Sarpogrelate Dilates Cerebral Arteries in the Absence of Exogenous Serotonin

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Abstract

Vasoconstriction of arteries induced by serotonin (5-hydroxytryptamine: 5-HT) is mediated by 5-HT_{2A} and 5-HT_{1B} receptors localized on smooth muscle. The present study investigated the impact of sarpogrelate, a 5-HT_{2A} receptor antagonist, on cerebral artery diameter in the presence and absence of exogenous 5-HT. Diameter measurements were obtained in vitro from rabbit cerebral arteries pressurized to 60 mmHg. In the absence of 5-HT, arteries exhibiting pressure-induced myogenic tone dilated to sarpogrelate in a concentration-dependent manner (half maximal inhibitory concentration [IC₅₀] $\approx$ 2.3 μ M). In a separate experimental series, exogenous application of 5-HT (0.01 μ M) caused further constriction of myogenically active arteries, decreasing cerebral artery diameter by an additional 25%. In the presence of 5-HT, sarpogrelate caused concentration-dependent vasodilation (IC₅₀ $\approx$ 2.3 μ M) that was similar to that observed in the absence of exogenous 5-HT. Dilation induced by sarpogrelate was not affected by physical removal of the endothelium or inhibition of nitric oxide synthase with *N* ω -nitro L-arginine. The highest concentration of sarpogrelate (100 μ M) induced near maximal dilation, comparable to dilation induced by the L-type voltage-dependent calcium channel antagonist diltiazem. These findings suggest that in rabbit cerebral arteries, sarpogrelate has direct vasodilator effects on vascular smooth muscle.

Key words: 5-hydroxytryptamine, cerebral artery, sarpogrelate, vascular smooth muscle, serotonin

Introduction

Receptors for serotonin (5-hydroxytryptamine: 5-HT) have been classified into seven subtypes, 5-HT₁₋₇, and 15 distinct receptor isoforms have been identified.⁷⁾ Activations of 5-HT_{1B} and 5-HT_{2A} receptor subtypes are thought to underlie vascular diameter changes induced by 5-HT in animals and humans.²⁹⁾ Studies using a variety of species, including rats, dogs, and pigs, have demonstrated that 5-HT-induced vasoconstriction is mediated through stimulation of smooth muscle 5-HT_{2A} receptors whereas vasodilation occurs via stimulation of 5-HT_{1B} receptors located in vascular endothelium.^{1,4,7)}

Ketanserin, classified as an antihypertensive agent by the World Health Organization³²⁾ was originally thought to be a selective 5-HT_{2A} receptor antagonist. However, ketanserin is now known to also exhibit significant antagonism of α_1 -adrenoceptors¹⁷⁾

and histamine H₁ receptors.¹⁰⁾ Sarpogrelate is a newly developed 5-HT_{2A} receptor antagonist that, unlike ketanserin, has little effect on α_1 -adrenoceptors.¹⁷⁾ Sarpogrelate has been shown to selectively inhibit 5-HT_{2A} receptors located on rabbit platelets, rat brain, and caudal arteries.^{16,20,28)} Sarpogrelate is clinically important because it blocks serotonin-induced platelet aggregation and has applications in the treatment of arteriosclerosis⁶⁾ including diabetes mellitus,^{18,21)} Buerger's disease,²⁴⁾ Raynaud's disease,⁹⁾ and coronary artery disease.^{13,25)} However, the actions of sarpogrelate on the cerebral vascular system have not been studied.

The present study examined responses to sarpogrelate in rabbit cerebral arteries exhibiting stable pressure-induced myogenic tone, and found that sarpogrelate caused significant concentration-dependent dilation in the absence of exogenous 5-HT. Our findings suggest that in addition to antagonism of 5-HT_{2A} receptors, sarpogrelate can induce cerebral artery dilation through a direct effect on smooth

muscle voltage-dependent Ca^{2+} channels.

