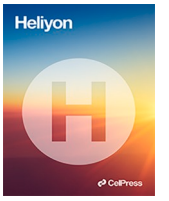
Heliyon 9 (2023) e18394

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



C-fos expression in the solitary nucleus medial region and reaction to acute hemorrhage in streptozotocin-stimulated diabetic rats

Aiji Sato (Boku)

Department of Anesthesiology, Aichi Gakuin University School of Dentistry, 2-11 Suemoridori, Chikusa-ku, Nagoya, Aichi, 464-8651, Japan

ARTICLE INFO

Keywords:
Autonomic nerve activity
Bezold–jarisch reflex
Acute hemorrhage
c-Fos expression

ABSTRACT

Examination of circulatory dynamics and autonomic nerve activity in acute hemorrhage in diabetic (DM) rats revealed that despite decreased receptor sensitivity to arterial blood pressure, the DM rats experienced a decline in the heart rate and acceleration of the parasympathetic nerve activity at the sympathoinhibitory phase in response to bleeding (Bezold–Jarisch [B–J] reflex). To elucidate the involvement of the B–J reflex as a reaction to acute hemorrhage in DM rats by assessing *c*-Fos-positive cell (*c*-Fos) expression in the nucleus of the solitary tract (SolM), the primary relay nucleus of the baroreflex, Streptozotocin-induced DM and non-DM rats underwent controlled-graded bleeding or continuous phenylephrine infusion under conscious state. Changes in hemodynamics and autonomous nervous system caused by acute hemorrhage and continuous phenylephrine infusion were examined by analyzing blood pressure–heart rate variability. Furthermore, effects of hemorrhage and phenylephrine infusion on the expression of *c*-Fos in SolM were examined. DM rats showed increased *c*-Fos expression in response to acute blood loss in the SolM. Non-DM rats showed the same phenomenon in response to continuous phenylephrine infusion in the SolM. Significant interactions between DM and Non-DM rats were observed among hemodynamic and autonomic response to acute hemorrhage and continuous phenylephrine infusion. DM rats were sensitive to acute blood loss, and the circulatory system easily collapsed with accelerating parasympathetic activity in the form of the B–J reflex.

1. Introduction

Neuropathy, a complication of diabetes, occurs at an early stage of diabetes. In particular, cardiovascular autonomic neuropathy (CAN) may cause tachycardia at rest, orthostatic hypotension, and exercise intolerance, sometimes resulting in sudden cardiac death [1,2]. Acute hemorrhage is a factor that has a significant impact on the circulatory dynamic and is known to be intricately regulated by the sympathetic and parasympathetic nervous systems, depending on the degree of hemorrhage [3]. However, diabetic patients with CAN may not have adequate autonomic-mediated compensatory mechanisms for acute hemorrhage. In our previous study, we examined the circulatory dynamics and autonomic nerve activity in acute hemorrhage in diabetic (DM) rats. Despite decreased receptor sensitivity to arterial blood pressure, the DM rats experienced a decline in the heart rate due to a considerable acceleration of the parasympathetic nerve activity at the sympathoinhibitory phase in response to the bleeding (Bezold–Jarisch reflex: B–J reflex). This indicated impairment in the compensatory mechanism against hemorrhage at an early stage [4].

This study examined the involvement of the B–J reflex [5–7] in response to acute hemorrhage in DM and control rats by comparing hemodynamic and autonomic changes and expression of *c*-Fos-positive cells (*c*-Fos) in the nucleus of the solitary medial region (SolM),

E-mail address: bokuaiji@dpc.agu.ac.jp.

https://doi.org/10.1016/j.heliyon.2023.e18394
Received 1 December 2022; Received in revised form 8 July 2023; Accepted 17 July 2023
Available online 17 July 2023

which is the primary relay nucleus of the baroreflex.

2. Materials and methods

Ethics approval

The study protocol for animal-based experimental studies received approval from the Institute of Experimental Animal Sciences, Osaka University Graduate School of Dentistry (22-011-0), and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23), revised in 1996. This study is also carried out according to ARRIVE guidelines (https://arriveguidelines.org), including the minimization of the number of animals and their suffering.

2.1. Animal preparation

For the experiment, we employed 12-week-old male Wistar–Kyoto (WKY) rats with the weight between 300 and 350 g, which had been raised in captivity with full access to nutrition. The animals were stored at a 12-h light/dark cycle and a constant temperature of ~25 pt.

