OPEN

The Novel Inodilator ORM-3819 Relaxes Isolated Porcine Coronary Arteries: Role of Voltage-Gated Potassium Channel Activation

Zoltán Márton, MPharm, PhD, János Pataricza, MD, PhD,* Piero Pollesello, MChem, PhD,† András Varró, MD, PhD, DSc,* and Julius Gy. Papp, MD, PhD, DSc‡*

Abstract: Relaxation and changes in the transmembrane potential of vascular smooth muscle induced by ORM-3819, a novel inodilating compound, were investigated in isolated porcine coronary arteries. Isometric tone was studied on arterial rings precontracted by KCl (30 mM), and resting membrane potential was investigated by a conventional microelectrode technique. ORM-3819 in the concentration range 0.38–230.6 μM evoked concentration-dependent relaxation with a maximum value of 58.1% and an effective concentration of the relaxing substance that caused 50% of maximum relaxation of 72.2 μM. The maximum hyperpolarization produced by ORM-3819 at a concentration of 120 μM (−2.6 ± 0.81 mV, N = 10) did not differ significantly from that induced by C-type natriuretic peptide (CNP), an endogenous hyperpolarizing mediator, at a concentration of 1.4 μM (−3.6 ± 0.38 mV, N = 17). The same effect elicited by the known inodilator levosimendan was less pronounced at a concentration of 3.7 μM: −1.82 ± 0.44 mV, N = 22 ($P < 0.05$ vs. CNP). The voltage-gated potassium channel inhibitor 4-aminopyridine, at a concentration of 5 mM, attenuated the relaxation induced by ORM-3819 at concentrations of 41.6 or 117.2 μM. These results suggest that ORM-3819 is a potent vasodilating agent able to relieve coronary artery vasospasm by causing hyperpolarization of vascular smooth muscle cells through processes involving activation of voltage-gated potassium channels.

Key Words: inodilator, hyperpolarization, vasodilation, coronary artery, voltage-gated potassium channel

(*J Cardiovasc Pharmacol*™ 2019;74:218–224)

Received for publication April 5, 2019; accepted June 4, 2019.

From the *Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary; ‡MTA-SZTE Research Group of Cardiovascular Pharmacology, Hungarian Academy of Sciences, University of Szeged, Szeged, Hungary; and †Orion Pharma, Espoo, Finland.

Supported by grants from the Hungarian Scientific Research Fund (Nos OTKA NK-104331 and ANN-113273) and by the Hungarian Academy of Sciences.

P. Pollesello is an employee of Orion Pharma, the company where ORM-3819 was discovered. The remaining authors report no conflicts of interest.

Reprints: Piero Pollesello, MChem, PhD, Orion Pharma, P.O.Box 65, Espoo FIN-02101, Finland (e-mail: piero.pollesello@orionpharma.com).

Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4. 0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

INTRODUCTION

It has been suggested that a positive inotrope needs to have a pleiotropic effect, such as peripheral vasodilation, to elicit further benefits in acute heart failure patients manifesting hypoperfusion and congestion.[1] Drugs possessing several mechanisms of action, such as levosimendan, have been described, which, in addition to a positive inotropic action through calcium sensitization,[2] dilates peripheral arteries and veins.[3–10] Levosimendan activates potassium channels in vascular smooth muscle (VSM) cells, a mechanism that hyperpolarizes the cell membrane and causes relaxation.[11] We demonstrated the direct vasodilating effect of levosimendan in porcine and human coronary artery preparations and suggested a hyperpolarizing mechanism of the inodilating drug through activation of potassium channels other than those modulated by adenosine triphosphate.[8,12]

Hyperpolarization of the membrane of smooth muscle cells closes voltage-dependent calcium channels and results in a decrease in vascular tone.[13] This represents an endogenous vasodilating mechanism that is independent of endothelium-derived nitric oxide in large epicardial coronary arteries under experimental conditions.[14–16] One candidate endogenous hyperpolarizing factor in the coronary arteries is C-type natriuretic peptide (CNP), which is present in atherosclerotic coronary artery tissue and exerts a vasodilating effect in the presence of damaged endothelium.[17–21]

We have recently studied the effect of a new inodilator, ORM-3819, which acts through 2 mechanisms: (1) calcium sensitization and (2) highly specific phosphodiesterase III inhibition.[22] The positive inotropic effect of this novel chemical entity was recently demonstrated both in vitro and in vivo,[22] but the potential effect of this compound on vascular tone and the mechanism underpinning that effect remain unrevealed.

The present investigation, performed in isolated porcine coronary arteries, was devised to determine the efficacy of ORM-3819 in decreasing vascular tone, to compare its hyperpolarizing effect with those of CNP and levosimendan and to explore the involvement of the voltage-gated potassium channel (Kv) in those effects.

METHODS

Tissue Preparation

Coronary arteries were obtained from porcine hearts harvested at a local abattoir. All animals received humane

care in accordance with the Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the ethical review board of the University of Szeged, Szeged, Hungary (approval no. XIII/1211/2012).

After harvesting, hearts were placed in ice-cold Krebs–Henseleit solution (see composition described below) and transported to the laboratory within 1 hour. Coronary arteries of the circumflex branch were dissected free from the surrounding connective tissue and cut into 5-mm-long rings while maintained in ice-cold Krebs–Henseleit solution.

