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The Effect of Maternal-Pup Separation on Maternal Systolic and Diastolic Blood Pressure in Sprague Dawley Rats

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Abstract

This study investigates changes in systolic and diastolic blood pressure in Sprague Dawley rat dams exposed to postpartum stress. Eighteen pregnant dams were randomly divided into three groups: Control (C, n = 6, no-stress), Maternal Separation (MS, n = 6), and Brief Maternal Separation (BMS, n = 6). The dams and pups in group C were housed under standard conditions in their cages at the animal facility during the postpartum period. From postpartum day 2 to day 14, the dams and pups in group MS and BMS were separated from each other every day for 3-h and 15-min, respectively. Systolic and diastolic blood pressures of the dams were recorded on postpartum days 2, 8, and 14 by tail-cuff method. The findings show that the 3-h dam-pup separation caused high systolic blood pressure in dams, compared to the control dams at all time points that blood pressure was recorded ($p<0.001$). However, this effect is significant only on postpartum days 2 ($p<0.01$) and 8 ($p<0.001$) when compared to the dams that were subjected to 15-min separation. In addition, the 3-h and 15-min dam-pup separations lead to high diastolic blood pressure when compared to the controls throughout the experiment. The 3-h separation caused high diastolic blood pressure only on day 2, when compared to the dams that were subjected to the 15-min pup separation ($p<0.01$). The obtained data indicate that postpartum dam-pup separation may lead to an increase in blood pressure in Sprague-Dawley rats.

Anahtar Kelimeler: Blood pressure, maternal separation, pup, rat, stress

Sprague Dawley Sıçanlarında Anne-Yavru Ayrılmasının Maternal Sistolik ve Diyastolik Kan Basıncı Üzerine Etkisi

Öz

Bu çalışma, doğum sonrası erken yaşam stresine maruz kalan Sprague Dawley anne sıçanlarda sistolik ve diyastolik kan basıncındaki değişiklikleri araştırmaktadır. On sekiz gebe sıçan rastgele Kontrol (K, n: 6), Anne ayırma (AA, n: 6) ve Kısa Anne Ayırma (KAA, n: 6) gruplarına ayrıldı. Grup K'de (ellenmemiş), anne sıçanlar ve yavrular postnatal 2-14 gün boyunca standart hayvan yetiştirme koşulları altında rahatsız edilmeden barındırıldı. AA grubunda anne sıçanlar ve yavrular postnatal 2. ve 14. günlerde her gün 3 saat birbirinden ayrıldı. Grup KAA'da anne sıçanlar ve yavrular postnatal 2. ve 14. günlerde her gün 15 dk birbirinden ayrıldı. Anne sıçanların sistolik ve diyastolik kan basınçları postnatal 2., 8. ve 14. günlerde tail-cuff yöntemi ile kaydedildi. AA grubunda sistolik kan basıncı tüm postnatal günlerde K grubuna göre daha yüksekti ($p<0.001$). KAA grubundaki sistolik kan basıncı postnatal 2. ve 8. günde AA grubuna göre sırasıyla daha düşüktü ($p<0.01$, $p<0.001$). Ancak postnatal 14. günde AA grubu ile KAA grupları arasında fark yoktu ($p>0.05$). Diyastolik kan basıncı AA grubunda doğum sonrası tüm günlerde K grubuna göre daha yüksekti ($p<0.001$, $p<0.01$, $p<0.001$). Benzer şekilde doğum sonrası tüm günlerde KAA grubu K grubundan yüksekti ($p<0.001$, $p<0.05$, $p<0.01$). Postnatal 2. günde KAA grubunun diyastolik kan basıncı AA grubuna göre sırasıyla daha düşüktü ($p<0.01$). Ancak AA grubu ile KAA grupları arasında postnatal 8. ve 14. günlerde fark yoktu ($p>0.05$). Sonuç olarak, erken yaşam stresi Sprague-Dawley anne sıçanlarda kan basıncını artırabilir.

Key Words: Kan basıncı, anneden ayrılma, yavru, sıçan, stres

INTRODUCTION

Newborn mammals naturally need their mother's care to survive. Specific needs vary depending on the species. In most species, the mother provides basic needs such as food, warmth, protection to her pup to survive and train them to develop social abilities and life skills. The mother-pup relationship is dynamic and reciprocal. In this relationship, the stimulus originating from one (mother or pup) affects the behavior and physiology of the other (1, 2). Maternal behavioral expressions in rodents are associated with pregnancy which are fully exhibited only after giving birth (3) and rapidly decreases at the end of the postpartum second week when pups open their eyes and become more independent (4).

Separating the pup from the mother for a few hours a day in the postpartum first week is called maternal separation (MS) and is widely used in laboratory animals, especially in mice and rats, as a model to study the effects of neonatal stress in adults (behavior, endocrine response). Many negative effects such as anxiety (5) and depression-like behaviors (6) and increase in the stress response intensity of the

Hypothalamic-Pituitary-Adrenal axis (HPA axis) (7) have been reported as negative consequences of MS.

