Immobilization Stress With α_2 -Adrenergic Stimulation Induces Regional and Transient Reduction of Cardiac Contraction Through Gi Coupling in Rats

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SUMMARY

Stress cardiomyopathy is characterized by transient apical hypokinesia related to catecholamine overflow. Recently, excessive epinephrine administration was shown to recapitulate stress cardiomyopathy through β_2 -adrenoceptor (AR)-inhibitory G protein (Gi) coupling in rats. We aimed to study whether α_2 -AR and Gi affect cardiac contraction in rats in which emotional stress was evoked using immobilization (IMO). Echocardiography results showed that when male rats were exposed to IMO for 30 minutes and then injected with the α_2 -AR agonist xylazine (Xy), ejection fraction and the movement of the anterior wall (AW) were suppressed, maximally at 5 minutes post-injection, whereas posterior wall (PW) movement was preserved. At the same time points, the phosphorylation of Ser282 in myosin-binding protein-C (MyBP-C-Ser282) was higher in the PW than in the AW. Pretreatment with the Gi inhibitor pertussis toxin (PTX) reversed the low contractility and MyBP-C-Ser282 phosphorylation in the AW, but induced lethal heart failure in 3 out of 11 rats. Moreover, at 5 minutes after Xy injection following 30 minutes of IMO, serum epinephrine levels were increased. Thus, in rats exposed to psychological stress, α_2 -AR stimulation triggered transient hypo-contraction and MyBP-C-Ser282 hypo-phosphorylation in the AW, in association with an epinephrine surge. PTX treatment reversed the AW hypo-contraction and MyBP-C hypo-phosphorylation, but induced acute heart failure. These findings suggest α_2 -AR/Gi-dependent signaling attenuates MyBP-C phosphorylation and contractility in the AW through an epinephrine surge in rats subjected to IMO and α_2 -AR stimulation. This model can recapitulate stress cardiomyopathy and thereby deepen our understanding of regional cardiac hypo-contraction and pro-survival mechanisms. (Int Heart J 2015; 56: 537-543)

Key words: Stress cardiomyopathy, α_2 -Adrenergic receptor, Myosin-binding protein-C (MyBP-C), Takotsubo cardiomyopathy, Emotional stress, Cardiac contractility, Calmodulin-dependent protein kinase II, Sudden cardiac death

Stress cardiomyopathy is characterized by transient apical hypo-contraction related to catecholamine overflow.¹⁻³⁾ Studies on psychological stress conducted using immobilization (IMO) in rats demonstrated the activation of the hypothalamo-pituitary-adrenocortical and sympatho-adrenomedullary axes.⁴⁻⁶⁾ This model served to establish the fight-and-flight doctrine of Hans Selye⁷⁾ and has contributed to studies on cardiovascular responses to emotional stress. We reported that IMO induces the expression of cardiac immediate-early genes through α - and β -adrenoceptors (ARs) in rats.⁸⁾ We also showed that whereas IMO induced enhanced gap-junction (GJ) coupling through α -AR, it triggered lethal ventricular arrhythmias when GJ was inhibited.^{9,10)}

Stress (takotsubo) cardiomyopathy is typically induced by psychological stress in postmenopausal women, and is characterized by apical myocardial akinesis and preserved basal movement, which is reflected by a takotsubo (Japanese oc-

topus trap)-like ventriculogram.¹¹⁾ Previously, Ueyama, *et al* reported that IMO induced takotsubo-like ventriculograms in ovariectomized rats.^{12,13)} Recently, Paur, *et al* demonstrated that excessive epinephrine exposure led to a negative inotropic effect and apical ballooning in rats, and that a Gi inhibitor or a β_2 -AR blocker reversed the effects of epinephrine.³⁾ High apical β_2 -AR density and enhanced Gi coupling have been proposed to trigger apical hypo-contraction during epinephrine overflow.^{2,3,14,15)} These studies have engendered the hypothesis that excessive epinephrine causes the switch from β_2 -AR coupling with stimulatory G proteins (GS) to inhibitory G proteins (Gi), and thereby offsets the β_1 -AR-dependent positive inotropic effect.^{2,3,14,15)} The β_1 -AR-dependent positive inotropic effect is mediated through the phosphorylation of troponin-I, myosin light chain, and myosin-binding protein-C (MyBP-C) by cyclic-AMP-dependent kinase (PKA) and calmodulin-dependent protein kinase II (CaMKII).¹⁶⁾ Conversely, reduced

