JPET Fast Forward. Published on January 23, 2024 as DOI: 10.1124/jpet.123.001918
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Efficacy of BAY 60-2770, a soluble guanylate cyclase activator, for coronary spasm in animal models

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Running title: BAY 60-2770 for coronary spasm

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Number of text pages: 31

Number of tables: 1

Number of figures: 5

Number of references: 45

Abstract: 212 words

Introduction: 526 words

Discussion: 1139 words

Abbreviations: ANOVA, analysis of variance; AVP, arginine vasopressin; DAP, diaminopyridine; DMSO, dimethyl sulfoxide; ECG, electrocardiography; ET-1, endothelin-1; HR, heart rate; H&E, hematoxylin-eosin; 5-HT, 5-hydroxytryptamine; INOCA, ischemia with non-obstructive coronary arteries; KCl, potassium chloride; MBP, mean blood pressure; NO, nitric oxide; NTG, nitroglycerin; pEC_{50}, the negative logarithm of the concentration that produces one-half the maximal response; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; SNP, sodium nitroprusside

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Recommended section: Cardiovascular

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Abstract

Ischemia with non-obstructive coronary arteries (INOCA), caused by coronary artery spasm, has gained increasing attention owing to the poor quality of life of impacted patients. Therapeutic options to address INOCA remain limited, and developing new therapeutic agents is desirable. Herein, we examined whether soluble guanylate cyclase (sGC) activators could be beneficial in preventing coronary spasms. In organ chamber experiments with isolated canine coronary arteries, prostaglandin $F_{2\alpha}$-, endothelin-1-, 5-hydroxytryptamine-, and potassium chloride-induced contractions were suppressed by the sGC activator BAY 60-2770 (0.1, 1, and 10 nM). In isolated pig coronary arteries, BAY 60-2770 (0.1, 1, and 10 nM) could prolong the cycle length of phasic contractions induced by 3,4-diaminopyridine, as well as lower the peak and bottom tension of the contraction in a concentration-dependent manner. Additionally, BAY 60-2770 (1 pM–0.1 μM) evoked a concentration-related relaxation to a greater extent in small (first diagonal branch) coronary arteries than in large (left anterior descending) coronary arteries. In vasopressin-induced angina model rats, pretreatment with BAY 60-2770 (3 μg/kg) suppressed electrocardiogram S-wave depression induced by arginine vasopressin without affecting changes in mean blood pressure and heart rate. These findings suggest that BAY 60-2770 could be valuable in preventing both large and small coronary spasms. Therefore, sGC activators could represent a novel and efficacious therapeutic option for INOCA.

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Significance Statement

The sGC activator BAY 60-2770 exerted antispastic effects on the coronary arteries in animal vasospasm models as proof-of-concept studies. These data can help to support potential clinical development with sGC activators, suitable for human use in patients with vasospastic angina.

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Introduction

Ischemia with non-obstructive coronary arteries (INOCA), a condition characterized by anginal attacks without substantial organic coronary stenosis, has gained increasing attention owing to poor prognosis (Kunadian et al., 2020). Although the incidence of INOCA remains unknown owing to challenges in precise diagnosis, it is estimated that this condition impacts approximately 3–4 million individuals in the United States (Herscovici et al., 2018), and it is believed that this number remains persistently elevated. The clinical phenotypes of INOCA include vasospastic angina (epicardial spasm), microvascular dysfunction (impaired coronary vasodilation, increased microvascular resistance, and microvascular spasm), or a mixture of the two (Takahashi et al., 2021; Mehta et al., 2022). Therefore, vasodilators are indispensable for the treatment of INOCA (Beltrame et al., 2021). Nitrates (nitric oxide (NO)-donor compounds) and calcium channel blockers are used clinically to prevent coronary artery constriction and for dilating constricted arteries (Ahlner et al., 1991; Zamponi et al., 2015). However, certain patients are refractory to these drugs (Beltrame et al., 2021), and treatment strategies for refractory patients remain an ongoing challenge, necessitating the development of new therapeutic agents.

Soluble guanylate cyclase (sGC), a molecular target of nitrates, is responsible for cGMP production and mediates vasodilation (Derbyshire and Marletta, 2012). In blood vessels, sGC exists in three forms: reduced sGC (with ferrous heme), oxidized sGC (with ferric heme), and apo-sGC (heme-free) (Tawa and Okamura, 2022). NO derived from nitrates enhances the enzymatic activity of reduced sGC. In recent years, novel drug types that act on these sGC in a NO-independent manner have been developed; sGC stimulators activate reduced sGC, and sGC activators activate oxidized/apo-sGC (Sandner et al., 2021). Given that the NO/sGC/cGMP pathway is well established as a key target in antianginal therapy (Steinhorn

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et al., 2015), sGC stimulators and sGC activators are considered promising new drug candidates to treat INOCA. Notably, one case report has indicated that the sGC stimulator riociguat was effective in patients with refractory vasospastic angina (Martínez Pereyra et al., 2021).

