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Persistence of Circadian Variation in Arterial Blood Pressure in β1/β2-Adrenergic Receptor-Deficient Mice

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Abstract

The β-adrenergic pathway has been considered one important effector of circadian variation in arterial pressure. Experiments were performed in β1/β2-adrenergic receptor-deficient mice (β1/β2ADR-/-) to assess whether this pathway is required for circadian variation in mean arterial pressure (MAP) and to determine the impact of its loss on the response to changes in dietary salt. Twenty-four hour recordings of MAP, heart rate (HR) and locomotor activity were made in conscious 16-17 week old mice (wild-type, WT, n = 7, β1/β2ADR-/- n=10) by telemetry. Both WT and β1/β2ADR-/- mice demonstrated robust circadian variation in MAP and HR, although 24 hour mean MAP was 10% lower (102.02 ± 1.81 vs. 92.11 ± 2.62 mm Hg) in β1/β2 ADR-/- than WT, HR was 16% lower and day-night differences reduced. Both WT and β1/β2ADR-/- adapted to changed salt intake without changed MAP. However, the β1/β2ADR-/- mice demonstrated a striking reduction in locomotor activity in light and dark phases of the day. In WT, MAP was markedly affected by locomotor activity, resulting in bimodal distributions in both light and dark. When MAP was analyzed using only intervals without locomotor activity, bimodality and circadian differences were reduced, and there was no significant difference between the two genotypes. The results indicate that there is no direct effect or role for the β-adrenergic system in circadian variation of arterial pressure in mice, aside from the indirect consequences of altered locomotor activity. Our results also confirm that locomotor activity contributes strongly to circadian variation in BP in mice.

Keywords

Salt intake; sympathetic nervous system

Introduction

The remarkable progress in delineating the molecular mechanisms responsible for circadian periodicity in biological functions (as reviewed in (16,27)) has not been accompanied by comparable progress in delineating the effector mechanisms that couple oscillations in expression of the primary clock genes with critical cardiovascular functions, such as heart rate, and arterial pressure. Although autonomous regulation of clock genes can be demonstrated in peripheral tissues (13), central control mechanisms appear to be critical for the circadian oscillations in arterial pressure, at least in rodents, since the diurnal variation in blood pressure has been reported to be virtually eliminated by lesions of the suprachiasmatic nucleus (14, 21), leading to the inference that the effector mechanisms for circadian arterial pressure regulation are not tissue autonomous, but rather require changes in autonomic activity or a circulating humoral factor triggered by CNS activity.

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A number of pharmacological and ablative strategies have been employed to study the mechanisms for circadian regulation of cardiovascular function (as reviewed in (14)). In the last five years, the availability of genetically modified mouse strains coupled with radiotelemetry has provided a new approach to this problem. No apparent alteration in the circadian rhythms of arterial pressure was detected in homozygous or heterozygous mice with deletions in angiotensin converting enzyme (2), suggesting that cyclic variation in the activity of the renin-angiotensin system is not required for circadian variation in arterial pressure. The vasodilatory effects of endothelial-derived nitric oxide would also appear not to be critical for diurnal blood pressure cycles since the effect of a targeted deletion of the gene for endothelial NOS is amplification, not a reduction, of the dark-light pressure difference (1,26). Circadian cycles of arterial pressure also persist in mice lacking cycloxygenase 1, although the magnitude of change is blunted modestly (12).

It has been widely assumed that variation in activity of the sympathetic nervous system (SNS) or circulating catecholamine levels underlie a major portion of circadian cardiovascular variation. The pharmacological evidence for this hypothesis is extensive although not, in fact, definitive (3,14,22). The present studies were undertaken to begin an exploration of the role of the adrenergic system in the generation of circadian variations of blood pressure and heart rate. In view of the importance of the β-adrenergic pathway for cardiovascular regulation the present studies investigated circadian rhythmicity in a mouse strain with a targeted deletion in the β1 and β2 adrenergic receptors (β1/β2 ADR-/-) (20). A second goal was to determine the impact of loss of this pathway on the response to variation in dietary salt or interruption of the renin-angiotensin system by enalapril. A third goal adopted during the course of the study was to describe and quantify the impact of locomotor activity cycles on blood pressure in mice. The major findings of these studies are that robust circadian rhythms in heart rate and arterial pressure persist in spite of the absence of the β1 and β2 adrenergic signaling pathway, and that spontaneous locomotor activity is strikingly reduced in β1/β2 ADR-/- mice.

Methods

Animals

We used male and female mice deficient in both β1 and β2 adrenergic receptors (β1/β2 ADR-/-) originally generated by Rohrer et al.(20). Mice were obtained from Jackson Laboratories and interbred to generate subsequent generations (Bar Harbor, ME). The background of these animals, as described in the original publication, contains contributions from FVB, C57BL/6 and 129SvJ strains. Mixed background mice need to be used with this double knockout line because it has been observed that β1/β2 ADR-/- mice in a pure genetic background show high perinatal mortality (20). To maximize the similarity of background, control animals (indicated throughout this report as wild type, WT) were generated from the F2 generation of a cross between β1/β2 ADR-/- mice and C57BL/6 animals, as recommended by Jackson laboratories.

Genotyping was done on tail DNA using PCR. All mice were maintained on a 12hour light-dark cycle and were kept on a standard rodent chow or high or low salt diets, as described below, and tap water. All diets were provided ad libitum. Animal care and experimentation was approved and carried out in accordance with NIH principles and guidelines for the Care and Use of Laboratory Animals.

Telemetry

The telemetry system of Data Sciences International (St Paul, MN) was used for these experiments. Each transmitter (model TA11PA-C10) was magnetically activated >24 hours before implantation. Mice were anesthetized with ketamine and xylazine (90 and 10 mg/kg, respectively), and the left carotid artery was isolated. The tip of the telemeter catheter was

inserted into the carotid artery and advanced into the aortic arch, with the telemeter body positioned in a subcutaneous pocket on the right flank. One day after surgery, each animal was returned to its home cage and provided with ad libitum food and water for the duration of the study. Telemeter calibration was performed following explantation and recorded blood pressure values were corrected for any drift in calibration that may have occurred (25). The telemeter signal was processed using a model RPC-1 receiver, a 20 channel data exchange matrix, APR-1 ambient pressure monitor, and a Data Quest ART Silver 2.3 acquisition system (Data Sciences International, St. Paul, MN). The implanted telemeter was activated on the morning of the 7 -10thday, with recording periods of a minimum of four days for each animal in each condition. The sampling intervals used for most of the studies presented here were as follows: at 2 min intervals, the system was set to sample the mean, systolic and diastolic blood pressure, pulse pressure, heart rate, and activity over a ten second interval and to record their average values. In most studies the recording room was maintained at 21-22 °C with a 12 h light/12h dark cycle (LD). In 4 WT and 4 β1/β2 ADR-/- mice LD conditions were followed by exposure to 24 h darkness for 7 days (DD). Measurements were made on days 5-7 of DD.