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This study was supported by JSPS KAKENHI grant number 25293162.

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Received for publication January 22, 2015. Revised and accepted February 25, 2015.

Released in advance online on J-STAGE September 4, 2015.

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phosphorylation of MyBP-C is accompanied by contractile dysfunction in heart failure.¹⁶⁾

α_2 -AR agonists induce central hypotension in association with an inhibition of catecholamine release from presynaptic adrenergic nerve terminals and the adrenal medulla.^{17,18)} Moreover, α_2 -AR agonists cause sedation,¹⁹⁾ stabilize circulatory dynamics, and improve cardiac oxygen consumption during surgery.^{20,21)} Recently, Ibacache, *et al* identified the cardiomyocyte α_{2A} - and α_{2C} -ARs that mediate the cardiac protection conferred by ischemic preconditioning in rats.²²⁾ α_2 -AR agonists have been widely used for echocardiography of rodents with fast heart rates.

Unexpectedly, in echocardiography studies, we have observed that the α_2 -AR agonist xylazine (Xy) induces a transient reduction in the anterior wall (AW) movement in rats following IMO; however, the posterior wall (PW) movement is maintained at the normal level. Based on an assumption that the regional hypo-contraction recapitulates stress cardiomyopathy, we aimed to clarify whether the α_2 -AR agonist induces regional hypo-contraction through Gi-dependent inhibition of the phosphorylation of contractile proteins such as MyBP-C¹⁶⁾ in rats exposed to IMO.

METHODS

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85–23, revised 1996). The protocol was approved by the Institutional Animal Care and Use Committee of the University of Tokyo. We purchased 8-week-old male Sprague-Dawley rats from Charles River Laboratories (Kanagawa, Japan) and allowed them free access to water and solid food (CRF-1, Oriental Yeast, Tokyo), and for 1 week before the experiments, maintained them under conditions of controlled temperature (21–25°C) and humidity (40–75%) and a 12-hour light/dark cycle.

Animal model and treatments: The first experimental procedure used is outlined in Figure 1A. Rats were assigned randomly to either the Xy group or the physiological saline group. Because injection with Xy before IMO rendered the rats insensitive to IMO as a result of sedation (data not shown), Xy was injected at the end of IMO. To obtain baseline data, rats were subjected to the 1st echocardiographic examination before and 5 minutes after intraperitoneal injection of Xy (6 mg/kg; DY006197, Bayer Health Care, Berlin, Germany) or saline under anesthesia, which was induced by using the inhalant 2% isoflurane at 1.5 L/minute and the evaporation anesthesia apparatus MK Vapo (Muromachi Kikai Co. Ltd., Tokyo). On the next day, the rats pretreated with Xy and saline were assigned randomly either to the IMO group or the free-moving (F) group. We used adhesion tape to secure the IMO rats in a supine position on a board for 30 minutes. The F rats were allowed free movement in a cage without access to food and water for the same duration as that of the IMO rats.

The 2nd echocardiographic examination was performed at 5, 20, or 35 minutes after injection with Xy and saline following the IMO and F procedures in the rats pretreated with Xy (Xy and IMO+Xy groups) and saline (F and IMO groups), respectively. To obtain samples for Western blotting and cate-

Figure 1. Experimental protocol. Immobilization (IMO) is indicated by the filled columns. The time points of drug administration are indicated by arrows; those of echocardiography by closed arrowheads; and those of Western blotting and catecholamine quantification by open arrowheads. F indicates free-moving rats; Xy, xylazine treatment; PTX, pertussis toxin treatment; and S, rats that survived after PTX injection (assay shown in Figure 2E).

cholamine quantification, some of the rats in each group were euthanized 5 minutes after the treatment.