After subjecting the rats to 12-h fasting, we administered streptozotocin (STZ), a pancreatic β-cell destruction agent, into their abdominal cavity (at 65 mg/kg) to induce type 1 DM [8]. At 5 days after the administration of STZ, blood samples were gathered from the caudal vein to determine blood glucose and blood ketone levels using MediSence Xtra™ (Abbott Japan). The rats with a former of ≥350 mg/Dl and a latter of ≥0.5 mmol/L were considered to have developed DM. Eleven days after STZ administration, each DM rat was placed under pentobarbital anesthesia, followed by the insertion of a polyethylene catheter into the femoral artery for measuring arterial pressure and blood removal, and another catheter into the jugular vein for drug infusion. Each catheter was placed subcutaneously to lead out from the dorsal region of the neck. To prevent the occlusion of the catheters, heparin-added physiological saline was poured into them at 12-h intervals until the day of the experiment [9]. Rats had 3 days to recover from surgery.

The control group encompassed WKY rats that had been administered normal saline instead of STZ. These were designated as non-DM rats. We also inserted catheters into these animals in the same manner as in the DM group.

2.2. Measurement of circulatory dynamics and analysis of autonomic nervous system activity

We connected the catheter inserted into the femoral artery to a pressure transducer and then amplified the resultant waveform of the arterial pressure with a blood pressure amplifier (LC210 A/Z, Unipulse). The signals generated from the amplifier were then transferred to a computer (VGN-K71B, SONY) via an analog-to-digital converter (KPCMCIA-16AI-C, KEITHLEY) at a frequency of 1 kHz. With the peak values of the arterial pressure waveform data defined as the systolic blood pressures (SBPs), we calculated the heart rate (HR) based on their intervals. To recognize the arterial pressure waveform and analysis of the autonomic nerve activity, we used a software product capable of full automatic analysis of circulatory dynamics and autonomic nervous system activity (Fluclet ™, Sumitomo Dainippon Pharma). We performed the frequency analysis based on the wavelet methodology, thereby calculating the fluctuation of the systolic blood pressure (SBP-LF) in the part with low frequency (0.25–0.75 Hz) and the fluctuation of the heart rate (HR–HF) in the high-frequency part (0.75–3.0 Hz). The SBP-LF and HR–HF have been used as indices of the sympathetic [10–14] and parasympathetic nervous system activities [15–19], respectively.

2.3. Measurement of circulatory dynamics and autonomic nervous system activity in acute hemorrhage

In this experiment, we used eight DM and non-DM rats each. After recording their SBP, HR, SBP-LF, and HR–HF at rest as control values, we extracted blood from each animal twice at a 20-min interval at an amount equivalent to ~6% (~1.2 mL) of the entire circulating volume. Next, we compared the mean arterial pressure (MAP), HR, SBP-LF, and HR–HF of each animal between the analytic and control values immediately after and at 10 min after the blood extraction.

2.4. Experimental procedure for continuous activation of the baroreflex

This experiment employed another eight DM and non-DM rats each. After recording their SBP, HR, SBP-LF, and HR–HF at rest as control values, each animal was subjected to continuous administration of phenylephrine for 30 min (at 0.75 μg/0.03 mL/min) through a syringe pump (CFV-3200™, NIHON KOHDEN) [20]. As a rough indication, a 15–30 mm Hg increase in average blood pressure was regarded as a significant increase in blood pressure. The volume was controlled through infusion of 0.9% saline (0.03 mL/min) for 30 min. We then compared the MAP, HR, SBP-LF, and HR–HF values of each animal between the analytic and control values at 10 and 30 min after the start of the phenylephrine administration, as well as at 10 min after the end thereof.

2.5. Immunohistochemical (c-Fos immunoreaction)

Two hours after the first blood extraction or 2 h after the end of the phenylephrine administration, we analyzed the immunoreactivity of the *c*-Fos protein [21], an index for neuron excitability, using immunohistochemistry of the circulatory reaction to the blood removal and phenylephrine infusion in the SolM. Under anesthesia with pentobarbital sodium (50 mg/kg, i. p.), each animal was