At the time of telemetry implantation, wild type mice averaged 28.6 ± 2.3 g and were 17.4 + 1.9 weeks old, while β1/β2 ADR-/- mice averaged 29.3 + 1.2 g and were 16.7 + 0.2 weeks of age. Telemetry studies were undertaken in 7 control animals (5 female, 2 male) and 10 β1/β2 ADR-/- mice (7 female, 3 male).

Salt Diets and Enalapril Administration—After baseline studies were obtained, five mice of each genotype were placed randomly on either a high (8.0%) or low (0.04%) NaCl in a random order. All diets were provided for a one week period prior to telemetry study. In three WT and 4 β1/β2 ADR-/- an additional telemetric study was performed in mice maintained on a low salt diet for one week with enalapril added to the drinking water (150 mg/l). This regimen provides an average of approximately 10 mg/kg/day of the drug.

Statistical Analyses—Initial telemetry data analyses were performed using the Analysis program of Dataquest A.R.T. 2.3 (DSI, St. Paul, MN). Data are expressed as means ± SE. Statistical comparisons were done by paired and unpaired student's t-test for comparisons of MAP, SAP, DAP, heart rate, pulse pressure and mean activity between WT and β1/β2ADR-/- mice. P values < 0.05 were considered to indicate a significant difference.

Results

Circadian rhythm of arterial pressure and heart rate

Both WT and β1/β2 ADR-/- mice demonstrated robust circadian variations of arterial pressure and heart rate. A representative recording from each strain is shown in Supplemental Fig. S1, and average hourly values are plotted in Fig. 1. While circadian periodicity was maintained, mean arterial pressure (MAP) and heart rate were significantly reduced in the β1/β2 ADR-/- mice, as summarized in Table 1 and shown in Fig. 2 for 24 hour mean values of individual animals. Average 24 hour values for MAP were 10% lower and values for heart rate were 16% lower in β1/β2 ADR-/- mice than WT mice. Of note, while circadian rhythms were present, the reduction in the absolute magnitude of the dark-light differences in mean, systolic and diastolic arterial pressure did achieve significance (see Table 1). As shown in Fig. 3, differences in MAP, heart rate, and activity between objective night time (6 PM - 6 AM) and objective day time (6 AM - 6 PM) were not different under LD and DD conditions.

A significant reduction in heart rate has been documented previously in the β1/β2 ADR-/- mice by telemetry (6). A small reduction in arterial pressure was also observed in the initial phenotypic description of the β1/β2 ADR-/- mice (20), but it did not achieve significance,

probably due to methodological differences; those measurements were made without the lengthy undisturbed observation possible with telemetry.

Locomotor activity

The β1/β2 ADR-/- mice also demonstrated a striking reduction in locomotor activity that has not previously been reported. A histogram depicting the distribution of activity counts is provided in Fig. 4 (individual activity profiles for 5 WT and 5 β1/β2 ADR-/- mice are given as Fig. S2). It can be seen that the most striking difference is the proportion of high activity intervals; the β1/β2 ADR-/- mice show no observation intervals with activity counts exceeding 40 (see inset Fig. 4).

Activity was reduced in both night and day phases of the circadian cycle (see Fig. 2 and Table 1). While some dark-light difference in activity persisted, the magnitude of the change was markedly reduced (Table 1).

Effect of locomotor activity on arterial pressure

Since β1/β2 ADR-/- mice showed markedly reduced locomotor activity, the impact of activity on blood pressure and blood pressure variability was assessed. We calculated the linear regression relationship between activity and MAP, using log(activity + 0.1) as the independent variable. The log transformation reduces the skewing of the activity distribution and addition of 0.1 allows log transformation of values with zero activity (26). Results were as follows:

WT: MAP= 8.9 log(activity+0.1) +92.3 (r^2 =0.387)

β1/β2 ADR-/-: MAP= 6.3 log(activity+0.1) +99.5 (r^2 =0.096).

The WT slope, 8.9 mm Hg per log activity, had a confidence interval 8.7 to 9.1, and differed significantly at the $p< 0.01$ level from the shallower slope, 6.4 mm Hg per log activity, (confidence interval 6.0 to 6.7) observed in the β1/β2 ADR-/- mouse, and the contribution of activity to variability was reduced in knockout animals.

We also compared the distribution of MAP measurements in all observation intervals and in intervals in which no locomotor activity was observed. This analysis was possible in five animals of each genotype in which recordings were made with an identical sampling protocol. When all observations were included, most WT animals showed a clear bi-modal pattern, as has been observed previously (26), with the higher MAP mode predominant in the dark or active phase and the lower MAP mode dominant in the light phase (Fig. S2, left panels). A tendency to bimodality was also present in several of the β1/β2 ADR-/- mice, but was less prominent (Fig. S3, right panels). The bimodal pattern was substantially less prominent when only intervals without activity were considered (Fig. S4) consistent with the earlier suggestion (25,26) that the high MAP mode reflects the impact of periods of high activity. Median values from these analyses are provided in Table 2.

Of significance for the present studies, when only inactive intervals were considered, the magnitude of the circadian shift in MAP was substantially reduced, suggesting that activity is a significant determinant of circadian blood pressure differences. Table 2 summarizes *median* values. In the WT animals the circadian shift in median MAP was reduced from a dark-light difference of 18.9 ±1.5 (all observations) to 7.6 ±2 mm Hg (inactive intervals only). The change in β1/β2 ADR-/- mice was more modest, reflecting lower activity levels, from 11.6 ±2.1 to 9.1 ±2.1 mm Hg. During the inactive intervals there was no significant difference between the two genotypes (see Table 2). This data suggest no direct effector role for the β-adrenergic system in circadian regulation of vascular variables, aside from for the indirect consequences of altered locomotor activity.

