76 (2026) 100085

Contents lists available at ScienceDirect

The Journal of Physiological Sciences

journal homepage: www.editorialmanager.com/jphyss/default2.aspx

Original article

Effects of serotonin transporters on myocardial cell injury during ischemia/reperfusion in rats

Yoshimasa Kishi [a], Shuji Shimizu [a,b,*], Yosuke Kuroko [a], Yasuhiro Kotani [a], Tsuyoshi Akiyama [c], Shingo Kasahara [a]

[a] Department of Cardiovascular Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital, Okayama, Japan
[b] Department of Research Promotion and Management, National Cerebral and Cardiovascular Center, Suita, Japan
[c] Department of Cardiac Physiology, National Cerebral and Cardiovascular Center, Suita, Japan

ARTICLE INFO

Keywords:
Myocardial ischemia/reperfusion injury
Platelets
Serotonin reuptake
Cardiac microdialysis
Myoglobin

ABSTRACT

Serotonin (5-HT) reuptake in the heart is mediated by the platelet serotonin reuptake transporter (SERT) and the cardiomyocyte plasma membrane monoamine transporter (PMAT). However, the specific roles of these transporters in myocardial cell injury during ischemia/reperfusion remain unclear. Sprague-Dawley rats underwent left coronary artery occlusion followed by reperfusion. Using cardiac microdialysis technique, fluoxetine (SERT inhibitor, 900 μM) or decynium 22 (PMAT inhibitor, 100 μM) was administered locally to the ischemic region. Myoglobin concentrations in the dialysate were measured during ischemia followed by reperfusion. In the fluoxetine-treated group, peak myoglobin concentration (14,889 ± 1364 ng/mL) during 0–15 min of reperfusion was significantly higher compared to both the control group (9800 ± 1627 ng/mL) and the decynium 22-treated group (8172 ± 877 ng/mL) ($P < 0.01$ for both). Local SERT inhibition exacerbated myocardial cell injury during ischemia/reperfusion. However, co-inhibition of PMAT reduced myocardial cell injury under SERT inhibition to the control level.

Introduction

Reducing myocardial ischemia/reperfusion injury in patients with acute myocardial infarction remains a clinical challenge [1,2]. Myocardial reperfusion injury refers to irreversible myocardial damage occurring when blood flow is restored after myocardial ischemia, and is caused by multiple factors including oxidative stress and inflammatory responses. Serotonin (5-HT) is stored in high concentrations within platelet granules. During myocardial ischemia/reperfusion, platelets become activated and release large amounts of 5-HT, which has been reported to exacerbate myocardial ischemia/reperfusion damage [3,4]. The cytotoxic effects of 5-HT on the myocardium are primarily mediated via two mechanisms. The first is a receptor-dependent effect involving vasoconstriction and platelet aggregation mediated by 5-HT receptors. The second is a monoamine oxidase (MAO)-dependent effect, in which 5-HT taken up into cardiomyocytes is metabolized by MAO, generating hydrogen peroxide and contributing to oxidative stress [5–7].

The platelet serotonin transporter (SERT) is the major transporter mediating 5-HT uptake into platelets, and is inhibited by selective serotonin reuptake inhibitors (SSRIs) [8,9]. Fluoxetine is an SSRI with a high affinity for SERT, with a human inhibition constant (Ki) and a rat 50% inhibitory concentration (IC50) of 1.4 and 70 nM, respectively [10,11]. Our previous study demonstrated that locally administered fluoxetine significantly increased myocardial interstitial 5-HT levels before ischemia and decreased 5-HT levels during ischemia without the influences on interstitial 5-Hydroxyindoleacetic acid (5-HIAA) levels [12].

The plasma membrane monoamine transporter (PMAT)—a low-affinity and high-capacity transporter—is a 5-HT uptake pathway in cardiomyocytes and may play an important role in environments with high 5-HT concentrations. PMAT is reported to be inhibited by decynium 22, with a human IC50 of 0.1 μM [13]. Inhibiting PMAT limits 5-HT uptake into cardiomyocytes and may reduce oxidative stress by decreasing MAO-dependent hydrogen peroxide production [5,6]. Our previous study demonstrated that locally administered decynium 22 significantly increased myocardial interstitial 5-HT levels and decreased 5-HIAA levels before ischemia and during ischemia/reperfusion [14].

* Correspondence to: Department of Research Promotion and Management. National Cerebral and Cardiovascular Center, 6-1 Kishibe-Shimmachi, Suita, Osaka 564-8565, Japan.
E-mail address: shujismz@ncvc.go.jp (S. Shimizu).

https://doi.org/10.1016/j.jphyss.2026.100085
Received 18 March 2026; Received in revised form 17 June 2026; Accepted 27 June 2026
Available online 30 June 2026

Cardiac microdialysis technique is an experimental method that allows real-time monitoring of bioactive substances in the myocardial interstitium [15]. Our previous study demonstrated that dialysate myoglobin concentration monitored by cardiac microdialysis is a more sensitive marker of local cell injury than blood myoglobin concentration during myocardial ischemia [16]. This technique also enables local drug administration directly to ischemic regions where blood flow is interrupted. Using this technique, we locally administered fluoxetine (an SSRI) and decynium 22 (a PMAT inhibitor) to the ischemic myocardium and investigated their effects on myocardial cell injury using myoglobin levels as an indicator, during ischemia/reperfusion.

Materials and methods

Ethical approval

All animal experiments were conducted in accordance with the Act on Welfare and Management of Animals in Japan and the guidelines of the Physiological Society of Japan and Okayama University. The protocol was approved by the Animal Research Committee of Okayama University (approval number: OKU−2024–268).

Animals and group assignment

Seventy-eight male Sprague-Dawley rats aged 12–17 weeks and weighing 342–519 g (Japan SLC, Shizuoka, Japan) were used in this study. Eighteen rats died during the experiment due to ventricular fibrillation after myocardial ischemia (5 rats in the control group, 10 in the decynium 22-treated group, and 3 in the fluoxetine-treated group). The data from the remaining 60 rats were included in analysis. Twenty rats were included in each of the following three groups: control, fluoxetine-treated, and decynium 22-treated groups.

