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β-Arrestin-2 Deficiency Alters Fluid Homeostasis and Blood Pressure Regulation

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Abstract

Background: G protein-coupled receptors (GPCRs) are implicated in blood pressure (BP) and fluid intake regulation. There is a developing concept that these effects are mediated by both canonical G-protein signaling and non-canonical β-arrestin mediated signaling, but the contributions of each remain largely unexplored. Here, we hypothesized that β-arrestin contributes to fluid homeostasis and blood pressure (BP) regulation in deoxycorticosterone acetate (DOCA)-salt hypertension, a prototypical model of salt-sensitive hypertension.

Methods: Global β-arrestin1 (*Arrb1*) and β-arrestin2 (*Arrb2*) knockout (KO) mice were employed to evaluate drinking behavior, and BP was evaluated in *Arrb2*-KO mice. Age- and sex-matched C57BL/6 mice served as controls. We measured intake of water and different sodium chloride solutions and BP employing a two-bottle choice paradigm with and without DOCA.

Results: Without DOCA (baseline), *Arrb2*-KO mice exhibited a significant elevation in saline intake with no change in water intake. With DOCA treatment, *Arrb2*-KO mice exhibited a significant increase in both saline and water intake. Although, *Arrb2*-KO mice exhibited hypernatremia at baseline conditions, we did not find significant changes in total body sodium stores or sodium palatability. In a separate cohort, BP was measured via telemetry in *Arrb2*-KO and C57BL/6 mice with and without DOCA. *Arrb2*-KO did not exhibit significant differences in BP before DOCA treatment when provided water alone, or when provided a choice of water and saline. However, *Arrb2*-KO exhibited an increased pressor response to DOCA-salt.

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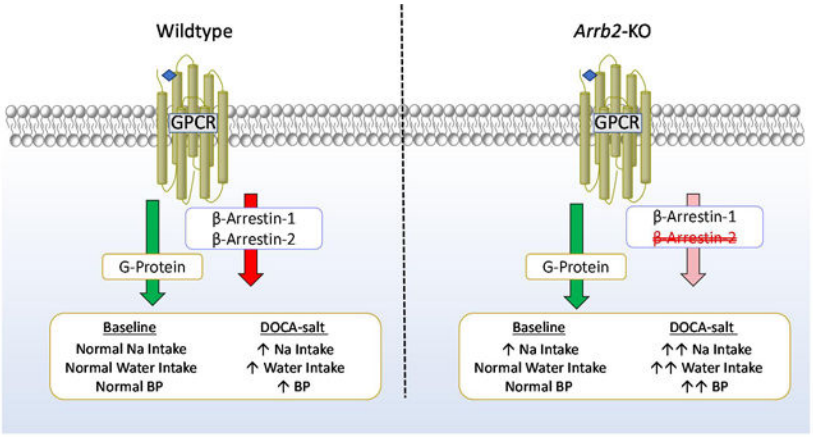
Conflicts of interest

CDS was a member of a Scientific Advisory Board for Ionis Pharmaceuticals in 2020. His contributions to that board are unrelated to the content of this manuscript. There are no other conflicts of interest.

Conclusions: These findings suggest that in salt-sensitive hypertension, ARRB2, but not ARRB1, might counterbalance the canonical signaling of GPCRs.

Graphical Abstract



Keywords

hypertension; fluid intake; G protein coupled receptor; β-arrestin; signaling

Introduction

Hypertension (HTN) is a major risk factor for cardiovascular disease, renal failure, stroke, myocardial infarction, and death. As reported by the American College of Cardiology and the American Heart Association, nearly half of the adults in the United States have HTN.[1] Despite the variety of treatments currently available to lower blood pressure (BP), many patients do not respond to antihypertensive therapy and thus have resistant HTN.[2] Resistant HTN is defined as patients taking three or more anti-hypertensive medications (including a diuretic) with no change in BP. Studies have shown that resistant HTN is associated with an altered autonomic function.[3]

It is well established that excessive sodium intake is associated with an increase in BP and therefore could be linked to the onset of HTN.[4] Meta-analyses have shown that a modest decrease in sodium intake has a beneficial effect of lowering BP in normotensive, hypertensive, and resistant-hypertensive patients.[5] This suggests that excessive sodium intake may contribute to the elevation of BP not only in patients with essential HTN but also in patients with resistant HTN. Blood pressure sensitivity to sodium intake varies among individuals. It is reported that 30–50% of the hypertensive population are salt sensitive, meaning that changes in BP are directly related to salt consumption.[6] The increase in BP due to high sodium intake is linked to aging, increased vascular peripheral resistance, endothelial dysfunction, cardiomyocyte remodeling leading to fibrosis and hypertrophy, and modulation of autonomic function.[7] A more recent concept also indicates that excess sodium may be stored in osmotically inactive pools which could serve as a buffering system to modulate BP in response to a sodium load.[8] This concept remains controversial and has been challenged.[9,10]