In a separate experiment (Figure 1B), rats were intravenously injected with pertussis toxin (PTX; 25 mg/kg; #168-22471, Wako, Osaka, Japan) (IMO+Xy+PTX) or saline (IMO+Xy) 48 hours before IMO followed by Xy treatment. The F rats were subjected to neither PTX pretreatment nor Xy posttreatment. Echocardiographic examination was performed on the rats at 5 minutes of Xy posttreatment and then they were sacrificed to obtain samples for Western blot analysis. Some of the rats (Figure 2E) were subjected to echocardiography at 35 minutes after Xy treatment following IMO and then euthanized to obtain samples for Western blotting. At 25–30 minutes after Xy injection, 3 out of 11 IMO+Xy+PTX rats died, and their hearts were harvested shortly postmortem.

Echocardiography: Echocardiography was performed using a ProSound $\alpha 10$ echocardiograph (Aloka Co., Ltd., Tokyo) after

Figure 2. Effects of G_i and β_2 -adrenoceptor inhibition on anterior wall (AW) hypo-contraction. The results show the effects of the G_i inhibitor pertussis toxin (PTX) applied with immobilization (IMO) and xylazine (Xy) on ejection fraction (EF; **A**), AW movement (**B**), posterior wall (PW) movement (**C**), and MyBP-C phosphorylation (**D**). **E** shows the difference in MyBP-C phosphorylation between the surviving rats (s) and the rats that died (d) after PTX injection. Effects of the β_2 -blocker ICI-118,551 used with Xy on EF (**F**) and AW movement (**G**) are shown. Numbers in parentheses indicate the numbers of rats used in the analysis. F indicates free-moving rats. Data are presented as means $\pm$ SEM. * $P < 0.05$ versus F of the corresponding wall region or treatment group.

Xy injection and isoflurane inhalation. Appropriate 2D parasternal short-axis views at the level of the left ventricular (LV) papillary muscle were acquired and recorded. We analyzed 2 consecutive heartbeats in each frame in order to measure the heart rate (HR), LV AW thickness in diastole (LVAWd) and systole (LVAWs), LV PW thickness in diastole (LVPWd) and systole (LVPWs), end-diastolic LV internal dimensions (LVDd), and end-systolic LVD (LVDs). LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVDSV) were calculated using Pombo's method.²³ As an index for LV systolic performance, the LV ejection fraction (EF) was calculated as LVEDV minus LVESV normalized for LVEDV. AW and PW movements were quantified as Δ AW (LVAWs – LVAWd)

and Δ PW (LVPWs – LVPWd), respectively. Transmittal Doppler echocardiography was performed to assess the ratio of the initial (E) and second (A) blood-flow velocities (E/A ratio), and the E velocity acceleration and deceleration times were normalized for the second root of the R-R interval to correct for the influence of HR.

Western blot analysis and serum catecholamine measurement: The specimens used for Western blot analyses and catecholamine quantification were harvested from rats shortly after pentobarbital overdose. Frozen heart tissue (~0.1 g) was thawed and homogenized in 1 mL of ice-cold STE buffer containing 320 mM sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 50 mM NaF, 1 mM Na_3VO_4 , and Complete Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany). We measured the concentrations of homogenate proteins by using a Coomassie Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA), and then used 10% and 7.5% SDS-PAGE to resolve samples for Western blotting examination of CaMK-II and MyBP-C, respectively. The blots were incubated with primary antibodies (diluted 1:1,000) against CaMK (rabbit polyclonal, sc-9035) or CaMK phospho-Thr286 (rabbit polyclonal, sc-12886-R) purchased from Santa Cruz Biotechnology (Dallas, TX); MyBP-C (rabbit polyclonal, C16776) from Assay Biotechnology (San Francisco, CA); or MyBP-C phospho-Ser282 (rabbit polyclonal, ALX-215-057) from Enzo Life Sciences (Farmingdale, NY). Peroxidase-conjugated anti-rabbit IgG secondary antibodies (1:5,000; Promega, Madison, WI) were used for chemiluminescence detection. The band densities were quantified using an ImageQuant™ LAS 4000 mini (GE Healthcare UK Ltd., Buckinghamshire, England). Serum catecholamine concentration measurements were outsourced to SRL Inc. (Tokyo).