Impact of low and high salt diet and low salt diet plus enalapril

Fig. 5 depicts the circadian pattern for MAP, HR and activity measured in both genotypes after animals were maintained on a low salt diet or a high salt diet for one week. Table 3 summarizes mean 24 hour values. Neither the WT nor the β1/β2 ADR-/- mice demonstrated salt sensitivity of arterial pressure with a high salt diet, and both strains compensated for a low salt dietary intake without a fall in MAP. Of note the low salt diet provoked a significant increase in nocturnal activity in the WT and a high salt diet significantly reduced HR, but neither change occurred in the β1/β2 ADR-/- mice, suggesting that both responses reflect altered β-adrenergic activation. Other variables did not change significantly.

We also assessed the ability of animals of both genotypes to compensate for the additional stimulus of renin-angiotensin system blockade with enalapril in addition to a low salt diet (Fig. 6). MAP fell to a similar level in the two strains, and circadian variation was maintained.

Discussion

In the present study, we utilized radiotelemetry to explore the role of the β-adrenergic pathway as a potential effector mechanism in circadian rythmicity of cardiovascular function. The most important finding is the persistence of strong diurnal cyclical variations in arterial pressure and heart rate in both male and female animals lacking β1- and β2-adrenergic receptors (β1/β2 ADR-/-), the β-adrenergic receptor classes most critical for cardiovascular regulation. The initial hypothesis of these studies, that the sympathetic nervous system through mediation of β-adrenergic receptors is a critical direct effector of the circadian variation of arterial pressure, either through direct effects on vascular tone or by alteration in heart rate and cardiac output, was supported by substantial indirect evidence. A number of experimental approaches have established that sympathetic activity is greater in the active phase of the circadian cycle, including direct measurement of stellate ganglion activity (11), and spectral analysis of heart rate variability in humans and rodents (7,28). Circulating catecholamines are higher in the active phase, during the day in humans and during the night in rodents (4,8). Many pharmacological studies that have compared the effect of administration of β-blockers on arterial pressure in different phases of the circadian cycle have demonstrated that the agents produce larger effects in the active phase (9,14). Nevertheless, while we also demonstrated a somewhat greater dark-light difference in arterial pressure between the wild type and the genetically “β -blocked” animals, the persistence of robust rhythmic variations in pressure and heart rate indicates that there must be other major effector pathways. Our data indicate that locomotor activity is an important determinant of the circadian variation in arterial pressure in animals of both genotypes, and that the modest reduction in the amplitude of circadian arterial pressure cycles in the β1/β2 ADR-/- mice is largely a consequence of reduced locomotor activity.

While diurnal cycles in arterial pressure and heart rate were only modestly affected by genetic interruption of the β-adrenergic pathway, locomotor activity was strikingly reduced in the β1/β2 ADR-/- mice, particularly in the active phase. The mechanisms responsible for the relative inactivity are unclear. Specifically, our studies do not permit inferences about whether the reduced locomotor activity in the β1/β2 ADR- /- mice is a central or peripheral effect. Nonetheless it is of note that a previous study, which carefully assessed exercise in the β1/β2 ADR-/- mouse found no defect in maximal exercise capacity (20). Review of the neurophysiological literature finds substantial support for central effects of the β-adrenergic system on the regulation of wakefulness and activity. β-receptor blocking agents have been shown to depress motor activity in rats and primates (15,24). Several laboratory groups have examined the effect of disruption of the dopamine β-hydroxylase gene on locomotor activity, sleep phases and the transitions between wakefulness and sleep (10,19,23). This genetic manipulation appears to produce increased non-REM sleep (19), but inconsistent effects on

locomotor activity, with a telemetric study demonstrating decreased activity (23) while videometric monitoring did not detect differences in activity (19). We did not undertake to assess sleep phases, but would infer, based on the absence of bouts of relatively high locomotor activity in the β1/β2 ADR-/- mice, that the differences in activity are not solely accounted for by differences in the portion of time spent asleep.

In the present studies we observed that changes in dietary salt intake were surprisingly well compensated in face of genetic interruption of β-adrenergic signaling, with little resultant effects on MAP, heart rate or circadian variation after one week of dietary manipulation. The mixed background animals used as control for this strain also showed no evidence of salt-induced elevations of blood pressure, suggesting that the absence of salt sensitivity in the knockout mice is not due to absence of β-adrenergic receptor signaling. In view of the higher C57Bl/6 component of WT compared to knockout mice this conclusion is somewhat tentative, since the salt effects on blood pressure in mice are likely to be influenced by genetic background. When we superimposed interruption of the renin-angiotensin system by administration of enalapril, MAP fell to comparable levels in the two genotypes, with preserved circadian rhythms, again indicating the robustness and redundancy of the regulatory pathways controlling arterial pressure.

Perspective

Our primary goal in the present studies was to determine the role of the β-adrenergic pathway in circadian variations of arterial pressure. Circadian cycles in arterial pressure are of substantial clinical interest; observational studies in patients indicate that loss of the normal fall in arterial pressure with sleep (non-dipping status) is associated with poorer health outcomes, for example with increased stroke and cardiovascular mortality (5,18) and increased progression of diabetic renal disease (17). It is therefore important to understand the effector mechanisms for circadian blood pressure cycles, since preservation of normal daily rhythms may potentially offer health benefits. The present studies provide evidence that even complete absence of β-adrenergic receptors does not have significant impact on the circadian rhythm of arterial pressure. Whether α-adrenergic receptors may play a role in diurnal blood pressure variations needs to be explored in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Arraj M, Lemmer B. Circadian rhythms in heart rate, motility, and body temperature of wild-type C57 and eNOS knock-out mice under light-dark, free-run, and after time zone transition. Chronobiol Int 2006;23:795–812. [PubMed: 16887749]
2. Carlson SH, Oparil S, Chen YF, Wyss JM. Blood pressure and NaCl-sensitive hypertension are influenced by angiotensin-converting enzyme gene expression in transgenic mice. Hypertension 2002;39:214–218. [PubMed: 11847186]
3. Carlson SH, Roysomutti S, Peng N, Wyss JM. The role of the central nervous system in NaCl-sensitive hypertension in spontaneously hypertensive rats. Am J Hypertens 2001;14:155S–162S. [PubMed: 11411751]
4. De Boer SF, Van der Gugten J. Daily variations in plasma noradrenaline, adrenaline and corticosterone concentrations in rats. Physiology & behavior 1987;40:323–328. [PubMed: 3659148]

