Changes of Blood Pressure Rhythm and Clock Protein Expression Levels in Spontaneously Hypertensive Rats After Ischemia-Reperfusion

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Research Article

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Abstract

Objective

A variety of circadian patterns of blood pressure after ischemic stroke in patients with essential hypertension appear to be a potential risk of stroke recurrence, but the mechanism is still unclear. This study intends to reveal the changes in blood pressure rhythm and circadian clock protein expression levels in spontaneously hypertensive rats (SHR) after ischemia-reperfusion, and the relationship between the two.

Methods

Using the SHR middle cerebral artery occlusion experimental model, the systolic blood pressure was continuously monitored for 24 hours after the operation to observe the blood pressure rhythm. The rat tail vein blood was taken every 3h, and the serum CLOCK, BMAL1, PER1 and CRY1 protein expression levels were detected by Elisa. Pearson correlation analysis counted the relationship between SHR blood pressure rhythm and circadian clock protein fluctuation after ischemia-reperfusion.

Results

The proportion of abnormal blood pressure patterns in the SHR + tMCAO group was significantly higher than that in the SHR group, the serum CLOCK expression was relatively constant, and the circadian rhythm of BMAL1, PER1 and CRY1 protein expression changed significantly. Pearson analysis showed that PER1 protein level was negatively correlated with dipper ($r = -0.565, P = 0.002$) and extreme-dipper ($r = -0.531, P = 0.001$) blood pressure, and was significantly positively correlated with non-dipper blood pressure ($r = 0.620, P < 0.001$).

Conclusion

The rhythm pattern of blood pressure after ischemia-reperfusion in SHR is obviously disordered, and it is closely related to the regulation of Per1 gene.

Introduction

Ischemic stroke has become a major disease threatening human life and health with its high morbidity, high disability and high mortality rate, and it has caused a serious burden on patients’ families and society (Ma et al. 2020). However, stroke is preventable and controllable. A large number of clinical studies and practices have shown that the morbidity, disability and mortality rate will be greatly reduced by intervening or blocking the effect of its pathogenic factors (Sarikaya et al. 2015; Guzik and Bushnell 2017). Leonardi-Bee and other studies have shown that 75% of stroke patients have a history of
hypertension (Leonardi-Bee et al. 2002), and hypertension is the primary risk factor for stroke. It has been confirmed that both the increase in blood pressure and the increase in blood pressure variability (BPV) are independent risk factors for the occurrence and development of stroke. BPV is not only significantly related to stroke prognosis and cognitive dysfunction after stroke, but the increase in BPV at night increases the risk of stroke recurrence (Pringle et al. 2003). With the application of 24h ambulatory blood pressure monitoring, the influence of blood pressure circadian rhythm changes on stroke has gradually attracted attention. The circadian rhythm of blood pressure is repeated every 24h in healthy people, mostly in the dipper blood pressure mode (the night blood pressure drops by 10%-20%) (Dadlani et al. 2019). When the blood pressure rhythm variation leads to the disappearance of the normal blood pressure rhythm of the human body, there is an excessive decrease in the night, a reverse increase, or a low daytime blood pressure difference (referred to as extreme-dipper, reverse-dipper, and non-dipper blood pressure modes, respectively). The blood pressure rhythm pattern will affect the self-regulation of human hemodynamic function, leading to target organ damage. Many scholars define extreme-dipper type as night blood pressure drop > 20%, non-dipper type is defined as night blood pressure drop < 10%, and reverse-dipper type is defined as night blood pressure higher than daytime (Shimamoto et al. 2014; Song et al. 2015; Cuspidi et al. 2019). However, there is still a lack of in-depth understanding of the causes and mechanisms of these abnormal blood pressure rhythms.

Recent studies have found that mammalian circadian clock genes play a key role in the regulation of various physiological cycles (including blood pressure rhythm) (Hastings et al. 2019). The core component of the circadian clock is a set of transcription factors that regulate gene expression. They mainly play a role in a series of feedback loops to drive the circadian expression of core clock genes and a large number of target genes. The mammalian clock genes Bmal1 and Clock constitute a positive feedback loop for circadian rhythm regulation, driving the rhythmic transcription of Period (encoding PER1-3) and Cryptochrome (encoding CRY1, 2) (Pilorz et al. 2018). In a negative feedback loop, PER and CRY can be phosphorylated by casein kinase 1 (CK1) and other kinases used for degradation, and combine to form a CRY-PER-CK1 protein complex to antagonize the effect of BMAL1/CLOCK (Husse et al. 2015). The suprachiasmatic nucleus of the hypothalamus is an advanced center that regulates the rhythm of the biological clock (Husse et al. 2015).