Materials and Methods

New Zealand White rabbits (male, 2.0–3.0 kg) were used in this study. Rabbits were euthanized (pentobarbital 150 mg/kg, intravenous), then decapitated. Brains were then removed and immersed in a dissection dish filled with cold (4°C), oxygenated (20% O_2 , 5% CO_2 , 75% N_2) physiological saline solution (PSS) of the following composition (in mM); 118.5 NaCl, 4.7 KCl, 24 NaHCO_3 , 1.18 KH_2PO_4 , 2.5 CaCl_2 , 1.2 MgCl_2 , 0.023 ethylenediaminetetraacetic acid, and 11 glucose. Cerebral artery segments obtained from branches of cerebellar or posterior cerebral arteries (100–300 μm in diameter) were carefully dissected from the brain and cleaned of blood using a stereodissection microscope. Experimental protocols (#08–109) were approved by the Institution Animal Care and Use Committee of Sapporo Medical University.

Arteries were cannulated on glass pipettes mounted in a 5 ml myograph chamber (Living Systems Instruments, Burlington, Vermont, USA), superfused with PSS (pH 7.4) aerated with 20% O_2 , 5% CO_2 , and 75% N_2 and warmed to 37°C . Arterial diameter was measured using video edge detection equipment and recorded using data acquisition software (Lab-Trax® with Data-Trax® recording software; World Precision Instruments, Sarasota, Florida, USA). Artery viability was tested at the start of each experiment by exposing the arteries to 60 mM extracellular K^+ . Arteries were discarded if the constriction induced by 60 mM K^+ was less than a 50% decrease in diameter.

After an equilibration period of 30 minutes at 20 mmHg, intraluminal pressure was elevated to 60 mmHg. Once myogenic tone developed and stabilized, sarpogrelate at concentrations varying from 0.001 to 100 μM were administered in the presence and absence of 5-HT. Some arteries were denuded of vascular endothelium by placing an air bubble in the lumen for 1 minute, followed by luminal washing with distilled water for 30 seconds. Endothelial disruption was verified by the absence of dilator response to acetylcholine (1 μM and 100 μM) after myogenic tone had developed. To examine the impact of sarpogrelate on nitric oxide production, studies were also performed in the presence and absence of $\text{N}\omega$ -nitro-L-arginine (LNNA, 100 μM), a nitric oxide synthase inhibitor. At the end of each experiment, the passive (fully dilated) diameter was obtained with 50 μM diltiazem in Ca^{2+} -free PSS.

Results are expressed as mean $\pm$ standard error of the mean. ORIGIN® (version 7.5; OriginLab Cor-

poration, Northampton, Massachusetts, USA) and SPSS (SPSS Japan Inc., Tokyo) were used for statistical analysis. Statistical significance was considered at the level of $p < 0.05$ or $p < 0.01$ using either Student's *t* test or repeated measures analysis of variance.

Sarpogrelate was obtained from Tanabe-Mitsubishi Chemical Corporation, Tokyo. Sodium pentobarbital was obtained from Tokyo Chemical Industry Corporation, Tokyo. All other drugs were obtained from Sigma (St Louis, Missouri, USA).

Results

Initial studies were designed to examine the impact of sarpogrelate on pressure-induced constriction of rabbit cerebral arteries in the absence of exogenous 5-HT. Sarpogrelate (0.01 μM to 100 μM) caused concentration-dependent vasodilation (half maximal inhibitory concentration (IC_{50}) value of sarpogrelate 2.30 μM) of isolated cerebral arteries pressurized to 60 mmHg ($n = 5$) (Fig. 1A). The highest concentration of sarpogrelate (100 μM) caused near maximal dilation of arteries, to $97.0 \pm 0.1\%$ of their passive diameter (Fig. 1C). In a separate experimental series, pressurized cerebral arteries exhibiting myogenic tone were treated with 5-HT (0.01 μM) prior to the addition of sarpogrelate. At 60 mmHg, 5-HT exposure resulted in additional constriction representing a further decrease in diameter of $24.6 \pm 3.6\%$ (Fig. 1B). In the presence of 5-HT, sarpogrelate also caused concentration-dependent vasodilation with an IC_{50} value of 2.06 μM . Near maximum vasodilation was again observed with 100 μM sarpogrelate (Fig. 1D). These data demonstrate that sarpogrelate caused similar concentration-dependent dilation of myogenically active cerebral arteries in the absence and presence of 5-HT.

