Glucose infusion suppresses acute restraint stress-induced peripheral and central sympathetic responses in rats

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ABSTRACT

Background: Acute restraint stress (RS) induces sympathetic activation such as elevating plasma catecholamines, resulting in increase in blood glucose. We aimed to investigate whether glucose infusion affects the RS-induced sympathetic responses.

Methods: Plasma catecholamines were measured by high-performance liquid chromatography with electrochemical detection. Blood glucose levels were measured with a glucometer and a glucose assay kit. Cardiac parameters were measured by echocardiographic and hemodynamic analysis. Prostanoid levels in the paraventricular nucleus of hypothalamus (PVN) microdialysates were measured by liquid chromatography-ion trap tandem mass spectrometry analysis.

Results: RS significantly increased plasma noradrenaline and adrenaline. Intravenous infusion of a 5% glucose solution significantly attenuated the RS-induced elevation of plasma adrenaline but did not alter the plasma noradrenaline. Glucose administration during RS suppressed the progression of cardiac impairment by attenuating the decline rates in left ventricular diastolic, end-diastolic volume, stroke volume, fractional shortening, and ejection fraction. Both intravenous and intracerebroventricular infusion of glucose solution significantly attenuated the RS-induced elevation of thromboxane B2 (TxB2) (a metabolite of TxA2) levels in the PVN but did not alter prostaglandin E2 levels in the PVN.

Conclusion: Our results demonstrate that glucose infusion suppresses RS-induced elevation of plasma adrenaline and left ventricular dysfunction. In the brain, glucose infusion suppresses RS-induced production of TxA2 in the PVN.

1. Introduction

Takotsubo cardiomyopathy is characterized by acute and reversible left ventricular (LV) dysfunction, which is clinically similar to acute coronary syndrome but without obstructive coronary disease (Akashi et al., 2008). A striking elevation of plasma catecholamine levels, especially plasma adrenaline levels, is suggested to be the main cause of takotsubo cardiomyopathy (Wittstein et al., 2005). Mechanisms of onset for takotsubo cardiomyopathy have been investigated using the acute restraint stress (RS) rat model, which evokes profound sympathetic-adrenal activation (Akashi et al., 2008; Ueyama et al., 2002, 2003). However, most studies have focused on peripheral LV dysfunction, and it is still unclear how central sympathetic regulatory mechanisms play a role in the stress-induced takotsubo cardiomyopathy.

The paraventricular hypothalamic nucleus (PVN) is an important regulatory center for autonomic, neuroendocrine, and other physiological functions that maintain homeostatic conditions (Pyner, 2009; Swanson and Sawchenko, 1980). In addition, the PVN is well-known to be important for regulating stress responses (Swanson and Sawchenko, 1980; Myers et al., 2017). We previously reported that inhibitors of

Abbreviations: RS, restraint stress; PVN, paraventricular nucleus of hypothalamus; LV, left ventricular; COX, cyclooxygenase; PGE2, prostaglandin E2; TxA2, thromboxane A2; IVSTD, interventricular septum diameters; LVWpWd, left ventricular posterior wall thickness diameters; LVIDd, left ventricular diastolic diameters; LVIDs, systolic internal diameters; EF, ejection fraction; FS, fractional shortening; CSF, cerebrospinal fluid; HPLC, high-performance liquid chromatography.

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cyclooxygenase (COX) isozymes (COX-1 and COX-2) attenuate acute RS-induced elevation of plasma catecholamine levels and neuronal activation of presympathetic PVN neurons, suggesting that COX-1 and COX-2 in the PVN mediate acute RS-induced sympathetic activation (Yamaguchi et al., 2010). Since COX-1 and COX-2 catalyze the formation of prostanoids, these findings raise the possibility that production of prostanoids in the PVN can mediate RS-induced sympathetic activation. However, it is unknown whether stress-induced prostanoid production in the PVN mediates plasma catecholamine elevation and whether these factors are involved in the LV dysfunction found in takotsubo cardiomyopathy.