5. Dolan E, Stanton A, Thijs L, Hinedi K, Atkins N, McClory S, Den Hond E, McCormack P, Staessen JA, O'Brien E. Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin outcome study. Hypertension 2005;46:156–161. [PubMed: 15939805]
6. Ecker PM, Lin CC, Powers J, Kobilka BK, Dubin AM, Bernstein D. Effect of targeted deletions of beta1- and beta2-adrenergic-receptor subtypes on heart rate variability. Am J Physiol Heart Circ Physiol 2006;290:H192–199. [PubMed: 16113068]
7. Furlan R, Guzzetti S, Crivellaro W, Dassi S, Tinelli M, Baselli G, Cerutti S, Lombardi F, Pagani M, Malliani A. Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. Circulation 1990;81:537–547. [PubMed: 2297860]
8. Gold PW, Wong ML, Goldstein DS, Gold HK, Ronsaville DS, Esler M, Alesci S, Masood A, Licinio J, Geracioti TD Jr. Perini G, DeBellis MD, Holmes C, Vgontzas AN, Charney DS, Chrousos GP, McCann SM, Kling MA. Cardiac implications of increased arterial entry and reversible 24-h central and peripheral norepinephrine levels in melancholia. Proceedings of the National Academy of Sciences of the United States of America 2005;102:8303–8308. [PubMed: 15919819]
9. Grundt C, Meier K, Lemmer B. Gender dependency of circadian blood pressure and heart rate profiles in spontaneously hypertensive rats: effects of beta-blockers. Chronobiol Int 2006;23:813–829. [PubMed: 16887750]
10. Hunsley MS, Palmiter RD. Altered sleep latency and arousal regulation in mice lacking norepinephrine. Pharmacology, biochemistry, and behavior 2004;78:765–773.
11. Jung BC, Dave AS, Tan AY, Gholmieh G, Zhou S, Wang DC, Akingba AG, Fishbein GA, Montemagno C, Lin SF, Chen LS, Chen PS. Circadian variations of stellate ganglion nerve activity in ambulatory dogs. Heart Rhythm 2006;3:78–85. [PubMed: 16399059]
12. Kawada N, Solis G, Ivey N, Connors S, Dennehy K, Modlinger P, Hamel R, Kawada JT, Imai E, Langenbach R, Welch WJ, Wilcox CS. Cyclooxygenase-1-deficient mice have high sleep-to-wake blood pressure ratios and renal vasoconstriction. Hypertension 2005;45:1131–1138. [PubMed: 15883230]
13. Ko CH, Takahashi JS. Molecular components of the mammalian circadian clock. Hum Mol Genet 2006;15(Spec No 2):R271–277. [PubMed: 16987893]
14. Lemmer B. The importance of circadian rhythms on drug response in hypertension and coronary heart disease--from mice and man. Pharmacol Ther 2006;111:629–651. [PubMed: 16480770]
15. Lemmer B, Neumann G. Circadian phase dependency of the effects of different beta-receptor blocking drugs on motor activity of rats. Importance of drug lipophilicity. Arzneimittel-Forschung 1987;37:321–325. [PubMed: 2885007]
16. Lowrey PL, Takahashi JS. Mammalian circadian biology: elucidating genome-wide levels of temporal organization. Annu Rev Genomics Hum Genet 2004;5:407–441. [PubMed: 15485355]
17. Lurbe E, Redon J, Kesani A, Pascual JM, Tacons J, Alvarez V, Batlle D. Increase in nocturnal blood pressure and progression to microalbuminuria in type 1 diabetes. The New England journal of medicine 2002;347:797–805. [PubMed: 12226150]
18. Metoki H, Ohkubo T, Kikuya M, Asayama K, Obara T, Hara A, Hirose T, Hashimoto J, Totsune K, Hoshi H, Satoh H, Imai Y. Prognostic significance of night-time, early morning, and daytime blood pressures on the risk of cerebrovascular and cardiovascular mortality: the Ohasama Study. Journal of hypertension 2006;24:1841–1848. [PubMed: 16915034]
19. Ouyang M, Hellman K, Abel T, Thomas SA. Adrenergic signaling plays a critical role in the maintenance of waking and in the regulation of REM sleep. Journal of neurophysiology 2004;92:2071–2082. [PubMed: 15190089]
20. Rohrer DK, Chruscinski A, Schauble EH, Bernstein D, Kobilka BK. Cardiovascular and metabolic alterations in mice lacking both beta1- and beta2-adrenergic receptors. J Biol Chem 1999;274:16701–16708. [PubMed: 10358009]
21. Sano H, Hayashi H, Makino M, Takezawa H, Hirai M, Saito H, Ebihara S. Effects of suprachiasmatic lesions on circadian rhythms of blood pressure, heart rate and locomotor activity in the rat. Jpn Circ J 1995;59:565–573. [PubMed: 7474301]
22. Sica DA. What are the influences of salt, potassium, the sympathetic nervous system, and the renin-angiotensin system on the circadian variation in blood pressure? Blood Press Monit 1999;4(Suppl 2):S9–S16. [PubMed: 10822417]

23. Swoap SJ, Weinshenker D, Palmiter RD, Garber G. Dbh(-/-) mice are hypotensive, have altered circadian rhythms, and have abnormal responses to dieting and stress. American journal of physiology 2004;286:R108–113. [PubMed: 12969876]

24. Turkkan JS, Hienz RD, Allen RP, Bezold HJ. Beta-blocker effects on 24-h activity in normotensive and renovascular hypertensive baboons. Pharmacology, biochemistry, and behavior 1992;42:465–471.