exsanguinated from the left ventricle with 100 mL of 0.02-M phosphate-buffered saline (PBS: pH 7.4), followed by perfusion with 500 mL of 0.1-M phosphate buffer (PB: pH 7.4) having 4% paraformaldehyde. After being removed from the lower brainstem, the upper portion of the cervical spinal cord was placed in a fixative solution for 3 h and then immersed in a 30% sucrose solution (at 4 °C). Subsequently, the removed cervical spinal cord was sliced into continuous transverse 60-μm-thick sections, which were then frozen and stored within a PB solution. After washing with PBS for 20 min, the sections were pretreated with 1% normal goat serum (Vectastain; Vector Laboratories, Burlingame, CA, USA) for another 20 min before being incubated with a rabbit *anti-c*-Fos antibody (1:7000 dilution; Santa Cruz Biotech, Santa Cruz, CA, USA) for 12 h. After washing with PBS, the samples were saturated with a biotinylated goat antirabbit antibody for 2 h, washed again, and finally incubated with an avidin–biotin–peroxidase combination solution (Vectastain; VectorLaboratories, Burlingame, CA, USA) for 1 h. To visualize peroxidase, the samples were allowed to react with a 0.05-M trishydrochloric acid buffer solution (pH 7.2) having 0.05% diaminobenzidine tetrahydrochloride (DAB), 0.1% ammonium nickel sulfate, and 0.01% hydrogen peroxide. Each resultant section was washed with PB and distilled water, placed on a gelatin-coated slide, and embedded in a mounting agent (Permount; Fisher Scientific, New Jersey, CA, USA). The specificity of each antibody was confirmed by the negativity of the marker when the primary antibody was not applied.

We observed the *c*-Fos immunoreaction in the SolM using a macroscopic latch as an anchoring point (with the upper end of the area postrema regarded as the latch). In the 360-μm section toward the tail from the latch, we counted the number of visible *c*-Fos cells using a microscope fitted with a × 10 objective lens, thereby calculating the mean value of the eight sections.

2.6. Statistical analysis

For statistical analysis, we carried out a paired *t*-test, two-way analysis of variance, and multiple comparisons in repeated measurements using SPSSII for Windows. Dunnett's method was employed for the multiple comparisons. The results were indicated by mean values ± standard deviations, with the significance level of <5% regarded as statistically significant.

3. Results

3.1. Hemodynamic and autonomic response to acute hemorrhage

Significant interactions were observed among MAP, HR, SBP-LF, and HR-HF (Figure.