Circulating catecholamines induce adaptive responses to stress exposure to maintain homeostasis via activation of the sympathetic nervous system (Kvetnansky et al., 1992). The stress-induced elevation of plasma catecholamines, especially adrenaline, increases blood glucose levels by promoting glycogenolysis and gluconeogenesis in the liver (Exton, 1985; Pilkis et al., 1988); however, sustained elevation of circulating catecholamines evoked by stress exposure can elicit takotsubo cardiomyopathy (Tank and Lee Wong, 2015). Blood glucose levels are regulated by various metabolic and non-metabolic factors including the nervous system (Bich et al., 2020), and hypoglycemia induces sympathetic activation such as the increase in adrenaline secretion to elevate blood glucose levels. However, it is unclear whether high levels of blood glucose can suppress an elevation of plasma catecholamine levels, and LV dysfunction of takotsubo cardiomyopathy. Given the negative feedback mechanism induced by glucose (the end-product of sympathetic activation) of regulating stress-induced adrenaline secretion, one can speculate that stress-induced LV dysfunction could be prevented by intervening in this signaling pathway.

In this study, we examined effects of intravenous glucose infusion on RS-induced elevation of plasma catecholamine levels. Furthermore, we examined whether intravenously administered glucose can ameliorate RS-induced LV dysfunction by echocardiography. We also examined effects of intravenous or intracerebroventricular glucose infusion on RS-induced elevation of prostaglandin E$_2$ (PGE$_2$) and thromboxane A$_2$ (TxA$_2$) levels in the PVN and plasma levels of catecholamines.

2. Materials and methods

2.1. Animals

Male Wistar rats (total number of rats = 103) weighing approximately 380 g were maintained in a temperature-controlled room at 22–24 °C under a constant day-night rhythm for at least 3 weeks and given food and water ad libitum. All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by Aichi Medical University (No. 2017-21, 2018-14 and 2019-1) and with ARRIVE guidelines.

2.2. Experimental procedure

To study the effects of continuous infusion of glucose on RS-induced sympathetic activation and LV dysfunction in a model of takotsubo cardiomyopathy, we first assessed the optimal dose of glucose for suppressing of RS-induced elevation of plasma catecholamine. Glucose solutions at doses of 1%, 5% or 10% for intravenous infusion were used in the first experiment (Experiment 1; Fig. 1).

Next, we examined effects of intravenous glucose infusion on RS-induced LV dysfunction. Echocardiographic analysis, as described below, was performed on stressed rats with or without glucose infusion (Experiment 2; Fig. 1).

Furthermore, to clarify whether exogenously infused glucose can exert inhibitory actions on RS-induced sympathetic activation in the brain, we examined effects of glucose infusion on RS-induced elevation of prostanoid production in the PVN and plasma catecholamine levels (Experiment 3; Fig. 1). To examine this, we compared the inhibitory effects of glucose between intravenous infusion and intracerebroventricular infusion. In addition, we observed glucose uptake in

Fig. 1. Experimental schedule. Arrows indicate the timing of blood sampling (Experiment 1 and 3) and echocardiographic assessment (Experiment 2). Gray squares on the top of Experiment 3 show dialysate collection every 20 min. RS, restraint stress.
2.3. Exposure to RS

Each rat was restrained by taping its body trunk and four limbs to a metal mesh according to our published methods, with slight modifications (Yamaguchi et al., 2010). We previously reported the effect of RS on Fos expression in the presympathetic PVN neurons, and the peak response to RS was observed at 60 min after the start of RS exposure (Yamaguchi et al., 2010). Therefore, we performed the stress paradigm for 120 min in this study.

2.4. Echocardiographic and hemodynamic analysis

Conventional echocardiographic study was performed using rats with consciousness subjected to RS. An XSV color Doppler ultrasound system (SonoScape Medical Corporation, China) with a 16-MHz scan probe was used to obtain 2D transthoracic echocardiograms. Using 2D parasternal long-axis imaging, we obtained left ventricular M-mode tracings, based on which the end-diastolic interventricular septum (IVSd), left ventricular posterior wall thickness (LVPWd), and left ventricular diastolic (LVIDd) or systolic internal diameters (LVIDs) were measured. The ejection fraction (EF) and fractional shortening (FS) were calculated according to standard formulas.