To examine the role of the vascular endothelium in sarpogrelate-induced vasodilation, responses were examined in endothelial-denuded cerebral arteries. Endothelium removal was confirmed by the absence of dilation induced by acetylcholine (ACh, 1 μM or 100 μM). The diameter of endothelial-denuded arteries was $119.3 \pm 8.8 \mu\text{m}$ prior to ACh administration, and was $123.8 \pm 8.1 \mu\text{m}$ and $118.9 \pm 9.4 \mu\text{m}$ in the presence of 1 μM and 100 μM ACh, respectively. Sarpogrelate-induced (1 μM and 100 μM) vasodilation of endothelium-denuded vessels was comparable to responses in endothelial intact arteries (Fig. 2). These data indicate that sarpogrelate causes vasodilation via a direct effect on the vascular smooth muscle cells within the arterial wall.

To examine the potential involvement of enhanced nitric oxide production in sarpogrelate-in-

Fig. 1 A, B: Representative experiments showing the effect of sarpogrelate on arterial diameter in the absence (A) and presence (B) of 0.01 μM 5-hydroxytryptamine (5-HT). MT: myogenic tone. C, D: Effects of increasing concentrations of sarpogrelate on luminal diameter of rabbit cerebral arteries at 60 mmHg. Sarpogrelate caused concentration-dependent dilation of cerebral arteries in physiological saline solution in the absence of 5-HT (C). Sarpogrelate caused concentration-dependent dilation of cerebral arteries in physiological saline solution containing 5-HT (D). *p < 0.05, **p < 0.01. Dotted line in D indicates mean diameter in the absence of 5-HT.

Fig. 2 Effect of sarpogrelate on endothelium-denuded arteries. Sarpogrelate (1 μM and 100 μM) significantly dilated pressure-induced constriction of cerebral arteries denuded of vascular endothelium (**p < 0.01). PSS: physiological saline solution.

duced vasodilation, studies were performed using the inhibitor of nitric oxide synthase, LNNA (100 μM). Sarpogrelate significantly dilated endothelial intact arteries from a mean diameter of 171.5 ± 10.4 μm to a mean diameter of 185.0 ± 14.1 μm. Subsequent addition of LNNA did not alter the diameter (185.4 ± 13.9 μm) (Fig. 3A). Further, pre-treatment of an additional set of endothelial intact arteries with LNNA did not alter sarpogrelate-induced dilation (Fig. 3B). These data demonstrate that sarpogrelate dilated cerebral arteries in a manner independent of nitric oxide generation.

Cerebral artery dilation induced by 100 μM sarpogrelate was similar in magnitude to dilation caused by the removal of extracellular Ca²⁺ from the PSS. Furthermore, dilation induced by sarpogrelate and Ca²⁺-free PSS were not additive (Fig. 4), suggesting that sarpogrelate may impair the influx of Ca²⁺ into cerebral artery myocytes. Considering that voltage-dependent Ca²⁺ channels (VDCCs) represent

Fig. 3 Summary of the diameter measurements of rabbit cerebral arteries in the presence of sarpogrelate (100 μM) and *N*-nitro-L-arginine (LNNA, 100 μM), an inhibitor of nitric oxide synthesis. **A:** Sarpogrelate dilated arteries from 171.5 ± 10.4 to 185.0 ± 14.1 μm, and the diameter was similar after addition of LNNA (185.4 ± 13.9 μm) ($n = 6$). **B:** LNNA did not change the diameter (149.9 ± 8.5 to 149.6 ± 8.6 μm) or affect sarpogrelate-induced vasodilation (179.4 ± 9.2 μm) ($n = 6$). * $p < 0.05$, ** $p < 0.01$. NS: not significant, PSS: physiological saline solution.

the major Ca^{2+} influx pathway in these cells, comparisons were made between the actions of sarpogrelate and the VDCC inhibitor diltiazem. In a manner similar to the removal of extracellular Ca^{2+} , dilations to maximal concentrations of sarpogrelate (100 μM) and diltiazem (50 μM) were not additive (Fig. 5). The effect of a lower concentration of sarpogrelate (1 μM) was also studied on cerebral artery dilation induced by three distinct classes of VDCC inhibitors: benzothiazepine diltiazem, phenylalkylamine verapamil, and dihydropyridine nifedipine. Sarpogrelate caused a leftward shift of concentration-response curve of all three VDCC inhibitors. IC_{50} values changed from 1.8 ± 0.4 to 0.6 ± 0.2 μM with diltiazem, from 1.6 ± 0.6 to 0.4 ± 0.2 μM with