Drug preparation

For fluoxetine hydrochloride (molecular weight 345.79), 4 mg of fluoxetine hydrochloride (Tocris Bioscience, Bristol, UK) was dissolved in 1.29 mL of distilled water to prepare a 9.0 mM stock solution. This solution was divided into 100 μL aliquots and stored at −30 °C. Immediately before use, the stock solution was diluted 10-fold with Ringer's solution (Otsuka Pharmaceutical, Tokyo, Japan) to obtain a final concentration of 900 μM.

For decynium 22 (molecular weight 454.35), 4.54 mg of decynium 22 (Tokyo Chemical Industry, Tokyo, Japan) was dissolved in 1.0 mL of DMSO to prepare a 10 mM stock solution, divided into 100 μL aliquots, and stored at −30 °C. Immediately before use, the stock solution was diluted 100-fold with Ringer's solution to a final concentration of 100 μM.

These drug concentrations were determined based on previous studies. The local administration of 1 mM fluoxetine or 100 μM decynium 22 significantly increased baseline myocardial interstitial 5-HT level before ischemia [12,14].

Anesthesia and surgical procedures

Anesthesia was induced with 3–4% isoflurane. After tracheotomy and intubation, mechanical ventilation using a mixture of room air and 50% oxygen was started, and general anesthesia was maintained with 1–1.5% isoflurane. Body temperature was monitored with an esophageal thermometer and maintained at 37.5–38.5°C using a heating pad.

A 24-gauge catheter was inserted into the right common carotid artery for arterial pressure monitoring. The arterial line was continuously infused with 5 IU/kg/h of heparin sodium. Arterial pressure and heart rate were continuously recorded using a PowerLab Data Acquisition System (AD Instruments, Sydney, Australia).

Anesthetic depth was comprehensively assessed by responses to noxious stimuli, fluctuations in blood pressure and heart rate, and the presence of spontaneous respiration. The isoflurane concentration was adjusted in 0.5% increments as required.

Myocardial ischemia/reperfusion model and cardiac microdialysis technique

With the rat placed in right lateral decubitus position, the heart was exposed through a left thoracotomy via the 3rd to 6th intercostal spaces. The left coronary artery (LCA) was identified approximately 1–2 mm from the left atrial appendage, and a 4–0 silk suture was placed around the LCA to induce 30 min of myocardial ischemia followed by 30 min of reperfusion. The ischemic region was visually identified by change in the cardiac surface color during temporary coronary artery occlusion. The ischemic area was confirmed to involve 30–50% of the left ventricle.

The dialysis probe consisted of a dialysis fiber with a pore size of 300 Å, measuring 8.0 mm in length, 0.215 mm in outer diameter and 0.175 mm in inner diameter (Evaflux 5 A, Kuraray Medical, Tokyo, Japan) and a polyethylene tubing measuring 0.5 mm in outer diameter and 0.2 mm in inner diameter (Natsume Seisakusho, Tokyo, Japan) [5]. The dialysis probe was carefully inserted into the left ventricular wall within the LCA perfusion area to a depth of 2–3 mm, taking care not to penetrate into the ventricular cavity. The probe was continuously perfused with 5 μL/min of Ringer's solution or Ringer's solution containing a drug (fluoxetine or decynium 22) using a microinjection pump (CMA 102; CMA Microdialysis, Stockholm, Sweden) (Fig. 1). After the experiment, animals were euthanized with a high concentration of isoflurane. An autopsy confirmed that the dialysis probe had not penetrated into the left ventricular cavity.

Experimental protocol

The experimental protocol is illustrated in Fig. 2. The experiment consisted of three periods: stabilization, ischemia, and reperfusion.

Stabilization period (120 min): In the control group, the dialysis probe was continuously perfused with Ringer's solution. In the drug treatment groups, the dialysis probe was perfused with Ringer's solution for the first 60 min. Then, the perfusate was switched to Ringer's solution containing either 900 μM fluoxetine or 100 μM decynium 22. The probe was perfused with drug solution for 60 min prior to baseline sampling.

Ischemia period (30 min): Myocardial ischemia was induced by occluding the LCA with a ligature while the dialysis probe was perfused with the same solution.

Reperfusion period (30 min): The ligature was released to initiate

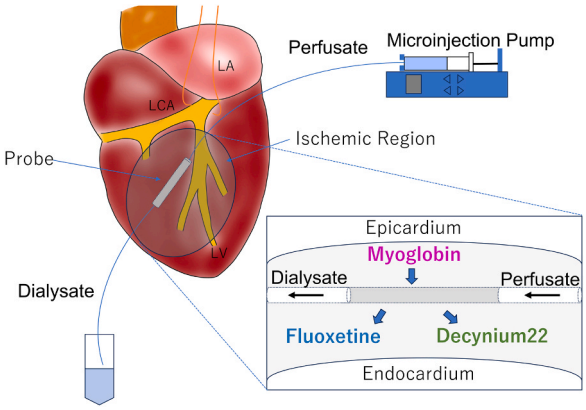

Fig. 1. Schematic illustration of the cardiac microdialysis technique. The dialysis probe is placed in the left coronary artery-perfused area of the left ventricular wall. When a drug (fluoxetine or decynium 22) is locally administered to the myocardial interstitium via the probe, myoglobin released due to myocardial cell injury is simultaneously collected in the dialysate.