Measurements of Isometric Tone

Ring segments were mounted on a pair of stainless-steel hooks and placed in water-thermostated (37°C) organ chambers containing 2 mL of Krebs–Henseleit solution. The solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at pH 7.4. One of the hooks was anchored inside the organ chamber, and the other was connected to a force-displacement transducer (Experimetria, Budapest, Hungary) to measure changes in isometric tension. Mechanical responses of the arterial rings were recorded by means of a pen recorder (Type 175; KUTESZ, Budapest, Hungary) as described previously.[15] The rings were stretched up to a tension of 29.4 mN and equilibrated for 90 minutes. During this period, the tension was continuously readjusted to 29.4 mN, and the medium was refreshed every 15 minutes.

Protocol for Studying the Effect of ORM-3819 on Isometric Tone

All the experiments were performed using intact vascular samples isolated from the same porcine hearts. Coronary artery rings were contracted with 30 mM KCl. When the contraction reached a stable plateau, ORM-3819 was administered at 5 stepwise increasing concentrations (0.38–230.6 μM; N = 7) into the organ baths. In a separate group of experiments, the effect of the solvent was investigated (N = 7). The effects of the "solvent alone" were always measured, and those values subtracted from the "solvent and agent" measured values at each set of data.

Measurements of Resting Membrane Potential

A ring segment of the coronary artery was prepared as described previously, slit longitudinally, and pinned to the Sylgard base of a 0.5-mL chamber with the intimal surface upward. The isolated vascular segments were continuously superfused with Krebs–Henseleit solution aerated with 95% O_2 and 5% CO_2 at a constant rate of 2 mL/min, and the temperature was maintained at 37°C (pH 7.4).

The transmembrane potential of smooth muscle cells was measured using a conventional microelectrode technique. An intracellular glass microelectrode filled with 3 M KCl (tip resistance 30–40 MΩ) was connected to the headstage of a recording amplifier (Intra 767; World Precision Instruments, Sarasota, FL) with capacitance neutralization. An Ag/AgCl pellet in contact with the bathing solution and directly connected to the amplifier served as a reference electrode. The signal was continuously monitored and recorded on a paperless recorder (Type 80807; Cole-Parmer International, Vernon Hills, IL). Microelectrodes were impaled in a smooth muscle cell from the intimal side, and successful impalements were signaled by a sudden negative shift in voltage, followed by a stable negative voltage for at least 2 minutes and an instantaneous return to the previous voltage level on dislodgement of the microelectrode.

Protocol for Studying the Effects of ORM-3819, CNP, and Levosimendan on Resting Membrane Potential

For detecting the successful impalement of the smooth muscle cell with the electrode, 5-μM pinacidil was added to the organ bath. Pinacidil is known to cause hyperpolarization in smooth muscle cells, but not endothelial cells, under resting conditions.[23] Data collection was restricted to those experiments, in which pinacidil exhibited a hyperpolarizing effect.

In pinacidil-positive preparations, the resting membrane potential of the smooth muscle cells was recorded after equilibration for 60 minutes. CNP (1.4 μM; N = 17), ORM-3819 (60, 120 or 180 μM; N = 14, 10, and 12, respectively), or the known hyperpolarizing inodilator levosimendan (1.8, 3.7, or 5.5 μM; N = 18, 22, and 15, respectively) were then added to the bath as bolus injections. The effect of solvents were tested in case of each agent at each mentioned concentration (N = 5).

Protocol for Studying the Effect of 4-Aminopyridine (4-AP) on ORM-3819–Induced Relaxation

Two endothelium-intact coronary artery rings were mounted in parallel in separate organ baths. One of the rings was pretreated with 4-AP (5 mM), while the other was preincubated with the same volume of 4-AP solvent (20 μL of distilled water). After 10 minutes, both rings were precontracted with 30 mM KCl, and then ORM-3819 was administered stepwise at increasing concentrations (41.6–230.6 μM). As the solvent of ORM-3819 exerted a significant relaxing effect at and above volumes corresponding to 117.2 and 230.6 μM ORM-3819 (90 and 180 μL, respectively), the mean effects of the solvent were ascertained separately and subtracted from the effects of ORM-3819.

Drugs

The composition of Krebs–Henseleit solution (mM) was as follows: NaCl, 120; KCl, 4.2; $CaCl_2$, 1.5; $NaHCO_3$, 20; $MgCl_2$, 1.2; KH_2PO_4, 1.2; and glucose, 11. The components of Krebs–Henseleit solution, including KCl, were obtained from Reanal (Budapest, Hungary).

The structure of the investigational compound ORM-3819, which has the chemical formula (L)-6-{4-[N′-(4-hydroxy-3-methoxy-2-nitro-benzylidene)-hydrazino]-phenyl}-5-methyl-4,5-dihydro-2H-pyridazin-3-one, is depicted in Figure 1. ORM-3819 and levosimendan were obtained from Orion-Pharma (Espoo, Finland). 4-AP, CNP, and pinacidil were purchased from Sigma (St. Louis, MO). ORM-3819 was prepared daily by dissolving it in a solution of 50%

Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

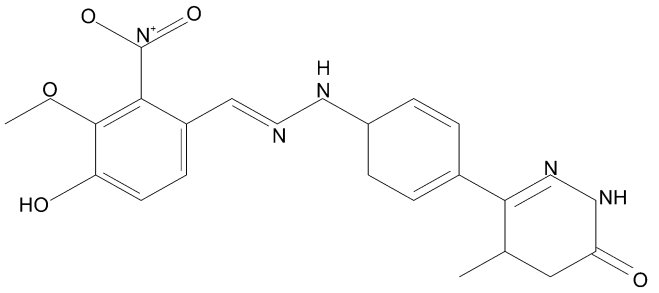

FIGURE 1. Structure of ORM-3819, a novel inodilating compound.