Reactive oxygen species (ROS), the principal mediators of oxidative stress, are closely associated with the pathophysiology of INOCA. For example, patients with vasospastic angina and microvascular dysfunction exhibit markedly increased expression of ROS markers, including thiobarbituric acid-reactive substances and thioredoxin (Miwa et al., 1999; Erdamar et al., 2009; Miwa et al., 2003). Moreover, ROS reportedly mediate coronary hyperconstriction in patients with vasospastic angina (Kugiyama et al., 1998). Importantly, under oxidative stress conditions, sGC in coronary arteries undergoes oxidation from a reduced to an oxidized/apo form (Tawa and Okamura, 2016). Although sGC activators exert stronger effects on coronary arteries than on other arteries, such as those in the kidneys and mesentery, under physiological conditions (Tawa et al., 2019), these activators may function more efficiently in coronary arteries exhibiting coronary spasms and microvascular dysfunction. Therefore, sGC activators may be more compatible with INOCA than sGC stimulators. However, data on the antispastic effects of sGC activators on coronary arteries remain limited. To establish the usefulness of sGC activators as therapeutic agents for INOCA, we examined the protective effects of the sGC activator BAY 60-2770 on coronary spasms in several experimental models. Among various sGC activators, BAY 60-2770 was selected based on availability during experiments.

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Materials and Methods

Animals

To undertake *in vitro* studies with isolated coronary arteries, we employed 6 male beagle dogs (7–8 years old; 9–12 kg) and 31 adult domestic pigs of either sex (about 6 months old; the males were castrated shortly after birth). The dogs were housed individually in cages under controlled humidity, temperature, and light conditions. A commercial dog food diet was provided daily, and water was provided *ad libitum*. To perform an *in vivo* study, an angina model was established using 20 male Wistar rats (9–10 weeks old; 360–440 g; Charles River Laboratories Japan, Inc., Kanagawa, Japan). The rats had free access to food and water in a light-controlled room with a 12-h light-dark cycle. There was no special reason for the selection of age and sex, but they were based on availability at the time of the experiments. The animal study was approved by the Animal Care and Use Committee at Shiga University of Medical Science (Permit No:2013-5-2) and the Experimental Animal Committee at the Faculty of Pharmacy, Osaka Medical and Pharmaceutical University (Permit No:92/2022).

Vasoconstrictor-induced tonic contraction

Under deep general anesthesia using ketamine (10 mg/kg, intramuscular) and sodium pentobarbital (40 mg/kg, intravenous), the dog's heart was harvested, isolated left circumflex coronary artery preparations were prepared, and changes in isometric tension were measured as described previously (Tawa et al., 2015). The contractile response to 30 mM potassium chloride (KCl) was measured twice, followed by repeated washout with a physiological solution. The second response was considered a 100% contraction. After equilibration, the preparations were exposed for approximately 20 min to BAY 60-2770 (0.1, 1 or 10 nM) or its solvent (referred to as "control") in a bathing solution. Concentration-response curves for

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prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), endothelin-1 (ET-1), and 5-hydroxytryptamine (5-HT) were then obtained by adding the drug directly to the bathing solution in cumulative concentrations. Alternatively, the response to 30 mM KCl was recorded.

3,4-Diaminopyridine (DAP)-induced phasic contraction

The pig hearts originated from a single farm were obtained from a local abattoir. Briefly, pigs were electrically stunned and then exsanguinated. The hearts were excised and immediately placed in cold Krebs–Ringer bicarbonate solution (118.5 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 10.0 mM glucose) perfused with a gas mixture containing 95% O_2 and 5% CO_2 (pH 7.4) for transportation to the laboratory. Isolated left anterior descending coronary artery preparations were prepared, and changes in isometric tension were measured as described previously (Tawa et al., 2020). The time from excision to immersion in the solution was ≤ 5 min, from immersion to finishing the transportation was ≤ 25 min, and from finishing the transportation to completion of coronary artery preparations was ≤ 30 min. The preparations were exposed to 10 mM 3,4-DAP, and those that exhibited phasic contractions were used for subsequent experiments. Once phasic contraction with stable amplitude and cycle length were sustained for 45 min (referred to as “pre”), BAY 60-2770 (0.1, 1, and 10 nM) was added cumulatively from the lowest concentration to the bathing solution; the response was observed for 45 min per concentration (1st 45 min, 0.1 nM; 2nd 45 min, 1 nM; 3rd 45 min, 10 nM). The preparations treated with solvent instead of BAY 60-2770 were referred to as “control”. The cycle length, developed tension, peak tension, and bottom tension were averaged for each 45-min period per preparation, and the values obtained were used to calculate group means.

Vasodilator-induced relaxation

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The left anterior descending coronary artery (segment #6 in AHA classification, referred to as “large”) and its first diagonal branch (segment #9 in AHA classification, referred to as “small”) were isolated from pig hearts and cut into 3–5-mm rings. Vascular rings were used for organ chamber experiments, as described previously (Tawa et al., 2020). The preparations, with the resting tension adjusted to 2.0 g for large and 1.0 g for small coronary arteries, were initially exposed to 30 and 60 mM KCl, respectively, to test the functional integrity. After washout and equilibration, the arterial rings were partially contracted using ET-1 (3 nM). Once the contraction reached a plateau, concentration-response curves for BAY 60-2770, nitroglycerin (NTG; organic nitrate), and sodium nitroprusside (SNP; NO donor) were obtained by adding the cumulative concentrations of the drug. Papaverine (0.1 mM) was then added to induce maximal relaxation, considering 100% relaxation induced by agonists.