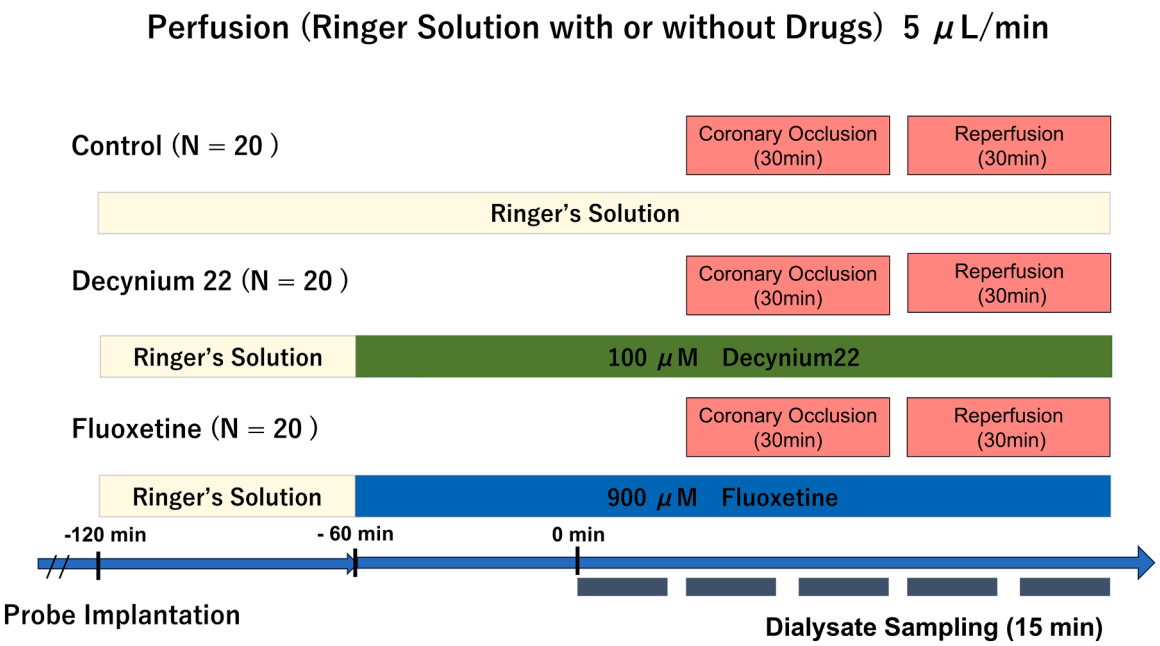

Fig. 2. Illustration of experimental protocol. After 15-min baseline dialysate sampling, 15-min dialysate samples were collected during the ischemia period of 30 min followed by reperfusion period of 30 min. In the control group, the dialysis probe was continuously perfused with Ringer's solution throughout the experiment. In the drug-treated groups, the dialysis probe was perfused with Ringer's solution for the first hour, followed by the drug solution for one hour prior to the first sampling.

reperfusion while the dialysis probe was perfused with the same solution.

Dialysate was sampled over a 15-min period prior to the onset of ischemia to obtain a baseline sample. Subsequent samples were collected over successive 15-min periods during both the 30-min ischemia and reperfusion phases. Myoglobin concentrations were measured immediately after sampling.

Additional experimental protocol

As an additional experiment, we tested the combined effects of SERT and PMAT inhibitors on myoglobin efflux during myocardial ischemia/reperfusion in 14 male Sprague-Dawley rats aged 12–14 weeks and weighing 333–461 g (Japan SLC). The dialysis probe was perfused with Ringer's solution containing both 900 μM fluoxetine and 100 μM decynium 22 for 60 min prior to baseline sampling and throughout the experiment. After 15 min baseline sampling, subsequent samples were collected over successive 15-min periods during 30-min ischemia followed by 30-min reperfusion.

Myoglobin measurement

Because smaller molecules diffuse more quickly across the damaged cell membranes, myoglobin could be a faster indicator of myocardial cell injury than troponin T and creatine kinase MB. Thus, as an index of myocardial cell injury, dialysate myoglobin concentration was measured using a Cobas h232 plus (Roche Diagnostics, Basel, Switzerland) [16].

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Two-way repeated measures analysis of variance (ANOVA) with Greenhouse-Geisser correction was performed, followed by post-hoc Holm's test for multiple comparison correction (JASP version 0.95, University of Amsterdam, Holland). $P < 0.05$ was considered statistically significant.

Results

Physiological parameters

Heart rate and mean arterial pressure (MAP) are shown in Table 1. Two-way repeated measures ANOVA demonstrated no significant differences in heart rate across time or among groups, including the co-treated group with fluoxetine and decynium 22. Two-way repeated measures ANOVA also demonstrated no significant differences in MAP among four groups but revealed significant differences across the time course ($P < 0.01$).

In the control group, MAP decreased significantly after ischemia onset (79.8 ± 4.5 mmHg during 0–15 min of ischemia) compared to baseline (90.0 ± 4.5 mmHg, $P < 0.01$). This decrease in MAP relative to baseline was sustained throughout the experiment ($P < 0.01$ during 15–30 min of ischemia, and during 0–15 min and 15–30 min of reperfusion).

In the fluoxetine-treated group, MAPs decreased significantly after ischemia onset (82.4 ± 3.6 mmHg) compared to baseline (88.3 ± 2.7 mmHg, $P < 0.05$), and this decrease was sustained during 15–30 min of ischemia (81.5 ± 4.2 mmHg, $P < 0.05$).

In the decynium 22-treated group, MAP decreased significantly after ischemia onset (77.4 ± 3.7 mmHg) compared to baseline (90.1 ± 4.2 mmHg, $P < 0.01$), and this decrease was sustained during 15–30 min of ischemia (75.7 ± 4.1 mmHg, $P < 0.01$).

In the co-treated group with fluoxetine and decynium 22, MAP decreased significantly after ischemia onset (76.4 ± 3.8 mmHg) compared to baseline (83.3 ± 3.6 mmHg, $P < 0.05$), and this decrease was sustained during 15–30 min of ischemia (74.3 ± 3.8 mmHg, $P < 0.05$).

Myoglobin efflux during myocardial ischemia/reperfusion

Dialysate myoglobin concentrations during myocardial ischemia/reperfusion in the four groups, control, fluoxetine-treated, decynium 22-treated and co-treated groups, are shown in Fig. 3. Two-way repeated measures ANOVA revealed significant effects among groups and across time, as well as an interaction between group and time ($P < 0.01$ for all).

Table 1
Hemodynamic parameters during myocardial ischemia/reperfusion in control, fluoxetine-treated, decynium 22-treated and co-treated groups.