Fig. 4 Summary of the diameter of rabbit cerebral arteries in the presence of sarpogrelate and calcium-free physiological saline solution (PSS). **A:** Sarpogrelate dilated arteries from 139.1 ± 21.4 to 182.4 ± 12.9 μm, and the diameter was similar after addition of calcium free PSS (182.5 ± 14.9 μm) ($n = 5$). **B:** Calcium-free PSS dilated arteries from 120.6 ± 15.0 to 155.6 ± 16.0 μm, and the diameter was similar after subsequent addition of sarpogrelate (156.6 ± 16.7 μm) ($n = 6$). * $p < 0.05$, ** $p < 0.01$. NS: not significant.

verapamil, and from 0.4 ± 0.1 to 0.2 ± 0.1 μM with nifedipine (Fig. 6). These data suggest that sarpogrelate may act through inhibition of VDCCs to reverse pressure-induced constriction of rabbit cerebral arteries.

Discussion

This study provides evidence indicating that the purported 5-HT_{2A} receptor antagonist sarpogrelate can dilate rabbit cerebral arteries via a mechanism independent of 5-HT_{2A} receptors. Specifically, we observed that: In the absence of 5-HT, sarpogrelate dilated cerebral arteries exhibited pressure-induced myogenic tone; the efficacy of sarpogrelate to induce cerebral artery vasodilation was similar in the

Fig. 5 Summary of the diameter of rabbit cerebral arteries in the presence of sarpogrelate and diltiazem. **A:** Sarpogrelate dilated arteries from 153.5 ± 14.3 to $180.9 \pm 12.6 \mu\text{m}$, and the diameter was similar after addition of diltiazem ($182.3 \pm 12.9 \mu\text{m}$). **B:** Diltiazem dilated arteries from 157.8 ± 10.6 to $179.7 \pm 5.5 \mu\text{m}$, and the diameter was similar after addition of sarpogrelate ($179.5 \pm 5.5 \mu\text{m}$). * $p < 0.05$, ** $p < 0.01$. NS: not significant, PSS: physiological saline solution.

presence and absence of exogenous 5-HT; sarpogrelate exhibited direct vasodilator effects on cerebral artery smooth muscle independent of nitric oxide and vascular endothelium; and maximal dilations to sarpogrelate and VDCC inhibitors were not additive and sarpogrelate caused a leftward shift in the IC_{50} curves of three types of calcium antagonists without changing maximum arterial dilation. In summary, these findings demonstrate that sarpogrelate can dilate cerebral arteries through a mechanism independent of antagonism of 5-HT_{2A} receptors.

5-HT is a powerful vasomotor agent causing increased cytoplasmic calcium levels in the cerebral artery myocytes assessed by radioactive ^{45}Ca influx and efflux measurements.³¹⁾ Among the various 5-HT receptor subtypes, 5-HT_{2A} receptor has been

Fig. 6 Effect of sarpogrelate ($1 \mu\text{M}$) on calcium channel blocker-induced (diltiazem, verapamil, and nifedipine) vasodilation in rabbit cerebral arteries. **A:** Dose-response curves of diltiazem ($n = 9$). Half maximal inhibitory concentration (IC_{50}) of diltiazem changed from 1.8 ± 0.4 to $0.6 \pm 0.2 \mu\text{M}$ after addition of sarpogrelate. **B:** Dose-response curves of verapamil ($n = 8$). IC_{50} of verapamil changed from 1.6 ± 0.6 to $0.4 \pm 0.2 \mu\text{M}$ after addition of sarpogrelate. **C:** Dose-response curves of nifedipine ($n = 9$). IC_{50} of nifedipine changed from 0.4 ± 0.1 to $0.2 \pm 0.1 \mu\text{M}$ after addition of sarpogrelate.