2.5. Microdialysis and intracerebroventricular infusion

The microdialysis experiment and intracerebroventricular administration were performed according to our published methods (Tachi et al., 2020; Yamaguchi et al., 2019a,b). Briefly, under anesthesia as described above, rats were placed in a stereotoxic apparatus, and then guide canulae for the dialysis probe (0.5 mm o.d.; Eicom, Kyoto, Japan) and intracerebroventricular infusion (0.71 mm o.d.; Plastics One, Roanoke, VA, USA) were implanted according to the rat brain atlas (Paxinos and Watson, 2005) 5 days before a microdialysis experiment.

On the day of a microdialysis experiment, a microdialysis probe (0.22 mm o.d., 1.0 mm membrane length; Eicom, Kyoto, Japan) and an internal cannula for intracerebroventricular infusion (0.36 mm o.d.; Plastics One, Roanoke, VA, USA) were inserted into the right side of the PVN (probe tip coordinates: AP = −1.8 mm from the bregma, L = 0.4 mm from the midline, V = 8.1 mm below the brain surface) and the left lateral ventricle (cannula tip coordinates: AP = −0.7 mm, L = 1.5 mm, V = 4.0 mm), respectively, under anesthesia with a 30% isoflurane solution.

The PVN was perfused with sterile Ringer’s solution (147 mM NaCl, 2.5 mM KCl and 2.3 mM CaCl 2 ) with a flow rate of 2 μL/min. After 4 h of stabilization, intracerebroventricular infusion of vehicle or glucose (2 mmol/L, 0.1 μL/min) was started and rats were exposed to RS for 120 min. Dialysis samples were collected in a tube every 20 min including three consecutive dialysates before glucose infusion and then frozen at −80 °C until analysis.

In addition, to measure glucose levels in the cerebrospinal fluid (CSF), the CSF was collected by cisternal puncture in unstressed rats treated with vehicle, unstressed rats infused intracerebroventricularly with glucose solution (2 mmol/L, 0.1 μL/min) for 60 min, and rats treated with vehicle during RS exposure for 60 min.

We used a 2 mmol/L glucose solution for intracerebroventricular infusion based on the previous report that showed intracerebroventricular glucose infusion was effective against severe hypoglycemia-induced arrhythmias (Reno et al., 2013).

2.6. Measurement of plasma catecholamines

Catecholamine levels in plasma samples were assessed as described in our previous reports (Yamaguchi et al., 2019a; Okada and Yamaguchi, 2017). In brief, plasma catecholamines and a standard mixture were extracted using alumina, and the extracted samples were assayed by high-performance liquid chromatography (HPLC) with electrochemical detection (HTEG-510, Eicom, Kyoto, Japan).

2.7. Measurement of glucose levels in the blood and the CSF

Blood glucose levels were measured with a glucometer (Medisafe Fit; Terumo Corporation, Tokyo, Japan), and CSF glucose levels were measured using a glucose assay kit (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) in accordance with the manufacturer’s instructions.

2.8. Measurement of prostanoids in the PVN microdialysate

PGE 2 and TxB 2 , a metabolite of TxA 2 , in the PVN microdialysates were analyzed as described in our previous reports (Yamaguchi et al., 2019b; Tachi et al., 2014). Briefly, 40 μL of dialysate was extracted by ethyl acetate and the ethyl acetate phase was collected and dried with a centrifugal vacuum system. The residue was dissolved in 50 μL of 25% methanol containing 0.1% formic acid. Each mixture was used for liquid chromatography-ion trap tandem mass spectrometry (LC-ITMS) analysis by the ACCELA HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) and LTQ Velos (Thermo Fisher Scientific, Waltham, MA, USA). To measure prostanoid concentrations in the dialysates, the peak area ratio relative to an internal standard was calculated and determined from the corresponding calibration curve.