ethanol (Etax AaS; Primalco, Rajamäki, Finland) in a sodium bicarbonate buffer ($NaHCO_3$ analytical reagent, Riedel-de-Haën, Seelze, at pH 9.6 containing 5% D(+)-glucose (anhydrous for biochemistry, Merck, Darmstadt, Germany). The concentration of the ORM-3819 stock solution was 1 mg/mL, which was further diluted with the same solvent to reach lower concentrations (0.1 and 0.01 mg/mL). The stock solution was stored at room temperature. Levosimendan was dissolved in 70% ethanol and diluted further in Krebs–Henseleit solution. Pinacidil was prepared in 50% ethanol. 4-AP and CNP were dissolved in distilled water. The dose range of ORM-3819 in the current experiments was selected on the basis of the previous experience with the in vitro and ex vivo effects of the drug.[22]

Analysis of Data

The increase in basal coronary tone and the tone induced by 30 mM KCl were expressed in millinewtons. Relaxations caused by ORM-3819 were calculated and expressed as the percentage of the KCl-induced steady-state contraction amplitude of the same preparation. All values are reported as mean ± standard error of the mean, where N represents the number of arterial samples tested. The effective concentration of the relaxing substance which caused 50% of maximum relaxation (E_{max}) was defined as EC_{50}. The logistic equation $(a \times x)/(x + b)$ was fitted to the mean values for calculating the values of E_{max} (1) and EC_{50} (2). The Wilcoxon rank-sum test or the Mann–Whitney–Wilcoxon test was used to determine significant differences. A linear correlation between hyperpolarization and relaxation was investigated (y = slope; x + y = intercept). Comparisons between samples were conducted using 1-way analysis of variance and the Newman–Keuls multiple-range test. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of ORM-3819 on Isometric Tone

ORM-3819 elicited concentration-dependent relaxation in the isolated porcine coronary artery. Figure 2 shows the magnitude of this relaxation, expressed as a percentage of the KCl-induced precontraction, corrected for the relaxation induced by the solvent. Fitting the equation $(a \times x)/(x + b)$ to the mean values of the relaxations, the calculated maximum relaxation (a) induced by ORM-3819 was 58.1% of KCl-induced tone and the EC_{50} value (b) was 72.2 μM (Fig. 2).

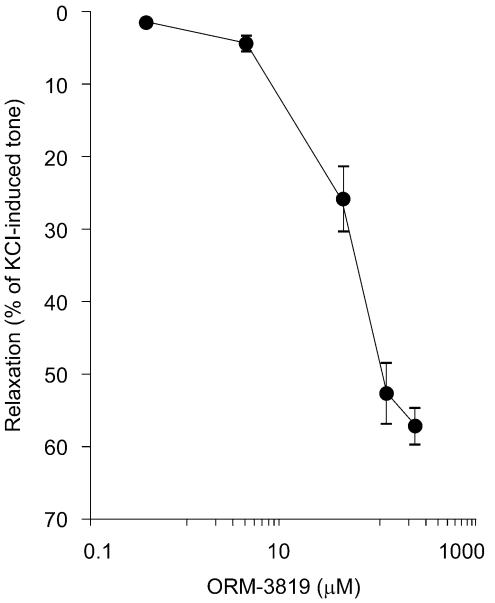

FIGURE 2. Concentration–response relationship of the inodilator ORM-3819 in porcine coronary arteries. In the concentration range 0.38–230.6 μM, ORM-3819 relaxed coronary arteries contracted by 30 mM KCl. The results are expressed as percent relaxation of KCl-evoked tone and in the form mean ± standard error of the mean, representing the net relaxing effect of the inodilator, ie, the effect of the solvent was deducted from that obtained with the corresponding concentration of ORM-3819. Five to 7 experiments were performed.

Effects of ORM-3819 in Comparison With Those of CNP and Levosimendan on the Membrane Potential of Coronary Artery Smooth Muscle Cells

Figures 3 and 4 (original recordings) demonstrate the hyperpolarizing effects evoked by ORM-3819, CNP, and levosimendan. Figure 4 summarizes the mean changes in membrane potentials obtained from 10 to 22 independent electrode impalements. Before addition of CNP, the resting membrane potential of coronary smooth muscle cells was −49.9 ± 0.92 mV (N = 17). CNP (1.4 μM) caused a mean hyperpolarization of −3.6 ± 0.38 mV. Resting membrane potentials were determined before administration of each concentration of ORM-3819 (60, 120, and 180 μM). These membrane potentials were −48.8 ± 1.15 (N = 14), −48.8 ± 0.88 (N = 10), and −50.3 ± 0.41 mV (N = 12), respectively, and the corresponding magnitude of changes in hyperpolarization induced by 60, 120, and 180 μM ORM-3819 was −1.8 ± 0.35, −2.6 ± 0.81, and −2.3 ± 0.99 mV, respectively. The hyperpolarizing effect of 60 μM ORM-3819 was calculated to be significantly less than that obtained with 1.4 μM CNP. Resting membrane potentials measured before the application of 1.8, 3.7, and 5.5 μM levosimendan were −49.7 ± 0.79 (N = 18), −50.8 ± 0.96 (N = 22), and −50.9 ± 1.18 mV (N = 15), respectively. Maximum hyperpolarization by this inodilator (−1.82 ± 0.44 mV) was obtained at 3.7 μM. All the concentrations of levosimendan produced significantly lower hyperpolarizations than those obtained with CNP.

Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

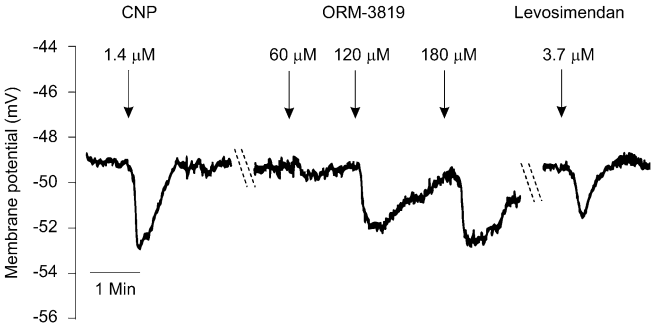

FIGURE 3. Original recordings of hyperpolarization in smooth muscle cells of porcine epicardial coronary arteries induced by CNP, ORM-3819, and levosimendan. Resting membrane potentials are depicted from 3 independent impalements. These values were −49.2, −49.2, and −49.0 mV before addition of CNP, ORM-3819, and levosimendan, respectively. CNP, an endogenous hyperpolarizing mediator, served as reference compound. CNP caused a maximum −3.7 mV change in resting membrane potential. ORM-3819 exerted a maximum change of −2.8 mV at a concentration of 120 μM. Levosimendan, a known hyperpolarizing inodilator, resulted in a change in membrane potential of −2.2 mV at a concentration of 3.7 μM.

Effects of 4-AP on ORM-3819–Induced Relaxation

Pretreatment of the coronary preparations with 5 mM 4-AP for 10 minutes resulted in a moderate but not significant enhancement of KCl-induced tone (control, 48.6 ± 9.83 mN; 4-AP, 73.4 ± 10.29 mN; N = 5) but decreased the extent of solvent-corrected coronary artery relaxation induced by ORM-3819 from 44.0 ± 8.52% to 30.3 ± 5.17% at a concentration of 41.6 μM ($P < 0.05$; N = 5) and from 65.6 ± 1.34% to 52.3 ± 3.15% at a concentration of 117.2 μM ($P < 0.05$; N = 5) (Fig. 5). At the highest concentration of ORM-3819 (230.6 μM), 4-AP did not influence relaxation (Fig. 5). 4-AP had no effect on the relaxations induced by the solvent itself (N = 5).

Correlation Between Hyperpolarization and Relaxation Induced by ORM-3819

The magnitude of ORM-3819–induced hyperpolarization (at a concentration of 60 μM), expressed as changes in membrane potential in millivolts, showed a correlation with the extent of relaxation evoked by ORM-3819 (Fig. 6). As the effect of this dose fits with the rise in the concentration–response curve of the compound (Fig. 2), a linear correlation between the individual relaxing values and hyperpolarization was investigated. The equation for this correlation was $y = 13.4 \times x + 18.7$ ($r = 0.75$, $P < 0.01$). The 4-AP–induced decrease in ORM-3819–evoked relaxation was calculated from the values obtained during the interaction of the 2 substances and found to be 1/3.2 of the relaxation produced by ORM-3819 alone. The slope value (13.4) divided by 3.2 shows that 1-mV hyperpolarization is responsible for 4.2% of relaxation. The intercept indicated that this relation is valid only above 18.7% relaxation by ORM-3819 (Fig. 6).

DISCUSSION

We demonstrated the coronary artery-dilating effect of ORM-3819 in vitro in partially depolarizing KCl solution. The potency of ORM-3819 was lower than that of the inodilator levosimendan under comparable experimental conditions.[8,12] Hyperpolarization induced by ORM-3819 was compared with that induced by levosimendan and CNP, an endogenous vasoactive regulator. The maximum increase in resting membrane potential induced by ORM-3819 was similar to that obtained with CNP, while the effect of levosimendan was smaller than that of CNP.

Hyperpolarization is an efficient vasodilating mechanism regulating the tension of conduit-type coronary arteries.[24] A nitric oxide– and prostaglandin-independent endothelium-derived hyperpolarizing factor (EDHF) has been demonstrated in the regulation of porcine, canine, and human coronary artery tones.[14,15,24,25] EDHF has been proposed to relax coronary arteries in experimental heart failure and coronary angioplasty under pathological conditions in which nitric oxide production is impaired.[26–28] These findings provide a basis and rationale for the development of hyperpolarizing coronary artery vasodilators.

ORM-3819 is a somewhat less potent vasodilator than levosimendan. However, the maximum hyperpolarizing effect of ORM-3819 is larger than that measured with levosimendan. The effects of both synthetic inodilators were compared with those of CNP because this natriuretic peptide has been

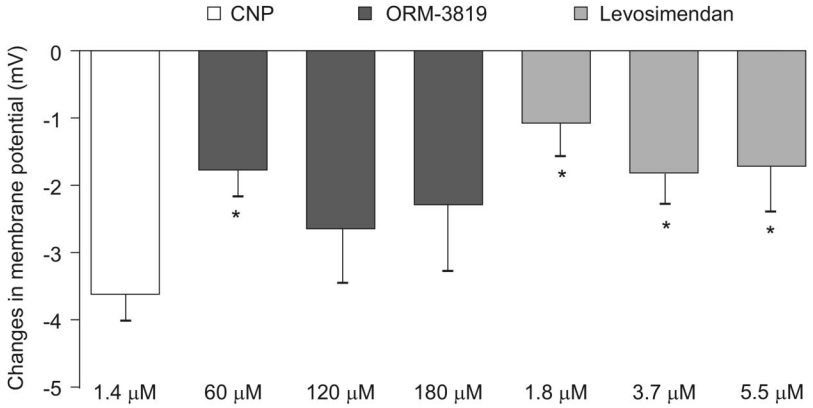

FIGURE 4. Changes in resting membrane potential in vascular smooth muscle cells of porcine epicardial coronary arteries after administration of CNP, ORM-3819, and levosimendan. Values are expressed in the form mean ± standard error of the mean, representing hyperpolarizations of 1.4 μM CNP (N = 17), 60 μM ORM-3819 (N = 14), 120 μM ORM-3819 (N = 10), 180 μM ORM-3819 (N = 12), 1.8 μM levosimendan (N = 18), 3.7 μM levosimendan (N = 22), and 5.5 μM levosimendan (N = 15). The effect of the solvent was deducted from that obtained with the corresponding concentration of ORM-3819. *$P < 0.05$ compared with the effect of the reference compound, CNP.

Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

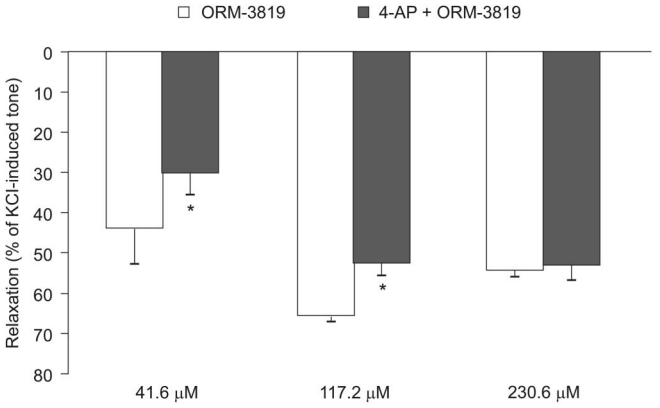

FIGURE 5. Influence of 4-aminopyridine (4-AP) on ORM-3819–induced relaxation of porcine epicardial coronary arteries. Relaxation by ORM-3819, at concentrations of 41.6 and 117.2 μM, was decreased by the voltage-gated potassium channel blocker, 4-AP, at a concentration of 5 mM. The effect of the solvent was subtracted from the relaxing effect of ORM-3819 at the concentrations studied (4-AP had no effect on the solvent). The results are expressed as percent relaxation of 30 mM KCl-evoked tone and in the form mean ± standard error of the mean, representing 5 experiments for each concentration of ORM-3819. *$P < 0.05$ for the difference between the effects of ORM-3819 and ORM-3819 + 4-AP.

shown to act as an EDHF in rat mesenteric and human penile resistance arteries; CNP also acts as an endothelium-independent endogenous hyperpolarizing mediator in several human conduit arteries.[18,29,30] CNP is a potential endogenous hyperpolarizing mediator in the epicardial coronary artery of the pig; it also plays an important role in the pathophysiology of human coronary arterial stenosis and in chronic heart failure.[17,21,31,32] Cardiac production of CNP and expression of its receptor, natriuretic peptide receptor B, are increased in heart failure, suggesting that CNP is released as a cytoprotective mechanism.[33,34] Exogenous administration of CNP, in the same concentration range that we applied in vitro ($\approx 10^{-6}$ M), results in a positive lusitropic effect, an observation that reinforces the significance of CNP in the setting of heart failure.[35]

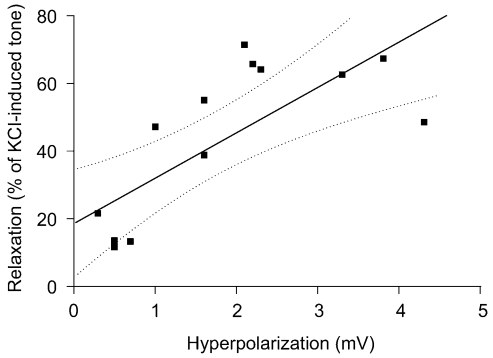

FIGURE 6. Correlation between hyperpolarization and relaxation induced by ORM-3819 in the isolated porcine coronary artery. The magnitude of hyperpolarization showed a positive correlation with relaxation induced by 60 μM ORM-3819 ($r = 0.75$, $P < 0.01$). Individual values are from the same coronary artery samples.

In the porcine coronary artery, the maximum response to CNP was −3.3 mV at a concentration of 1.4 μM.[36] The hyperpolarization induced by 1.4 μM CNP in this study (mean −3.6 mV) is comparable with those obtained in other experiments with this peptide (≈ −4 to −5 mV) or with the adenosine triphosphate–sensitive potassium channel opener levcromakalim (≈ −4 mV).[17,37] Therefore, we only used this concentration of CNP as a control for comparing the magnitude of hyperpolarization induced by ORM-3819 or levosimendan.

Our investigations have produced the first evidence that at the maximal vasorelaxing concentrations of levosimendan,[8] ≈1–3 μM, it hyperpolarizes the large epicardial coronary artery. Similar concentrations of levosimendan have previously been reported to hyperpolarize resistance arteries (EC_{50} = 2.9 μM).[11] It is important to note, however, that the magnitude of hyperpolarization is much less in our epicardial coronary artery preparations than in resistance arteries.[11,29] Some millivolt changes in the membrane potential might considerably influence vascular tone. In this study, observations on the correlation between the hyperpolarizing and relaxing effects of ORM-3819 showed that an increase in membrane potential of 1 mV corresponded to a relaxation of 4.2%. This value is close to that found in rat mesenteric artery (4.3%/mV).[38]

ORM-3819, as levosimendan, could also have pleiotropic effects in addition to the influence on the voltage-gated potassium channels, causing hyperpolarization and thus vasodilation, K_{ATP}-channel opening, amplification of BKCa channel function, and/or phosphodiesterase inhibition being the most probable ones. With the present research, we demonstrated the role of voltage-gated potassium channel activation in the vasodilatory effects of ORM-3819.