In some experiments, the arteries were exposed to the heme oxidant ODQ (10 μM; referred to as “ODQ (+)”) or its solvent (referred to as “ODQ (−)”) for 15 min. Subsequently, vascular reactivity to BAY 60-2770 (1 nM) and SNP (1 μM) was observed in ET-1-contracted preparations, as described above. The concentration of vasodilators was determined based on the respective pEC_{50} values.

Hematoxylin-eosin (H&E) staining

Redundant portions of large and small coronary arteries were fixed with 10% formaldehyde and embedded in paraffin. The samples were cut into 3-μm sections and stained with H&E according to standard procedures.

Vasopressin-induced angina model

The rat angina model was constructed by infusing vasopressin, as previously described

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by Fujisawa et al. (2013). Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg) and subcutaneously injected with meloxicam (1.0 mg/kg). During the experiment, body temperature was maintained using a heating pad (KN-475, Natsume Seisakusho Co., Ltd., Tokyo, Japan). A polyethylene catheter was inserted into the femoral artery for hemodynamic monitoring and in the femoral vein for drug injection. The surface lead II electrocardiogram was measured using a biological amplifier (FE132 Bio Amp, AD Instruments, Sydney, Australia). Electrocardiography (ECG) and hemodynamics were continuously recorded using a PowerLab data acquisition system (PowerLab/4sp, AD Instruments). After an equilibrium period, BAY 60-2770 (3 μg/kg) or its solvent, dimethyl sulfoxide (DMSO, 0.5 μL/kg), was injected. In our preliminary study (n = 2), an intravenous injection of 10 μg/kg could induce hypotension; hence, we used 3 μg/kg in the current study. After 8 min, arginine vasopressin (AVP) was infused at a rate of 0.3 μg/kg/min for 8 min using a syringe pump (CFV-3200, Nihon Kohden, Tokyo, Japan). The infusion dose and rate were determined based on the results of a pilot study (n = 2). The difference in the amplitude of the ECG S-wave before administering BAY 60-2770 or its solvent injection (referred to as "pre") and before and after AVP infusion was presented as the depression of the S-wave (ΔS-wave); the S-wave after AVP infusion was documented at the time when the amplitude was maximum. At each evaluation point, five consecutive beats were analyzed and averaged to obtain the S-wave amplitude.

Drugs

The following drugs were used: BAY 60-2770 (purity ≥ 95%; kindly provided by Dr. Johannes-Peter Stasch and Dr. Peter Sandner, Pharmaceuticals R&D, Pharma Research Center, Bayer AG, Wuppertal, Germany); KCl (Kanto Chemical Co. Inc., Tokyo, Japan); $PGF_{2\alpha}$ (Pharmacia-Upjohn, Tokyo, Japan); ET-1 and AVP (Peptide Institute Inc., Osaka, Japan);

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5-HT and SNP (Nacalai Tesque, Kyoto, Japan); 3,4-DAP (Sigma-Aldrich Co. LLC, St. Louis, MO, USA); NTG (Nihonkayaku Co., Tokyo, Japan); papaverine hydrochloride and sodium pentobarbital (Dainippon-Sumitomo Pharma Co., Osaka, Japan); ODQ (Cayman Chemical Co., Ann Arbor, MI, USA); ketamine (Sankyo, Tokyo, Japan); meloxicam (Virbac Japan Co., Ltd., Osaka, Japan). Unless otherwise indicated, all chemicals were of the highest purity available. DMSO and ethanol were used as solvents for preparing stock solutions of BAY 60-2770 and ODQ, respectively. $PGF_{2\alpha}$ was dissolved in sodium bicarbonate buffer (pH 9.2). These solvents did not considerably impact the vascular response at concentrations used in the present study. Distilled water was used to dissolve all other drugs and prepare serial dilutions as required from stocks on the day of the experiment.

Statistics

All values are expressed as the mean ± SEM. The concentration-response curves were fitted by nonlinear regression analysis using Graph Pad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA). The negative logarithm of the concentration that produces one-half the maximal response (pEC_{50}) was obtained. Unless otherwise indicated, data were analyzed using a two-way repeated-measures analysis of variance (ANOVA) and Bonferroni *post hoc* tests. Responses to KCl and vasodilators in the presence of ODQ were assessed using one-way and two-way ANOVA, respectively, and Bonferroni *post hoc* tests. pEC_{50} values were compared using an unpaired two-tailed Student's *t* test. Differences were considered statistically significant at $P < 0.05$.

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Results

Effects of BAY 60-2770 on vasoconstrictor-induced tonic contraction

The addition of $PGF_{2\alpha}$ at concentrations of 10 nM–10 μM produced concentration-dependent contractions in dog coronary arteries, and we demonstrated that BAY 60-2770 could significantly attenuate this contraction in a concentration-dependent manner (Fig. 1A). Likewise, contractile responses to ET-1 (30 pM–30 nM) and 5-HT (1 nM–1 μM) were suppressed in the presence of BAY 60-2770 in a concentration-dependent manner (Figs. 1B and 1C). Furthermore, BAY 60-2270 significantly attenuated KCl (30 mM)-induced depolarizing contraction (Fig. 1D).