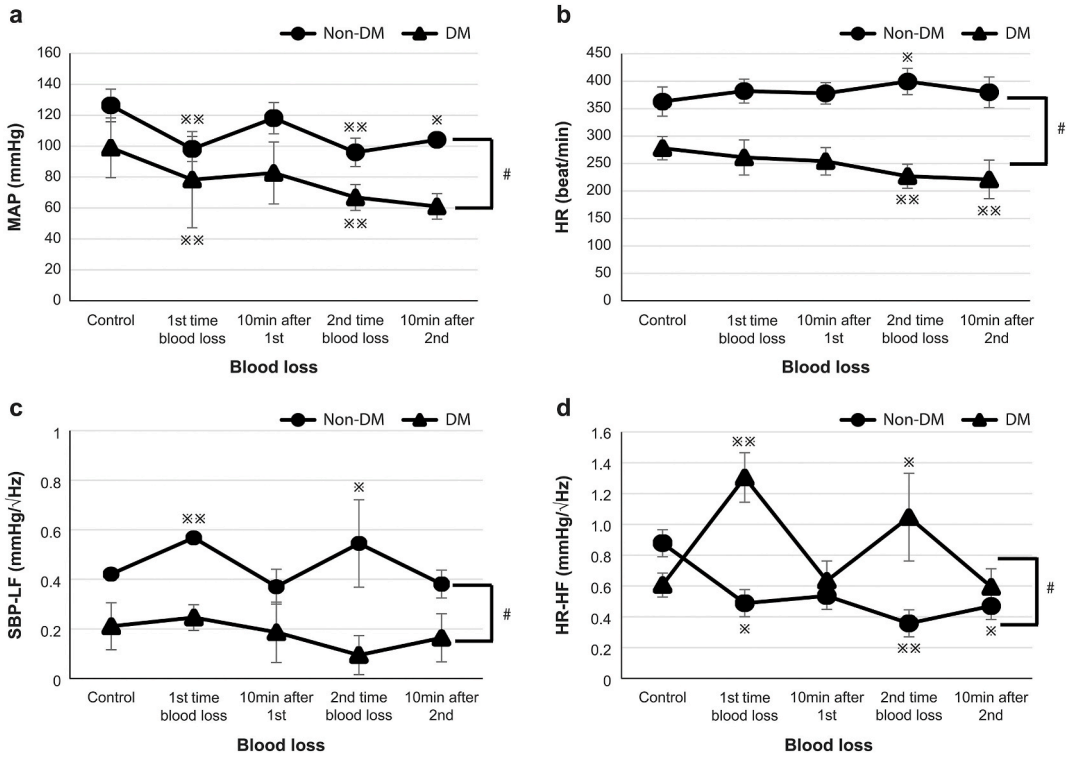


Fig. 1. Changes in MAP (a), HR (b), SBP-LF (c), and HR-HF (d) response to acute hemorrhage. Values are shown as mean ± SD (n = 8 in each group), ※※ $p < 0.01$ vs. control, ※ $p < 0.05$ vs. control, #$p < 0.05$ significant interaction (different response between DM and non-DM as evaluated using repeated measures analysis of variance). 【MAP: p = 0.002, F = 5.34, F (0.95) = 2.75, HR: p = 0.04, F = 3.12, F (0.95) = 2.75, SBP-LF: p = 0.04, F = 2.86, F (0.95) = 2.75, HR-HF: p = 0.00003, F = 10.56, F (0.95) = 2.75】.

1). In DM rats, MAP and HR continuously decreased during blood loss. However, in non-Dm rats, both MAP and HR were recovered to control even in 10 min after 2nd blood loss. SBP-LF increased after 1st time blood loss and 2nd time blood loss in non-DM rats but not in DM rats. Conversely, HR–HF increased after 1st time blood loss and 2nd time blood loss in DM rats but not in non-DM rats (see Fig. 1).

3.2. Hemodynamic and autonomic response to continuous phenylephrine infusion

Significant interactions were observed among MAP, SBP-LF, and HR-HF (Fig. 2). In DM rats, MAP continuously increased after phenylephrine infusion. In non-DM rats, HR significantly decreased 30 min after phenylephrine infusion; however, this was not observed in DM rats. SBP-LF significantly increased at 10 and 30 min after phenylephrine administration in non-DM rats but not in DM rats. HR–HF significantly increased at 10 and 30 min after phenylephrine infusion in non-DM rats and at 30 min after phenylephrine infusion in DM rats.

3.3. Effects of hemorrhage and continuous phenylephrine infusion on c-Fos in the SolM

C-Fos expression during blood loss and continuous phenylephrine infusion in the SolM are described in Fig. 3. The number of Fos expressions during blood loss was 62.8 ± 6.8 in non-DM rats (Fig. 3**a**) and 84.5 ± 11.7 in DM rats (Fig. 3**b**), which was significantly different. Additionally, the number of Fos expressions during continuous phenylephrine infusion was 104.25 ± 8.1 in non-DM rats (Fig. 3**c**) and 62.0 ± 11.5 in DM rats. (Fig. 3**d**), which was significantly different.

4. Discussion

This study aimed to elucidate the involvement of the B–J reflex as a reaction to an acute hemorrhagic event in DM and non-DM rats by comparing the expression of *c*-Fos in the SolM, the primary relay nucleus of the pressure receptor reflex. Injection of STZ is a well-recognized technique for inducing DM in rats. Indeed, this model has been commonly applied to study the pathological mechanisms of