25. Van Vliet BN, Chafe LL, Antic V, Schnyder-Candrian S, Montani JP. Direct and indirect methods used to study arterial blood pressure. J Pharmacol Toxicol Methods 2000;44:361–373. [PubMed: 11325579]

26. Van Vliet BN, Chafe LL, Montani JP. Characteristics of 24 h telemetered blood pressure in eNOS-knockout and C57Bl/6J control mice. J Physiol 2003;549:313–325. [PubMed: 12665600]

27. Wager-Smith K, Kay SA. Circadian rhythm genetics: from flies to mice to humans. Nat Genet 2000;26:23–27. [PubMed: 10973243]

28. Witte K, Engelhardt S, Janssen BJ, Lohse M, Lemmer B. Circadian and short-term regulation of blood pressure and heart rate in transgenic mice with cardiac overexpression of the beta1-adrenoceptor. Chronobiol Int 2004;21:205–216. [PubMed: 15332342]

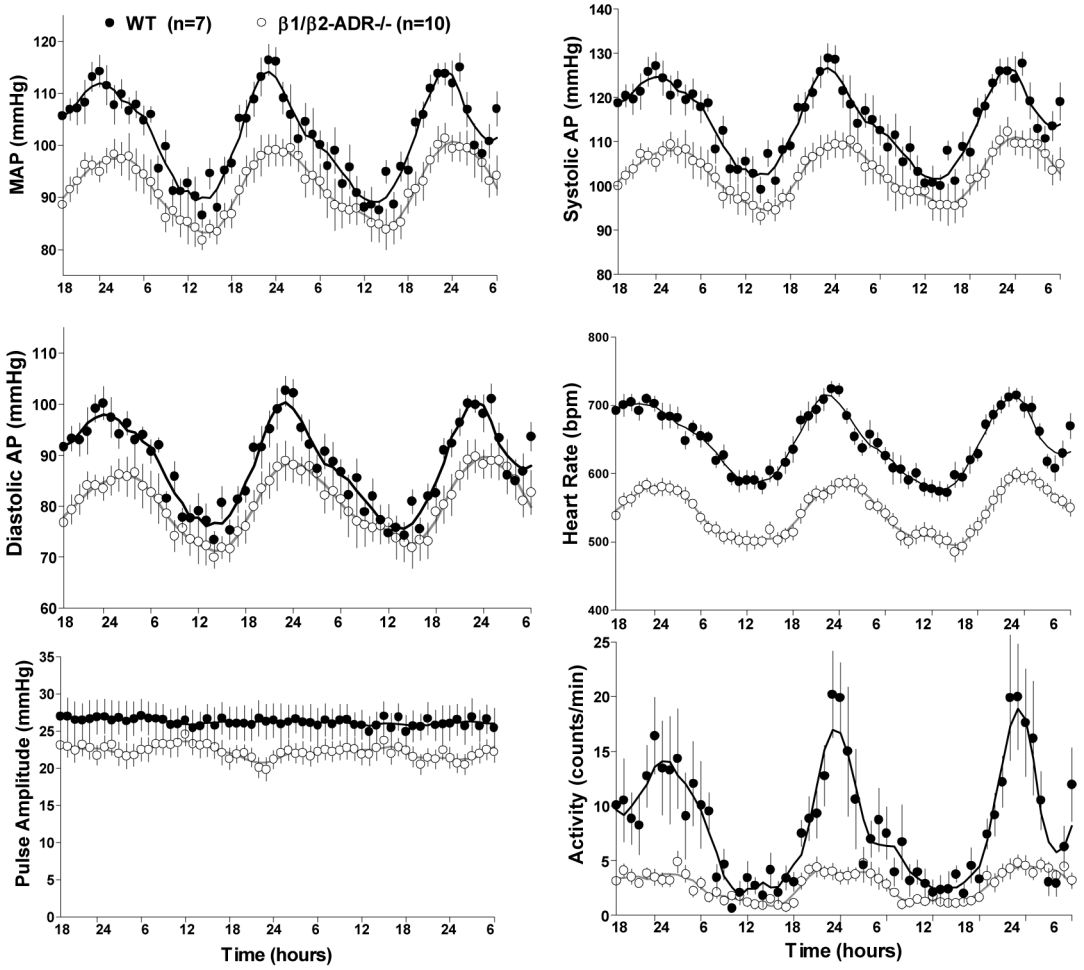

Figure 1. Circadian patterns in WT and β1/β2 ADR-/- mice on 12h light/12h dark light cycle

Graphs depict mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and pulse pressure (PP), all in mmHg, and heart rate (BPM) and activity (counts per observation interval). Mice were studied on a normal salt diet. Values plotted are hourly means measured over sixty hours. Lines represent data smoothing using the weighted average of the 9 nearest points.

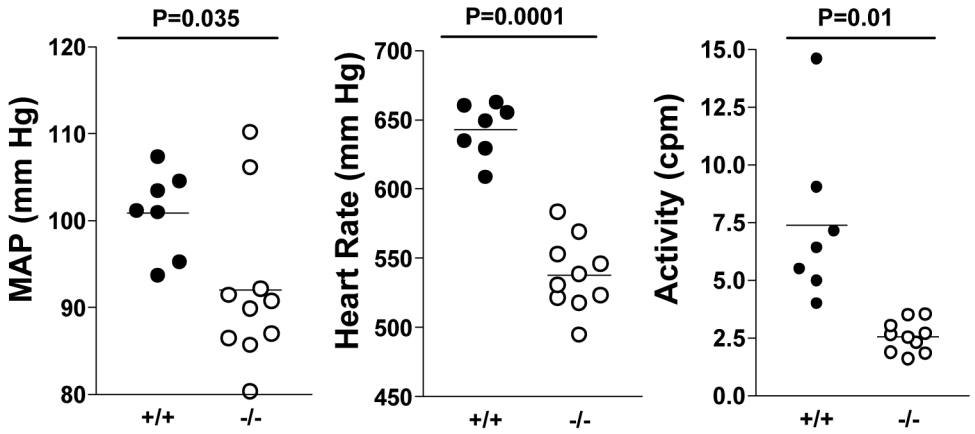

Figure 2. Comparison of the individual animal 24 h parameter means in β1/β2 ADR-/- and WT mice

The mean value for each group is represented by a horizontal line. (WT-dark circle and KO-open circle, n=10 and 7 per group)