Heart rate and mean arterial pressure					
Control (N = 20)					
	Baseline	Ischemia 0–15 min	Ischemia 15–30 min	Reperfusion 0–15 min	Reperfusion 15–30 min
HR (bpm)	376 ± 6	378 ± 7	374 ± 7	365 ± 10	357 ± 14
MAP (mmHg)	90.0 ± 4.5	79.8 ± 4.5**	79.0 ± 4.2**	73.7 ± 3.7**	71.9 ± 4.0**
Fluoxetine (N = 20)					
	Baseline	Ischemia 0–15 min	Ischemia 15–30 min	Reperfusion 0–15 min	Reperfusion 15–30 min
HR (bpm)	382 ± 7	389 ± 7	398 ± 8	393 ± 8	391 ± 8
MAP (mmHg)	88.3 ± 2.7	82.4 ± 3.6*	81.5 ± 4.2*	77.2 ± 4.1	76.4 ± 4.2
Decynium 22 (N = 20)					
	Baseline	Ischemia 0–15 min	Ischemia 15–30 min	Reperfusion 0–15 min	Reperfusion 15–30 min
HR (bpm)	371 ± 7	374 ± 8	374 ± 8	371 ± 9	375 ± 9
MAP (mmHg)	90.1 ± 4.2	77.4 ± 3.7**	75.7 ± 4.1**	79.7 ± 9.0	80.6 ± 9.0
Additional Experiment					
Fluoxetine and Decynium 22 (N = 14)					
	Baseline	Ischemia 0–15 min	Ischemia 15–30 min	Reperfusion 0–15 min	Reperfusion 15–30 min
HR (bpm)	379 ± 9	376 ± 10	374 ± 10	369 ± 9	367 ± 10
MAP (mmHg)	83.3 ± 3.6	76.4 ± 3.8*	74.3 ± 3.8*	70.6 ± 3.6	67.1 ± 3.3

Data are expressed as mean ± standard error of the mean. HR, heart rate; MAP, mean arterial pressure. *P < 0.05 vs. baseline; **P < 0.01 vs. baseline. A two-way repeated measures analysis of variance revealed no significant difference in HR or MAP between the groups.

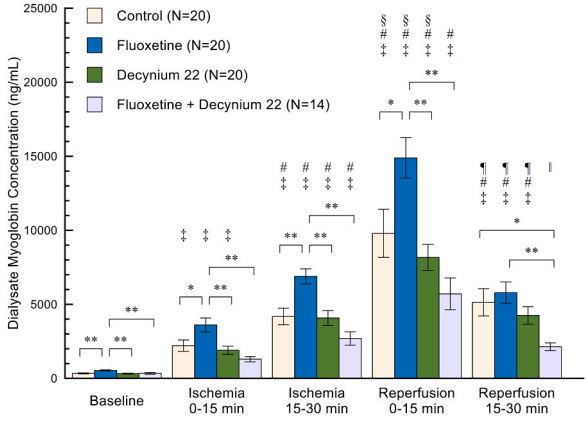

Fig. 3. Dialysate myoglobin concentrations during myocardial ischemia/reperfusion in control, fluoxetine-treated, decynium 22-treated, and co-treated groups. *P < 0.05; **P < 0.01; ‡P < 0.01 vs. baseline; #P < 0.01 vs. 0–15 min of ischemia; §P < 0.01 vs. 15–30 min of ischemia; ‖P < 0.05 and ¶P < 0.01 vs. 0–15 min of reperfusion.

In the control group, myoglobin concentration during 0–15 min of ischemia (2212 ± 384 ng/mL) increased significantly compared to baseline (337 ± 34.7 ng/mL, P < 0.01). Myoglobin concentration increased further during 15–30 min of ischemia (4187 ± 561 ng/mL), and was significantly elevated compared to baseline (P < 0.01) and 0–15 min of ischemia (P < 0.01). Myoglobin concentration reached a peak during 0–15 min of reperfusion (9800 ± 1627 ng/mL), and was significantly elevated compared to baseline, and 0–15 min and 15–30 min of ischemia (P < 0.01 for all). During 15–30 min of reperfusion, myoglobin concentration (5139 ± 921 ng/mL) decreased significantly compared to 0–15 min of reperfusion (P < 0.01), but remained significantly elevated compared to baseline and 0–15 min of ischemia (P < 0.01 for both).

In the fluoxetine-treated group, a significant increase in myoglobin concentration was observed during 0–15 min of ischemia (3609 ± 466 ng/mL) compared to baseline (537 ± 35.6 ng/mL, P < 0.01). Further significant elevation in myoglobin concentration was found during 15–30 min of ischemia (6890 ± 513 ng/mL; P < 0.01 vs. baseline and vs. 0–15 min of ischemia). Myoglobin concentration peaked during 0–15 min of reperfusion (14,889 ± 1364 ng/mL; P < 0.01 vs. baseline, and vs. 0–15 min and 15–30 min of ischemia). Thereafter, myoglobin concentration declined significantly during 15–30 min of reperfusion (5794 ± 718 ng/mL) compared to 0–15 min of reperfusion (P < 0.01), but remained higher than those at baseline and 0–15 min of ischemia (P < 0.01 for both).

In the decynium 22-treated group, a significant increase in myoglobin concentration was observed during 0–15 min of ischemia (1903 ± 275 ng/mL) compared to baseline (319 ± 30.7 ng/mL, P < 0.01). Myoglobin concentration continued to increase significantly during 15–30 min of ischemia (4079 ± 499 ng/mL; P < 0.01 vs. baseline and vs. 0–15 min of ischemia), and peaked during 0–15 min of reperfusion (8172 ± 877 ng/mL; P < 0.01 vs. baseline, and vs. 0–15 min and 15–30 min of ischemia). Myoglobin concentration declined significantly during 15–30 min after reperfusion (4255 ± 592 ng/mL) compared to 0–15 min of reperfusion (P < 0.01), but remained significantly elevated compared to baseline and 0–15 min of ischemia (P < 0.01 for both).