The latest research shows that tumors, immune abnormalities, endocrine diseases, etc. are all related to the circadian clock regulation function disorder (Gamble et al. 2014; Sulli et al. 2019; Lananna and Musiek 2020). Then, is there any relationship between the blood pressure rhythm disorder in patients with essential hypertension after stroke and the regulation of biological clock genes? Based on existing research results, we hypothesize that circadian clock genes are involved in the process of blood pressure disturbances in patients with essential hypertension after ischemic brain injury. To verify this hypothesis, this study uses SHR as a model of essential hypertension. We observed the changes in the blood pressure rhythm and the circadian clock protein expression level of different Zeitgeber Time (ZT) within 24 hours of SHR after transient middle cerebral artery occlusion (tMCAO) to clarify the characteristics of blood pressure rhythm changes after stroke and their relationship with the circadian rhythm changes in hypothalamic brain tissue and serum circadian clock key regulatory gene expression protein levels.
Materials And Methods

Animals and Groups

30 SPF male SHR rats aged 10 weeks, animal license number: SCXK (Beijing) 2016-0011, weighing 180g-220g, purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Place it in a standard 12h: 12h light-dark environment, maintain a room temperature of 20-25 ℃, relative humidity (50 ± 10)%., free drinking and eating. Turn on the lights at 7:00 in the morning and turn off the lights at 19:00 at night. ZT0 is defined as the time when the lights are turned on. Before entering the experimental protocol, all rats were fed continuously for 1 week to minimize stress to ensure consistency. In the experiment, the rats were randomly divided into 2 groups: SHR group, SHR+tMCAO group, 15 rats in each group. The protocol used in this study and the animal ethics involved have been approved by the Animal Ethics Committee of Xuzhou Medical University.

Experimental Reagents and Instruments

DEM animal physiological signal telemetry system (DSI, American); microplate reader, electrophoresis instrument, film transfer instrument (Bio-Rad, American); gel imaging analysis system (ProteinSimple, American). Rabbit anti-mouse CRY1, PER1, CLOCK and BMAL1 antibodies (Abclonal, China). FITC labeled goat anti-rabbit IgG (Shanghai Aibixin Biotechnology Co., Ltd.); BCA kit (Shanghai Biyuntian Biotechnology Co., Ltd.). PVDF membrane 0.45 μm (Santa Cruz, USA); ECL chemiluminescence kit (Shanghai Aibixin Biotechnology Co., Ltd.); PER1, BMAL1, CLOCK, CRY1 Elisa kit (Wuhan Yiaibo Company).

Ambulatory Blood Pressure Measurement

A blood pressure remote sensing monitoring system was established. The rats were anesthetized with 1% sodium pentobarbital (0.4 ml/100 g) intravenously, and an incision of about 2 to 3 cm was made at the midline of the abdomen to separate the abdominal aorta. Clamp the blood vessel temporarily, insert the needle into the artery, and insert the implant catheter into the abdominal aorta about 5~6 mm (against the direction of blood flow) along the hole. Fix the catheter with fiber sheet and adhesive glue, and then flush the blood vessel with 2% lidocaine to avoid vasoconstriction and spasm. The intestine segment is returned to the abdominal cavity, and the implant body is fixed on the abdominal wall and the abdomen is closed. After the operation, the rats were reared in a single cage and moved freely without restraint. After 7 to 10 days, the blood pressure and heart rate became stable. The cage was placed on the receiving board to collect the data, which was converted by the signal processor and analyzed by relevant software to monitor the changes in blood pressure. Since the circadian rhythm of rats is opposite to that of humans, the rate of blood pressure drop is (average night blood pressure-average day blood pressure)/average night blood pressure × 100%, which is a quantitative index to judge the circadian rhythm of blood pressure.