Effects of BAY 60-2770 on 3,4-DAP-induced phasic contraction

The pig coronary arteries began contracting approximately 60 min after administering 3,4-DAP (10 mM), which was followed by periodic and spontaneous contractions for more than 180 min (Supplemental Fig. 1). As shown in Fig. 2, the cycle length, peak tension, bottom tension, and developed tension in the control group remained constant during the experimental period. Treatment with 1 nM BAY 60-2770 significantly prolonged the cycle length, and this effect was more pronounced at 10 nM (Fig. 2A). Although the developed tension was not significantly reduced (Fig. 2B), treatment with 1 and/or 10 nM BAY 60-2770 could significantly reduce peak and bottom tensions (Figs. 2C and 2D).

Differences in vasodilator-induced relaxation between large and small coronary arteries

Fig. 3A illustrates the histology of the large and small coronary arteries. The addition of 1 pM–0.1 μM BAY 60-2770 induced concentration-dependent relaxation in pig coronary arteries, with stronger relaxation observed in small arteries than that in large arteries (Fig. 3B;

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Table 1). Conversely, small arteries exhibited weaker relaxant responses to NTG (1 nM–0.1 mM) and SNP (1 nM–0.1 mM) than large arteries (Figs. 3C and 3D; Table 1).

Vasodilator-induced relaxation in the presence of ODQ

ODQ enhanced relaxant responses to BAY 60-2770 in both pig large and small coronary arteries, and the difference in the degree of relaxation observed in the absence of ODQ disappeared (Fig. 4A). Similarly, the heterogeneity in SNP responsiveness was no longer observed in the presence of ODQ (Fig. 4B).

Effects of BAY 60-2770 on AVP-induced angina

Supplementary Figure 2 illustrates representative ECG recordings. In both the control and BAY 60-2770 groups, the S-wave amplitude gradually increased, reaching a maximum amplitude at approximately 3.5 min after initiating the AVP infusion (average time: control, 3.43 min; BAY 60-2770, 3.56 min). BAY 60-2770 at a dose of 3 μg/kg significantly inhibited AVP-induced ΔS-wave (Fig. 5A). BAY 60-2770 did not affect changes in mean blood pressure or heart rate after AVP infusion (Figs. 5B and 5C).

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Discussion

Studies using isolated coronary arteries from various species, including monkeys (Tawa and Okamura, 2016; Tawa et al., 2019), pigs (Schindler et al., 2006; Kollau et al., 2018; Hahn et al., 2021), cattle (Zhang et al., 2018), dogs (Tawa et al., 2015), rabbits (Tawa et al., 2021), and mice (Durgin et al., 2022), have demonstrated that sGC activators can exert relaxant effects on coronary arteries. However, there is limited evidence on the contraction-preventing effects of sGC activators in coronary arteries without obstructive lesions. The present study revealed that the sGC activator BAY 60-2770 could suppress both receptor-mediated ($PGF_{2\alpha}$-, ET-1-, and 5-HT-induced) and depolarization (KCl)-induced contractions in canine coronary arteries, suggesting that sGC activators exhibit considerable potential for preventing coronary spasms. This finding is consistent with that of previous reports, showing similar anti-contraction effects of NO donors and a cGMP analog (Walia et al., 2003; Lee et al., 2004). Accordingly, it can be established that the NO/sGC/cGMP pathway is a pivotal therapeutic target for preventing coronary spasms. Interestingly, the contraction-preventing effect of BAY 60-2770 on pig coronary arteries persisted after repeated washouts with the bathing solution for 4 h (Supplemental Fig. 3). Therefore, sGC activators could exert a prolonged duration of action.

Vasospastic angina attacks can occur repeatedly at short intervals of several tens of minutes (Murdoch et al., 2015; Alexander et al., 2016). Notably, spontaneous phasic contractions often occur without the addition of vasoactive drugs in coronary arteries collected from patients with coronary artery disease, with and without visible atherosclerotic lesions (Vedernikov, 1986; Cocks et al., 1993). Therefore, we next examined the effects of the sGC activator BAY 60-2770 on the phasic contraction of coronary arteries. Based on our findings, BAY 60-2770 could reduce the force and frequency of contraction induced by

3,4-DAP in pig coronary arteries, suggesting its potential for relieving sustained phasic contractions. This finding further supports the claim that sGC activators are highly attractive candidates for INOCA therapy.