2.9. Glucose uptake using 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG)

2-NBDG is a fluorescent glucose analog and can be used as a tool for monitoring glucose uptake in cells (Yamada et al., 2000; Kurabayashi et al., 2013). To examine effects of RS exposure on glucose uptake in the PVN, we used unstressed rats and stressed rats (n = 3 in each). All rats were infused intracerebroventricularly with glucose solution (2 mmol/L, 0.1 μL/min) for 60 min. Rats were sacrificed for brain sampling under deep anesthesia. Brains were removed without fixation, and then coronal sections were cut at 30 μm thickness using a cryostat. Brain sections were observed using a confocal laser scanning microscope (LSM710, Zeiss, Jena, Germany).

2.10. Data analysis and statistics

Data are expressed as the mean ± S.E.M. Data on plasma catecholamine levels and brain protonaloid levels are displayed as net change above the respective basal values, and those of glucose levels are displayed as actual values. Data were analyzed by two-way ANOVA to test for main effects of treatment and time, and their interaction (Figs. 1, 3 and 4), by one-way ANOVA (Fig. 5) followed by a post hoc analysis with the Bonferroni method, or by a Student’s t-test (Fig. 2) with SPSS v25.0 (IBM, New York, NY, USA). Data in Table 1 were analyzed by two-way ANOVA to test for evaluation of cardiac functions (Table 1), by one-way ANOVA followed by a post hoc analysis with SNK test. P values less than 0.05 indicate statistical significance.

3. Results

3.1. Effects of glucose infusion on the RS-induced elevation of plasma catecholamine levels

Two-way ANOVA indicated significant main effects of treatment [F
Fig. 2. Effects of glucose infusion on RS-induced elevation of plasma catecholamine. Glucose solution was infused throughout RS exposure. ▼, vehicle + RS (+) (n = 11); ◀, 1% glucose + RS (+) (n = 9); ▼, 5% glucose + RS (+) (n = 8); ▲, 10% glucose + RS (+) (n = 9). *Significantly different (P < 0.05) from 0 min; #significantly different (P < 0.05) from the vehicle + RS (+) group.

| Table 1 | Echocardiographic analysis of RS subjected rats with saline or glucose. |
|---------|------------------------------------------------------------------------|
|         | Pre | 30  | 60  | 90  | 120 min |
| RS with saline |     |     |     |     |         |
| LVIDd | 0.474 ± 0.020 | 0.460 ± 0.005 | 0.425 ± 0.014 | 0.410 ± 0.015 | 0.449 ± 0.020 |
| cm    | 0.071 ± 0.005 | 0.085 ± 0.008 | 0.070 ± 0.009 | 0.074 ± 0.007 | 0.093 ± 0.006 |
| EDV mL | 0.271 ± 0.029 | 0.238 ± 0.019 | 0.194 ± 0.018 | 0.180 ± 0.029 | 0.239 ± 0.039 |
| ESV mL | 0.002 ± 0.009 | 0.003 ± 0.009 | 0.001 ± 0.007 | 0.001 ± 0.006 | 0.004 ± 0.005 |
| EF %   | 99.149 ± 0.001 | 99.133 ± 0.002 | 99.348 ± 0.003 | 99.284 ± 0.004 | 98.555 ± 0.005 |
| SV mL | 0.268 ± 0.029 | 0.234 ± 0.008 | 0.194 ± 0.019 | 0.178 ± 0.025 | 0.234 ± 0.042 |
| FS %   | 85.192 ± 1.460 | 81.433 ± 0.234 | 83.499 ± 0.178 | 81.930 ± 0.234 | 78.974 ± 0.425 |
| RS with glucose |     |     |     |     |         |
| LVIDd | 0.513 ± 0.019 | 0.496 ± 0.013 | 0.507 ± 0.018 | 0.497 ± 0.020 | 0.524 ± 0.030 |
| cm    | 0.070 ± 0.009 | 0.063 ± 0.009 | 0.084 ± 0.011 | 0.073 ± 0.009 | 0.071 ± 0.005 |
| EDV mL | 0.328 ± 0.032 | 0.297 ± 0.023 | 0.321 ± 0.031 | 0.304 ± 0.033 | 0.350 ± 0.041 |
| ESV mL | 0.003 ± 0.002 | 0.001 ± 0.002 | 0.001 ± 0.001 | 0.001 ± 0.000 | 0.000 ± 0.000 |
| EF %   | 99.558 ± 0.134 | 99.703 ± 0.109 | 99.370 ± 0.263 | 99.571 ± 0.152 | 99.693 ± 0.047 |
| SV mL | 0.326 ± 0.032 | 0.297 ± 0.023 | 0.319 ± 0.030 | 0.300 ± 0.035 | 0.350 ± 0.044 |
| FS %   | 86.001 ± 0.032 | 87.524 ± 0.009 | 83.507 ± 0.030 | 85.550 ± 0.043 | 86.543 ± 0.041 |

