Effects of 4-Aminopyridine and Tetraethylammonium on Relaxant Responses of Isolated Dog Coronary Arteries to 2-Nicotinamidoethyl Nitrate (SG-75)

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SUMMARY

Effects of 4-aminopyridine (4-AP) and tetraethylammonium (TEA) on vasodilator actions of 2-nicotinamidoethyl nitrate (SG-75) were investigated using isolated dog coronary arteries. SG-75 10^{-8} \text{--} 10^{-4} \text{Gm/ml} produced concentration-dependent relaxations of coronary arterial strips which were contracted with potassium 30 mM or prostaglandin (PG) F_{2\alpha} 10^{-6} \text{Gm/ml}. Both 4-AP 5 \times 10^{-4} \text{Gm/ml} and TEA 1.6 \times 10^{-3} \text{Gm/ml} produced further contractions of the strips under potassium- or PGF_{2\alpha}-contracture. The potency to constrict the strips was greater in 4-AP than in TEA. 4-AP 5 \times 10^{-4} \text{Gm/ml} and TEA 1.6 \times 10^{-3} \text{Gm/ml} did not affect the SG-75-induced relaxations of the strips under potassium-contracture, but significantly depressed them under PGF_{2\alpha}-contracture. In the strips under a resting state, 4-AP 5 \times 10^{-4} \text{Gm/ml} increased tension and produced oscillations in their contractions, but TEA 1.6 \times 10^{-3} \text{Gm/ml} did not. Results suggest that SG-75 may display its relaxant effect on isolated dog coronary arteries in part through an increase in potassium conductance.

Additional Indexing Words:
Mechanism of action of SG-75 Potassium-contracture PGF_{2\alpha}-contracture Potassium conductance

IN 1978, Uchida, Yoshimoto, and Murao\(^1\) found that a new nitroester compound, 2-nicotinamidoethyl nitrate (SG-75), has a potent coronary vasodilator action. Though many investigations have been performed to explore the mechanisms of vasodilator action of SG-75,\(^2\)-\(^6\) the mode of action of which had not been fully clarified. Recently, Yanagisawa and Taira\(^7\) pro-
posed that the mechanism of action of SG-75 on left atrial muscle fibers of
the dog may be an increase in potassium conductance of the membrane.
Therefore, the present study was designed to investigate whether SG-75 can
affect on potassium conductance of the membrane of coronary arterial smooth
muscles producing coronary vasodilatation or not.

Methods

Experiments were carried out using adult mongrel dogs weighing 7.0–
17.0 Kg of either sex. After anesthetized with pentobarbital sodium 30 mg/
Kg i.v., animals underwent fourth intercostal thoracotomy under an artificial
ventilation. Then, the hearts were isolated and helical strips, 2.0 mm wide
and 20 mm long, were cut from left circumflex coronary arteries. The strips
were suspended in a 20-ml muscle bath filled with Krebs-Ringer bicarbonate
solution of the following millimolar composition: NaCl 117.7, KCl 4.7, CaCl₂
2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 24.4, and glucose 10.0. The solu-
tion was maintained at 37°C and aerated with a gas mixture of 95% O₂ and
5% CO₂. The oxygen tension of the solution was 600 mmHg and the pH
was 7.40 when measured by a blood-gas analyzer (Instrumentation Laboratory
Micro-13). The coronary arterial strips were connected to a force-displace-
ment transducer (Nihonkohden TB-611T) and isometric tension developments
were recorded on an ink-writing polygraph (Nihonkohden RJG-4004). An
initial or passive tension was adjusted to an optimal tension, about 1.0 Gm,
and the strips were allowed to equilibrate for 2 hrs before any experiments
were begun.

The following drugs were used in this experiment: 2-nicotinamidoethyl
nitrate (SG-75, Chugai), prostaglandin F₂α (PGF₂α, Ono), 4-aminopyridine
(4-AP, Sigma), tetraethylammonium chloride (TEA, Eastman-Kodak), and
diltiazem hydrochloride (Tanabe). All drugs were dissolved in physiological
saline solution, and the solvent had no effect. The drug solution with a
volume of 0.2 ml was added to the bath. The doses of the drugs were ex-
pressed as final bath concentrations of the salts in TEA and diltiazem and of
free forms in SG-75, PGF₂α, and 4-AP in terms of Gm/ml except potassium.