Statistical analysis: Data are expressed as the mean $\pm$ SEM. For comparison between 2 groups, Student's *t* test was used, whereas for comparisons between multiple groups, analysis of variance (ANOVA) was employed with Tukey's or Dunnett's *post hoc* test. Differences were considered significant at $P < 0.05$.

RESULTS

After IMO, the α_2 -AR agonist induced a transient reduction in AW movement: Echocardiographic analyses demonstrated that in the free-moving rats, EF, HR, and AW movement were lower in rats injected with Xy (F+Xy) than in rats injected with saline (F) at 5, 20, and 35 minutes after the injection (Figure 3A-E). IMO followed by saline injection (IMO) affected neither EF nor wall movement (Figure 3B, D, E). However, IMO followed by Xy injection (IMO+Xy) induced a transient reduction in EF (7.8% of pre-IMO levels) (Figure 3B) and AW movement (27.3% of pre-IMO levels) (Figure 3D), which peaked at 5 minutes and returned to baseline levels at 20 minutes after Xy injection. HR and PW movement did not differ between Xy and IMO+Xy rats (Figure 3C, E), and no difference was detected in diastolic function (evaluated using E/A) and the normalized speeds of acceleration (AC/√RR) and deceleration (DC/√RR) between F+Xy and IMO+Xy rats. Clonidine, which is another α_2 -AR agonist, exerted effects almost identical to those of Xy. Moreover, Xy treatment following 15, 45, or 60 minutes of IMO did not affect the echocardiographic

Figure 3. Echocardiographic analyses in free-moving (F) and immobilized (IMO) rats. The figure shows representative echocardiograms (A) and temporal changes in ejection fraction (EF; B), heart rate (HR; C), anterior wall (AW) movement (Δ AW; D), and posterior wall (PW) movement (Δ PW; E). Numbers in parentheses indicate the numbers of rats used in the analysis. Data are presented as the mean $\pm$ SEM. * $P < 0.05$ versus pre-IMO (pre) of corresponding treatment group. NS indicates not significant.

parameters (Data not shown).

Effect of IMO and Xy on serum catecholamine levels: The serum epinephrine level increased at a statistically insignificant level at 5 minutes after 30 minutes of IMO (IMO; Figure 4A), but increased substantially and significantly after Xy injection following IMO (IMO+Xy; Figure 4A). The level of norepinephrine increased at 5 minutes after Xy injection following 30 minutes of IMO, but it varied considerably and thus did not reach a statistically significant level (Figure 4B). Treatment with Xy alone did not affect serum catecholamine levels (data not shown).

Phosphorylation of CaMKII and MyBP-C: The autophosphorylation of CaMKII at Thr286 reflects activation of the enzyme.²⁴⁾ Phosphorylation was observed at 5 minutes after Xy injection following 30 minutes of IMO, at the time-point of the transient AW hypokinesia. CaMKII-Thr286 phosphorylation was enhanced following either IMO or Xy injection, and when both treatments were used, it was increased synergistically in the PW and to a lesser extent in the AW (Figure 5A). Phosphorylation of MyBP-C at Ser282 was significantly enhanced in the IMO+Xy group only in the PW (Figure 5B), but no such change in troponin-I phosphorylation occurred in the IMO+Xy group (data not shown). The lack of increase in MyBP-C phos-