In the co-treated group with fluoxetine and decynium 22, a significant increase in myoglobin concentration was observed during 15–30 min of ischemia (2694 ± 453 ng/mL) compared to baseline (342 ± 59.3 ng/mL, P < 0.01). Myoglobin concentration peaked during 0–15 min of reperfusion (5711 ± 1076 ng/mL; P < 0.01 vs. baseline, and vs. 0–15 min of ischemia, and P = 0.051 vs. 15–30 min of ischemia). Myoglobin concentration declined significantly during 15–30 min after reperfusion (2136 ± 260 ng/mL) compared to 0–15 min of reperfusion (P < 0.05).

Effects of drugs on myoglobin efflux during myocardial ischemia/reperfusion

Before ischemia onset (baseline), myoglobin concentration was significantly higher in the fluoxetine-treated group (537 ± 35.6 ng/mL) compared to control (337 ± 34.7 ng/mL, P < 0.01), the decynium 22-treated group (319 ± 30.7 ng/mL, P < 0.01) and the co-treated group with fluoxetine and decynium 22 (342 ± 59.3 ng/mL, P < 0.01).

During 0–15 min of ischemia, myoglobin concentration was significantly higher in the fluoxetine-treated group (3609 ± 466 ng/mL) compared to control (2212 ± 384 ng/mL, P < 0.05), the decynium 22-treated group (1903 ± 275 ng/mL, P < 0.01), and the co-treated group with fluoxetine and decynium 22 (1299 ± 176 ng/mL, P < 0.01).

A similar pattern was observed during 15–30 min of ischemia, with significantly higher myoglobin concentration in the fluoxetine-treated group (6890 ± 513 ng/mL) compared to control (4187 ± 561 ng/mL), decynium 22-treated group (4079 ± 499 ng/mL), and the co-treated group with fluoxetine and decynium 22 (2694 ± 453 ng/mL) (P < 0.01 for each).

During 0–15 min of reperfusion, myoglobin concentration reached a peak in all four groups. Peak myoglobin concentration was significantly

higher in the fluoxetine-treated group (14,889 ± 1364 ng/mL) than in control (9800 ± 1627 ng/mL, $P < 0.05$), decynium 22-treated group (8172 ± 877 ng/mL, $P < 0.01$), and the co-treated group with fluoxetine and decynium 22 (5711 ± 1076 ng/mL, $P < 0.01$).

During 15–30 min after reperfusion, myoglobin concentration was significantly lower in the co-treated group with fluoxetine and decynium 22 (2136 ± 260 ng/mL) than those in control (5139 ± 921 ng/mL, $P < 0.05$) and fluoxetine-treated groups (5794 ± 718 ng/mL, $P < 0.01$).

No significant differences in myoglobin concentration were observed between the control and decynium 22-treated group throughout the experiment.

Discussion

This study demonstrated that local SERT inhibition exacerbated myocardial cell injury as indicated by increased myoglobin efflux in myocardial interstitium. In contrast, PMAT inhibition had no effect on myoglobin efflux. In the fluoxetine-treated group, dialysate myoglobin concentrations were significantly higher than those in the control group throughout the experiment. All four groups showed a peak in dialysate myoglobin concentration during 0–15 min of reperfusion. The peak myoglobin concentration in the fluoxetine-treated group (14,889 ± 1364 ng/mL) was approximately 1.5-fold higher than that in the control group (9800 ± 1627 ng/mL, $P < 0.05$). In contrast, the peak myoglobin concentration in the decynium 22-treated group (8172 ± 877 ng/mL) did not differ significantly from the control. In addition, co-treatment with fluoxetine and decynium 22 reduced peak myoglobin levels to the same level as in the control and decynium 22-treated groups. These results suggest that platelet SERT plays a critical role in modulating injury during myocardial ischemia/reperfusion, and inhibiting SERT may enhance PMAT-dependent 5-HT reuptake into cardiomyocytes, resulting in further myocardial cell injury.

Role of serotonin reuptake transporter in myocardial ischemia/reperfusion

During myocardial ischemia/reperfusion, activated platelets release large amounts of 5-HT [1,3,4,17]. A portion of the released 5-HT is rapidly reuptaken via platelet SERT, which functions as a buffering mechanism to limit excessive effects of 5-HT (Fig. 4) [17,18]. SSRIs, such as fluoxetine, block this SERT-mediated buffering mechanism, leading to elevated plasma 5-HT concentrations. Previous animal study has also demonstrated that acute SSRI administration directly increases plasma 5-HT concentrations [19].

In this study, fluoxetine-treated animals exhibited elevated dialysate myoglobin concentrations compared to controls and decynium 22-treated animals, except during 15–30 min of reperfusion. High concentration of 5-HT secreted from platelets may accumulate in myocardial interstitium and microvessels (Fig. 5), and may exhibit vasoconstrictive effects, particularly on vascular endothelium damaged by ischemia [18–22]. These actions may contribute to microcirculatory disturbances, resulting in worsened reperfusion injury [23,24]. A human clinical study has reported elevated plasma 5-HT concentrations as an independent predictor of coronary microvascular dysfunction [25]. The findings of the present study may support the association between elevated plasma 5-HT levels and microcirculatory disturbances observed in clinical settings.

Fluoxetine acts on platelet SERT to inhibit 5-HT uptake, which may also promote platelet aggregation and thrombus formation [3,4]. Although 5-HT has vasodilatory effects on normal endothelium [20,21] and SSRIs have direct vasodilatory actions [26,27], local fluoxetine administration may exert a stronger effect on platelet aggregation, thereby exacerbating myocardial ischemia/reperfusion injury (Fig. 5).

Furthermore, our additional experiment revealed that PMAT inhibition significantly decreased myoglobin efflux at baseline and during ischemia/reperfusion under SERT inhibition by fluoxetine. These facts suggest that SERT inhibition enhances PMAT-induced 5-HT reuptake into cardiomyocytes. Because our previous study demonstrated that MAO inhibition significantly decreased myoglobin efflux during ischemia/reperfusion [5], the reuptake of 5-HT via PMAT can lead to excessive myocardial damage due to the generation of hydrogen peroxide from intracellular MAO-mediated metabolism (Fig. 5).

