amplitude but of 200 msec duration. One may still point out that the reduction of developed tension with the highest dose of SG-75 (3 mg/min) was far greater than that expected from the shortening of the ventricular action potential. Indeed, with the highest dose of SG-75 developed tension was decreased to about 22% of control. If SG-75 causes a generalized increase in potassium conductance of the membrane of ventricular muscle, this would not only shorten the action potential and thereby reduce the time during which the slow inward current flows but oppose membrane depolarization especially at phase 2 and thereby interfere with the full development of the slow inward current. Voltage clamp experiments will provide a satisfactory explanation of the negative inotropic action of SG-75. Before such sophisticated experiments we should examine with conventional microelectrode techniques whether or not SG-75 shortens the action potential of canine ventricular muscle.

All the findings obtained in the present experiments strongly suggest that the basic mechanism of action of SG-75 involves an increase in potassium conductance in the membrane of cardiac muscle and that this action is independent of stimulation of muscarinic receptors. This mechanism would also be responsible for the vasodilator action of this drug.

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REFERENCES

1) UCHIDA, Y., YOSHIMOTO, N. AND MURAO, S.: Effect of 2-nicotinamidethyl nitrate (SG-75) on coronary circulation. Japan. Heart J. 19, 112–124 (1978)

2) TAIRA, N., SATOH, K., YANAGISAWA, T., IMAI, Y. AND HIWATARI, M.: Pharmacological profile of a new coronary vasodilator drug, 2-nicotinamidoethyl nitrate (SG-75). Clin. exp. Pharmacol. Physiol. 6, 301–315 (1979)

3) FLECKENSTEIN, A.: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. A. Rev. Pharmacol. 17, 149–166 (1977)

4) CHARLIER, R.: Nitrates. Antianginal drugs. Pathophysiological, haemodynamic, methodological, pharmacological, biochemical and clinical basis for their use in human therapeutics. Handbook of Experimental Pharmacology, Vol. 31, p. 118–150, Springer-Verlag, Berlin-Heidelberg-New York (1971)

5) ENDOH, M. AND HASHIMOTO, K.: Pharmacological evidence of autonomic nerve activities in canine papillary muscle. Am. J. Physiol. 218, 1459–1463 (1970)

6) Taira, N., Iijima, T., Narimatsu, A., Satoh, K. AND YANAGISAWA, T.: Effects on atrio-ventricular conduction of propranolol, pindolol and carteolol in the dog heart in situ as assessed by automated devices. Japan. J. Pharmacol. 28, 473–483 (1978)

7) FLECKENSTEIN, A.: Specific inhibitors and promoters of calcium action in the excitation-contraction coupling of heart muscle and their role in the prevention or production of myocardial lesions. Calcium and the Heart. Edited by HARRIS, P. AND OPIE, L., p. 135–188, Academic Press, London and New York (1971)

8) Narimatsu, A. AND TAIRA, N.: Effects on atrio-ventricular conduction of calcium-antagonistic coronary vasodilators, local anaesthetics and quinidine injected into the posterior and the anterior septal artery of the atrio-ventricular node preparation of the dog. Arch. Pharmacol. 294, 169–177 (1976)

9) Himori, N., Ono, H. AND TAIRA, N.: Simultaneous assessment of effects of coronary vasodilators on the coronary blood flow and the myocardial contractility by using the blood-perfused canine papillary muscle. Japan. J. Pharmacol. 26, 427–435 (1976)

10) Rosenblueth, A.: The mechanism of auricular flutter and auricular fibrillation. Circulation 7, 612–613 (1953)

11) Farges, J.P., Faucou, G., Lievre, M. AND Ollagnier, M.: Relationship between atrial and ventricular rates of fibrillation and cardiac contractile tissue effective refractory periods in the dog. Brit. J. Pharmacol. 63, 587–591 (1978)

12) Endoh, M., Kimura, T. AND HASHIMOTO, K.: Effect of manganese ions on the contraction and automaticity of the blood-perfused canine papillary muscle. Japan. J. Pharmacol. 24, 771–778 (1974)

13) Endoh, M., YANAGISAWA, T. AND TAIRA, N.: Effects of calcium-antagonistic coronary vasodilators, nifedipine and verapamil, on ventricular automaticity of the dog. Arch. Pharmacol. 302, 235–238 (1978)

14) Tsien, R.W.: Effects of epinephrine on the pacemaker potassium current of cardiac Purkinje fibers. J. gen. Physiol. 64, 293–319 (1974)

15) Noble, D.: Potassium currents and pacemaker activity. The Initiation of the Heartbeat, p. 89–102, Oxford University Press, London (1975)

16) Tse, W.W., Han, J. AND Yoon, M.S.: Effect of acetylcholine on automaticity of canine Purkinje fibers. Am. J. Physiol. 230, 116–119 (1976)

17) Gadsby, D.C., Wit, A.L. AND CRANEFIELD, P.F.: The effects of acetylcholine on the electrical activity of canine cardiac Purkinje fibers. Circulation Res. 43, 29–35 (1978)

18) Hoffman, B.F. AND Suckling, E.E.: Cardiac cellular potentials: effect of vagal stimulation and acetylcholine. Am. J. Physiol. 173, 312–320 (1953)

19) Beeler, G.W. AND Reuter, H.: The relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. J. Physiol. 207, 211–229 (1970)