The data were statistically analyzed with Student’s t-test.

Results

SG-75 10⁻⁸–10⁻⁴ Gm/ml produced concentration-dependent relaxations
of the isolated dog coronary arterial strips (n=6) which were contracted with
potassium 30 mM. When the mean values were calculated, they reached
100±0% of the control value (100%=808±134 mg, n=6) with SG-75 10^{-8} Gm/ml, 97±1% with 10^{-7} Gm/ml, 80±4% with 10^{-6} Gm/ml, 45±10% with 10^{-5} Gm/ml, and -11±3% with 10^{-4} Gm/ml, respectively (Fig. 1). Diltiazem 10^{-4} Gm/ml produced no more changes in these strips (Fig. 1). 4-AP 5×10^{-4} Gm/ml further increased tension of the coronary arterial strips (n=4) under potassium-contracture to 1640±234 mg (203±29% of the control), but did not change relaxant responses of the strips (n=4) to SG-75 10^{-8}–10^{-5} Gm/ml (Fig. 1). Only the value obtained with SG-75 10^{-4} Gm/ml after treatment of 4-AP (21±11%) was significantly different from those before 4-AP (−11±3%). In these strips, diltiazem 10^{-4} Gm/ml produced further relaxations of the strips reaching −9±3% (Fig. 1). TEA 1.6×10^{-3} Gm/ml also produced further but slight increases in tension of the strips under potassium-contracture (n=4) which reached 832±8 mg (103±1% of the control), but did not induce any change in relaxant responses to SG-75 10^{-8}–10^{-4} Gm/ml (Fig. 1). Diltiazem 10^{-4} Gm/ml did not affect on the relaxations with SG-75 10^{-4} Gm/ml (Fig. 1).

Fig. 1. Effects of SG-75 on isolated dog coronary arterial strips under potassium-contracture. Open circles: control (n=6). Solid triangles: after 4-AP 5×10^{-4} Gm/ml (n=4). Solid circles: after TEA 1.6×10^{-3} Gm/ml (n=4). Open circle in circle, solid triangle in circle, and solid circle in circle represent the effect of diltiazem 10^{-4} Gm/ml on control-strips, 4-AP-treated strips, and TEA-treated strips, respectively. 100%=808±134 mg in control-strips (n=5), 1640±234 mg in 4-AP-treated strips (n=4), and 832±8 mg in TEA-treated strips (n=4). * p<0.05 vs control. Each point indicates means±S.E.
SG-75 $10^{-8}-10^{-4}$ Gm/ml relaxed concentration-dependently the isolated dog coronary arterial strips (n=6) which were contracted with PGF$_{2\alpha}$ $10^{-6}$ Gm/ml (Fig. 2B and 3). When the mean values were calculated in 6 pre-

![Figure 2](image_url)

**Fig. 2.** Typical recordings of effects of SG-75 on isolated dog coronary arterial strips under PGF$_{2\alpha}$-contracture. A: effect of TEA $1.6\times10^{-3}$ Gm/ml. B: control. Intervals between left and right panel in each recording are 30 min.

![Figure 3](image_url)