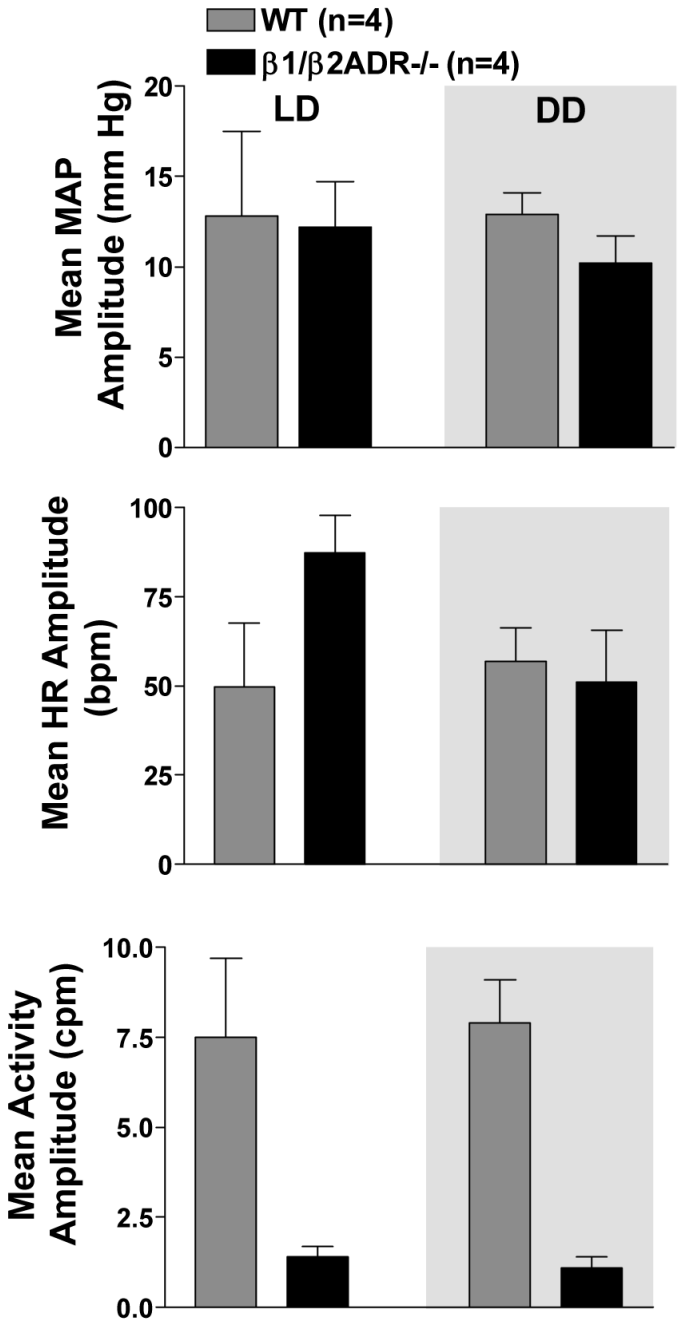

Figure 3. Circadian amplitudes of MAP, heart rate, and activity under 12h light/12h dark (LD) and 24h dark conditions (DD) in 4 WT and 4 β1/β2 ADR-/- mice

Amplitudes represent the differences in mean 12 h values in the 6 PM-6 AM period (dark) and 6 AM - 6 PM period (light in LD and dark in DD).

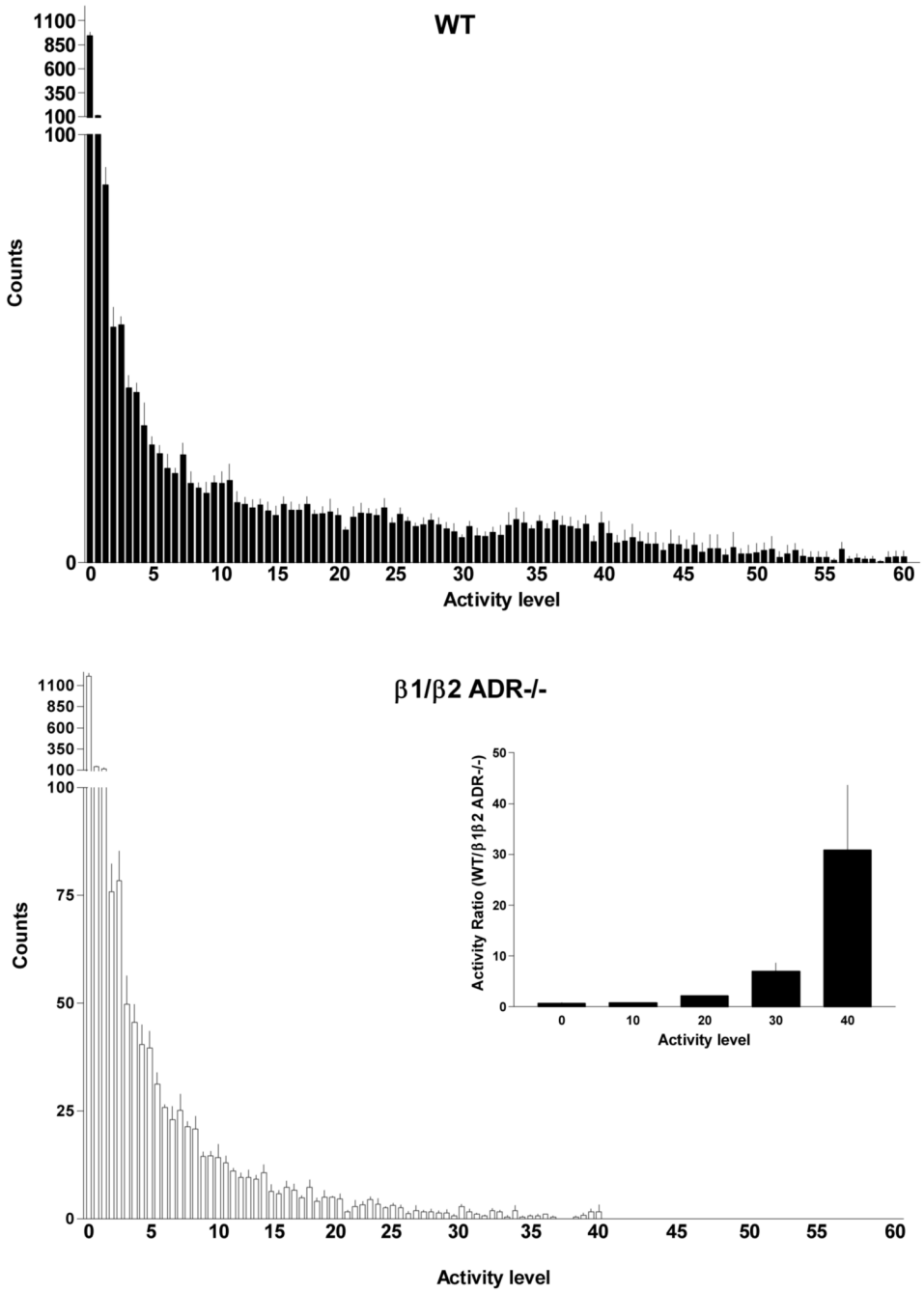

Figure 4. Histograms depicting distribution of locomotor activity counts in WT (upper panel) and β1/β2 ADR-/- (lower panel) mice

A ten-second observation interval was utilized. The inset show the ratio (WT/ β1/β2 ADR-/-) of observation intervals in each decade.