Figure 4. Effects of immobilization (IMO) and xylazine (Xy) on serum catecholamine levels. Serum epinephrine (A) and norepinephrine (B) levels in rats subjected to IMO for 30 minutes and then Xy treatment for 5 minutes are shown. Numbers in parentheses indicate the numbers of rats used in the analysis. Data are presented as means $\pm$ SEM. * $P < 0.05$ versus F. NS indicates not significant.

phorylation only in the AW in the IMO+Xy rats is consistent with the hypo-contraction of the AW (Figure 3D, E).

Effect of Gi and β_2 -AR inhibition on AW hypo-contraction: To examine whether enhanced Gi coupling underlies the AW hypo-contraction observed in the IMO+Xy rats, we examined the effect of the widely used Gi inhibitor PTX. Treatment with PTX reversed the reduction in EF and AW movement at 5 minutes after Xy injection following IMO for 30 minutes (Figure 2A, B); however, the PW movement was preserved (Figure 2C). In the IMO+Xy rats, PTX increased MyBP-C phosphorylation in the AW to levels comparable to those in the PW, but PTX did not affect the enhanced phosphorylation of MyBP-C in the PW (Figure 2D). In the PTX-injection group, within 30 minutes of Xy injection following IMO (30 minutes), 3 out of 11 rats died of heart failure, which was detected based on echocardiography and pleural effusion, whereas the other rats survived. In the AW but not the PW, MyBP-C phosphorylation was higher in the rats that died than it was in the rats that survived (Figure 2E). The β_2 -AR blocker ICI-118,551 did not reverse the reduction in EF (Figure 2F) or AW movement (Figure 2G) at 5 minutes after Xy injection following IMO for 30 minutes. These findings suggest that a negative inotropic and pro-survival role of α_2 -AR-Gi coupling offsets the β_1 -AR-Gs path-

Figure 5. Phosphorylation of CaMKII and MyBP-C in immobilized (IMO) rats. The results show the effects of IMO for 30 minutes and subsequent xylazine (Xy) treatment on the phosphorylation of CaMKII (**A**) and MyBP-C (**B**) in the anterior wall (AW) and posterior wall (PW). F indicates free-moving rats. Numbers in parentheses indicate the numbers of rats used in the analysis. Data are presented as the mean $\pm$ SEM. * $P < 0.05$ versus F of the corresponding wall region (AW or PW), # $P < 0.05$ versus AW of the corresponding treatment group.

ways, and this is reflected by the hypo-phosphorylation of MyBP-C in the AW of IMO+Xy rats.

DISCUSSION

This is the first report showing that the α_2 -AR agonist Xy transiently suppressed contractility and MyBP-C phosphorylation selectively in the AW by enhancing Gi coupling in rats exposed to psychological stress evoked by IMO. This model could recapitulate the regional hypo-contractility that occurs in

stress cardiomyopathy.

In the rats subjected to IMO for 30 minutes and Xy treatment for 5 minutes, the EF and AW movement were reduced, whereas PW movement was preserved (Figure 3). Consistently, the phosphorylation of MyBP-C-Ser282 was enhanced in the PW, but not in the AW (Figure 5). HR was reduced at 5 minutes after Xy treatment, but it did not differ between the IMO and F groups (Figure 3C). Thus, bradycardia does not explain the reduction in AW contractility and EF in the IMO+Xy group.