In the porcine coronary artery, voltage-gated potassium channels regulate tone both at rest and under stimulated conditions.[39,40] ORM-3819 seems to trigger the activation of 4-AP–sensitive voltage-gated potassium channels. In this respect, the drug resembles levosimendan.[41] 4-AP–sensitive potassium channels have been proposed to play a role in the vasomotor tone of coronary arteries under pathological conditions.[42] Both ORM-3819 and levosimendan have been shown to elicit beneficial hemodynamic effects in canine pathological cardiac models[22,43] and, in the case of levosimendan, in human severe heart failure.[44–46] The activation of voltage-gated potassium channels is significantly involved in the vasodilatory mechanism induced by ORM-3819. The hyperpolarizing property of this new compound on arterial vessel walls, at concentrations close to the EC_{50} value, suggests the involvement of other important ionic mechanism(s) that beneficially influence the vascular tone.

In our experiments, high concentration of KCl would attenuate the hyperpolarizing and relaxant effect of ORM-3819 by abolishing the Kv channel–related effects—in case, ORM-3819 was to be a selective Kv channel opener. Such experiments are warranted to reveal other possible mechanism(s) involved in the effect of ORM-3819. Such further studies aimed to complete the characterization of the vasodilatory effects of the new drug candidate, including patch-

Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

clamp studies with isolated coronary VSM cells, would corroborate that the ORM-induced increase in outward potassium current is indeed due predominantly to Kv channel activation.

A word or 2 have to be spent on the possible role of cAMP in the ORM-3819–induced vasodilation. cAMP induces vasodilatation when produced in VSM cells,[47] and both BKCa and Kv potassium channels are involved in cAMP-induced vasodilation.[48] The level of cAMP is regulated through the control of both synthesis and degradation, with the latter being controlled by PDE3A and PDE4 in VSM cells. The phosphodiesterase inhibitor and inodilator milrinone were indeed shown to interfere with the BKCa channels.[49]

In a previous article,[22] we tested the PDE inhibitory effect of ORM-3819 on purified PDEIII and PDEIV isozymes and found that ORM-3819 is a very selective inhibitor of PDEIII, with a selectivity versus PDEIV of more than 12,000-fold. In comparison, such selectivity was 8000 for levosimendan[50] but only 14 for milrinone (see Table 2 on page 11 in de Cheffoy de Courcelles et al[51]). As stated by Szilágyi et al regarding levosimendan,[50] both PDEIII and PDEIV have the power to prevent intracellular cAMP accumulation. Therefore, to achieve an increase of cAMP (with all its consequences), both isozymes need to be blocked. At therapeutic doses, levosimendan fails to inhibit PDEIV, and several reports have in fact shown that levosimendan does not increase the intracellular Ca2+ concentration to levels high enough to account fully for the drug's positive inotropic or vasodilatory effects.[52–55]

Being as the PDEIII and PDEIV IC50 values of ORM-3819 are in the same range as the levosimendan values, and being as the PDEIII versus PDEIV selectivity of ORM-3819 is 1.5-fold the one found for levosimendan, we are not expecting ORM-3819 to induce an accumulation of cAMP, which would participate in BKCa and Kv potassium channel activation. Nevertheless, we cannot fully discount this possibility, and we warrant for further studies to investigate a possible contribution of PDE inhibition on the vasodilatory effect of ORM-3819.

CONCLUSIONS

ORM-3819 is structurally and functionally similar to levosimendan and shares its main mechanisms of action, including—as we hereby demonstrated—the vasodilatory one. This, in conjunction with the positive inotropic property of ORM-3819, may be relevant to its therapeutic potential in ischemic heart disease. Different magnitude of hyperpolarization/relaxation may be advantageous during development of agents with different pleiotropic effects. Finally, despite both the structure and the pharmacology of ORM-3819 and levosimendan are related, the pharmacokinetic behavior of the molecules differs considerably, thus justifying a development plan for a better drug.