Among INOCA, microvascular dysfunction and spasms remain areas of unmet medical need (Rahman et al., 2019). The vasodilatory effects of nitrates, the mainstay of medical therapy for vasospastic angina, are more substantial in large coronary arteries than in small coronary arteries (Matsumoto et al., 1997; Ying et al., 2012). However, the existence of heterogeneous vasorelaxant responses in coronary arteries to sGC activators remains unexplored. To the best of our knowledge, the present study is the first report demonstrating that the sGC activator BAY 60-2770 could evoke greater relaxation in pig small coronary arteries than in large coronary arteries. As described above, under oxidative stress, reduced sGC, a target of nitrates, undergoes alteration to oxidized/apo-sGC, a target of sGC activators (Tawa and Okamura, 2016). Accumulating evidence suggests the presence of higher flow velocity and wall shear stress in the distal coronary branches than in the proximal coronary arteries (Soulis et al., 2006; Schultz et al., 2023). Additionally, the subendocardial flow was assumed to be pulsatile, generating oscillatory shear stress (Sorop et al., 2003). Furthermore, oscillatory shear stress can increase ROS production (Takabe et al., 2011). Therefore, small coronary arteries may be under higher oxidative stress than large coronary arteries, resulting in increased oxidized/apo-sGC expression. This theory is supported by the finding that differences in BAY 60-2770 and SNP responsiveness between large and small coronary arteries disappeared in the presence of the heme oxidant ODQ. Moreover, sGC activators were shown to reduce coronary perfusion pressure in the Langendorff system, which primarily reflects coronary microvascular function (Mourmoura et al., 2013), in several studies (Stasch et al., 2002; Hahn et al., 2021). Collectively, these results suggest that sGC activators could be valuable in treating vasospastic angina caused by small coronary artery

spasms.

AVP is a potent coronary vasoconstrictor that preferentially acts on the arterioles rather than on the arteries (Lamping et al., 1989). Therefore, AVP-induced angina characterized by S-wave depression is widely used as a model of subendocardial ischemia due to small coronary artery constriction (Fujisawa et al., 2013). Herein, the sGC activator BAY 60-2770 inhibited AVP-induced S-wave depression in rats. Considering the above-mentioned discussion, this result can be attributed to the BAY 60-2770-mediated suppression of AVP-induced coronary artery contraction. Conversely, BAY 60-2770 did not inhibit blood pressure elevation or associated reflex bradycardia. Given that BAY 60-2770 exerted greater effects on the coronary arteries than on other peripheral arteries (Tawa et al., 2019), the BAY 60-2770 dose used in the present study may have induced only local effects on coronary circulation. This characteristic is important for the development of therapeutic drugs for diseases with lesions in coronary arteries. Taken together, sGC activators could prevent small coronary artery spasms without lowering blood pressure, thereby affording attractive new therapeutic candidates for INOCA.

Prolonged coronary spasms can lead to myocardial infarction in distal areas. We focused mainly on the preventive effects of BAY 60-2770 against coronary spasms and did not address its effects on spasm-related cardiac events. Interestingly, administering BAY 58-2667, an sGC activator, 15–30 min before 30 min of ischemia was shown to reduce infarct size 2–3 h after reperfusion in a coronary artery ligation model in rabbits (Salloum et al., 2012) and mice (Salloum et al., 2012; Frankenreiter et al., 2017). Therefore, if sGC activators are administered to prevent angina attacks, it is assumed that even if a prolonged coronary spasm occurs, the associated ischemic myocardial damage can be minimized; this clarifies the recommendation of sGC activators as therapeutic agents for INOCA.

One limitation of the present study is that we did not examine the effects of BAY

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60-2770 using different administration methods and doses in AVP-infused experiments. Detailed verification of the optimal administration methods and dosages is essential for the clinical development of new drugs. Although BAY 60-2770 exhibits the typical mode of action of an sGC activator, it could not be advanced into clinical development due to physicochemical limitations of these first-generation sGC activators (Hahn et al., 2021). However, the objective of the present study was to clarify whether sGC activators, as a novel drug class, could exert antispastic effects on the coronary arteries as proof-of-concept studies. These data therefore can help to support the potential clinical development of other sGC activators suitable for human use in patients with vasospastic angina. Recently, second-generation sGC activators that have overcome the limitations of first-generation drugs, such as BAY 60-2770, have been developed. For example, BAY 1101042 (runcaciguat) exhibits improved potency and absorption, distribution, metabolism, and excretion/pharmacokinetic properties (Hahn et al., 2021). It is crucial to establish the potential of these new sGC activators as therapeutic agents for INOCA. Future investigations should focus on identifying appropriate sGC activators for drug development, along with their optimal dosing regimens (Sandner et al., 2021).

In conclusion, the present study demonstrated that sGC activators could be explored as promising drugs for treating coronary spasms. Our findings will lead to the future expansion of treatment options for INOCA.

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Acknowledgments

The authors wish to thank Dr. Johannes-Peter Stasch and Dr. Peter Sandner (Pharmaceuticals R&D, Pharma Research Center, Bayer AG) for their gift of BAY 60-2770 and for reviewing the early drafts and providing valuable comments. We thank Emeritus Professor Tomio Okamura and Dr. Takashi Shimosato (Shiga University of Medical Science) for their kind assistance.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author Contributions

Participated in research design: Tawa.

Conducted experiments: Tawa.

Performed data analysis: Tawa.

Wrote or contributed to the writing of the manuscript: Tawa, Nakagawa, and Ohkita.

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JPET Fast Forward. Published on January 23, 2024 as DOI: 10.1124/jpet.123.001918
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Footnotes

This study was partly supported by the President's Discretionary Fund from the Shiga University of Medical Science [Nos. 1515503BF and 1515503CJ to MT], the former affiliation of MT, and Grants-in-Aid for Scientific Research Program from the Japan Society for the Promotion of Science [No. 22K15299 to MT]. No author has an actual or perceived conflict of interest with the contents of this article.