Preparation and Verification of Rat tMCAO Model
SHR was anesthetized with 10% chloral hydrate (3.5mL/kg) intraperitoneally. Routinely disinfect the neck skin, cut along the midline of the neck. Separately expose the left common carotid artery, left internal carotid artery, and left external carotid artery. The vascular clamp temporarily clamps the left internal carotid and common carotid artery, and advances the thread tether through the incision on the left external carotid artery and inserts it into the left internal carotid artery until a slight resistance is felt. After 2 hours, the thread plug was withdrawn, and the cerebral blood flow began to reperfusion spontaneously. The operation of the rats in the sham operation group was similar to that of the ischemia-reperfusion group, but no thread plug was inserted into the left internal carotid artery. All experimental animals eat and drink freely after being awake. Nerve injury behavior score (Longa classification method) was used to score the two groups of rats. The specific scoring principles are as follows: 0 points: no defect; 1 point: the contralateral forelimb cannot be fully extended; 2 points: turning to the opposite side while walking; 3 points: falling to the opposite side while walking; 4 points: unable to walk spontaneously. TTC staining was used to evaluate the degree of cerebral infarction, red is normal brain tissue, and white is post-infarction brain tissue.

Elisa Detects the Content of Serum Clock Protein

Take blood samples every 3h. Fix the rat in a fixed rat cage, expose the tail of the rat outside the cage, and place it in hot water at 40°C for 5-10s. Sterilize the tail with an alcohol cotton ball, then pierce the intravenous needle a few centimeters up from the tip of the tail and pull it out immediately. Take 0.3 mL of blood each time, centrifuge at 4000 rpm for 15 minutes, take the serum and store in the refrigerator at -80°C. Take out the kit to equilibrate at room temperature for 30 minutes, and at the same time take out the blood sample and place it at room temperature. First configure the standard: Take 150μL of the standard and add 150μL of the standard diluent, and dilute 5 times in sequence. Add 50μL of the standard and 40μL of the sample to each reaction well. Make a duplicate hole for the standard and 3 holes for the sample. Add 10 μL of antibody to the sample wells. Add 50μL of streptavidin-HRP to the standard and sample respectively, cover with sealing film, shake gently to mix, and incubate at 37°C for 1h. When the time is up, carefully uncover the sealing film, discard the liquid, and spin dry. Add 200μL of washing solution to each well, let it stand for 30s and discard it, repeat this 4 times, and pat dry. Then add 100 μL of color developing solution to each well, gently shake and mix, incubate at 37°C for 30 minutes in the dark, and then add stop solution. Measure the absorbance of each well at 450nm wavelength with a microplate reader, and process the data with Excel.

Statistical Methods

Continuous variables are expressed as mean±standard deviation (Mean±SD). Use one-way analysis of variance to compare the differential expression of proteins between groups. The relationship between the two variables uses Pearson correlation analysis. Two-sided test $P<0.05$, indicating that there are statistical differences between the two groups. All statistical analyses were performed using SPSS 26.0.

Results
Establishment and Verification of tMCAO-SHR Experimental Model

The reliability of the animal experimental model was verified by two methods: neurological evaluation and TTC staining of brain slices. SHR rats showed significant neurological deficit symptoms after tMCAO, manifested in severe walking obstacles, falling to the opposite side, and flexion of the fore and hind limbs on the opposite side of ischemia (Fig. 1). After statistics of behavioral scores, it was found that the scores of rats in the SHR group were 0 at each time (Table 1). The rats in the SHR + tMCAO group had neurological impairment at the three time points, with varying degrees. Compared with the SHR group, \( P < 0.05 \). The brain was taken out, cut into 5 2mm thick coronal sections, and stained with TTC, the results showed obvious cerebral infarction lesions (Fig. 1D).

| Groups            | Number | 6h          | 12h          | 24h          |
|-------------------|--------|-------------|--------------|--------------|
| SHR               | 15     | 0           | 0            | 0            |
| SHR + tMCAO       | 15     | 2.19 ± 0.39*| 2.44 ± 0.50* | 1.81 ± 0.63* |

Compared with SHR group, \( *P < 0.05 \).

Tmcao-shr Presents A Variety Of Blood Pressure Rhythm Disorders

Monitor the 24h ambulatory blood pressure of the two groups of rats, each start time is 7:00, day (7:00–19:00), night (19:00–7:00 the next day), the main monitoring parameter is Systolic blood pressure. In the SHR group, a variety of blood pressure patterns can be observed in rats, including dipper (53%), non-dipper (27%), extreme-dipper (13%), and reverse-dipper (7%). SHR is dominated by dippers, with an average blood pressure value of 167.11 mmHg in 24 hours (Table 2). The blood pressure during the daytime rest period was significantly lower than that during the nocturnal period \( (P < 0.01) \), and the blood pressure drop rate during the day was 13.71%. The average systolic blood pressure at night was 178.88 mmHg, there were two peaks of systolic blood pressure around 21:00 and 2:00, and a trough appeared around 14:00 (Fig. 2).