Deoxycorticosterone acetate (DOCA)-salt is a widely used model of salt-dependent HTN.[11] DOCA-salt HTN is initiated by an increase in vascular resistance without an increase in cardiac output.[12] Secondary to the excess of sodium intake and during the early stages, an increase in sympathetic outflow contributes to the increased BP, like what is observed in resistant HTN.[13] Among the systems involved in the development and maintenance of high BP in the DOCA-salt model, endothelin, vasopressin and angiotensin II (Ang II) have been reported to play a crucial role.[11,14] These hormones act through their corresponding G protein-coupled receptor (GPCR) subtypes. In particular, Ang II-mediated signaling during DOCA-salt has been reported to be increased in the brain, implicating the overactivation of the brain renin angiotensin system (RAS) in this model.[15] Within the brain, Ang II has been described to increase sympathetic outflow, sodium and water retention, and vasopressin release.[16,17] Further, pharmacological blockade of Ang II production or action within the brain can prevent and reverse DOCA-salt HTN in rodent models.[18–20]

Over the past decade, it has been established that in addition to G protein signaling, GPCRs including the Ang II type 1 receptor (AT_1R) can also signal via non-canonical or G-protein-independent mechanisms through β-arrestin. β-arrestins are recognized as GPCR adaptor proteins that are able to terminate G-protein signaling by mediating receptor internalization and signal desensitization.[21] In addition, β-arrestins have been reported to mediate G protein-independent signaling.[22] Two non-visual arrestins have been identified: β-arrestin-1 (ARRB1) and β-arrestin-2 (ARRB2). Both isoforms differentially modulate the signaling of GPCRs. At the level of the AT_1R, activation of ARRB1 and ARRB2 can either be beneficial or detrimental. Balanced agonists such as Ang II can activate both G-protein and β-arrestin pathways. Biased agonists are pharmacological agents which can preferentially activate either the G-protein or β-arrestin pathway, but not both. Activation of β-arrestin using biased agonists has been shown to lower BP and increase cardiac contractility.[23–25] Studies in several disease models suggests that ARRB2 can contribute to aortic aneurysms.[26,27] Moreover, specific activation of ARRB1 in the adrenal gland has been reported to promote aldosterone production leading to heart hypertrophy and fibrosis.[28,29]

Selective agonists have emerged which exhibit biased GPCR signaling. TRV120027 (TRV027) is a biased agonist which selectively engages β-arrestin downstream of the AT_1R. Our group recently reported that chronic intracerebroventricular (ICV) infusion of TRV027 increased salt aversion (reduced preference ratio for NaCl versus water) and increased water intake in DOCA-salt HTN.[25] In addition, and consistent with the literature, we also showed that acute ICV injection of TRV027 lowered BP.[23,25] These results might suggest that biased activation of β-arrestin downstream of the AT_1R can prevent and/or reverse the detrimental effects of the G-protein signaling pathway during hypertension. However, the involvement of each arrestin has not been well established. In the present study, we investigate the distinct roles of ARRB1 and ARRB2 on sodium intake and the role of ARRB2 in BP in mice subjected to DOCA-salt HTN.

Methods

Top Guidelines:

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

Procedures for animal breeding, two-bottle experimental design, water- and salt-intake measurements, urine and plasma analysis, Na challenge, transdermal glomerular filtration rate (tGFR), body composition, lickometer, and BP are described in the accompanying Online Supplemental Methods.[25,30–33]

Animals: This study was conducted in C57BL/6J (Stock 000664), β-Arrestin1-KO (*Arrb1*-KO; Stock 011131) and β-Arrestin2-KO (*Arrb2*-KO; Stock 011130) mice obtained from the Jackson Laboratories (Bar Harbor, ME). All experiments were conducted in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" and were approved by the Medical College of Wisconsin Animal Care and Use Committee.

Statistics: All data are presented as mean ± standard error of the mean (SEM). Parametric analyses were used throughout, including 3- or 2-way ANOVA with or without repeated measures and followed by selected (Sidak) or all pairwise (Tukey) multiple comparison procedures, independent *t*-test, or one-sample *t*-test. $P<0.05$ was considered statistically significant.

Results

Ablation of β-arrestin-2 Results in Increased Preference for Saline

To evaluate the role of β-arrestin signaling in males and females in the regulation of water and sodium intake, global *Arrb1*-KO, *Arrb2*-KO, and C57BL/6 control mice were subjected to the two-bottle choice paradigm at baseline and after 2 weeks with DOCA treatment. In each condition, mice were presented with two burettes containing water and increasing concentrations of saline (0.15, 0.30, or 0.45 M NaCl) (Figure 1A). First, mice were presented with a choice of two tap water-filled burettes. There was no difference in the side bias (left vs right burette) among the groups, averaging ~50% during both baseline and DOCA conditions (Figure 1B). At baseline, there was no difference in daily water intake among the groups. However, DOCA induced a significant increase in daily water intake in all groups when compared to baseline. Global deletion of ARRB2, but not ARRB1, further increased water intake compared to controls after DOCA ($P<0.01$) (Figure 1C).