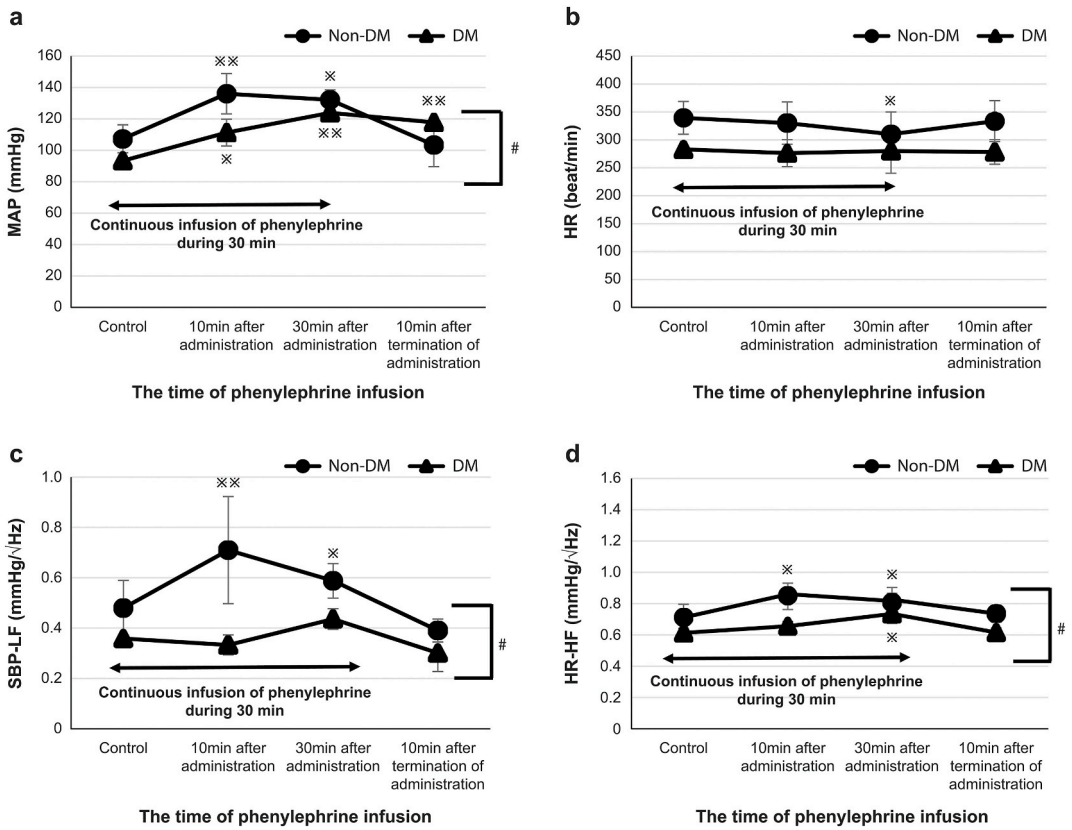


Fig. 2. Changes in MAP (a), HR (b), SBP-LF (c), and HR-HF (d) response to continuous phenylephrine infusion. Values are shown as mean ± SD (n = 8 in each group), ※※ $p < 0.01$ vs. control, ※ $p < 0.05$ vs. control, #$p < 0.05$ significant interaction (different response between DM and non-DM as evaluated using repeated measures analysis of variance). 【MAP: $p = 0.007$, $F = 5.72$, $F(0.95) = 3.28$, HR: $p = 0.80$, $F = 0.24$, $F(0.95) = 3.28$, SBP-LF: $p = 0.02$, $F = 4.18$, $F(0.95) = 3.28$, HR-HF: $p = 0.0001$, $F = 12.7$, $F(0.95) = 3.28$】.

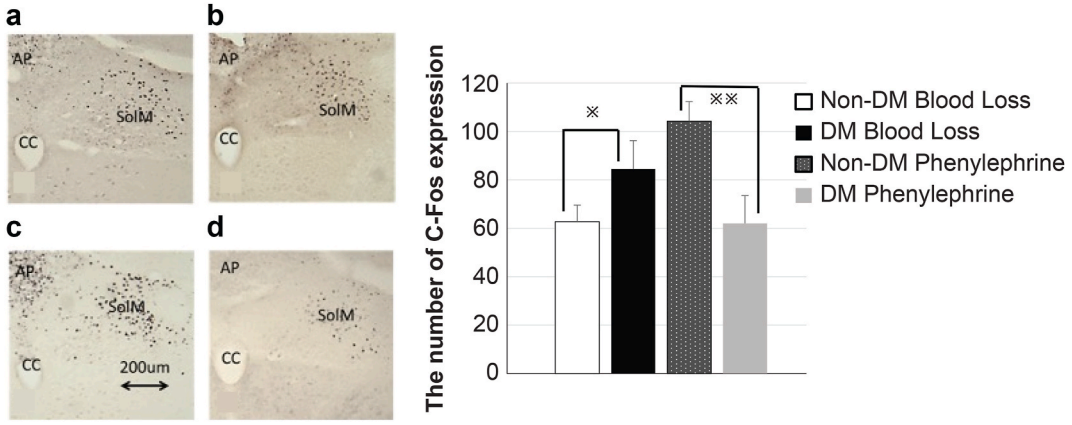


Fig. 3. (Left) Effect of hemorrhage and continuous phenylephrine infusion on expression of *c*-FOS in the SolM. (a) *c*-Fos to blood loss in non-DM; (b) *c*-Fos to blood loss in DM; (c) *c*-Fos to continuous infusion of phenylephrine in non-DM; (d) *c*-Fos IR to continuous infusion of phenylephrine in DM; AP: Area postrema; CC: Central canal. (Right) Effect of hemorrhage and continuous phenylephrine infusion on *c*-Fos. Expression in the SolM. Values are shown as mean ± SD (n = 8 in each group), ※※ $p < 0.01$ WKY vs. DM, ※ $p < 0.05$ WKY vs. DM.

different adverse events, which includes autonomic neuropathy [8].