JPET Fast Forward. Published on January 23, 2024 as DOI: 10.1124/jpet.123.001918
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Figure Legends

Figure 1. Effects of BAY 60-2770 on tonic contraction induced by $PGF_{2\alpha}$ (A), ET-1 (B), 5-HT (C), and KCl (D) in dog coronary arteries. The contraction was presented as values relative to that induced by 30 mM KCl. Each point and bar represent the mean ± SEM of 6 (A, B, and D) or 3 experiments (C). *P < 0.05 and **P < 0.01, compared with the control. Statistical analysis was performed using two-way repeated ANOVA with Bonferroni *post hoc* test (A–C) or one-way ANOVA with Bonferroni *post hoc* test (D). Abbreviations: $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; ET-1, endothelin-1; 5-HT, 5-hydroxytryptamine; KCl, potassium chloride; BAY 60, BAY 60-2770.

Figure 2. Effects of BAY 60-2770 on cycle length (A), developed tension (B), peak tension (C), and bottom tension (D) of phasic contraction induced by 3,4-DAP in pig coronary arteries. Each point and bar represent the mean ± SEM of 9 experiments. **P < 0.01, compared with the control. Statistical analysis was performed using two-way repeated ANOVA with Bonferroni *post hoc* test. Abbreviations: 3,4-DAP, 3,4-diaminopyridine; BAY 60, BAY 60-2770.

Figure 3. Typical images of pig large (right side in upper panel, bottom right panel) and small coronary arteries (left side in upper panel, bottom left panel). Scale bars, 1 mm (upper panel), 50 μm (bottom left panel), and 100 μm (bottom right panel). Vasorelaxant responses of pig large and small coronary arteries to BAY 60-2770 (B), NTG (C), and SNP (D). The relaxation was presented as values relative to that induced by 0.1 mM papaverine. Each point and bar represent the mean ± SEM of 6 experiments. *P < 0.05 and **P < 0.01, compared with the

large. Statistical analysis was performed using two-way repeated ANOVA with Bonferroni *post hoc* test. Abbreviations: NTG, nitroglycerin; SNP, sodium nitroprusside.

Figure 4. Vasorelaxant responses of pig large and small coronary arteries to BAY 60-2770 (A) and SNP (B) in the presence or absence of ODQ. The relaxation was presented as values relative to that induced by 0.1 mM papaverine. Each column and bar represent the mean ± SEM of 8 experiments. $^{**}P < 0.01$, compared with the respective large; $\dagger\dagger P < 0.01$, compared with the respective ODQ (−). Statistical analysis was performed using two-way ANOVA with Bonferroni *post hoc* test. Abbreviations: SNP, sodium nitroprusside.

Figure 5. Effects of BAY 60-2770 on changes in S-wave (A), MBP (B), and HR (C) after AVP infusion in rats. Each point and bar represent the mean ± SEM of 8 experiments. $^{**}P < 0.01$, compared with the control. Statistical analysis was performed using two-way repeated ANOVA with Bonferroni *post hoc* test. Abbreviations: MBP, mean blood pressure; HR, heart rate; AVP, arginine vasopressin; BAY 60, BAY 60-2770.

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Table 1. pEC_{50} to Vasodilators

	BAY 60-2770	NTG	SNP
Large	9.32 ± 0.17	6.44 ± 0.11	6.01 ± 0.14
Small	10.07 ± 0.09 **	5.96 ± 0.17 *	5.50 ± 0.14 *

Data are the mean ± SEM of 6 experiments. *P < 0.05 and **P < 0.01, compared with the large. Statistical analysis was performed using an unpaired two-tailed Student's *t* test. Abbreviations: pEC_{50}, the negative logarithm of the concentration that produces one-half the maximal response; NTG, nitroglycerin; SNP, sodium nitroprusside.