**Fig. 3.** Effects of SG-75 on isolated dog coronary arterial strips under PGF$_{2\alpha}$-contracture. Open circles: control (n=6). Solid triangles: after 4-AP $5\times10^{-4}$ Gm/ml (n=9). Solid circles: after TEA $1.6\times10^{-3}$ Gm/ml (n=6). Open circle in circle, solid triangle in circle, and solid circle in circle represent the effect of diltiazem $10^{-4}$ Gm/ml on control-strips, 4-AP-treated strips, and TEA-treated strips, respectively. 100% = 767±67 mg in control-strips (n=6), 3421±790 mg in 4-AP-treated strips (n=9) and 936±123 mg in TEA-treated strips (n=6). * p<0.05 and ** P<0.01 vs control. Each point indicates means±S.E.
parations, they reached 100±0% of the control (100%=767±67 mg) with SG-75 10^{-8} \text{Gm/ml}, 87±4% with 10^{-7} \text{Gm/ml}, 51±9% with 10^{-6} \text{Gm/ml}, 5±5% with 10^{-5} \text{Gm/ml}, and \ -16±3% with 10^{-4} \text{Gm/ml}, respectively (Fig. 3). Diltiazem 10^{-4} \text{Gm/ml} did not produce any more relaxations of the strips (Fig. 3). 4-AP 5\times10^{-4} \text{Gm/ml} did not produce any more relaxations of the strips (Fig. 3). Diltiazem 10^{-4} \text{Gm/ml} further constricted the coronary arteries (n=9) under PGF_{2\alpha}-contracture to 3421±790 mg (446±103% of the control), and significantly depressed the relaxant responses to SG-75 10^{-7}-10^{-4} \text{Gm/ml} (Fig. 3). Diltiazem 10^{-4} \text{Gm/ml} further relaxed these coronary arterial strips from 7±6% with SG-75 10^{-4} \text{Gm/ml} to \ -8±2\% (Fig. 3). TEA 1.6\times10^{-8} \text{Gm/ml} produced more increases in PGF_{2\alpha}-contracture which reached 936±123 mg (122±16% of the control), and significantly depressed the SG-75 (10^{-7}-10^{-4} \text{Gm/ml})-induced relaxations of the strips (n=6) as shown in Fig. 2A and 3. Diltiazem 10^{-4} \text{Gm/ml} further relaxed the strips from \ -7±3\% with SG-75 10^{-4} \text{Gm/ml} to \ -16±5\% (Fig. 3).

In 3 coronary arterial strips under a resting state, 4-AP 5\times10^{-4} \text{Gm/ml} itself produced increases in tension with oscillations in their contractions (Fig. 4A). On the other hand, TEA 1.6\times10^{-3} \text{Gm/ml} did not produce any change in the strips under a resting state. SG-75 10^{-8}-10^{-4} \text{Gm/ml} concentration-dependently relaxed the coronary arterial strips under 4-AP-contracture or under a resting state (Fig. 4A and B).

![Fig. 4. Typical recordings of effects of SG-75 on isolated dog coronary arterial strips under 4-AP-contracture (A) and under a resting state (B).](image)

**DISCUSSION**

4-AP^{8)-11)} and TEA^{12)-14)} are known to inhibit potassium conductance in excitable membrane of various tissues. If SG-75 increases potassium conductance in the cell membrane of coronary arteries, the vasodilator action of SG-75 may be depressed by prior treatment of 4-AP or TEA. The present results indicated that the relaxant effects of SG-75 on isolated dog coronary arteries under PGF_{2\alpha}-contracture were significantly depressed by 4-AP or
TEA, although the effects of SG-75 on potassium-contracted arteries were not influenced by 4-AP or TEA. This suggests the possibility that SG-75 may produce its relaxant effect on coronary arteries through an increase in potassium conductance of the membrane of vascular smooth muscles. It will not be impossible to consider that the result, in which 4-AP and TEA could not significantly depress the SG-75-induced relaxations of the strips contracted with potassium, may be related to an abundance of extracellular potassium ion level (30 mM). Further extended studies will be required in this point. Maybe, it is plausible that potassium-contraction will be an inadequate condition to estimate the mode of action of drugs on potassium conductance in vascular smooth muscles.

In this study, diltiazem was applied at the end of each experiment. This procedure should be useful to confirm the ability of further relaxations in coronary arterial strips tested. The present results showed that the strips had enough responsiveness to the drug at the end of each experiment or were maximally relaxed.

In the present results, 4-AP increased tension to 203±29% of the control in potassium-contracted arteries and to 446±103% in PGF$_2$-contracted arteries, while TEA increased it to 103±1% in potassium-contracted arteries and to 122±16% in PGF$_2$-contracted arteries. These constrictor effects of 4-AP and TEA may be due to an increase in Ca$^{++}$ availability. In fact, such an action of 4-AP or TEA has been reported on contractility in avian muscle.15),16) Judging from the values of increased tension, Ca$^{++}$-available effect seems to be greater in 4-AP than in TEA. The observation that TEA could not produce any change in the coronary arterial strips under a resting state in contrast with marked contractions induced by 4-AP will support the view.

In conclusion, it was suggested from the present study that SG-75 would act on coronary arteries in part through an increase in potassium conductance.

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REFERENCES