Effects of 2-Nicotinamidoethyl Nitrate (SG-75, Nicorandil) on Indomethacin-Induced Contractions of Isolated Dog Coronary Arteries

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SUMMARY

Effects of 2-nicotinamidoethyl nitrate (SG-75) on contractile responses of dog coronary arteries to indomethacin were investigated in vitro. Indomethacin (3×10⁻⁸ and 3×10⁻⁷ Gm/ml) produced contractions of isolated coronary arterial strips, which were reproduced by successive administration of the drug. SG-75 (10⁻⁵ Gm/ml) administered 5 min prior to indomethacin, significantly depressed indomethacin-induced contractions of the strips. In coronary arterial strips under potassium-contracture, SG-75 (10⁻⁸-10⁻⁴ Gm/ml) produced concentration-dependent relaxations, which were not affected by prior administration of indomethacin (3×10⁻⁶ Gm/ml). Tranylcypromine (10⁻⁴ Gm/ml) did not influence the relaxant responses of the strips to SG-75 (10⁻⁸-10⁻⁵ Gm/ml) but significantly depressed them to SG-75 (10⁻⁴ Gm/ml). Results indicate that large doses of SG-75 will induce a relaxant effect on isolated dog coronary arteries through activation of intravascular biosynthesis or release of prostacyclin from vascular tissues.

Additional Indexing Words:
Mode of action of SG-75 Relaxation Tranylcypromine Participation of prostacyclin biosynthesis

SINCE the first report of the potent coronary vasodilator effect of 2-nicotinamidoethyl nitrate (SG-75),¹ several investigations have attempted to clarify the mode of action of SG-75.²⁻⁶ However, the mechanisms of action of the drug have not been fully established to this time. In our previous work,⁷ we suggested that indomethacin produces contractions of dog coronary arteries through inhibition of endogenous prostaglandin biosyn-

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thesis in vascular tissues. Furthermore, it was reported by Neichi et al\textsuperscript{8)} that SG-75 accelerates the conversion of prostaglandin H\textsubscript{2} to prostacyclin in the coupled system of platelets and aortic microsomes. Therefore, the present study was designed to estimate the participation of increased intravascular prostacyclin biosynthesis in the coronary vasodilator action of SG-75, by examining effects of SG-75 on indomethacin-induced contractions of isolated dog coronary arteries.

**METHODS**

Experiments were carried out using adult mongrel dogs of either sex, weighing 8.0–16.0 Kg. The animals were anesthetized intravenously with sodium pentobarbital 30 mg/Kg. The chest was opened widely with a fourth intercostal thoracotomy under artificial ventilation, and the heart was isolated. The left circumflex coronary artery was carefully cut into helical strips, 2.0 mm wide and 20 mm long, which 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, CaCl\textsubscript{2} 2.5, MgSO\textsubscript{4} 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 24.4, and glucose 10.0. The solution in the bath was maintained at 37°C and aerated continuously with a gas mixture of 95% O\textsubscript{2} and 5% CO\textsubscript{2}. When measured by means of a blood-gas analyzer (Instrumentation Laboratory Micro-13), the oxygen tension of the solution was 600 mmHg and the pH was 7.40. The strips were connected to an isometric transducer (Nihon Kohden TB-611T) and the tension development was recorded on an ink-writing recticorder (Nihon Kohden RJG-4004). Resting tension was adjusted to an optimal tension, about 1.0 Gm, and the strips were allowed to equilibrate for 2 hrs before each experiment.

The drugs used in this experiment were 2-nicotinamidoethyl nitrate (SG-75, Chugai), indomethacin (Sigma), and tranylcypromine dihydrochloride (Sigma). Indomethacin was dissolved in ethanol and equimolar sodium bicarbonate, and diluted with physiological saline solution. Other drugs were dissolved in physiological saline solution. Application of the solvents had no effect. A volume of 0.2 ml of the drug solutions was added to the bath solution. The doses of drugs listed in this report correspond to the final bath concentrations of the salt of tranylcypromine and of free forms of SG-75 and indomethacin, and are expressed in terms of Gm/ml. Statistical analysis of the data was done with Student's t-test.
RESULTS

Indomethacin \((3 \times 10^{-8} - 3 \times 10^{-7} \text{Gm/ml})\) produced concentration-dependent contractions of the isolated dog coronary arterial strips in the resting state (Fig. 1). Indomethacin-induced contractions began 2 min after administration of the drug and reached maximal level about 20 min later (Fig. 1). These contractile responses of the strips were reproducible, since a second administration of indomethacin 1 hr after the initial administration produced the same contractile responses. SG-75 \((10^{-5} \text{Gm/ml})\) relaxed the coronary

![Fig. 1. Typical recordings of indomethacin-induced contractions of isolated dog coronary arterial strips. Upper trace: control (indomethacin \(3 \times 10^{-7} \text{Gm/ml}\)). Middle trace: after SG-75 application \((10^{-5} \text{Gm/ml})\). Lower trace: 1 hr after washing out the drug solution.](image)