In contrast, the degree of blood pressure disorder in the SHR + tMCAO group was significantly increased, mainly non-dipper type, the proportion was as high as about 40%. The proportions of extreme-dipper and reverse-dipper have also increased significantly, 27% and 13% respectively. The proportion of dipper blood pressure dropped to 20% (Table 2). In the dipper mode, the blood pressure fluctuation rhythm of the SHR + tMCAO group is similar to that of the SHR group, showing a "double peak and one valley" rhythm pattern, with peaks at around 8:00 and 12:00, and troughs at around 13:00, which is slightly earlier than
the SHR group as a whole (Fig. 2A). In the non-dipper mode, the difference between the day and night systolic blood pressure of the two groups was significantly reduced, and the daytime blood pressure drop rate (3.91%) of the SHR + tMCAO group was lower than that of the SHR group (6.35%), and the diurnal fluctuation range was greater (Fig. 2B). In the extreme-dipper mode, the night systolic blood pressure of the SHR + tMCAO group and the SHR group increased significantly, with an average value of 192.88 mmHg and 190.42 mmHg, respectively. The blood pressure drop rate during the day was 21.54% and 21.11%, and the peak value of the SHR + tMCAO group appeared earlier. Blood pressure fluctuations are more obvious (Fig. 2C). In the reverse-dipper mode, the systolic blood pressure of the two groups during the day exceeded that of the night, showing a phenomenon of high daytime and low night (Table 2).

| Groups     | Type       | N(%) | 24hSBP(mmHg) | dSBP(mmHg) | nSBP(mmHg) | dBPDR (%) |
|------------|------------|------|--------------|------------|------------|-----------|
| SHR        | Dipper     | 8(53%) | 167.11 ± 13.24 | 154.35 ± 4.42 | 178.88 ± 5.52 | 13.71     |
|            | Non-dipper | 4(27%) | 166.65 ± 6.82  | 161.69 ± 3.96  | 171.23 ± 5.58  | 5.57      |
|            | Extreme-dipper | 2(13%) | 171.12 ± 20.78 | 150.21 ± 6.07 | 190.42 ± 4.49 | 21.11     |
|            | Reverse-dipper | 1(7%)  | 166.76 ± 7.06  | 170.00 ± 7.47  | 163.77 ± 5.06  | -3.81     |
| SHR + tMCAO| Dipper     | 3(20%) | 168.84 ± 12.46 | 157.58 ± 6.47 | 179.23 ± 5.92 | 12.08     |
|            | Non-dipper | 6(40%) | 168.49 ± 9.33  | 166.00 ± 9.80  | 170.79 ± 8.23  | 2.81      |
|            | Extreme-dipper | 4(27%) | 172.94 ± 23.33 | 151.33 ± 9.86 | 192.88 ± 11.35 | 21.54     |
|            | Reverse-dipper | 2(13%) | 169.32 ± 6.82  | 172.46 ± 6.99  | 166.42 ± 5.19  | -3.63     |

24hSBP: all-day average systolic blood pressure, dSBP: daytime average systolic blood pressure, nSBP: nighttime average systolic blood pressure; dBPDR: Daytime blood pressure drop rate.