Figure 1

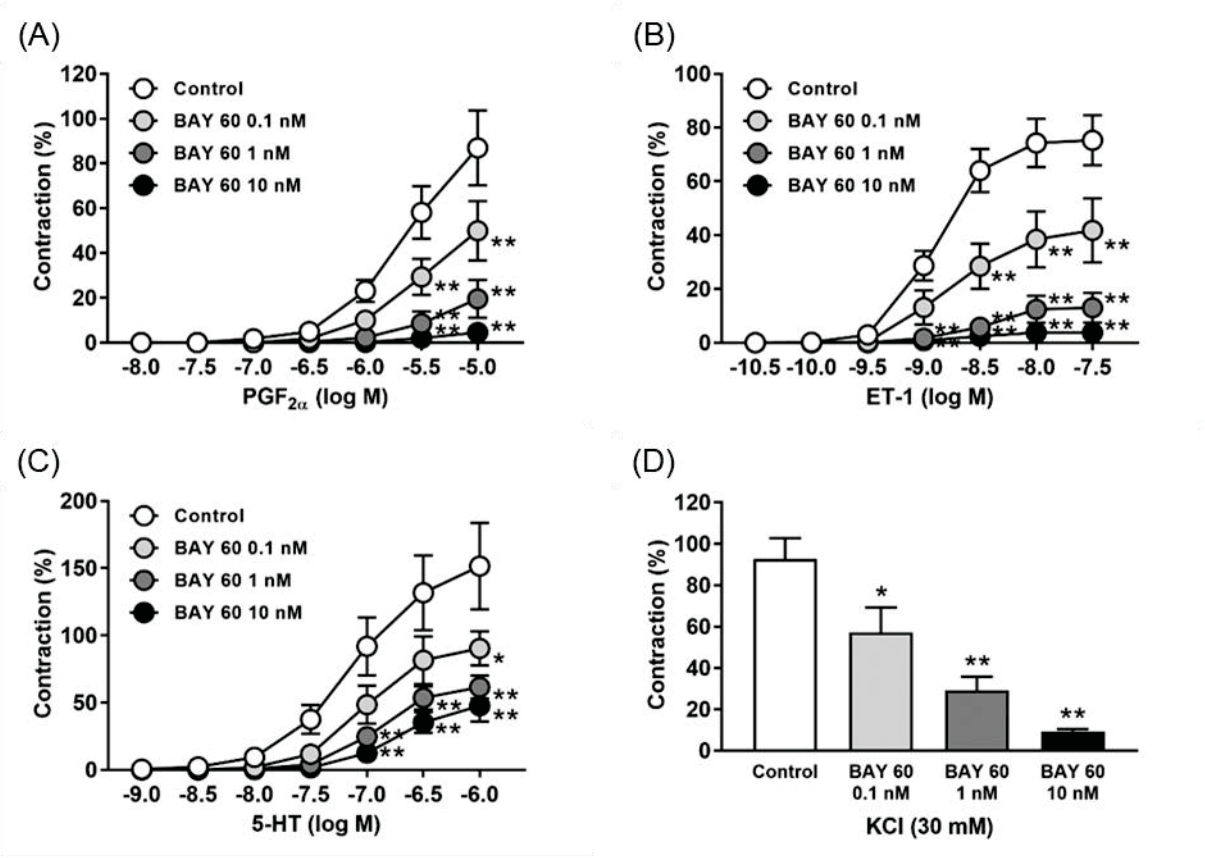

Figure 2

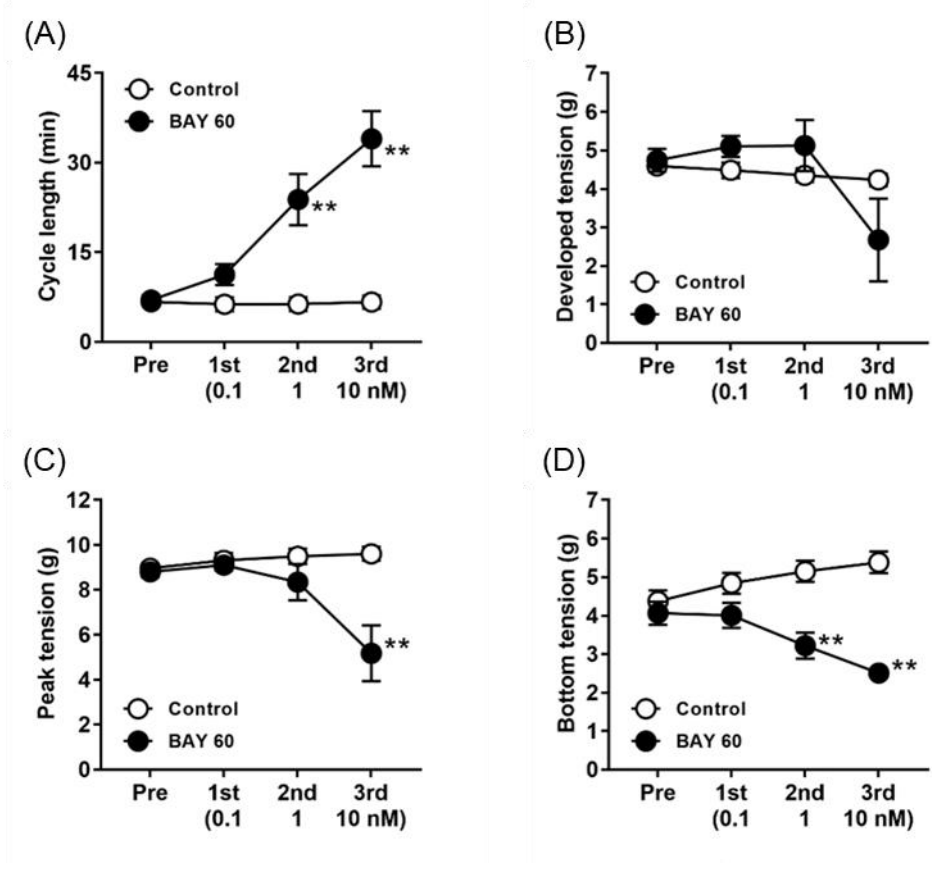
(A)
Cycle length (min)
45
30
15
0
Control
BAY 60
**
**
Pre
1st (0.1
2nd 1
3rd 10 nM)
(B)
Developed tension (g)
7
6
5
4
3
2
1
0
Control
BAY 60
Pre
1st (0.1
2nd 1
3rd 10 nM)
(C)
Peak tension (g)
12
10
8
6
4
2
0
Control
BAY 60
**
Pre
1st (0.1
2nd 1
3rd 10 nM)
(D)
Bottom tension (g)
7
6
5
4
3
2
1
0
Control
BAY 60
**
**
Pre
1st (0.1
2nd 1
3rd 10 nM)