1. Vs. pre RS level, P < 0.05.
2. Vs. RS with saline, P < 0.01.
3. (3,198) = 3.874, P < 0.01 and time (F(3,198) = 24.463, P < 0.01) on plasma noradrenaline levels, but no significant interaction was found. There were significant main effects of treatment (F(3,198) = 15.287, P < 0.01) and time (F(5,198) = 17.835, P < 0.01) on plasma adrenaline level, but there was no significant interaction. Similarly, there were significant main effects on blood glucose level [treatment, F(3,198) = 47.080, P < 0.01], but there was no significant interaction. RS exposure significantly increased plasma levels of noradrenaline and adrenaline, and these responses were sustained for 120 min in vehicle-treated rats (Fig. 2). Continuous glucose infusion did not affect the RS-induced elevation of plasma noradrenaline level, regardless of glucose concentration (1%, 5%, or 10%). In contrast, continuous glucose infusion of a 5% solution (at 5, 30, 60 and 120 min) and a 10% solution (at 30 and 120 min) significantly suppressed the RS-induced elevation of plasma adrenaline compared to the vehicle + RS-treated group (Fig. 2). In the subsequent experiments, a 5% glucose solution was used.

The actual values for plasma noradrenaline and adrenaline at 0 min were 165.8 ± 38.1 and 319.0 ± 50.4 pg/ml for the vehicle + RS-treated group (n = 11), 114.1 ± 29.0 and 285.3 ± 96.1 pg/ml for the 1% glucose + RS-treated group (n = 9), 126.5 ± 28.9 and 382.6 ± 98.3 pg/ml for the 5% glucose + RS-treated group (n = 8), and 126.4 ± 14.6 and 275.2 ± 86.1 pg/ml for the 10% glucose + RS-treated group (n = 9), respectively.

3.2. Impaired cardiac function induced by RS is improved by glucose infusion

Baseline parameters of cardiac function as well as heart rate demonstrated to be comparable between saline- and glucose-treated rats before RS (LVIDd: 0.474 ± 0.020 cm vs. 0.513 ± 0.019 cm; EDV: 0.271 ± 0.024 mL vs. 0.328 ± 0.032 mL; SV: 0.268 ± 0.029 mL vs. 0.326 ± 0.032 mL; FS: 85.192 ± 1.460% vs. 86.00 ± 1.904; EF: 99.149 ±
0.503% vs. 99.558 ± 0.134%; HR: 390 ± 7 vs. 370 ± 17, n = 8–9 in each group). The alteration of cardiac function by RS with or without glucose administration was demonstrated in Table 1. RS with saline significantly reduced EDV and SV at 60 and 90 min, compared with pre-RS levels (EDV: F(4,32) = 2.956, P = 0.017; SV: F(4,32) = 2.549, P = 0.029); however, RS with glucose clearly blunted reduction in EDV and SV. Furthermore, compared with saline, glucose-treated rats significantly sustained EDV at 60 and 90 min [F(9,67) = 3.950, P = 0.0002] as well as SV at 90 min [F(9,67) = 3.823, P = 0.0003].