Role of plasma membrane monoamine transporter on myocardial ischemia/reperfusion

Unlike SERT inhibition, inhibition of PMAT—the 5-HT uptake pathway in cardiomyocytes—did not significantly affect myocardial cell injury in this study. This result provides important insights into understanding the mechanisms of 5-HT-mediated injury during myocardial ischemia/reperfusion. Although studies have shown that PMAT inhibition suppresses 5-HT uptake into cardiomyocytes and reduces oxidative stress by decreasing hydrogen peroxide generated from intracellular MAO-mediated metabolism (MAO-dependent effect) [5,6,28–31], local decynium 22 administration did not reduce cardiomyocyte injury as indicated by myoglobin release (Fig. 6).

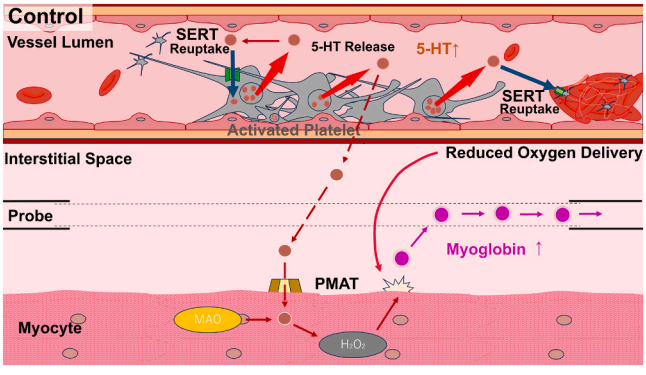

Fig. 4. Mechanism of serotonin transporter regulation in myocardial ischemia/reperfusion injury in control rat. During myocardial ischemia/reperfusion, 5-HT released from activated platelets is partially reuptaken via platelet serotonin reuptake transporter (SERT), suppressing excessive 5-HT accumulation. Plasma membrane monoamine transporter (PMAT) mediates 5-HT uptake into cardiomyocytes. Monoamine oxidase (MAO) degrades 5-HT in cardiomyocytes, producing hydrogen peroxide (H_2O_2).

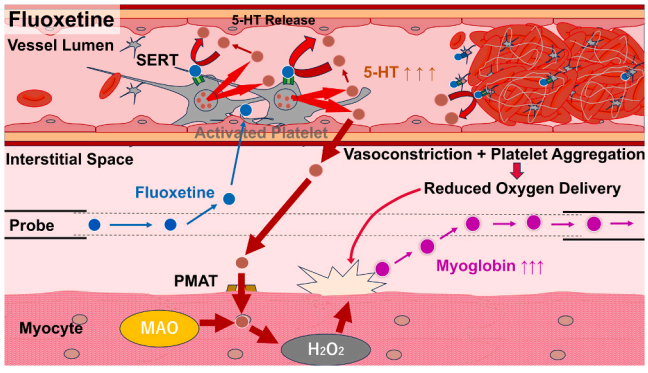

Fig. 5. Mechanism of serotonin transporter regulation in myocardial ischemia/reperfusion injury in fluoxetine-treated rat. Serotonin reuptake transporter (SERT) inhibition nullifies the platelet reuptake mechanism, resulting in marked 5-HT accumulation. Excessive 5-HT causes vasoconstriction and platelet aggregation through receptors, leading to microcirculatory disturbance and reduced oxygen supply. Because plasma membrane monoamine transporter (PMAT)-mediated uptake into cardiomyocytes is maintained, SERT inhibition by fluoxetine may exacerbate myocardial cell injury in the ischemic period and dramatically increase myoglobin release during early reperfusion (0–15 min of reperfusion). MAO, monoamine oxidase; H_2O_2, hydrogen peroxide.

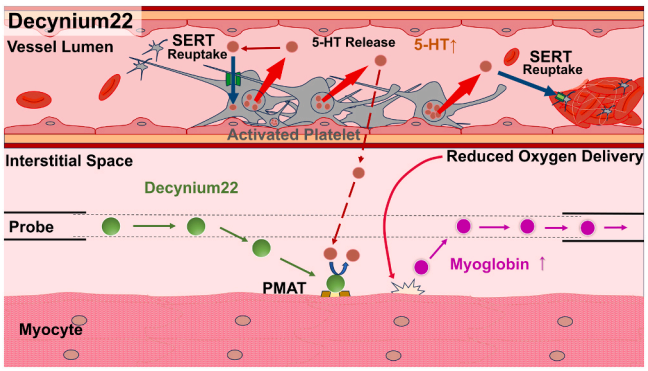

Fig. 6. Mechanism of serotonin transporter regulation in myocardial ischemia/reperfusion injury in decynium 22-treated rat. Plasma membrane monoamine transporter (PMAT) inhibition blocks 5-HT uptake into cardiomyocytes, but reuptake via platelet serotonin reuptake transporter (SERT) is maintained, preventing excessive 5-HT accumulation. Myoglobin efflux is similar to that of the control group, suggesting that the effect of 5-HT reuptake into cardiomyocytes on myocardial ischemia/reperfusion injury is weaker than that of SERT-mediated platelet 5-HT reuptake.

5-HT reuptake via platelet SERT may be the primary mechanism regulating plasma and interstitial 5-HT levels during myocardial ischemia/reperfusion, with PMAT in cardiomyocytes playing a lesser role in the normal condition. However, if SERT-dependent reuptake of 5-HT is impaired, the main pathway of 5-HT metabolism will become PMAT-dependent 5-HT reuptake into cardiomyocytes, causing critical damages to the myocardium during ischemia/reperfusion.

Acute versus chronic SSRI administration

It is important to note that this study examined acute SSRI administration under experimental conditions, which differs from clinical use in which SSRIs are administered orally over the long term [32]. While acute SSRI administration rapidly increases extracellular 5-HT concentration, chronic administration leads to adaptive changes, including downregulation of transporters [33,34] and depletion of platelet 5-HT stores [17,18]. These chronic effects may produce different results from those observed in this study. Although several clinical reports have focused on the long-term effects of fluoxetine, the results are still controversial. Further experimental investigations are therefore needed to evaluate the long-term effects of fluoxetine.