implicated in a variety of cellular processes such as vascular smooth muscle contraction,²⁾ non-vascular smooth muscle contraction (including uterine contraction),⁸⁾ and platelet aggregation.¹⁵⁾ Sarpogrelate has been reported to be a specific antagonist of the 5-HT_{2A} receptor, that unlike ketanserin, has no inhibitory effect on α_1 -adrenoceptors.³⁾ Our in vitro data demonstrate that 10 μ M sarpogrelate abolished 5-HT-induced cerebral artery constriction, which is consistent with a previous study of the human thoracic artery.¹¹⁾ However, we observed sarpogrelate was equally effective in causing vasodilation of cerebral arteries in the absence of 5-HT (Fig. 1). To our knowledge, this is the first study to report sarpogrelate-induced cerebral artery dilation not only in the presence of 5-HT but also in the absence of 5-HT. Moreover, in the present study, the dilatory effects of sarpogrelate were preserved after endothelial denudation or inhibition of nitric oxide synthase, suggesting that the actions of sarpogrelate are independent of vascular endothelium-derived signals.

Platelets contain 5-HT, and activation of 5-HT_{2A} receptors by platelet-derived 5-HT has been implicated in platelet aggregation.¹⁵⁾ In vitro, ex vivo, and in vivo studies have demonstrated that sarpogrelate acts not only on vascular smooth muscle cells but also on platelets.⁵⁾ One possibility to explain the sarpogrelate-induced vasodilation which we observed in the absence of exogenous 5-HT would be antagonism of platelet-derived 5-HT. However, in our experimental system, arteries were cannulated and blood flushed from the lumen prior to the initiation of diameter measurements. Thus, the actions of sarpogrelate in the present study were unlikely to reflect antagonism of endogenous platelet-derived 5-HT.

5-HT induced contractions are blocked by L-type calcium channel antagonists such as diltiazem, verapamil, and nifedipine.^{12,31)} 5-HT_{2A} receptor activation may increase intracellular Ca²⁺ levels through both release of intracellular stores of Ca²⁺ and activation of L-type Ca²⁺ channels.³⁰⁾ In our study, sarpogrelate caused a leftward shift of the concentration-effect curves of calcium channel blockers, the IC₅₀ of diltiazem, verapamil, and nifedipine, without suppressing the maximum response. These findings suggest that sarpogrelate can also act as a competitive antagonist of voltage-dependent calcium channels. Consistent with inhibition of voltage-dependent calcium channels by sarpogrelate, we observed that this compound caused a concentration-dependent reversal of pressure-induced myogenic tone in the absence of 5-HT.

Experimentally, inhibition of L-type calcium

channels has shown promise in minimizing damage caused by cerebral ischemia.¹⁴⁾ However, clinically, most L-type calcium blockers have failed to demonstrate benefits.²⁷⁾ Only nimodipine has been established as a neuroprotectant after subarachnoid hemorrhage.²²⁾ In addition, L-type calcium channel blockers have potential therapeutic uses for subcortical vascular dementia¹⁹⁾ and Parkinson's disease.²³⁾ Thus, as a L-type calcium channel antagonist, sarpogrelate may represent a new and important therapeutic agent in the treatment of cerebral ischemia, subarachnoid hemorrhage, dementia, and Parkinson's disease.

In summary, we report that sarpogrelate abolished cerebral artery constriction in both the absence and presence of exogenous 5-HT. These findings suggest that sarpogrelate acts not only as a 5-HT_{2A} receptor antagonist in smooth muscle cells of rabbit cerebral arteries, but also as a calcium channel blocker. Importantly, voltage-dependent calcium channel blockade may account for the clinical utility of sarpogrelate in the treatment of vascular diseases such as atherosclerosis including diabetes mellitus, Buerger's disease, Raynaud's disease, and coronary artery disease. Furthermore, these findings suggest sarpogrelate may have additional clinical benefits such as acting as a neuroprotectant during cerebral ischemia, dementia, and Parkinson's disease.

Acknowledgments

This study was supported by Sapporo Medical University Foundation for Promotion of Medical Science, Japan (#20) and Tanabe-Mitsubishi Chemical Corporation, Tokyo. The authors wish to thank Dr. N. Tohse for helpful comments on this study.

Conflicts of Interest Disclosure

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices in the article. All authors who are members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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