Figure 3

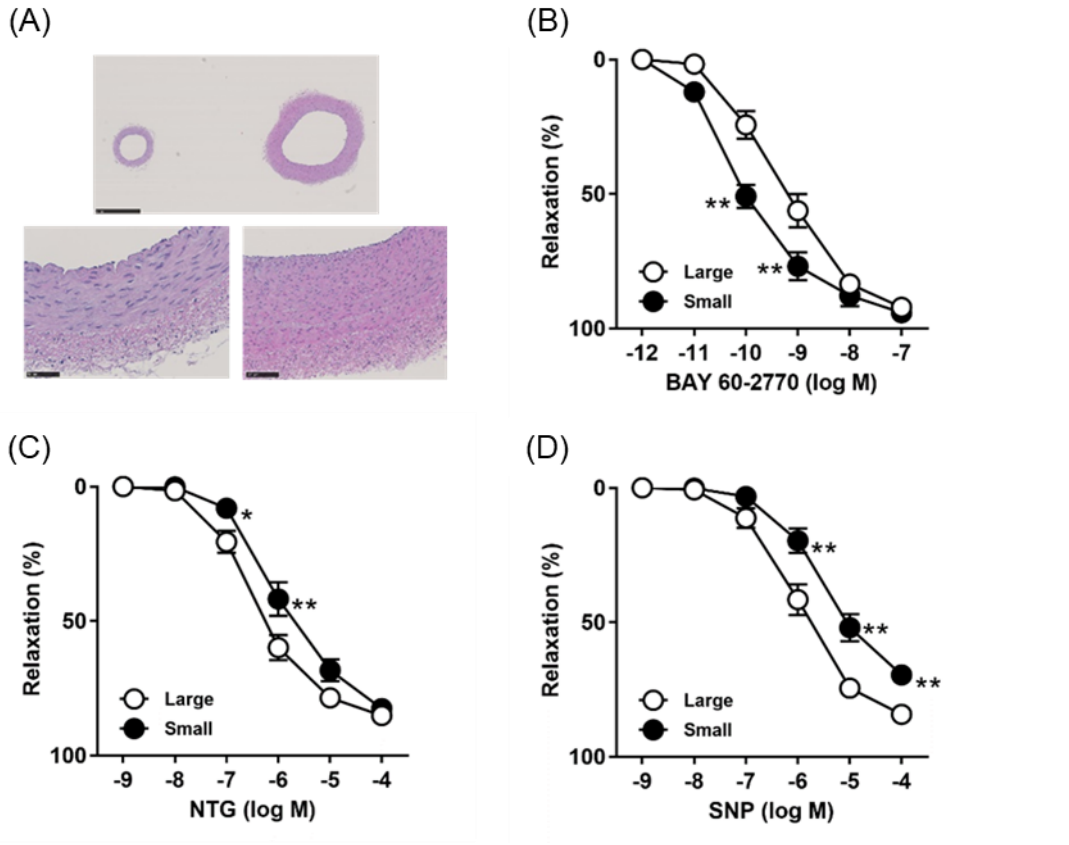
(A)
(B)
Relaxation (%)
0
50
100
-12
-11
-10
-9
-8
-7
BAY 60-2770 (log M)
Large
Small
**
**
(C)
Relaxation (%)
0
50
100
-9
-8
-7
-6
-5
-4
NTG (log M)
Large
Small
*
**
(D)
Relaxation (%)
0
50
100
-9
-8
-7
-6
-5
-4
SNP (log M)
Large
Small
**
**
**

Figure 4

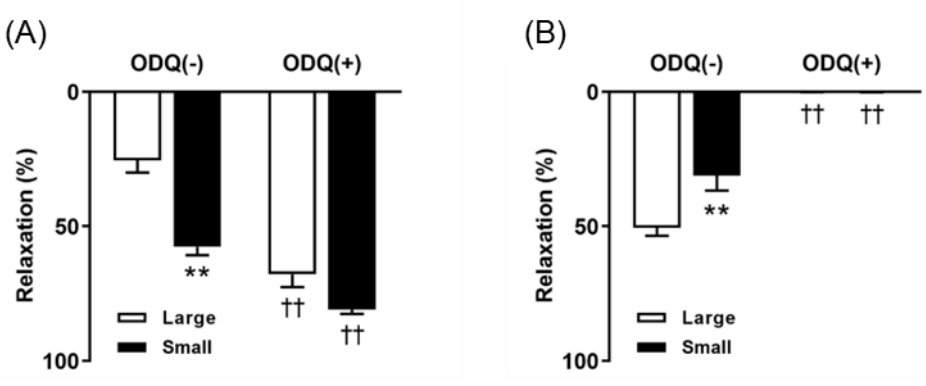
(A)
ODQ(-)
ODQ(+)
0
50
100
Relaxation (%)
**
††
††
Large
Small
(B)
ODQ(-)
ODQ(+)
0
50
100
Relaxation (%)
**
††
††
Large
Small

Figure 5