Arrb1-KO, *Arrb2*-KO and C57BL/6 mice were next presented with a choice of tap water vs. isotonic saline (0.15M NaCl). Males and females were analyzed separately. Whereas the patterns of fluid intake were similar, there were sex-specific differences as noted below. At baseline, there were no differences in water intake, saline intake, and fluid intake, defined as total ingested volume (Figure 2). However, there was a modest increase in total saline preference (defined as the percentage of ingested saline volume from total ingested volume) in both female ($P=0.15$) and male ($P=0.09$) *Arrb2*-KO mice compared with C57BL/6 mice that did not reach statistical significance. The increase in saline preference in male *Arrb2*-

KO was significant when examined by a one sample *t*-test compared to a saline preference of 50% where mice drank equally from water and saline. When treated with DOCA, all groups exhibited higher water intake, saline intake, and total fluid intake compared to same genetic group under baseline conditions ($P<0.01$). Global deletion of ARRB2, but not ARRB1, further increased water intake compared to wildtype control in females ($P<0.01$) and modestly increased in males ($P=0.22$). In addition, male and female *Arrb2*-KO mice, but not *Arrb1*-KO mice, exhibited a significant increase in saline intake and total fluid intake compared to the control group after DOCA. Despite saline intake being higher in each genotype after DOCA treatment, water intake was also higher, and thus, animals did not exhibit a preference for saline as it remained under 50%. Indeed, the female C57BL/6 and *Arrb2*-KO mice and male C57BL/6, *Arrb1*-KO, and *Arrb2*-KO mice exhibited a modest aversion to saline after DOCA treatment as the mean saline preference was significantly lower than 50% (according to a one sample *t*-test).

Next, mice were presented with a choice of tap water vs. two more concentrated saline solutions (0.3 M, Figure S1) and (0.45 M NaCl, Figure S2). When presented tap water vs. 0.30 M saline, *Arrb2*-KO males and females exhibited an increase in saline intake at baseline, but this did not reach significance. When treated with DOCA, all groups exhibited higher water intake, saline intake, and total fluid intake compared to the same genetic group at baseline ($P<0.01$). Global deletion of ARRB2, but not ARRB1, in both sexes exacerbated DOCA-induced water intake, saline intake, and total fluid intake compared to C57BL/6 mice ($P<0.01$). Despite increased saline intake in all the DOCA groups, mice drank twice as much water, thus animals exhibited saline aversion (decreased saline preference) (Figure S1).

Finally, when presented tap water vs. 0.45 M saline, there was no difference in water intake, salt intake or total fluid intake at baseline among the groups (Figure S2). When treated with DOCA, global deletion of ARRB2, but not ARRB1, in females increased water intake, and total fluid intake compared to wildtype ($P<0.01$). All groups exhibited proportionally higher intake of water over saline (Figure S2).

The results from the 3-way ANOVA comparing C57BL/6 to *Arrb2*-KO (Table S1) and C57BL/6 to *Arrb1*-KO (Table S2) are tabulated. Importantly, sex-specific and genotype-specific differences, and an interaction between sex and genotype were noted for a number of fluid intake parameters in *Arrb2*-KO mice (Table S1). Fewer differences were noted in *Arrb1*-KO mice with most related to saline intake and preference (Table S2).

All data from males and females were combined since they had very similar drinking behaviors at all concentrations of saline (Figure 3). Under both baseline and DOCA conditions, we observed that as salinity increased, all animals tended to drink more water, reduce saline intake, modestly decrease total fluid intake, and develop an aversion to saline. There were no differences between *Arrb1*-KO and C57BL/6 in terms of water intake, saline intake, total fluid intake, and saline preference at any given saline concentration during either baseline or DOCA condition, as the curves are largely superimposable. The exception to this was a modest decrease in saline preference at 0.3 M saline compared with C57BL6. In contrast, *Arrb2*-KO mice exhibited a significant increase in saline intake compared to control mice when presented with 0.15 M and 0.30 M, but not 0.45 M saline. This response

was not observed in *Arrb1*-KO mice. Although *Arrb2*-KO mice showed the highest fluid intake at baseline, this did not reach significance compared to the control group. Finally, *Arrb2*-KO mice exhibited a saline preference at baseline when presented with 0.15 M saline (one-sample *t*-test, compared to 50%) and when compared to control mice. This comparison among different saline concentrations suggests a significant aversion to 0.45 M saline (Figure 3A). Given the increase in saline intake, it is not surprising that the amount of sodium ingested from saline is elevated in *Arrb2*-KO mice when presented with 0.15 M and 0.30 M, but not 0.45 M saline when compared to C57BL/6 mice (Figure 3B). Under DOCA, only *Arrb2*-KO mice exhibited a significant increase in saline intake when presented with 0.15 M and 0.30 M, but not 0.45 M saline when compared with wildtype. In addition, *Arrb2*-KO mice showed a significant increase in fluid intake when presented to 0.15 M, 0.30 M and 0.45 M saline. Moreover, there was no significant difference in saline preference at any saline concentration among the groups after DOCA (Figure 3C–D).