The principal finding was that the expression of *c*-Fos in the SolM in response to acute blood loss was increased in DM rats. The same phenomenon was observed in non-DM rats in response to continuous phenylephrine infusion.

During blood loss, a significant reduction in MAP was detected in DM rats. This phenomenon indicated on a transient reduction in the sympathetic drive. On the contrary, MAP was recovered after blood loss by activating the sympathetic drive in the non-DM rats. This result is consistent with our previous study [4].

DM rats showed significant bradycardia during blood loss, and this phenomenon was connected with elevated parasympathetic drive. This early parasympathetic drive in response to acute hemorrhage differed from our previous study [4]. However, an increase in HR was found in non-DM rats, which was linked to a transient deactivation of parasympathetic pathway and sympathetic stimulation. This difference in reaction between non-DM and DM rats connected with diabetic autonomic neuropathy may be provoked by the B–J reflex. The B–J reflex was originally believed to serve for protection, as it has anti-ischemic action during a severe hypovolemic state by decreasing its workload [3,22]. However, a previous report demonstrated that facilitated B–J reflex is observed in severe symptomatic hypotension with bradycardia during dialysis and that the patients who were susceptible to dialysis hypotension have a history of diabetes [23]. This phenomenon proposes that diabetic neuropathy may be linked to the strengthened B–J reflex and that excessive activation of the B–J reflex may cause severe bradycardia hypotension [4].

During continuous phenylephrine infusion, the increase in MAP in non-DM rats was associated with a transient increase in the sympathetic drive, whereas a significant reduction in HR was simultaneously detected due to the parasympathetic activation. This phenomenon can be explained by the baroreflex reaction (BRS). BRS is one of the defense reflexes that form a short-term regulatory system for cardiovascular stability [24].

Conversely, in DM rats, the MAP and HR response to continuous phenylephrine infusion were not similar to those in non-DM rats. We described the reduction of the baseline value of SBP-LF and HR–HF in a previous study [4], and some reports indicated an impairment of BRS caused because of diabetes [25–27]. This study showed that in emergencies, such as an elevation of blood pressure, the normally functional parasympathetic activity essentially did not function due to diabetic neuropathy.

In DM rats, *c*-Fos in the SolM increased in response to hemorrhage compared with non-DM rats. This phenomenon indicated that parasympathetic activity was accelerated in a critical situation, such as hemorrhage, without accelerating sympathetic activity, and this was distinguished by noticeable parasympathetic activity (HR–HF). This finding says in favor for an enhancement of the B–J reflex in DM rats. Moreover, this finding proves that the afferent fibers are affected by autonomic neuropathy. Under normal circumstances, the B–J reflex has a stimulative impact on the circulatory system. A previous report indicated that dogs with bradycardia had better outcomes than dogs with tachycardia that were experiencing massive hemorrhage [3]. However, the B–J reflex may lead to a critical situation under abnormal circumstances such as autonomic neuropathy in this study. This study proved that DM does not equally impair all the arterial reflexes. This finding corresponds to the literature that the impairments in vascular reactivity due to DM may be different depending on the site of the artery or gender [28,29].

A similar *c*-Fos increase in response to acute blood loss in DM rats was seen in response to continuous phenylephrine infusion in non-DM rats. This immunoreaction in non-DM rats indicated the parasympathetic acceleration resulting from the acute elevation of BP, which is a defense reflex.

There are some limitations in this study. First, in DM rats, the reaction to acute blood loss in SBP-LF, HR-HF, and HR differed from our previous study [4]. Particularly, the acceleration of HR–HF occurred much earlier. As a result, the HR reaction to blood loss was seen at an early stage. Although I could not obtain the same result, this was due to the severity of the autonomic neuropathy. Second, it is difficult to directly apply these results in a clinical setting because the activation of DM in rats causes a single insult to the pancreatic β-cells and transitory hyperglycemia, whereas in humans, the causes of DM vary and most patients with DM maintain control of their

blood glucose levels.

5. Conclusions

In our previous study showed that DM rats were sensitive to acute blood loss, and the circulatory system easily collapsed with accelerating parasympathetic activity. In addition to the previous study, this study elucidated the involvement of the B–J reflex in response to acute hemorrhage in DM and control rats by comparing hemodynamic and autonomic changes and expression of *c*-Fos in the nucleus of the solitary medial region SolM.

Funding

This work was supported by JSPS KAKENHI Grant Number JP23792335.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contribution statement

Aiji Sato (Boku): Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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.

Acknowledgements

The author would like to thank Enago (www.enago.jp) for the English language review.