REFERENCES

1. Pollesello P, Papp Z, Papp JGY. Calcium sensitizers: what have we learned over the last 25 years? *Int J Cardiol.* 2016;203:543–548.
2. Papp Z, Édes I, Fruhwald S, et al. Levosimendan: molecular mechanisms and clinical implications: consensus of experts on the mechanisms of action of levosimendan. *Int J Cardiol.* 2012;159:82–87.
3. Ambrus N, Szolnoky J, Pollesello P, et al. Prolonged antispasmodic effect in isolated radial artery graft and pronounced platelet inhibition induced by the inodilator drug, levosimendan. *Basic Clin Pharmacol Toxicol.* 2012;110:269–274.
4. Gruhn N, Nielsen-Kudsk JE, Theilgaard S, et al. Coronary vasorelaxant effect of levosimendan, a new inodilator with calcium-sensitizing properties. *J Cardiovasc Pharmacol.* 1998;31:741–749.
5. Höhn J, Pataricza J, Petri A, et al. Levosimendan interacts with potassium channel blockers in human saphenous veins. *Basic Clin Pharmacol Toxicol.* 2004;94:271–273.
6. Kaheinen P, Pollesello P, Levijoki J, et al. Levosimendan increases diastolic coronary flow in isolated Guinea-pig heart by opening ATP-sensitive potassium channels. *J Cardiovasc Pharmacol.* 2001;37:367–374.
7. Labriola C, Siro-Brigiani M, Carrata F, et al. Hemodynamic effects of levosimendan in patients with low-output heart failure after cardiac surgery. *Int J Clin Pharmacol Ther.* 2004;42:204–211.
8. Pataricza J, Krassói I, Höhn J, et al. Functional role of potassium channels in the vasodilating mechanism of levosimendan in porcine isolated coronary artery. *Cardiovasc Drugs Ther.* 2003;17:115–121.
9. Pataricza J, Szolnoky J, Krassói I, et al. Vasorelaxing effect of levosimendan against 5-hydroxytryptamine-induced contractions in isolated human conduit bypass grafts. *J Pharm Pharmacol.* 2006;58:1107–1112.
10. Pathak A, Lebrin M, Vaccaro A, et al. Pharmacology of levosimendan: inotropic, vasodilatory and cardioprotective effects. *J Clin Pharm Ther.* 2013;38:341–349.
11. Yokoshiki H, Katsube Y, Sunagawa M, et al. Levosimendan, a novel Ca2+ sensitizer, activates the glibenclamide-sensitive K+ channel in rat arterial myocytes. *Eur J Pharmacol.* 1997;333:249–259.
12. Krassói I, Pataricza J, Kun A, et al. Calcium-dependent vasorelaxant capacity of levosimendan in porcine and human epicardial coronary artery preparations. *Cardiovasc Drugs Ther.* 2000;14:691–693.
13. Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science.* 1992;256:532–535.
14. Fujioka H, Ayajiki K, Shinozaki K, et al. Mechanisms underlying endothelium-dependent, nitric oxide- and prostanoid-independent relaxation in monkey and dog coronary arteries. *Naunyn Schmiedebergs Arch Pharmacol.* 2002;366:488–495.
15. Krassói I, Pataricza J, Torday LL, et al. Improvement by phosphoramidon of damaged endothelial function in porcine coronary artery. *Ann Thorac Surg.* 2000;70:878–882.
16. Ng KFJ, Leung SWS, Man RYK, et al. Endothelium-derived hyperpolarizing factor mediated relaxations in pig coronary arteries do not involve Gi/o proteins. *Acta Pharmacol Sin.* 2008;29:1419–1424.
17. Barton M, Bény JL, d'Uscio LV, et al. Endothelium-independent relaxation and hyperpolarization to C-type natriuretic peptide in porcine coronary arteries. *J Cardiovasc Pharmacol.* 1998;31:377–383.
18. Kun A, Kiraly I, Pataricza J, et al. C-type natriuretic peptide hyperpolarizes and relaxes human penile resistance arteries. *J Sex Med.* 2008;5:1114–1125.
19. Márton Z, Pataricza J, Krassói I, et al. NEP inhibitors enhance C-type natriuretic peptide-induced relaxation in porcine isolated coronary artery. *Vascul Pharmacol.* 2005;43:207–212.
20. Naruko T, Ueda M, van der Wal AC, et al. C-type natriuretic peptide in human coronary atherosclerotic lesions. *Circulation.* 1996;94:3103–3108.
21. Wei CM, Hu S, Miller VM, et al. Vascular actions of C-type natriuretic peptide in isolated porcine coronary arteries and coronary vascular smooth muscle cells. *Biochem Biophys Res Commun.* 1994;205:765–771.
22. Nagy L, Pollesello P, Haikala H, et al. ORM-3819 promotes cardiac contractility through Ca^{2+} sensitization in combination with selective PDE III inhibition, a novel approach to inotropy. *Eur J Pharmacol.* 2016;775:120–129.
23. Lückhoff A, Busse R. Activators of potassium channels enhance calcium influx into endothelial cells as a consequence of potassium currents. *Naunyn Schmiedebergs Arch Pharmacol.* 1990;342:94–99.
24. Bychkov R, Gollasch M, Steinke T, et al. Calcium-activated potassium channels and nitrate-induced vasodilation in human coronary arteries. *J Pharmacol Exp Ther.* 1998;285:293–298.

Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

25. Pataricza J, Toth GK, Penke B, et al. Effect of selective inhibition of potassium channels on vasorelaxing response to cromakalim, nitroglycerin and nitric oxide of canine coronary arteries. *J Pharm Pharmacol.* 1995;47:921–925.
26. Gschwend S, Buikema H, Henning RH, et al. Endothelial dysfunction and infarct-size relate to impaired EDHF response in rat experimental chronic heart failure. *Eur J Heart Fail.* 2003;5:147–154.
27. Malo O, Carrier M, Shi YF, et al. Specific alterations of endothelial signal transduction pathways of porcine epicardial coronary arteries in left ventricular hypertrophy. *J Cardiovasc Pharmacol.* 2003;42:275–286.
28. Thollon C, Fournet-Bourguignon MP, Saboureau D, et al. Consequences of reduced production of NO on vascular reactivity of porcine coronary arteries after angioplasty: importance of EDHF. *Br J Pharmacol.* 2002; 136:1153–1161.
29. Chauhan SD, Nilsson H, Ahluwalia A, et al. Release of C-type natriuretic peptide accounts for the biological activity of endothelium-derived hyperpolarizing factor. *Proc Natl Acad Sci USA.* 2003;100:1426–1431.
30. Kelsall CJ, Chester AH, Sarathchandra P, et al. Expression and localization of C-type natriuretic peptide in human vascular smooth muscle cells. *Vascul Pharmacol.* 2006;45:368–373.
31. Kalra PR, Clague JR, Bolger AP, et al. Myocardial production of C-type natriuretic peptide in chronic heart failure. *Circulation.* 2003;107:571–573.
32. Naruko T, Itoh A, Haze K, et al. C-Type natriuretic peptide and natriuretic peptide receptors are expressed by smooth muscle cells in the neointima after percutaneous coronary intervention. *Atherosclerosis.* 2005;181:241–250.
33. Del Ry S, Cabiati M, Lionetti V, et al. Expression of C-type natriuretic peptide and of its receptor NPR-B in normal and failing heart. *Peptides.* 2008;29:2208–2215.
34. Lumsden NG, Khambata RS, Hobbs AJ. C-type natriuretic peptide (CNP): cardiovascular roles and potential as a therapeutic target. *Curr Pharm Des.* 2010;16:4080–4088.
35. Moltzau LR, Aronsen JM, Meier S, et al. SERCA2 activity is involved in the CNP-mediated functional responses in failing rat myocardium. *Br J Pharmacol.* 2013;170:366–379.
36. Márton Z, Pataricza J, Krassói I, et al. Hyperpolarizing effect of CNP—a model for studying novel coronary artery dilators. *Fundam Clin Pharmacol.* 2004;18:54.
37. Burnham MP, Bychkov R, Félétou M, et al. Characterization of an apamin-sensitive small-conductance Ca^{2+}-activated K^+ channel in porcine coronary artery endothelium: relevance to EDHF. *Br J Pharmacol.* 2002;135:1133–1143.
38. Cheung DW, Chen G, MacKay MJ, et al. Regulation of vascular tone by endothelium-derived hyperpolarizing factor. *Clin Exp Pharmacol Physiol.* 1999;26:172–175.
39. O'Rourke ST. Effects of potassium channel blockers on resting tone in isolated coronary arteries. *J Cardiovasc Pharmacol.* 1996;27:636–642.
40. Shimizu S, Yokoshiki H, Sperelakis N, et al. Role of voltage-dependent and Ca^{2+}-activated K^+ channels on the regulation of isometric force in porcine coronary artery. *J Vasc Res.* 2000;37:16–25.
41. Yokoshiki H, Sperelakis N. Vasodilating mechanisms of levosimendan. *Cardiovasc Drugs Ther.* 2003;17:111–113.
42. Uchida Y, Nakamura F, Tomaru T, et al. Phasic contractions of canine and human coronary arteries induced by potassium channel blockers. *Jpn Heart J.* 1986;27:727–740.
43. Udvary E, Papp JGY, Végh Á. Cardiovascular effects of the calcium sensitizer, levosimendan, in heart failure induced by rapid pacing in the presence of aortic constriction. *Br J Pharmacol.* 1995;114:656–661.
44. Nieminen MS, Fruhwald S, Heunks LM, et al. Levosimendan: current data, clinical use and future development. *Heart Lung Vessel.* 2013;5: 227–245.
45. Oner E, Erturk M, Birant A, et al. Assessment of sustained effects of levosimendan and dobutamine on left ventricular systolic functions by using novel tissue Doppler derived indices in patients with advanced heart failure. *Cardiol J.* 2015;22:87–93.
46. Põder P, Eha J, Sundberg S, et al. Pharmacokinetic-pharmacodynamic interrelationships of intravenous and oral levosimendan in patients with severe congestive heart failure. *Int J Clin Pharmacol Ther.* 2003;41:365–373.
47. Berg T, Degerman E, Tasken K. Increased cAMP signaling can ameliorate the hypertensive condition in spontaneously hypertensive rats. *J Vasc Res.* 2009;46:25–35.
48. Pintérová M, Behuliak M, Kuneš J, et al. Involvement of BKCa and KV potassium channels in cAMP-induced vasodilatation: their insufficient function in genetic hypertension. *Physiol Res.* 2014;63:275–278.
49. Zhu S, White RE, Barman SA. Role of phosphodiesterases in modulation of BKCa channels in hypertensive pulmonary arterial smooth muscle. *Ther Adv Respir Dis.* 2008;2:119–127.
50. Szilágyi S, Pollesello P, Levijoki J, et al. The effects of levosimendan and OR-1896 on isolated hearts, myocyte-sized preparations and phosphodiesterase enzymes of the Guinea pig. *Eur J Pharmacol.* 2004;486: 67–74.
51. de Cheffoy de Courcelles D, de Loore K, Freyne E, et al. Inhibition of human cardiac cyclic AMP-phosphodiesterases by R 80122, a new selective cyclic AMP-phosphodiesterase III inhibitor: a comparison with other cardiotonic compounds. *J Pharmacol Exp Ther.* 1992;263:6–14.
52. Brixius K, Reicke S, Schwinger RH. Beneficial effects of the Ca(2+) sensitizer levosimendan in human myocardium. *Am J Physiol Heart Circ Physiol.* 2002;282:H131–H137.
53. Lancaster MK, Cook SJ. The effects of levosimendan on [Ca2+]i in Guinea-pig isolated ventricular myocytes. *Eur J Pharmacol.* 1997;339: 97–100.
54. Hasenfuss G, Pieske B, Castell M, et al. Influence of the novel inotropic agent levosimendan on isometric tension and calcium cycling in failing human myocardium. *Circulation.* 1998;98:2141–2147.
55. Sato S, Talukder MA, Sugawara H, et al. Effects of levosimendan on myocardial contractility and Ca2+ transients in aequorin-loaded right-ventricular papillary muscles and indo-1-loaded single ventricular cardiomyocytes of the rabbit. *J Mol Cell Cardiol.* 1998;30:1115–1128.

Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.