![Fig. 2. Effects of SG-75 on indomethacin-induced contractions of isolated dog coronary arterial strips. Open squares: responses to a \(3 \times 10^{-7} \text{Gm/ml}\) dose of indomethacin \((n = 4)\). Solid squares: responses to a \(3 \times 10^{-8} \text{Gm/ml}\) dose of indomethacin \((n = 4)\). C: control. SG-75: treatment with SG-75 \((10^{-5} \text{Gm/ml})\). Wash: 1 hr after washing out the drug solution. * \(p < 0.05\) and ** \(p < 0.01\) vs control. Each point indicates the mean±SE.](image)
arterial strips by $131\pm20$ mg ($n=8$) from a resting level (Fig. 1), and suppressed the contractile responses to indomethacin. The mean values of the contractions in 4 preparations reached $81\pm15$ mg with indomethacin ($3\times10^{-8}$ Gm/ml) and $200\pm54$ mg with $3\times10^{-7}$ Gm/ml of indomethacin in control preparations (Fig. 2). During treatment with SG-75 ($10^{-6}$ Gm/ml), they reached $5\pm5$ mg ($p<0.01$) in the presence of $3\times10^{-8}$ Gm/ml and $11\pm11$ mg ($p<0.05$) with $3\times10^{-7}$ Gm/ml of indomethacin, respectively (Fig. 2). When the preparations were equilibrated with oxygenated Krebs-Ringer bicarbonate solution for 1 hr after washing out the drug solution, the responses were restored to $25\pm10$ mg ($p<0.05$) with a $3\times10^{-8}$ Gm/ml dose and to $56\pm12$ mg ($p<0.05$) with a $3\times10^{-7}$ Gm/ml dose of indomethacin (Fig. 2).

SG-75 ($10^{-8}-10^{-4}$ Gm/ml) elicited a concentration-dependent relaxation of isolated dog coronary arterial strips ($n=6$), contracted in the presence of $30$ mM potassium (Fig. 3). When potassium-induced maximal tensions were defined as $100\%$ ($=808\pm134$ mg, $n=6$), the relaxations of the strips with SG-75 reached $100\pm0\%$ at a concentration of $10^{-8}$ Gm/ml, $97\pm1\%$ at $10^{-7}$ Gm/ml, $80\pm4\%$ at $10^{-6}$ Gm/ml, $45\pm10\%$ at $10^{-5}$ Gm/ml, and $-11\pm3\%$ at $10^{-4}$ Gm/ml, respectively (Fig. 3). In 4 coronary arterial strips under potassium-induced contraction, indomethacin ($3\times10^{-8}$ Gm/ml), ad-
administered 20 min prior to application of SG-75, augmented the potassium-induced contractions by about 20% but did not significantly affect the concentration-dependent relaxation of the strips induced by SG-75 in concentrations of $10^{-8}-10^{-4}$ Gm/ml (Fig. 3A).

The effects of tranylcypromine on SG-75-induced relaxation were observed in 6 coronary arterial preparations under potassium-induced contraction. Tranylcypromine ($10^{-4}$ Gm/ml) augmented the potassium-induced contractions by about 30% in 2 out of 6 preparations, while the other 4 preparations did not respond to the drug. The relaxant responses of the strips ($n=6$) to SG-75 ($10^{-8}-10^{-5}$ Gm/ml) were not influenced by prior administration of tranylcypromine ($10^{-4}$ Gm/ml) (Fig. 3B), but responses to a $10^{-4}$ Gm/ml dose of SG-75 were significantly depressed by tranylcypromine ($10^{-4}$ Gm/ml) from $-11\pm3\%$ to $10\pm5\%$ ($p<0.001$).

DISCUSSION

In our previous work,7) we proposed that indomethacin produces a decrease in coronary blood flow and contractions of coronary arteries of dogs through inhibition of biosynthesis of endogenous prostaglandins, especially vasodilating prostaglandins such as prostaglandin E$_1$ and/or prostacyclin. The present results indicate that SG-75 can depress the contractile responses of isolated dog coronary arterial strips to indomethacin. Since indomethacin-induced contractions have been shown to be depressed by such drugs as arachidonate and phospholipase A$_2$, which accelerate biosynthesis or release of prostaglandins,7) the depression of indomethacin-induced contractions by SG-75 may be related to a similar action. In fact, Neichi et al8) have reported that SG-75 increases the prostacyclin biosynthesis in a coupled system of rat platelets and pig aortic microsomes.

In the present study, tranylcypromine, an inhibitor of prostacyclin biosynthesis,9),10) could significantly depress only the relaxation induced by a $10^{-4}$ Gm/ml dose of SG-75. This implies that SG-75 is only able to accelerate prostacyclin biosynthesis in coronary arteries at a dose of $10^{-4}$ Gm/ml. The dose required in this experiment is consistent with that obtained from the biochemical data reported by Neichi et al.8) On the other hand, the present observations show that SG-75 depresses the indomethacin-induced contractions of coronary arteries at a dose of $10^{-5}$ Gm/ml. It is plausible that this discrepancy in the dose levels ($10^{-5}$ Gm/ml versus $10^{-4}$ Gm/ml) is caused by actions of SG-75 other than prostaglandin biosynthesis. At least two possible actions of SG-75 have been suggested: one is a nitroglycerin-like action4),5) the another is an increase in potassium conductance.6) Therefore, it is rea-
sonable to consider that SG-75 produces its vasodilator effect through combined actions of enhanced prostacyclin biosynthesis, increased potassium conductance, and an unknown mechanism such as a nitroglycerin-like action.

In the present study, a prior administration of indomethacin did not influence the relaxant responses of SG-75 on isolated dog coronary arterial strips. Unlike tranylcypromine, indomethacin is known to block the activity of cyclooxygenase, which is an enzyme for synthesis of prostaglandin G₂ from arachidonic acid.¹¹,¹² Therefore, indomethacin should not affect the conversion of prostaglandin H₂ to prostacyclin in vascular tissues, resulting in no influence on SG-75-induced relaxations.

In conclusion, the present study indicates that SG-75 may produce coronary vasodilatation through activation of either intravascular prostacyclin biosynthesis or release from the tissues when large doses are given.

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