References

[1] N. Singh, Diabetes, heart rate and mortality, J. Cardiovasc. Pharmacol. Therapeut. 7 (2002) 117–129, https://doi.org/10.1177/107424840200700208.
[2] I.V. Aoron, Z. Dan, Diabetic cardiovascular autonomic neuropathy, Circulation 115 (2007) 387–397, https://doi.org/10.1161/CIRCULATIONAHA.106.634949.
[3] M. Kawase, T. Komatsu, K. Nishiwaki, T. Kimura, Y. Fujiwara, T. Takahashi, Y. Shimada, Heart rate variability during massive hemorrhage and progressive hemorrhagic shock in dogs, Can. J. Anaesth. 47 (2000) 807–814, https://doi.org/10.1007/BF03019486.
[4] A. Boku, M. Sugimura, Y. Morimoto, H. Hanamoto, H. Niwa, Hemodynamic and autonomic response to acute hemorrhage in streptozotocin-induced diabetic rats, Cardiovasc. Diabetol. 9 (2010) 78, https://doi.org/10.1186/1475-2840-9-78.
[5] N.H. Secher, J. Jacobsen, D.B. Friedman, S. Matzen, Bradycardia during reversible hypovolaemic shock: associated neural reflex mechanisms and clinical implications, Clin. Exp. Pharmacol. Physiol. 19 (1992) 733–743, https://doi.org/10.1111/j.1440-1681.1992.tb00411.x.
[6] A.L. Mark, The Bezold-Jarisch reflex revisited: clinical implications of inhibitory reflexes originating in the heart, J. Am. Coll. Cardiol. 1 (1983) 90–102, https://doi.org/10.1016/S0735-1097(83)80014-X.
[7] S.M. Kinsella, J.P. Tuckey, Perioperative bradycardia and asystole: relationship to vasovagal syncope and the Bezold-Jarisch reflex, Br. J. Anaesth. 86 (2001) 859–868, https://doi.org/10.1093/bja/86.6.859.
[8] P. Dall'Ago, V.O. Silva, K.L. De Angelis, M.C. Irigoyen, R. Fazan, H.C. Salgado, Reflex control of arterial pressure and heart rate in short-term streptozotocin diabetic rats, Braz. J. Med. Biol. Res. 35 (2002) 843–849, https://doi.org/10.1590/S0100-879X2002000700013.
[9] R. Nagai, S. Nagata, F. Fukuya, J. Higaki, H. Rakugi, T. Ogihara, Changes in autonomic activity and baroreflex sensitivity with the hypertension process and age in rats, Clin. Exp. Pharmacol. Physiol. 30 (2003) 419–425, https://doi.org/10.1046/j.1440-1681.2003.03852.x.
[10] D.R. Brown, L.V. Brown, A. Patwardhan, D.C. Randall, Sympathetic activity and blood pressure are tightly coupled at 0.4 Hz in conscious rats, Am. J. Physiol. 267 (1994) R1378–R1384, https://doi.org/10.1152/ajpregu.1994.267.5.R1378.
[11] C. Cerutti, M.P. Gustin, C.Z. Paultre, M. Lo, C. Julien, M. Vincent, J. Sassard, Autonomic nervous system and cardiovascular variability in rats: a spectral analysis approach, Am. J. Physiol. 261 (1991) H1292–H1299, https://doi.org/10.1152/ajpheart.1991.261.4.h1292.
[12] M. Pagani, F. Lombardi, S. Guzzetti, O. Rimoldi, R. Furlan, P. Pizzinelli, G. Sandrone, G. Malfatto, S. Dell'orto, E. Piccaluga, Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog, Circ. Res. 59 (1986) 178–193, https://doi.org/10.1161/01.res.59.2.178.
[13] P.B. Persson, H. Stauss, O. Chung, U. Wittmann, T. Unger, Spectrum analysis of sympathetic nerve activity and blood pressure in conscious rats, Am. J. Physiol. 263 (1992) H1348–H1355, https://doi.org/10.1152/ajpheart.1992.263.5.H1348.
[14] J. Hayano, S. Mukai, M. Sakakibara, A. Okada, K. Takata, T. Fujinami, Effects of respiratory interval on vagal modulation of heart rate, Am. J. Physiol. 267 (1994) H33–H40, https://doi.org/10.1152/ajpheart.1994.267.1.H33.
[15] G.G. Berntson, J.T. Cacioppo, K.S. Quigley, Respiratory sinus arrhythmia: autonomic origins, physiological mechanisms, and psychophysiological implications, Psychophysiology 30 (1993) 183–196, https://doi.org/10.1111/j.1469-8986.1993.tb01731.x.
[16] D.L. Eckberg, Human sinus arrhythmia as an index of vagal cardiac outflow, J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 54 (1983) 961–966, https://doi.org/10.1152/jappl.1983.54.4.961.
[17] D.C. Randall, D.R. Brown, R.M. Raisch, J.D. Yingling, W.C. Randall, SA nodal parasympathectomy delineates autonomic control of heart rate power spectrum, Am. J. Physiol. 260 (1991) H985–H988, https://doi.org/10.1152/ajpheart.1991.260.3.H985.
[18] D.C. Randall, W.C. Randall, D.R. Brown, J.D. Yingling, R.M. Raisch, Heart rate control in awake dog after selective SA-nodal parasympathectomy, Am. J. Physiol. 262 (1992) H1128–H1135, https://doi.org/10.1152/ajpheart.1992.262.4.H1128.