We measured sodium intake from food in a sub-cohort of the mice in Figure 3. These data show that *Arrb2*-KO and control mice exhibited the same overall level of sodium intake from food at all concentrations of saline (Figure S3). Thus, changes in sodium intake from saline were not compensated by changes of the sodium intake from food.

At the end of the experimental protocol of drinking measurements after the incremental saline challenges, mice were shifted back to a 2-bottle choice of drinking water and 0.15 M saline for 2 days after which mice were sacrificed and plasma electrolyte levels and organ masses were measured. *Arrb2*-KO females, but not males, exhibited a significant increase in plasma Na electrolyte levels compared to wildtype controls (P=0.03) (Figure S4). There were no differences in K, Cl, iCa, glucose, blood urea nitrogen (BUN), creatinine, and hematocrit between *Arrb1*-KO and C57BL/6 and between *Arrb2*-KO and C57BL/6 in either sex. *Arrb2*-KO females also exhibited: 1) a decrease in relative adrenal mass, 2) increase in relative kidney mass, and 3) an increase in relative inguinal white adipose tissue (iWAT) compared to C57BL/6 (P=0.01, 0.04 and 0.001; respectively) (Figure S5). There was no difference in relative organ weight in *Arrb2*-KO males compared to C57BL/6.

Hypernatremia and Elevated Sodium Excretion in β-Arrestin2-KO Mice

We next evaluated whether *Arrb2*-KO mice have altered basal urine and plasma sodium levels. To accomplish this, an independent cohort of *Arrb1*-KO, *Arrb2*-KO, and C57BL/6 mice were placed in metabolic cages for 24-hour urine collection and were provided drinking water (no saline). We did not observe any significant changes in urine Na and K concentration, nor urine osmolality, between C57BL/6 and *Arrb1*-KO or *Arrb2*-KO mice (Figure 4A). Urine potassium was slightly lower in *Arrb1*-KO compared to *Arrb2*-KO and C57BL/6 mice. We did not observe significant changes in plasma osmolality. However, consistent with the results above, we observed a significant increase in plasma sodium concentration in the *Arrb2*-KO group compared to the C57BL/6 group (P<0.01) (Figure 4B). There was no change in plasma potassium or plasma osmolarity in any group (Figure 4B). There was no difference in sodium consumption from food (Figure S6A), nor any change in urine sodium balance between groups (Figure S6B and S6C). Based on these results, we measured urinary copeptin, a metric of vasopressin release rate, and aldosterone. Although

there was a trend towards a decrease in urine copeptin in *Arrb2*-KO mice, this did not reach statistical significance (Figure S7A). There was also no difference in urine aldosterone (Figure S7B).

We next evaluated the capacity of *Arrb2*-KO mice to excrete a sodium load. *Arrb2*-KO and C57BL/6 mice were injected with a volume of isotonic NaCl equivalent to 10% of body weight into the intraperitoneal space, and urine was collected after 4 hours. *Arrb2*-KO mice exhibited a significantly higher sodium excretion to the urine compared to wildtype (Figure 4C). There were no differences between in potassium excretion *Arrb2*-KO and C57BL/6 mice in this experiment (Figure 4D). There was no evidence of renal dysfunction in *Arrb2*-KO mice as transcutaneous glomerular filtration rate (tGFR) showed no significant differences between *Arrb2*-KO and controls (Figure 4E).

No Alteration in Total Body Na Stores in *Arrb2*-KO Mice

To further explore whether *Arrb2*-KO mice exhibit changes in total body Na homeostasis, we next examined body fluid compartmentalization in a new cohort of mice. Briefly, although differences between sexes were observed for many endpoints, no effect of genotype or interaction between genotype and sex were observed for the following: body mass, body fat, total body water or tissue hydration, osmotically-active Na, osmotically-inactive Na, or total body Na pools (Table 1). Similarly, there were no changes in total body, osmotically-active or osmotically inactive potassium (data not shown). These findings indicate that although plasma Na concentration is elevated in *Arrb2*-KO mice, that total body Na stores are normal in these animals.