Since peaked time-points of downregulated cardiac parameters varied among individual rats, the reduction rate from a pre-stress level was calculated to compare saline and glucose groups. Compared with saline-treated rats, glucose-treated rats showed attenuation of decline rate in LVIDd (26.0 ± 4.2% vs 7.6 ± 2.2%, t = 3.817, P < 0.01, n = 8–9), EDV (52.1 ± 6.3% vs 24.2 ± 3.3%, t = 2.825, P < 0.05), SV (47.3 ± 7.2% vs. 10.7 ± 4.6%, t = 4.292, P < 0.01), FS (16.1 ± 2.6% vs 8.1 ± 1.4%, t = 2.564, P < 0.05), and EF (0.98 ± 0.29% vs 0.29 ± 0.1%, t = 2.240, P = 0.05; Fig. 3). These results suggest that glucose administration during RS suppressed the progression of cardiac impairment.

### 3.3. Effects of intravenous and intracerebroventricular infusion of glucose on RS-induced elevation of plasma catecholamine levels

Two-way ANOVA revealed significant main effects of treatment [F (3,100) = 31.905, P < 0.01] and time [F (4,100) = 19.921, P < 0.01] on plasma noradrenaline levels, and a significant interaction between treatment and time [F(12,100) = 2.320, P < 0.05]. There were significant main effects of treatment [F (3,100) = 34.963, P < 0.01] and time [F (4,100) = 8.895, P < 0.01] on plasma adrenaline level, and a significant interaction between treatment and time [F(12,100) = 2.874, P < 0.01]. There were significant main effects on blood glucose levels [treatment, F (3,60) = 12.037, P < 0.01; time, F (2,60) = 20.192, P < 0.01], and a significant interaction between treatment and time [F (6,60) = 4.600, P < 0.01]. Neither intravenous nor intracerebroventricular infusion of glucose solution affected the RS-induced elevation of plasma noradrenaline (Fig. 4). On the other hand, the RS-induced elevation of plasma adrenaline was suppressed not only by intravenous infusion but also by intracerebroventricular infusion of glucose solution (Fig. 4).

The actual values for plasma noradrenaline and adrenaline at 0 min were 128.7 ± 38.6 and 285.4 ± 80.9 pg/ml for the vehicle + RS (-)-treated group (n = 6), 266.4 ± 71.1 and 239.6 ± 106.3 pg/ml for the vehicle + RS (+)-treated group (n = 7), 179.6 ± 23.9 and 139.2 ± 25.6 pg/ml for the glucose (i.c.v., 2 mmol/L solution) + RS (+)-treated group (n = 5), and 151.3 ± 33.7 and 265.0 ± 25.8 pg/ml for the glucose (i.c.v., 2 mmol/L solution) + RS (-)-treated group (n = 6), respectively.

### 3.4. Effects of intravenous and intracerebroventricular infusion of glucose on prostanoid levels in the PVN

There were significant main effects of treatment [F(3,139) = 5.042, P < 0.01] and time [F(6,139) = 4.026, P < 0.01] on PGE_2 levels, but there was no significant interaction. There were significant main effects of treatment [F(3,139) = 13.638, P < 0.01] and time [F(6,139) = 3.323, P < 0.01] on TxB_2 levels, and there was a significant interaction between treatment and time [F(18,139) = 2.379, P < 0.01]. The mean baseline levels (before RS exposure; indicated as 0 min in Fig. 5) of PGE_2 and TxB_2 in the microdialysates were 0.013 ± 0.002 and 0.046 ± 0.020 pg/20 min-fraction, respectively. Administration of vehicle did not affect the baseline levels of PGE_2 and TxB_2 in the PVN dialysates. RS exposure significantly increased PGE_2 and TxB_2 levels in the PVN at 20 min (dialysate was collected from 0 to 20 min; indicated as 20 min in Fig. 5) and 40 min (dialysate was collected from 20 to 40 min; indicated as 40 min in Fig. 5). The intravenous infusion of glucose solution significantly suppressed the RS-induced elevation of TxB_2 levels in the PVN, whereas the treatment did not affect PGE_2 levels in the PVN. Furthermore, the intracerebroventricular infusion of glucose solution significantly suppressed the RS-induced elevation of both PGE_2 and TxB_2 levels in the PVN.