The brief maternal separation (BMS) can decrease anxiety-like behavior, HPA-axis tone, and stress response in adulthood compared to MS (7, 8). This short separation period mimics the natural conditions in which the mother leaves her nest to seek food (9). In their natural environment, domestic rats stay with their pup for more than 12-h every day and postpartum 25–35th days and show intensive care behaviors until weaning (4).

It has been reported that the long-term MS causes disorders such as depression and anxiety in the mother, but brief maternal separation increases maternal behavior (10, 11). Kurata et al. (12) found that MS reduced maternal active breastfeeding time in rodents. The response of the maternal HPA axis to stressors is significantly reduced during breastfeeding and pregnancy. This adaptive reaction leads to alleviate the possible negative effects of stress in mothers and infants (13).

It is widely stated in the current literature that mother separation causes stress on mothers. However, studies investigating the effect of maternal separation and brief maternal separation which both of them are early life stress models on systolic and diastolic blood pressures in Sprague-Dawley rats are limited. Dams may learn that they cannot control access to their pups, and thus they may decrease active maternal behaviors such as licking in MS. This induction of learned helplessness can cause a depression-like state in dams (10). We hypothesized that maternal separation causes depression-like behavior and increases blood pressure in the dams. This study aimed to investigate changes in systolic and diastolic blood pressures in Sprague-Dawley dams exposed to MS and BMS.

MATERIAL AND METHOD

The study was carried out in Adnan Menderes University Veterinary Faculty Experimental Animal Breeding and Experimental Research Unit. The study was approved by Adnan Menderes University Animal Experiments Local Ethics Committee (ADÜ-HADYEK) (approval no: 64583101/2016/174). Rats were housed in the controlled experimental room (50%-70% humidity, 22 ± 1°C room temperature, 12:12 light/dark-light cycle) throughout the study. A total of 24 healthy Sprague Dawley rats (18 females + 6 males) weighing 230±10 g at the age of 3 months were used in the study. After one week of environmental adaptation, they were crossed in macrolon type IV cages with 1 male + 3 females for 10 days. Pregnant females were placed into single cages. After parturition, 18 dams were equally divided into 3 groups: Group 1 (Control group - non-handling (C)): In the control group, dams and pups were constantly housed together in their cages. Cage cleaning and handling were not performed until weaning. Rats were not subjected to any stressors except for short-term blood pressure recording. Blood pressure measurements and analyzes were done according to the manufacturer's procedure. Systolic and diastolic blood pressures were recorded from the tail of mother rats by non-invasive indirect method (NIBP200A, Commat, BIOPAC®, Turkey) on postpartum day 2, 8 and 14. Group 2 (Maternal separation group (MS)): The maternal separation protocol for dams in this group was as described before (15, 16); briefly, the pups were separated from their mother for 3 hours each day between 9:00 am and 12:00 pm from postpartum day 2 to day 14. Bedding was changed every day to remove odors during the separation process. The pups were brought to another experimental room to cut off ultrasonic sounds between the pups and the dams. During separation, the pups were housed at room temperature 32 ± 1 °C to prevent hypothermia. After the 3-h separation time, they were brought back to their dams' cage and remained together until the next separation period.

Group 3 (Brief Maternal Separation group (BMS)): Brief maternal separation procedure was performed as described previously (7). To do this, the dams and the pups were separated for 15 minutes every day. The separation was done the same way with group MS.

Systolic and diastolic blood pressures were recorded in dams on postpartum days 2, 8 and 14. After recording, the dam was rejoined with her pups.

Blood pressure measurements were recorded in accordance with the manufacturer's protocol. Systolic and diastolic blood pressures in dams were measured from the tail by the noninvasive indirect method (NIBP200A, Commat, BIOPAC®, Turkey). Biopac Version 3.7.2 program was used for data evaluation (BIOPAC Devices, Inc., Aero Camino, USA).

Statistically Analyses

The data were analyzed in SPSS version 26 (SPSS Inc., Chicago IL, USA) package program using repeated-measures analysis of variance and *post hoc* Bonferroni tests. Values are expressed as mean ± standard error. P value <0.05 was considered statistically significant.

RESULTS

Systolic and diastolic blood pressures are shown in Table 1. Systolic blood pressure was higher in the group MS than that of control group on all postpartum days ($p<0.001$). Systolic blood pressure of rats in the group BMS was lower than that of group MS on postpartum days 2 and 8, respectively ($p<0.01$, $p<0.001$) while on the postpartum day 14, there was no difference between groups MS and BMS ($p>0.05$). Diastolic blood pressure was higher in the MS group than that of control group on all postpartum days, respectively ($p<0.001$, $p<0.01$, $p<0.001$). Similarly, the rats in group BMS had higher diastolic blood pressure than that of controls on all postpartum days ($p<0.001$, $p<0.05$ and $p<0.01$ for postpartum day 2, 8, and 14, respectively). On postpartum day 2, diastolic blood pressure of group BMS was lower than in the group MS ($p<0.01$). However, there was no difference between groups MS and BMS on postpartum days 8 and 14 ($p>0.05$).