Arrb2-KO Mice Exhibit Normal Licking Responses During Brief Access to 0.15 M NaCl

To probe whether the increase in 0.15 M saline intake by *Arrb2*-KO mice was associated with a gross deficit in the ability to detect (taste) sodium, *Arrb2*-KO and C57BL/6 mice that had never previously exposed to 0.15 M saline were subjected to a two-bottle choice test in lickometer-equipped cages. Volumes of water and saline intake, and licking events were recorded for two hours. When the animals were presented with water and 0.15 M saline, we did not observe changes in the number of licking events that occurred during the first bout (*i.e.*, the first minute after the first lick during the testing period, which is most closely associated with taste hedonics) with either the water or saline burette (Figure 5). Likewise, there were no differences in the total number of licking bouts within the 2 hour experiment with either the water or the saline burette. When saline preference was calculated over the entire 2 hour testing period, both *Arrb2*-KO (P=0.05) and C57BL/6 (P=0.05) exhibited a preference for saline over water. Collectively, these results provide preliminary evidence arguing against a gross deficit in the ability to detect sodium in *Arrb2*-KO mice.

Effect of Global Deletion of ARRB2 on Blood Pressure

We next evaluated whether the elevated saline intake observed in *Arrb2*-KO mice is associated with an increase in arterial BP. We did not examine BP in *Arrb1*-KO mice as there were minimal effects on saline intake. Male and female *Arrb2*-KO and C57BL/6 were implanted with radiotelemeters and BP was continuously recorded under three different conditions. In period 1, animals were presented two water-filled burettes. In period 2,

animals were presented with one burette of water and one burette of 0.15 M saline. In period 3, animals were implanted with a subcutaneous DOCA pellet, and presented with one burette of water and one burette of 0.15 M saline (Figure 6A). During period 1 (water only), *Arrb2*-KO mice exhibited a mild elevation of SBP and lower heart rate (HR) (Figure 6B, Figure S8). However, this did not reach statistical significance. During period 2 (water vs 0.15M saline), no changes were observed in either BP or HR in *Arrb2*-KO mice despite the observation that this cohort (like those studied above) consumed 55% saline and 45% water. Lastly, in period 3, when animals were subjected to DOCA-salt, *Arrb2*-KO exhibited an exacerbated pressor response compared to controls. The increase was particularly notable during days 17–20 and 28–32, which correspond to the first and third weeks of DOCA treatment (Figure 6C). Although we did not find a significant sex x genotype interaction, when analyzed separately, female mice exhibited an increase in BP at all three time points post-DOCA treatment, but only a non-significant trend was observed in male mice (Figure S9). Hourly analysis of BP and HR recordings revealed that there was a slightly larger increase in SBP during the dark cycle compared to the light cycle during the baseline and saline conditions (Figure 6D). DOCA-salt increased BP more during the dark cycle compared to the light cycle, although the effect of DOCA was significant during both cycles (Figure 6D–E). Effects of genotype were noted in the 2-way ANOVA for daytime and nighttime SBP and for daytime HR (Table S3). There was also a significant effect of DOCA treatment. Because the evaluation included the entire time course of the study, there were no genotype x treatment interactions.

Discussion

The present study provides evidence implicating ARRB2 in the regulation of BP and fluid homeostasis. Our results demonstrate that mice carrying a global deletion of ARRB2 exhibit: 1) elevated saline preference under baseline conditions, 2) elevated water, saline and total fluid intake in response to DOCA, and 3) an exacerbated pressor response to DOCA-salt treatment. These results support the protective role of ARRB2 in mechanisms involved in salt-dependent HTN by attenuating sodium consumption and reducing BP. Although β-arrestins have been reported to be protective and beneficial for cardiovascular disease, β-arrestins can also be detrimental in other conditions such as opioid abuse, in which β-arrestin-dependent receptor internalization decreases analgesia and increases opioid tolerance.[34]

Our results showed that *Arrb2*-KO mice exhibited higher daily saline intake and pressor responses to DOCA-salt and are consistent with our previous studies examining the effects of an AT_1R β-arrestin biased agonist, TRV027.[25] In that study, stimulation of AT_1R-mediated β-arrestin signaling in the brain using TRV027 induced an aversion to saline and decreased BP in DOCA-salt HTN. Essentially, the effects of activating β-arrestin signaling downstream of the AT_1R were opposite to the effects of deleting ARRB2. Taken together, these results suggest that the modulatory effects of TRV027 on salt intake and BP may be mediated primarily by ARRB2, and less so by ARRB1.

Other researchers have evaluated the role of Ang II analogs known to be β-arrestin biased agonists such as SII (Sar1, Ile4, Ile8) and bovine serum albumin conjugated to Ang II (BSA-

Ang II) in the regulation of water and salt intake. It is notable that the acute injection of these analogs induced excessive saline intake.[35–37] It is possible that the differences between these acute studies and our results in a chronic setting indicate that long-term activation of β-arrestins could induce distinct molecular signals that differ from the short-term effects. Indeed, it was reported that different biased agonists of the AT_1R mediate subtly different intracellular signals.[38]