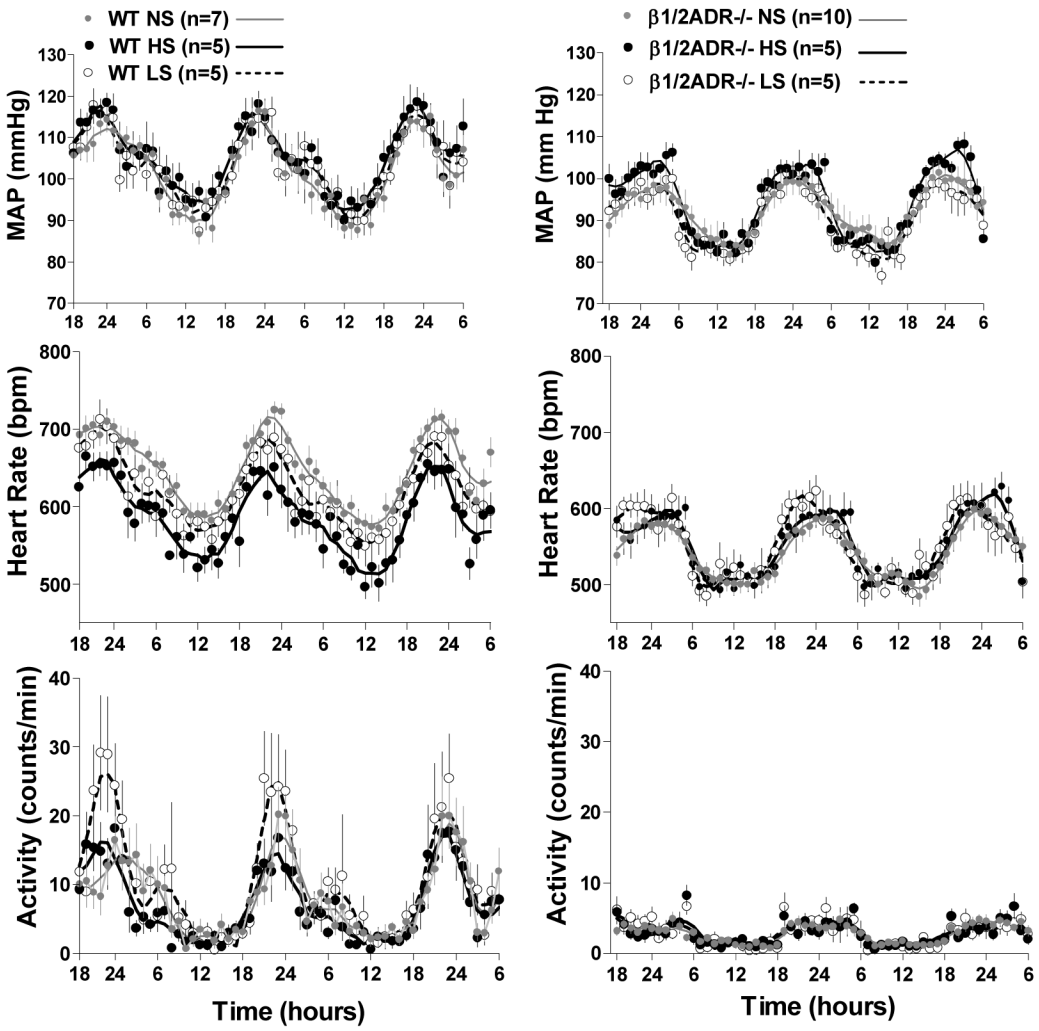

Figure 5. Effect of one week of high or low salt diet on circadian patterns in mean arterial pressure (MAP in mmHg), heart rate (beats per minute, BPM) and activity

Values measured in WT mice are shown on the left and β1/β2 ADR-/- mice on the right. Observations on a high salt diet are shown with closed symbols, and low salt with open symbols. Shown for comparison by the gray symbols and gray lines are the values measured on the control diet, which were also presented in Figure 2. Values plotted are hourly means measured over 60 hours. Lines represent data smoothing using the weighted average of the 9 nearest points.