Circadian Rhythm Changes Occur in tMCAO-SHR Serum Clock Protein Levels

In order to observe the changes of circadian clock proteins after SHR ischemia-reperfusion, this experiment performed tMCAO operation at ZT0, and Elisa was used to detect the changes of core circadian clock proteins CLOCK, BMAL1, PER1 and CRY1. The results showed that the average CLOCK protein levels of the SHR group and SHR + tMCAO group were similar, 6.18 ± 0.57ng/mL and 6.19 ± 0.78ng/mL, respectively, with relatively constant protein expression levels within 24h (Fig. 3A). The BMAL1 protein level fluctuates circadian rhythm. The SHR group has a peak around ZT12 (Fig. 3B), with a peak of 6.72 ± 0.29ng/mL. The peak of BMAL1 protein in the SHR + tMCAO group was advanced and appeared around ZT6 (13:00, 6h after ischemia) (P < 0.01), with a peak value of 7.87 ± 0.5ng/mL.
followed by ZT24 (7:00 the next day, 24h after ischemia) dropped to a trough (P < 0.01). The PER1 protein level in the SHR group gradually decreased during the ZT0-ZT15 period, dropped to a minimum at ZT15, and then gradually increased (Fig. 3C). In the SHR + tMCAO group, the PER1 protein level of rats continued to increase during ZT6-ZT12 (Fig. 3C), and reached a peak around ZT12 (19:00, 12h after ischemia) (P < 0.001), and then Decrease gradually. The CRY1 protein level in the SHR group gradually increased during the ZT3-ZT15 period, ZT15 reached the peak, and then gradually decreased. The expression of CRY1 protein in the SHR + tMCAO group was higher at ZT0, and then gradually decreased with time (Fig. 3D).

The Relationship Between SHR Serum Clock Protein Level and Blood Pressure Circadian Rhythm After Ischemia-Reperfusion

In order to further explore the mechanism of circadian rhythm disturbance in SHR blood pressure after ischemia-reperfusion, this study analyzed the correlation between the expression levels of serum core clock proteins CLOCK, BMAL1, CRY1, PER1 and blood pressure circadian fluctuations. Take the blood pressure and circadian clock protein values of rats in the SHR + tMCAO group recorded every 3h during the time period ZT0-ZT24 for statistical analysis. The results showed that the changes in CLOCK, BMAL1, and CRY1 protein levels after ischemia-reperfusion were not correlated with the four blood pressure patterns in rats. However, changes in PER1 protein levels were negatively correlated with dipper blood pressure ($r=-0.565$, $P=0.002$), negatively correlated with extreme-dipper blood pressure ($r=-0.531$, $P=0.001$), and significantly positive with non-dipper blood pressure Correlation ($r=0.620$, $P=0.002$), has nothing to do with reverse-dipper blood pressure ($P>0.05$) (Fig. 4).

Discussion

The pathophysiological process of blood pressure circadian rhythm disturbance in patients with essential hypertension stroke is composed of several molecular events that lead to brain damage. In order to explore the specific mechanism of blood pressure disorder after stroke, this study uses SHR to simulate human essential hypertension and tMCAO to simulate human ischemic stroke. Based on the current research results, SHR shows obvious neurological damage and cerebral infarction after ischemia-reperfusion. Compared with the SHR group, the proportion of abnormal blood pressure patterns in the SHR + tMCAO group was significantly increased. And in the dipper and extreme-dipper mode, the blood pressure peak time is slightly earlier, the blood pressure fluctuates more day and night, and the rhythm disorder is more severe. A similar phenomenon exists in human hypertensive patients. Salwa et al. conducted 24-hour ambulatory blood pressure monitoring on 161 hypertensive patients and found that more than 50% of hypertensive patients developed circadian blood pressure abnormalities, of which 21.1% were non-dipper type, 32.3% were extreme-dipper type, and 1.9% were reverse-dipper type (Salwa et al. 2014). The circadian rhythm of blood pressure is affected by many factors and is closely related to the regulation of genes.
Previous studies have confirmed that biological clock genes control the body's circadian rhythm, and are closely related to processes such as sleep-wake cycle, behavioral cognition, cardiovascular function, and metabolism (Riedel et al. 2018; Baschieri and Cortelli 2019). In order to explore the mechanism of circadian rhythm disturbance in blood pressure of spontaneously hypertensive rats after ischemia-reperfusion, this study analyzed the expression of key circadian clock proteins CLOCK, BMAL1, PER1 and CRY1 in the serum within 24 hours after ischemia-reperfusion of SHR. Elisa test results showed that the expression of CLOCK protein was relatively stable within 24 hours, and there was no time-dependent change, while Beker et al. fluctuated the most in the rat striatum after 18:00-induced ischemia (Beker et al. 2018). The difference between the two results may be due to the different animal species used or the inconsistent time point of ischemia-reperfusion. Compared with CLOCK protein, BMAL1 protein fluctuates significantly day and night, and the expression level of BMAL1 protein increases significantly 6 hours after SHR ischemia-reperfusion. At the same time, the circadian rhythm of PER1 protein also changes after SHR ischemia-reperfusion, and its expression peaks at 12h after ischemia. This is consistent with the results of a previous study. Tischkau et al. (Tischkau et al. 2007) induced transient global cerebral ischemia in rats at three different time points in a day. The rhythm of the PER1 transcript was changed, and the peak appeared at 6h after ischemia. Although the time of the peak of PER1 protein expression is different, this may be affected by animal species, but both indicate that ischemia changes the circadian rhythm of PER1 protein. In addition, this study showed that the expression of CRY1 protein gradually decreased over time after SHR ischemia-reperfusion.