Previous studies have shown that activation of β-arrestin reduced surface expression of different Na transporters in the kidneys such as the Na/K pump, and sodium-hydrogen antiporter 3 and 5.[39–41] Moreover, other studies have shown that TRV027 decreases tubular Na reabsorption in a canine model of heart failure.[42,43] Based on this, we would anticipate that *Arrb2*-KO mice would exhibit increased sodium reabsorption. Interestingly, the elevated BP and saline intake in *Arrb2*-KO mice were associated with hypernatremia. However, that renal function (as measured by GFR) and natriuresis (as measured by response to acute volume expansion) were preserved in *Arrb2*-KO mice suggests that the hypertensive phenotype may not be largely driven by a renal mechanism. *Arrb2*-KO mice exhibited higher Na and K urinary excretion compared to *Arrb1*-KO, and increased Na excretion in response to an acute increase in volume suggesting that the renal compensatory mechanism to BP elevation is maintained in these mice. We recognize that renal function and electrolyte balance were measured only at the endpoint and a more comprehensive approach would be necessary for a definitive conclusion.

To explore potential mechanisms driving increased saline intake behavior in *Arrb2*-KO mice, we examined total body sodium stores and compartmentalization using bioimpedance and ashing methods. These studies indicated that although plasma sodium concentrations were increased in *Arrb2*-KO mice, total body sodium, and its distribution between osmotically-active and -inactive pools were essentially normal. Further, experiments using a lickometer indicated that preference for 0.15M saline drink solution is essentially intact, and that the preference was indistinguishable from control mice in the relatively brief-access paradigm that was utilized. These results support the concept that the *Arrb2*-KO mice are able to detect sodium in the saline drink solution, and that sodium taste / perception is not grossly altered. Thus, there does not appear to be a physiological need for sodium, nor a loss of the ability to detect sodium. Instead, these results hint that *Arrb2*-KO mice may exhibit a change in post-ingestive responses to saline consumption. They may not experience the same negative consequences of consuming large volumes of saline, or they may exhibit deficits in learning and memory functions regarding consummatory behaviors. Future studies to explore these concepts might include assays of conditioned taste aversion to sodium, studies of saline consumption when a sodium taste inhibitor (*e.g.*, amiloride) is present in the drink solution, and other behavioral and electrophysiological approaches.

Given the apparent increase in saline intake in *Arrb2*-KO mice, we tested the hypothesis that offering the mice free access to a 0.15M NaCl drink would be sufficient to cause BP elevation. Our results indicate that *Arrb2*-KO mice show a slight (but not statistically significant) increase in BP at baseline as previously reported.[26,27] Contrary to our hypothesis, there was no augmentation of the BP response when the *Arrb2*-KO were presented with a choice of water and 0.15 M saline. This is probably explained by a

preservation of renal function. However, when the animals were subjected to DOCA-salt, mice lacking ARRB2 showed an exacerbated pressor response. These results are also consistent with our previous studies showing that infusion of TRV027 to the brain decreased SBP in DOCA-salt.[25] This suggests that the protective effects of TRV027 in decreasing BP might be mediated by the activation of ARRB2.

All our experiments used males and females to account for sex variabilities. Our results did not show sex x genotype, nor sex x genotype x DOCA-salt interactions in drinking behavior, indicating that the altered ingestive behaviors observed in the *Arrb2*-KO mouse are independent of sex. Nonetheless, when analyzed independently, the magnitude of the BP increase after DOCA may be modestly greater in females than males. Moreover, it is tempting to speculate therefore that the effects we observed in this study are due to a loss of ARRB2, specifically in the brain. Whereas, this speculation is consistent with our data, direct studies employing conditional brain-specific deletion of ARRB2 would be needed to perform to test this experimentally. Such studies are currently in progress using both $Arrb1^{Flox}$ and $Arrb2^{Flox}$ mice bred to neuron-specific Cre-recombinase driver mice, or with brain region-specific injection of AAV^{Cre}. It is notable in this regard that intracerebroventricular administration of TRV027 lowered BP and improved autonomic function in spontaneously hypertension rats.[23] Lastly, over-expression of either ARRB1 or ARRB2 in the rostral ventrolateral medulla of spontaneously hypertension rats lowered BP.[44,45]

Perspectives

β-arrestin biased agonist such as TRV027 have been shown to elicit beneficial cardiovascular effects, not only in rodent models but also in clinical settings. Administration of TRV027 attenuated high blood pressure in healthy patients with elevated renin angiotensin system levels.[46] Whereas TRV027 did not show a significant clinical benefit in patients an acute heart failure in the BLAST-AHF trial,[47] a *post-hoc* analysis, in which these patients were stratified based on their baseline SBP, supports the concept that TRV027 reduced 180-day all-cause mortality, cardiovascular death, or readmission in the two higher BP tertiles.[48] That activation of β-arrestin using biased ligands reduced BP and decreased sodium intake in salt-sensitive model of HTN suggests that the β-arrestin pathway must be further investigated. One possible interpretation of our previous and current studies is that the beneficial effects of TRV027 may be mediated by ARRB2 associated with the AT_1R. This concept serves as a foundation to develop more specific and efficacious β-arrestin biased ligands for the treatment of salt-sensitive or resistant HTN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ANG	Angiotensin
ARRB1	β-arrestin 1
ARRB2	β-arrestin 2
***Arrb1*-KO**	β-arrestin 1 knockout
***Arrb2*-KO**	β-arrestin 2 knockout
AT_1R	angiotensin II AT1 receptor
BP	blood pressure
BSA-Ang II	Bovine Serum Albumin Conjugated Angiotensin II
DOCA	Deoxycorticosterone acetate
GPCR	G protein coupled receptor
HR	heart rate
HTN	hypertension
iWAT	inguinal white adipose tissue
RAS	renin-angiotensin system
SII	(Sar1, Ile4, Ile8) Angiotensin
tGFR	transdermal glomerular filtration rate
TRV027	TRV120027