β_1 -AR-dependent MyBP-C-Ser282 phosphorylation by PKA or CaMKII accelerates cross-bridge kinetics and enhances contractility in response to catecholamine.¹⁶⁾ By contrast, MyBP-C dephosphorylation is associated with contractile dysfunction in heart-failure patients.²⁵⁾ In the IMO+Xy rats, CaMKII phosphorylation was high in the PW and AW (PW > AW), which contrasted the high PW and marginal AW phosphorylation of MyBP-C (Figure 5). This discrepancy might be caused by the distinct localization or activation kinetics of CaMKII and MyBP-C, or by the requirement of an adjunct protein for CaMKII-dependent MyBP-C phosphorylation. In the AW, MyBP-C phosphorylation in the IMO+Xy rats was similar to that in the F rats, but contractility was diminished. Conversely, in the PW, MyBP-C phosphorylation in the IMO+Xy rats was higher than that in the F rats, but contractility was similar. Thus, in the IMO+Xy rats, enhanced MyBP-C phosphorylation would be required to maintain baseline contractility in order to overcome the negative inotropy induced through unknown mechanisms. In other experiments, we observed that PKA-dependent troponin-I phosphorylation did not differ between the IMO+Xy and other groups (data not shown).

β_1 -AR-Gs signaling enhances catecholamine-induced cardiac contractility through PKA and CaMKII.^{24,26,27)} The Gi inhibitor PTX increased the contractility and MyBP-C phosphorylation in the AW at 5 minutes after Xy injection following 30 minutes of IMO (Figure 2); this effect was also exerted by another α_2 -AR agonist, clonidine. These results suggest that α_2 -AR-Gi-mediated inhibition of β_1 -AR-Gs signaling occurs in the AW. Notably, PTX induced lethal heart failure in 3 of 11 rats within 30 minutes in the IMO+Xy group. In the rats that died, MyBP-C phosphorylation in the AW was not suppressed but rather enhanced, which contrasted with the results obtained for the rats that survived (Figure 2). These findings support the view that the regional hypo-contractility and high survival rate in stress-cardiomyopathy cases are recapitulated in the IMO+Xy rats through the α_2 -AR-Gi-coupling pathway identified in this study, and this is reflected by the lowering of MyBP-C phosphorylation.

Recently, β_2 -AR-Gi coupling has been implicated in the apical-mid-layer hypokinesia and sudden cardiac death that occur in rats injected with excessive epinephrine, because the β_2 -AR blocker ICI-118,551 or PTX reversed the hypokinesia, but induced sudden death.³⁾ In contrast to these previous findings, we detected no effect of ICI-118,551 on AW hypo-contractility or survival in the IMO+Xy rats (Figure 2). Our results indicate that α_2 -AR-Gi coupling, independently of β_2 -AR-Gi, mediates AW hypo-contractility by inhibiting β_1 -AR-Gs-dependent phosphorylation of MyBP-C and protecting against lethal heart failure in the IMO+Xy rats.

α_2 -AR agonists are widely recognized to inhibit stimulation-evoked release of norepinephrine and epinephrine from

presynaptic adrenergic nerve terminals¹⁸⁾ and the adrenal medulla.¹⁹⁾ During the progression of congestive heart failure, negative-feedback inhibition through presynaptic α_2 -AR is potentiated, which attenuates excessive norepinephrine release in patients²⁸⁾ and in mice exposed to pressure overload.²⁹⁾ Consistent with these findings, sustained (30 minutes) IMO did not increase norepinephrine secretion, possibly reflecting endogenous α_2 -AR-mediated feedback inhibition (Figure 4B). Unexpectedly, injection of the α_2 -AR-agonist Xy following IMO for 30 minutes increased the serum levels of epinephrine (Figure 4A). The epinephrine surge is consistent with the hypo-contraction and impaired MyBP-C phosphorylation in the AW (Figures 3, 5), given that excessive epinephrine enhances Gi coupling and reduces cardiac contractility in mouse cardiomyocytes³⁰⁾ and rat hearts.³⁾ A line of evidence suggested that the epinephrine surge at 5 minutes after Xy injection following 30 minutes of IMO was enhanced through adrenal Ca^{2+} influx. Because Ca^{2+} and calmodulin activate CaMKII,²⁴⁾ Xy likely increased Ca^{2+} concentrations not only in the myocardium, but also in the adrenal medulla. In agreement with this interpretation, α_2 -AR agonists elevate intracellular Ca^{2+} by increasing Ca^{2+} mobilization through phospholipase C in HEK-293 cells,³¹⁾ or by stimulating Ca^{2+} influx through L-type Ca^{2+} channels in the rat aorta.³²⁾ Moreover, Ca^{2+} influx through L-type Ca^{2+} channels was shown to promote the release of norepinephrine and epinephrine in adrenal chromaffin cells.³³⁾