In order to further analyze the relationship between circadian clock proteins and blood pressure circadian rhythm disturbances after ischemia-reperfusion, this study used Pearson's linear relationship was used to analyze the correlation between the changes in serum CLOCK, BMAL1, PER1, CRY1 protein levels and the four blood pressure patterns after SHR ischemia-reperfusion. The results showed that the change of serum PER1 protein level was negatively correlated with dipper and extreme-dipper blood pressure patterns, and was significantly positively correlated with non-dipper blood pressure patterns. A previous animal study also reported the relationship between PER1 protein and blood pressure. Alli et al. used Per1 knockout mice and pharmacological effects to indirectly inhibit the Per1 gene, and found that changes in the expression of Per1 target genes are related to lower blood pressure. The gene knockdown method directly inhibits the Per1 gene in the distal nephron cells, which proves that the decrease of Per1 gene expression is related to the decrease of sodium channel protein activity (Alli et al. 2019). Therefore, the Per1 gene may play a role in blood pressure regulation by regulating the renin-angiotensin-aldosterone system of renal sodium ion reabsorption and the circadian rhythm of tissue-specific physiological functions. This may be a potential regulatory mechanism closely related to changes in PER1 protein levels and blood pressure circadian rhythm patterns.

In summary, the blood pressure rhythm pattern of spontaneously hypertensive rats is significantly disturbed after ischemia-reperfusion, and it is closely related to the regulation of Per1 gene. The findings of this study lay a theoretical foundation for further research on the mechanism of circadian clock-mediated blood pressure rhythm disturbance after stroke. However, this study also has some shortcomings. For example, the hypothalamus is the regulating center of the circadian clock. After
ischemia-reperfusion, is the change of circadian clock protein in the hypothalamus also related to the corresponding blood pressure pattern? Due to the limitation of the sample size of rats, the materials were not collected one by one for statistical analysis, which will be further clarified in future studies.

Declarations

Ethics approval and consent to participate: The protocol used in this study and the animal ethics involved have been approved by the Animal Ethics Committee of Xuzhou Medical University.

Consent for publication: The manuscript is approved by all authors for publication.

I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

Availability of data and materials: All data generated or analysed during this study are included in this article.

Competing interests: The authors have no competing interests.

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Authors' contributions: Jing Jin and Mingli He designed the research; Jing Jin, Yumeng Liu and JingHuang performed animal experiments; Jing Jin performed laboratory analyses and drafted the article; Dong Zhang and Jian Ge conducted statistical analyses; Jing Jin and Mingli He wrote the manuscript, with edits from other authors.

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Declaration of Competing Interest

The authors have no competing financial interests.

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Figures

Figure 1

Behavioral performance and TTC staining of brain tissue after SHR ischemia-reperfusion. A paralyzed forelimbs cannot be fully extended during the tail suspension. B spontaneously circle to the paralyzed side. C The grip of the right forelimb is reduced. D 24 hours after SHR undergoing tMCAO, the brain was taken out and cut into 5 coronal brain sections. Cerebral infarction lesions appeared after staining with TTC.
Figure 2

Circadian rhythm patterns of blood pressure in rats in SHR and SHR+tMCAO groups. A Dipper blood pressure B Non-dipper type C Extreme-dipper type D Reverse-dipper type.
Figure 3

ELISA detects the circadian rhythm changes of serum CLOCK, BMAL1, PER1 and CRY1 protein levels in the 2 groups. A-D are the dynamic level changes of CLOCK, BMAL1, PER1 and CRY1 protein at each time point in the 2 groups of rats (15 rats each). Compared with the previous point in time, *P <0.05; **P <0.01; ***P <0.001.
Figure 4

Pearson correlation analysis between SHR serum PER1 protein level and blood pressure after ischemia-reperfusion. A-D are the relationship between the PER1 protein level of SHR+tMCAO group and the blood pressure of dipper (3 rats), non-dipper (6 rats), extreme-dipper (4 rats), and reverse-dipper (2 rats).