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What is new?

- Global deletion of ARRB2 augmented fluid and saline intake in mice with and without DOCA-salt.
- Global deletion of ARRB2 in mice exhibited normal blood pressure at baseline with coincident bradycardia but exhibited an exacerbated pressor response after DOCA-salt.
- The role of ARRB2 in BP regulation and fluid homeostasis is largely sex independent.

What is relevant?

Activation of ARRB2 was shown to modulate salt intake and BP regulation. Therefore, targeting ARRB2 downstream of GPCRs that control fluid intake and BP such as the Ang II AT_1R might be useful for the development of novel and efficacious therapies against resistant and salt-sensitive hypertension.

Clinical/Pathophysiological Implications

Agonists of the AngII AT_1R which activate β-arrestin signaling in a biased manner lower arterial pressure in several models of HTN in rodents, and lower blood pressure in patients with a high index of renin-angiotensin activation. They have also been reported to be cardioprotective in preclinical models of heart failure and may have some benefit in heart failure patients that exhibit high BP. Other rodent studies suggest that some of these benefits may be derived from the activation of Ang II AT_1R β-Arrestin pathway in the central nervous system. Further study is clearly warranted to assess the full range of cardioprotection and the mechanisms by which this occurs.

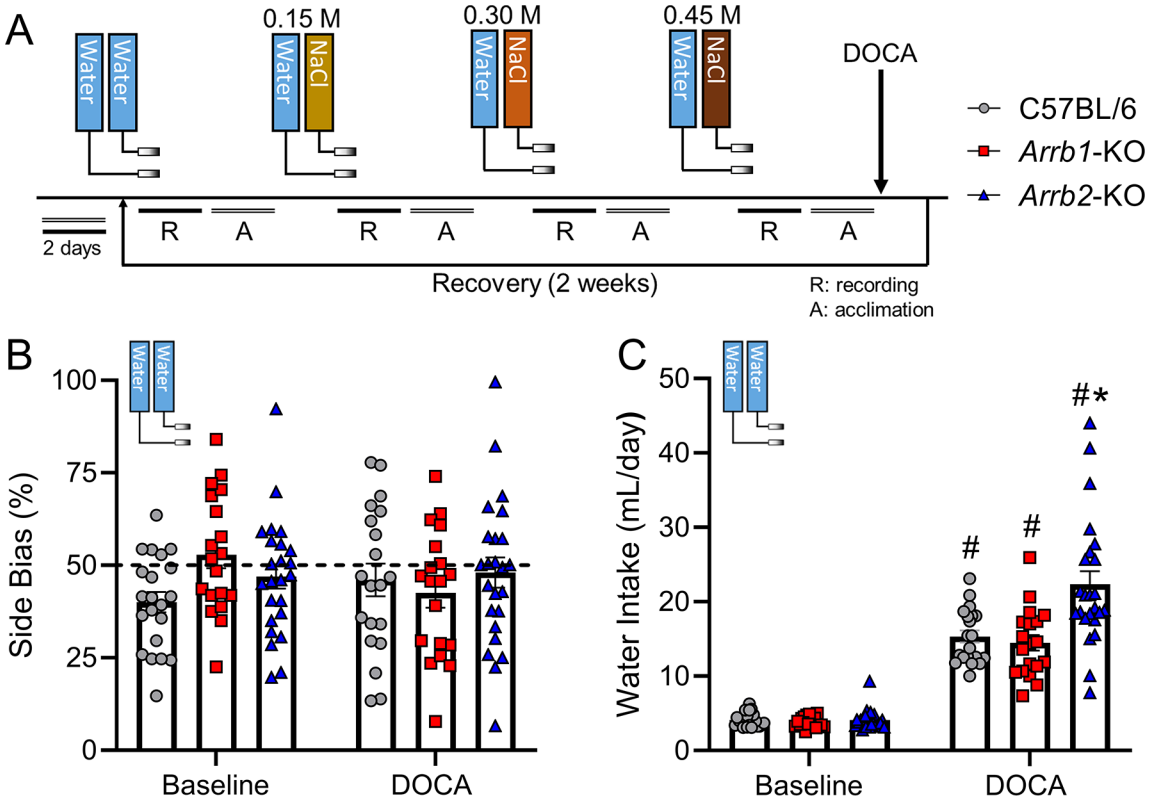

Figure 1. Ablation of β-Arrestin2 Exacerbates Water Intake with DOCA-salt.