Limitations

This study has several limitations. First, myocardial interstitial 5-HT concentrations were not measured. Therefore, the dynamics of interstitial 5-HT during the experiment were not fully elucidated. Second, cardiac function, infarct size and circulating biomarkers were not measured in this study. Although the pharmacological effects of drugs administered via a microdialysis probe are thought to be confined to the immediate vicinity of the probe, the effects on cardiac function, infarction size and circulating biomarkers cannot be ignored when drugs are administered systemically. Further research is required to evaluate these systemic effects. Third, decynium 22 inhibits organic cation transporter 3 (OCT−3) as well as PMAT. OCT−3 is also a pathway of 5-HT reuptake in cardiomyocytes. Therefore, it is difficult to determine which transporters primarily participate in 5-HT reuptake in cardiomyocytes in this study. Fourth, we hypothesize that one possible mechanism of 5-HT-induced cell injury is that reuptaken 5-HT via PMAT is degraded to hydrogen peroxide by mitochondrial MAO, resulting in mitochondrial dysfunction and apoptosis of cardiomyocytes. However, further investigations into ROS production and mitochondrial function are needed to prove this hypothesis. Fifth, even though the doses of the inhibitors that passed through the probe during the experiment were much lower than those administered intravenously to rats, it may not be possible to completely eliminate the systemic side effects.

Conclusions

Local administration of a SERT inhibitor, fluoxetine, to the myocardial ischemia/reperfusion region significantly exacerbated myocardial cell injury, as indicated by myoglobin efflux. In addition, PMAT inhibition can reduce myocardial cell injury during ischemia/reperfusion under SERT inhibition. These findings highlight the pivotal role of 5-HT reuptake via platelet SERT and myocardial PMAT in the pathophysiology of myocardial ischemia/reperfusion injury.

CRediT authorship contribution statement

Shingo Kasahara: Writing – review & editing, Supervision, Investigation. **Tsuyoshi Akiyama:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Yasuhiro Kotani:** Writing – review & editing, Supervision, Investigation. **Yosuke Kuroko:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Shuji Shimizu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yoshimasa Kishi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Funding acquisition, Formal analysis, Data curation.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by the Animal Research Committee of Okayama University (approval number: OKU−2024–268).

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used Gemini (Google LLC) to check English grammar.

Funding

This study was supported by Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (JSPS KAKENHI Grant Numbers 22K09182, 25K12272 and 25K12229).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Mauler M, Herr N, Schoenichen C, Witsch T, Marchini T, Härdtner C, et al. Platelet Serotonin Aggravates Myocardial Ischemia/Reperfusion Injury via Neutrophil Degranulation. Circulation 2019;139:918–31. https://doi.org/10.1161/CIRCULATIONAHA.118.033942.

[2] Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. The Journal of Clinical Investigation 2013;123:92–100. https://doi.org/10.1172/JCI62874.

[3] Turetta L, Donella-Deana A, Folda A, Bulato C, Deana R. Characterisation of the serotonin efflux induced by cytosolic Ca2+ and Na+ concentration increase in human platelets. Cellular Physiology and Biochemistry 2004;14:377–86.

[4] Ziegler M, Wang X, Peter K. Platelets in cardiac ischaemia/reperfusion injury: a promising therapeutic target. Cardiovascular Research 2019;115:1178–88. https://doi.org/10.1093/cvr/cvz070.

[5] Inagaki T, Akiyama T, Du CK, Zhan DY, Yoshimoto M, Shirai M. Monoamine oxidase-induced hydroxyl radical production and cardiomyocyte injury during myocardial ischemia-reperfusion in rats. Free Radical Research 2016;50:645–53. https://doi.org/10.3109/10715762.2016.1162300.

[6] Bianchi P, Kunduzova O, Masini E, Cambon C, Bani D, Raimondi L, et al. Oxidative stress by monoamine oxidase mediates receptor-independent cardiomyocyte apoptosis by serotonin and postischemic myocardial injury. Circulation 2005;112:3297–305. https://doi.org/10.1161/CIRCULATIONAHA.104.528133.

[7] Shimizu Y, Minatoguchi S, Hashimoto K, Uno Y, Arai M, Wang N, et al. The role of serotonin in ischemic cellular damage and the infarct size-reducing effect of sarpogrelate, a 5-hydroxytryptamine-2 receptor blocker, in rabbit hearts. The Journal of the American College of Cardiology 2002;40:1347–55. https://doi.org/10.1016/s0735-1097(02)02158-7.

[8] Ni W, Watts SW. 5-hydroxytryptamine in the cardiovascular system: focus on the serotonin transporter (SERT). Clinical and Experimental Pharmacology and Physiology 2006;33:575–83. https://doi.org/10.1111/j.1440-1681.2006.04410.x.

[9] Mercado CP, Kilic F. Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. Molecular interventions 2010;10:231–41. https://doi.org/10.1124/mi.10.4.6.

[10] Owens MJ, Knight DL, Nemeroff CB. Second-generation SSRIs: human monoamine transporter binding profile of escitalopram and R-fluoxetine. Biological Psychiatry 2001;50:345–50. https://doi.org/10.1016/s0006-3223(01)01145-3.

[11] Wong DT, Bymaster FP, Engleman EA. Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. Life Sciences 1995;57:411–41. https://doi.org/10.1016/0024-3205(95)00209-o.

[12] Du CK, Zhan DY, Akiyama T, Inagaki T, Shishido T, Shirai M, et al. Myocardial interstitial levels of serotonin and its major metabolite 5-hydroxyindole acetic acid during ischemia-reperfusion. The American Journal of Physiology-Heart and Circulatory Physiology 2017;312:H60–7. https://doi.org/10.1152/ajpheart.00471.2016.

[13] Engel K, Wang J. Interaction of organic cations with a newly identified plasma membrane monoamine transporter. Molecular Pharmacology 2005;68:1397–407. https://doi.org/10.1124/mol.105.016832.