Previous work supports the view that hypo-contraction in the IMO+Xy rats is not caused by myocardial ischemia. In isolated guinea pig heart, the α_2 -AR-selective agonist clonidine induced coronary vasoconstriction, but not myocardial hypo-contraction.³⁴⁾ In healthy volunteers, another α_2 -AR agonist dexmedetomidine reduced coronary flow, but did not induce electrocardiographic and echocardiographic changes indicating myocardial ischemia.³⁵⁾ Furthermore, in contrast echocardiographic analysis, high-dose isoproterenol was found to induce apical ballooning in rat heart but not apical hypo-perfusion.³⁶⁾

A previous proposal was that β_2 -AR distribution is more dense at the apex than in the base, and that this induces apical hypokinesia following epinephrine overdose, but compelling evidence for this proposal remains to be documented.²⁾ Mantravadi, *et al* reported a basal-apical gradient in sympathetic nerve innervation in rabbit hearts,³⁷⁾ but untreated and IMO (2 hours) rats showed no regional differences in β -AR distribution.³⁸⁾ Notably, we confirmed the recent findings on the expression of α_{2A} -AR and α_{2C} -AR in cardiomyocytes.²²⁾ However, we detected no regional difference in the distribution of α_{2A} -AR, α_{2C} -AR, β_1 -AR, β_2 -AR, Gs, Gi, or G protein-coupled receptor kinase -2 in the F and IMO rats (data not shown). In ovariectomized rats, IMO was shown to induce apical ballooning,¹³⁾ and α_2 -AR stimulation was shown to enhance hemodynamic response through α_2 -AR overexpression.³⁹⁾ These results support a relationship between α_2 -AR levels and an elevated susceptibility of postmenopausal women to stress cardiomyopathy.

α_2 -AR agonists have been used as antihypertensive drugs, sedatives, and adjuncts in anesthesia and echocardiography.^{19,20)} However, the results of this study indicate that physicians must be cautious of excessive contractile depression following injection of α_2 -AR agonists in patients suspected of stress cardiomyopathy, and in patients who have experienced restraint or

psychological stress. The genotyping of α_2 -AR and β_2 -AR might facilitate individualized therapy for patients with distinct backgrounds who are suspected of stress cardiomyopathy or heart failure. Sudden deaths during restraint have been considered to result from asphyxia caused by chest compression, and this has led to the criminal conviction of people that forced the restraint. Previously, we demonstrated that IMO induced arrhythmic death when coupled with the GJ inhibition that frequently results from heart diseases.^{9,10)} This study has provided additional support for the hypothesis on the role of cardiogenic death in restraint.

The limitations of this study are that the causal relationship between the AW hypo-phosphorylation of MyBP-C and AW hypokinesia was verified only based on the effects of Xy on IMO rats, and that of PTX on IMO+Xy rats. Moreover, the mechanisms underlying the transience of AW hypokinesia and the pro-death effects of PTX on IMO+Xy rats remain to be addressed. Notably, however, we demonstrated the presence of α_2 -AR-Gi-dependent negative inotropy and pro-survival pathways in association with psychological stress.

In conclusion, we found that α_2 -AR-Gi-dependent suppression of β_1 -AR-Gs-dependent MyBP-C phosphorylation underlies regional hypo-contraction and survival in rats exposed to emotional stress and α_2 -AR stimulation. This model could contribute to enhancing our understanding of stress cardiomyopathy.

DISCLOSURE

Conflict of interest: There is no conflict of interest in this study.

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