### 3.5. Effects of glucose infusion and RS on glucose levels in the CSF

One-way ANOVA indicated a significant effect of treatment on glucose levels in the CSF [F(2,16) = 32.311, P < 0.01] (Fig. 6). Intracerebroventricular infusion of a glucose solution (2 mmol/L, 0.1 μl/min, 60 min) significantly reduced the glucose levels in the CSF in unstressed rats. RS exposure significantly increased the glucose levels in the CSF compared to that of vehicle-treated unstressed rats.

### 3.6. Effects of RS on glucose uptake in the PVN

In unstressed rats, relatively few 2-NBDG-positive cells were observed in the PVN. On the other hand, in rats exposed to RS for 60 min, 2-NBDG-positive cells were abundantly expressed in the PVN, especially the ventral part and lateral magnocellular part of the PVN (Fig. 7).

### 4. Discussion

As far as we are aware, the present results provide the first evidence...
that an intravenous 5% glucose infusion suppresses RS-induced elevation of plasma adrenaline and TxA₂ production in the PVN and attenuates the LV dysfunction of takotsubo cardiomyopathy induced by stress exposure (Fig. 7).

First, we examined whether continuous intravenous infusion of a glucose solution can affect RS-induced elevation of plasma catecholamine. In agreement with previous findings of the effects of RS on plasma catecholamine (Kvetnansky et al., 1992; Pajovic et al., 2006) and glucose (Park et al., 2016), exposure to RS increased plasma catecholamine and blood glucose levels in this study. Furthermore, we found that blood glucose levels after RS did not differ between groups for three doses (1%, 5% or 10%) of glucose solutions. Interestingly, continuous glucose infusion showed different effects on the plasma levels of catecholamines. That is, only the 5% glucose infusion significantly reduced RS-induced elevation of plasma adrenaline. Previously, Nijjima (1975) reported that intravenous injection of glucose decreases discharge rate of the adrenal nerve filaments but not the activity of renal nerve filaments. These observations are consistent with our results that glucose infusion affected elevation of plasma adrenaline, which is secreted from the adrenal medulla. In contrast, none of the concentrations of continuous glucose infusion altered RS-induced elevation of plasma noradrenaline. It is well established that plasma adrenaline is involved in systemic sympathetic regulation as a hormone, whereas plasma noradrenaline is mainly involved in local sympathetic regulation as a neurotransmitter in sympathetic postganglionic nerve terminals. Therefore, we hypothesized that under systemic emergency processes such as Takotsubo cardiomyopathy, plasma adrenaline plays a major role for sympathetic responses and is easily influenced by systemic infusion of glucose. Further studies are necessary to elucidate the underlying mechanisms. In the subsequent experiments, we used a 5% glucose solution.

In this study, our results showed that an intravenous 5% glucose infusion restored RS-induced LV dysfunction, a model for takotsubo cardiomyopathy, suggesting that glucose administration during RS exposure can suppress the progression of cardiac impairment. Pathologically high levels of plasma catecholamines, especially adrenaline, are associated with LV abnormalities found in takotsubo cardiomyopathy (Wittstein et al., 2005; Ueyama, 2004), and these responses are prevented by combined blockade of α- and β-adrenergic receptors (Ueyama, 2004). In fact, intraperitoneal administration of catecholamine induces takotsubo-like cardiac dysfunction in rats (Redfors et al., 2014). On the other hand, sympathetic activation induces adrenaline secretion from the adrenal medulla (Okada et al., 2003; Yamaguchi-Shima et al., 2007), while also increasing blood glucose levels via various pathways including adrenaline-dependent hepatic glucose production (Exton, 1985; Pilkis et al., 1988). In the present study, we found that exposure to RS induced a marked elevation of plasma adrenaline with LV dysfunction. Moreover, continuous 5% glucose infusion reduced the RS-induced elevation of plasma adrenaline and ameliorated LV dysfunction. It is known that blood glucose levels are maintained within a narrow range through negative feedback, mainly by modulation of insulin and glucagon release (Bich et al., 2020). Since it has been reported that glucose infusion attenuates intense exercise-induced elevation of plasma adrenaline (Manzon et al., 1998), our findings raise the possibility that exogenously administered glucose-induced hyperglycemia causes central negative feedback regulation through the sympathetic nervous system, and subsequent reduction of adrenaline secretion from the adrenal medulla, resulting in suppression of LV dysfunction.