[14] Sonobe T, Akiyama T, Du CK, Pearson JT. Serotonin uptake via plasma membrane monoamine transporter during myocardial ischemia-reperfusion in the rat heart in vivo. Physiological Reports 2019;7:e14297. https://doi.org/10.14814/phy2.14297.

[15] Van Wylen DG, Willis J, Sodhi J, Weiss RJ, Lasley RD, Mentzer RM. Cardiac microdialysis to estimate interstitial adenosine and coronary blood flow. The American Journal of Physiology 1990;258:H1642–9. https://doi.org/10.1152/ajpheart.1990.258.6.H1642.

[16] Kitagawa H, Yamazaki T, Akiyama T, Sugimachi M, Sunagawa K, Mori H. Microdialysis separately monitors myocardial interstitial myoglobin during ischemia and reperfusion. The American Journal of Physiology-Heart and Circulatory Physiology 2005;289:H924–30. https://doi.org/10.1152/ajpheart.01207.2004.

[17] Vanhoutte PM. Platelet-derived serotonin, the endothelium, and cardiovascular disease. Journal of Cardiovascular Pharmacology 1991;17(5):S6–12.

[18] Maurer-Spurej E. Serotonin reuptake inhibitors and cardiovascular diseases: a platelet connection. Cellular and Molecular Life Sciences 2005;62:159–70. https://doi.org/10.1007/s00018-004-4262-1.

[19] Rothman RB, Zolkowska D, Baumann MH. Serotonin (5-HT) transporter ligands affect plasma 5-HT in rats. The Annals of the New York Academy of Sciences 2008;1139:268–84. https://doi.org/10.1196/annals.1432.042.

[20] Golino P, Piscione F, Willerson JT, Cappelli-Bigazzi M, Focaccio A, Villari B, et al. Divergent effects of serotonin on coronary-artery dimensions and blood flow in patients with coronary atherosclerosis and control patients. The New England Journal of Medicine 1991;324:641–8. https://doi.org/10.1056/NEJM199103073241001.

[21] McFadden EP, Clarke JG, Davies GJ, Kaski JC, Haider AW, Maseri A. Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina. The New England Journal of Medicine 1991;324:648–54. https://doi.org/10.1056/NEJM199103073241002.

[22] Heusch G, Kleinbongard P, Böse D, Levkau B, Haude M, Schulz R, et al. Coronary microembolization: from bedside to bench and back to bedside. Circulation 2009;120:1822–36. https://doi.org/10.1161/CIRCULATIONAHA.109.888784.

[23] Kloner RA, Ganote CE, Jennings RB. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. The Journal of Clinical Investigation 1974;54:1496–508. https://doi.org/10.1172/JCI107898.

[24] Rezkalla SH, Kloner RA. No-reflow phenomenon. Circulation 2002;105:656–62. https://doi.org/10.1161/hc0502.102867.

[25] Odaka Y, Takahashi J, Tsuburaya R, Nishimiya K, Hao K, Matsumoto Y, et al. Plasma concentration of serotonin is a novel biomarker for coronary microvascular dysfunction in patients with suspected angina and unobstructive coronary arteries. The European Heart Journal 2017;38:489–96. https://doi.org/10.1093/eurheartj/ehw448.

[26] Ungvari Z, Pacher P, Kecskeméti V, Koller A. Fluoxetine dilates isolated small cerebral arteries of rats and attenuates constrictions to serotonin, norepinephrine, and a voltage-dependent Ca(2+) channel opener. Stroke 1999;30:1949–54. https://doi.org/10.1161/01.str.30.9.1949.

[27] van Melle JP, Buikema H, van den Berg MP, van Buiten A, van Veldhuisen DJ, Boonstra PW, et al. Sertraline causes strong coronary vasodilation: possible relevance for cardioprotection by selective serotonin reuptake inhibitors. Cardiovascular Drugs and Therapy 2004;18:441–7. https://doi.org/10.1007/s10557-004-6221-3.

[28] Kaludercic N, Carpi A, Menabò R, Di Lisa F, Paolocci N. Monoamine oxidases (MAO) in the pathogenesis of heart failure and ischemia/reperfusion injury. Biochimica et Biophysica Acta 2011;1813:1323–32. https://doi.org/10.1016/j.bbamcr.2010.09.010.

[29] Bianchi P, Pimentel DR, Murphy MP, Colucci WS, Parini A. A new hypertrophic mechanism of serotonin in cardiac myocytes: receptor-independent ROS generation. FASEB Journal 2005;19:641–3. https://doi.org/10.1096/fj.04-2518fje.

[30] Kaludercic N, Mialet-Perez J, Paolocci N, Parini A, Di Lisa F. Monoamine oxidases as sources of oxidants in the heart. Journal of Molecular and Cellular Cardiology 2014;73:34–42. https://doi.org/10.1016/j.yjmcc.2013.12.032.

[31] Pizzinat N, Copin N, Vindis C, Parini A, Cambon C. Reactive oxygen species production by monoamine oxidases in intact cells. Naunyn-Schmiedeberg's Archives of Pharmacology 1999;359:428–31. https://doi.org/10.1007/pl00005371.

[32] Sauer WH, Berlin JA, Kimmel SE. Selective serotonin reuptake inhibitors and myocardial infarction. Circulation 2001;104:1894–8. https://doi.org/10.1161/hc4101.097519.

[33] Benmansour S, Owens WA, Cecchi M, Morilak DA, Frazer A. Serotonin clearance in vivo is altered to a greater extent by antidepressant-induced downregulation of the serotonin transporter than by acute blockade of this transporter. Journal of Neuroscience 2002;22:6766–72. https://doi.org/10.1523/JNEUROSCI.22-15-06766.2002.

[34] Cremers TI, Spoelstra EN, de Boer P, Bosker FJ, Mørk A, den Boer JA, et al. Desensitisation of 5-HT autoreceptors upon pharmacokinetically monitored chronic treatment with citalopram. European Journal of Pharmacology 2000;397:351–7. https://doi.org/10.1016/s0014-2999(00)00308-3.