Table 1. Effect of MS and BMS on systolic and diastolic blood pressure in Sprague-Dawley dams during postpartum period.

Days	Groups	Systolic Blood Pressure	Diastolic Blood Pressure
Postpartum day 2	C	123.05 ± 3.18	72.70 ± 3.21
	MS	171.46 ± 3.15***	133.32 ± 1.79***
	BMS	145.55 ± 4.84**, ##	110.20 ± 5.62***, ##
Postpartum day 8	C	131.83 ± 3.77	82.39 ± 3.63
	MS	149.35 ± 7.94***	107.80 ± 2.24**
	BMS	134.25 ± 4.28###	98.73 ± 4.93*
Postpartum day 14	C	129.66 ± 2.80	80.36 ± 2.25
	MS	155.32 ± 5.26***	110.87 ± 2.83***
	BMS	148.81 ± 1.40**	102.43 ± 4.62**

The significance values are *p<0.05, **p<0.01, ***p<0.001 compared to C group, #p<0.05, ##p<0.01, ###p<0.001 compared to MS group using repeated-measures analysis of variance and *post hoc* Bonferroni tests.

DISCUSSION AND CONCLUSION

This study revealed that there are significant changes in systolic and diastolic blood pressure in Sprague Dawley rat dams exposed to maternal-pup separation. The obtained data indicate that MS clearly increased systolic and diastolic blood pressure in dams compared to control dams. It was observed that systolic blood pressure in BMS dams increased after giving birth compared to control dams but this increase was not as much as that of group MS. Diastolic blood pressure increased in group BMS compared to controls. Diastolic blood pressure did not increase as much in group BMS, compared to group MS. These results are consistent with previous studies (8, 9).

The relationship between mother and infant is complex and reciprocal; both have an inherent drive for each other. The triggers that exist in one influence the actions and physiology of the other and vice versa (1, 2). Repeated separation of mothers from pups during the postnatal time may cause emotional distress in mothers. It has been shown that MS changes maternal behavior towards pups during the first week of the stress protocol and triggers some characteristics related to the mother's anxiety (16). These findings indicate that maternal separation can also induce long-term changes in mothers.

Previous studies have reported that maternal separation increases plasma corticosterone (17). Mothers treated with corticosterone chronically spent more time outside the nest and spent less time breastfeeding their pups, suggesting a rise in depressive-like behavior (18). Kurata et al. (12) reported that the early maternal depression reduced maternal active nursing. Similarly, they found that repeated long-term MS during the postpartum two weeks caused an increase in depression-like behavior in female rats immediately after weaning (10). In parallel, we thought that repetitive long-term MS might induce emotional stress in mothers and increase plasma corticosterone levels that can alter maternal behavior and thus increase blood pressure. It is likely that MS stress leads to increased plasma corticosterone levels and sympathetic activation-induced decreasing sensitivity of arterial baroreflex, and this may cause stress-induced blood pressure increase. It is essential to further investigate and shed light on the underlying mechanisms.

Threatening stimuli alter the heart rate and blood pressure by activating the autonomic nervous system (19). The sympathetic nervous system plays an important role in stress-related changes in cardiovascular regulation and contributes to cardiovascular morbidity and mortality by inducing arrhythmia as well as vasoconstriction and tachycardia. Activation of the sympathetic nervous system plays a role in the etiology of hypertension as well (20). Blood pressure is controlled by the central autonomic effect on the baroreceptor system. Many brain areas, considered to be important players in the development and control of emotions, have been found to reduce the sensitivity of arterial baroreflex under stress (21). Based on these studies, researchers have suggested that brain abnormality is associated with exaggerated blood pressure responses to stress in groups at high risk of developing hypertension (22). In addition, the sensitization of Ang II signaling may lead to susceptibility to cardiovascular disease (24). It has been suggested that such cardiovascular responses may promote structural changes in vascular tissues and thus lead to the development of hypertension (23).

Few studies have investigated the effects of MS on cardiovascular parameters and/or functions. Loria et al. (24) found that 12-week-old male rats which were exposed to MS had no change in mean arterial pressure (MAP) and heart rate (HR). Sanders and Anticevic (25) reported that baseline levels of MAP and HR were similar between control and MS rats in the effects of early life stress on cardiovascular function in borderline-hypertensive rats; however, MS rats exposed to restraint stress showed an increased HR response, although they did not report a significant difference in blood pressure responses. These data were obtained when pups exposed to the MS protocol became adults. By contrast in our study, the cardiovascular reflection of MS stress in dams was investigated at the blood pressure level. Consistent with our study, we hypothesize that increased sympathetic nerve activation, associated reduced sensitivity of the arterial baroreflex and sensitization of Ang II signaling may be among the possible causes of increased systolic and diastolic blood pressure values due to maternal separation.

In conclusion, MS anxiety stress in dams may lead to an increase in systolic and diastolic blood pressure. However, the underlying mechanisms remain to be evaluated.

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