We previously reported roles of prostanoids in the brain in the regulation of plasma catecholamine levels: brain PGE₂ mediates noradrenaline release from sympathetic nerve endings and brain TxA₂ mediates noradrenaline and adrenaline secretion from the adrenal medulla (Okada et al., 2003; Yamaguchi-Shima et al., 2007), although the specific brain regions involved in this process were unclear. In this study, we examined changes in prostanoid production in the PVN after stress exposure, and showed that RS elevated PGE₂ and TxB₂, a metabolite of TxA₂, levels in the PVN in addition to elevating plasma...
catecholamines. Taken together, the present findings suggest that PGE$_2$ and TxA$_2$ produced in the PVN are involved in RS-induced elevation of plasma noradrenaline and adrenaline. Moreover, we showed that intravenous infusion of a glucose solution significantly attenuated the RS-induced elevation of plasma adrenaline and TxB$_2$ levels in the PVN, whereas the treatment did not alter plasma noradrenaline and PGE$_2$ levels in the PVN. Collectively, these results suggest that intravenous infusion of glucose can suppress the RS-induced production of TxB$_2$ in the PVN, preventing an increase in plasma adrenaline under stress exposure. Furthermore, intracerebroventricular administration of glucose solution also significantly reduced the RS-induced elevation of plasma adrenaline and TxB$_2$ levels in the PVN. Niijima (1975) previously revealed that spinal transection at the T5 level abolishes the adrenal nerve response to glucose injected intravenously, suggesting that effects of systemic administration of glucose on the activity of adrenal medulla are regulated via the brain. Furthermore, in our results, the effects of intracerebroventricular administration of glucose on plasma adrenaline and TxB$_2$ in the PVN were similar to those of intravenous administration. Taken together, our findings suggest that glucose exerts suppressive effects on RS-induced sympathetic activation via the central nervous system. Interestingly, Faour et al. (2008) reported that activation of AMP-activated protein kinase (AMPK), playing a maintenance for glucostasis, induces COX-2 expression levels, implying an existence of an exogenous glucose-induced AMPK-mediated signaling pathway in the PVN. Elucidation of the precise mechanisms will require further studies.

In our experiments that compared effects between intravenous and intracerebroventricular infusion of glucose solution on RS-induced changes in the levels of plasma catecholamine and prostanoids in the PVN, we found that not only intravenous but also intracerebroventricular infusion can affect these responses to RS. To examine whether the effects of administered glucose found in this study are caused by its central actions, we measured the concentration of glucose in the CSF. As expected, RS significantly increased the CSF glucose concentrations. On the other hand, unexpectedly, intracerebroventricular infusion of glucose reduced the CSF glucose concentrations. One explanation for this result is that the reduction might be due to transportation of glucose into cells in the brain as part of an energy restoration process. To explore this hypothesis, we observed glucose uptake into cells in the PVN using 2-NBDG, a fluorescent glucose analog that is taken up by cells via the glucose transporter. Our results showed that RS rats exhibited enhanced glucose uptake in the PVN compared to unstressed control rats. Together, our findings suggest that
administered glucose is taken up by cells in the brain, probably in the PVN, resulting in suppression of TxA2 production and plasma adrenaline levels.

In this study, we used acute RS rats as an animal model of takotsubo cardiomyopathy. In light of the present findings, one can assume that non-invasive administration of an appropriate amount of glucose may easily prevent the onset of Takotsubo cardiomyopathy. Accumulation of evidence from clinical studies will be essential in the future.

5. Conclusions

In summary, we demonstrated that intravenous glucose infusion suppresses RS-induced elevation of plasma adrenaline and LV dysfunction, which is a hallmark of takotsubo cardiomyopathy. Furthermore, in the brain, both intravenous and intracerebroventricular glucose infusion suppresses RS-induced production of TxA2 in the PVN. Our findings may provide a new insight for treatment of developing takotsubo cardiomyopathy.

Declaration of competing interest

The authors declare no conflict of interest.

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