[19] B. Pomeranz, R.J. Macaulay, M.A. Caudill, I. Kutz, D. Adam, D. Gordon, K.M. Kilborn, A.C. Barger, D.C. Shannon, R.J. Cohen, Assessment of autonomic function in humans by heart rate spectral analysis, Am. J. Physiol. 248 (1985) H151–H153, https://doi.org/10.1152/ajpheart.1985.248.1.H151.
[20] C. Cruz, L.G. Bonagamba, J.E. Stern, B.H. Machado, Fos expression in the NTS in response to peripheral chemoreflex activation in awake rats, Auton. Neurosci. 152 (2010) 27–34, https://doi.org/10.1016/j.autneu.2009.08.016.
[21] S.P. Hunt, A. Pini, G. Evan, Induction of c-fos-like protein in spinal cord neurons following sensory stimulation, Nature 328 (1987) 632–634, https://doi.org/10.1038/328632a0.
[22] A.I. Batchinsky, W.H. Cooke, T.A. Kuusela, B.S. Jordan, J.J. Wang, L.C. Cancio, Sympathetic nerve activity and heart rate variability during severe hemorrhagic shock in sheep, Auton. Neurosci. 136 (2007) 43–51, https://doi.org/10.1016/j.autneu.2007.03.004.
[23] M.G. Barnas, W.H. Boer, H.A. Koomans, Hemodynamic patterns and spectral analysis of heart rate variability during dialysis hypotension, J. Am. Soc. Nephrol. 10 (1999) 2577–2584, https://doi.org/10.1681/ASN.V10122577.
[24] P. Andriessen, B.J. Janssen, R.C. Berendsen, S.B. Oetomo, P.F. Wijn, C.E. Blanco, Cardiovascular autonomic regulation in preterm infants: the effect of atropine, Pedatric. Res. 56 (2004) 939–946. https://doi:10.1203/01.PDR.0000145257.75072.BB.
[25] C.Y. Maeda, T.G. Fernandes, H.B. Timm, M.C. Irigoyen, Autonomic dysfunction in short-term experimental diabetes, Hypertension 26 (1995) 1100–1104, https://doi.org/10.1161/01.hyp.26.6.1100.
[26] J.M. do Carmo, J.E. Hall, A.A. da Silva, Chronic central leptin infusion restores cardiac sympathetic-vagal balance and baroreflex sensitivity in diabetic rats, Am. J. Physiol. Heart Circ. Physiol. 295 (2008) H1974–H1981, https://doi.org/10.1152/ajpheart.00265.2008.
[27] K.S. Chang, D.D. Lund, Alterations in the baroreceptor reflex control of heart rate in streptozotocin diabetic rats, J. Mol. Cell. Cardiol. 18 (1986) 617–624, https://doi.org/10.1016/s0022-2828(86)80969-5.
[28] A.M. Gurney, F.C. Howarth, Effects of streptozotocin-induced diabetes on the pharmacology of rat conduit and resistance intrapulmonary arteries, Cardiovasc. Diabetol. 8 (2009) 4, https://doi.org/10.1186/1475-2840-8-4.
[29] P. Failli, C. Alfarano, S. Franchi-Micheli, E. Mannucci, E. Cerbai, A. Mugelli, L. Raimondi, Losartan counteracts the hyper-reactivity to angiotensin II and ROCK1 over-activation in aortas isolated from streptozotocin-injected diabetic rats, Cardiovasc. Diabetol. 8 (2009) 32, https://doi.org/10.1186/1475-2840-8-32.