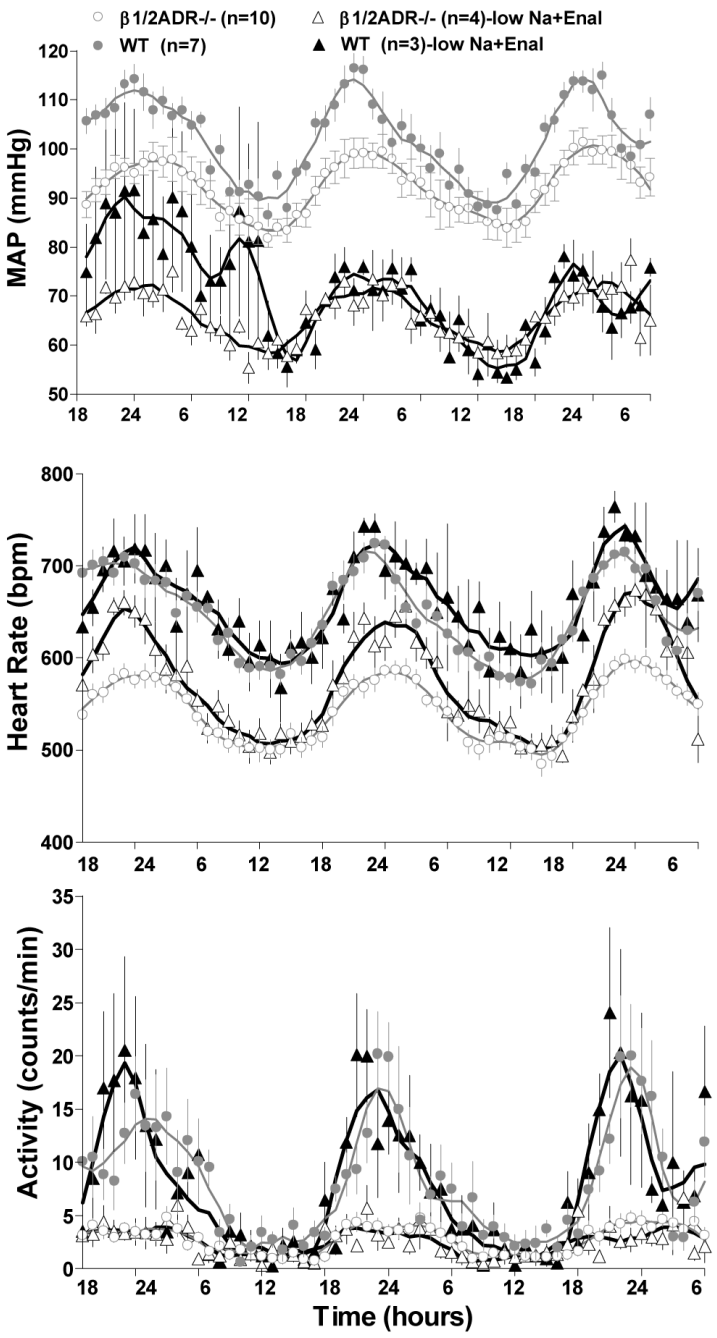

Figure 6. Circadian patterns in mean arterial pressure (MAP in mmHg), heart rate (beats per minute, BPM) and activity in WT and β1/β2 ADR-/- mice treated with a low salt diet and enalapril for one week

Values plotted are mean arterial pressure (MAP in mmHg), heart rate (beats per minute, BPM) and activity (counts per observation interval) in WT and β1/β2 ADR-/- mice. Shown for comparison by the gray symbols and gray lines are the values, also presented in Figure 2, measured on the control diet.

Table 1

Summary of hemodynamic values and locomotor activity for β1/β2 adrenergic (β1/β2 ADR -/-) and their control mice on a normal salt diet

Period	Variable	WT	β1/β2 ADR -/-	*P* value
24 h	SAP (mmHg)	114 ± 2	103 ± 3	0.02
	MAP (mmHg)	101 ± 2	92 ± 2	0.03
	DAP (mmHg)	87 ± 1.5	80 ± 3	0.12
	PP (mmHg)	26 ± 2	22 ± 1	0.09
	HR (beats min)	643 ± 7	538 ± 8	<0.001
	Mean activity	7.4 ± 1	2.6 ± 0.2	<0.001
Light period	SAP (mmHg)	106 ± 2	98 ± 3	0.043
	MAP (mmHg)	94 ± 2	87 ± 3	0.08
	DAP (mmHg)	80 ± 1	75 ± 3	0.21
	PP (mmHg)	26 ± 2	23 ± 1	0.15
	HR (beats min)	606 ± 8	511 ± 10	<0.001
	Mean activity	3.8 ± 0.7	1.5 ± 0.1	0.001
Dark period	SAP (mmHg)	120 ± 3	107 ± 3	0.008
	MAP (mmHg)	107 ± 2	96 ± 3	0.01
	DAP (mmHg)	94 ± 1	85 ± 3	0.07
	PP (mmHg)	26 ± 2	22 ± 1	0.13
	HR (beats min)	674 ± 7	566 ± 7	<0.001
	Mean activity	11 ± 2	3.7 ± 0.3	<0.001
Dark-light Difference	SAP (mmHg)	13.5 ± 0.9	8.4 ± 0.8	<0.001
	MAP (mmHg)	13.4 ± 0.8	9.5 ± 0.7	0.002
	DAP (mmHg)	13.1 ± 0.8	9.8 ± 0.7	0.008
	PP (mmHg)	0.34 ± 0.5	-0.5 ± 0.6	0.33
	HR (beats min)	68.4 ± 4.3	55.1 ± 6.5	0.14
	Mean activity	7.3 ±1.4	2.1 ± 0.2	<0.001

Table 2

Impact of Activity on MAP Frequency Distribution

	Dark Median (interquartile range)	Light Median (interquartile range)	Dark-Light Difference±SE
All observations			
WT	109.1 (96.7 - 115.0)	90.1 (84.0-102.8)	18.9 ± 1.52
β1/β2ADR-/-	94.9 (87.5-102.8)	83.4 (77.6-90.9)	11.6 ± 2.08
p values	(0.035)	NS	(0.021)
Observations from intervals with no activity			
WT	94.2 (88.9-101.2)	86.6 (84.3-91.9)	7.6 ± 2.01
β1/β2ADR-/-	90.8 (84.3-98.2)	81.7 (76.6-87.5)	9.1 ± 2.14
p values	NS	NS	NS

All values are in mm Hg. p values are for comparison of WT and β1/β2ADR-/- values.

Table 3

Summary of 24 hour mean values of hemodynamic variables and locomotor activity in WT (n=5) and β1/β2 ADR -/- mice (n=5) maintained on low or high NaCl diets

		WT		β1/β2 ADR -/-	
	Variable	**Low NaCl**	**High NaCl**	**Low NaCl**	**High NaCl**
24 h Mean	SAP (mmHg)	115 ± 3	117 ± 3	102 ± 2**	104 ± 1**
	MAP (mmHg)	102.5 ± 3	104 ± 1	90 ± 1.4**	93 ± 1**
	DAP (mmHg)	89 ± 2	90 ± 1.5	78 ± 1**	81 ± 1**
	PP (mmHg)	25 ± 3	27 ± 1	24 ± 1.5	23.5 ± 1
	HR (beats min)	622 ± 16	578 ± 7	553 ± 9**	548 ± 9*
	Mean activity	9.2 ± 2	6.4 ± 0.6#	2.7 ± 0.4*	2.7 ± 0.2**

** $p < 0.01$

* $p < 0.05$ compared to WT mice on same diet

# $p < 0.05$ compared to low NaCl