A) Schematic representation of the two-bottle choice experimental protocol. Mice were subjected to the two-bottle paradigm under baseline and DOCA conditions. At each condition, mice were subjected to 4 different trials in which mice were presented to two burettes containing: 1) water vs. water, 2) water vs. 0.15 M saline, 3) water vs. 0.30 M saline, and 4) water vs. 0.45 M saline. Burettes were switched every 24h to avoid a side bias and data were calculated as the average of 2 consecutive days. B and C) Side bias (B) and total daily water intake (C) when animals were presented with 2 burettes filled with water (water vs. water). Data are expressed as mean±SEM. Data were analyzed by 2-way ANOVA with Tukey multiple comparisons procedure. *P<0.05 compared with C57BL/6; $^{\#}P$<0.05 compared to same genetic groups at baseline. C57BL/6 (n=21), *Arrb1*-KO (n=19), and *Arrb2*-KO (n=24).

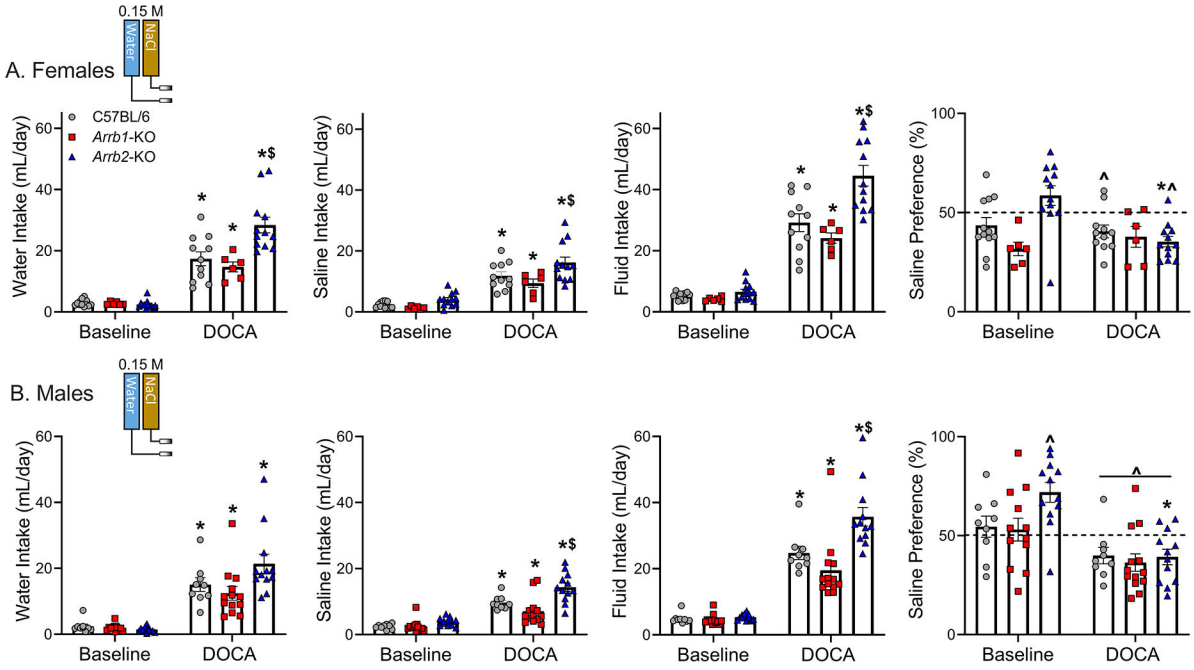

Figure 2. Drinking Responses to Two-bottle Choice: Water vs. 0.15M Saline

Female (A) and male (B) C57BL/6J, *Arrb1*-KO and *Arrb2*-KO mice were presented with a choice of water vs. 0.15 M saline at baseline and after 2 weeks of DOCA-salt. Total water intake, saline intake, fluid intake (calculated as total water intake plus total saline intake), and saline preference (calculated as the percentage of total saline intake over total fluid intake) are shown. Data are expressed as mean±SEM. Data were analyzed by 3-way ANOVA with Sidak multiple comparisons procedure. **P*<0.05 compared to same genetic group on baseline condition; $ *P*<0.05 *Arrb2*-KO+DOCA vs. C57BL/6+DOCA; ^ *P*<0.05 one sample *t*-test compared to 50%. C57 females (n=12), C57 males (n=9), *Arrb1*-KO females (n=6), *Arrb1*-KO males (n=13), *Arrb2*-KO females (n=12), and *Arrb2*-KO males (n=12).

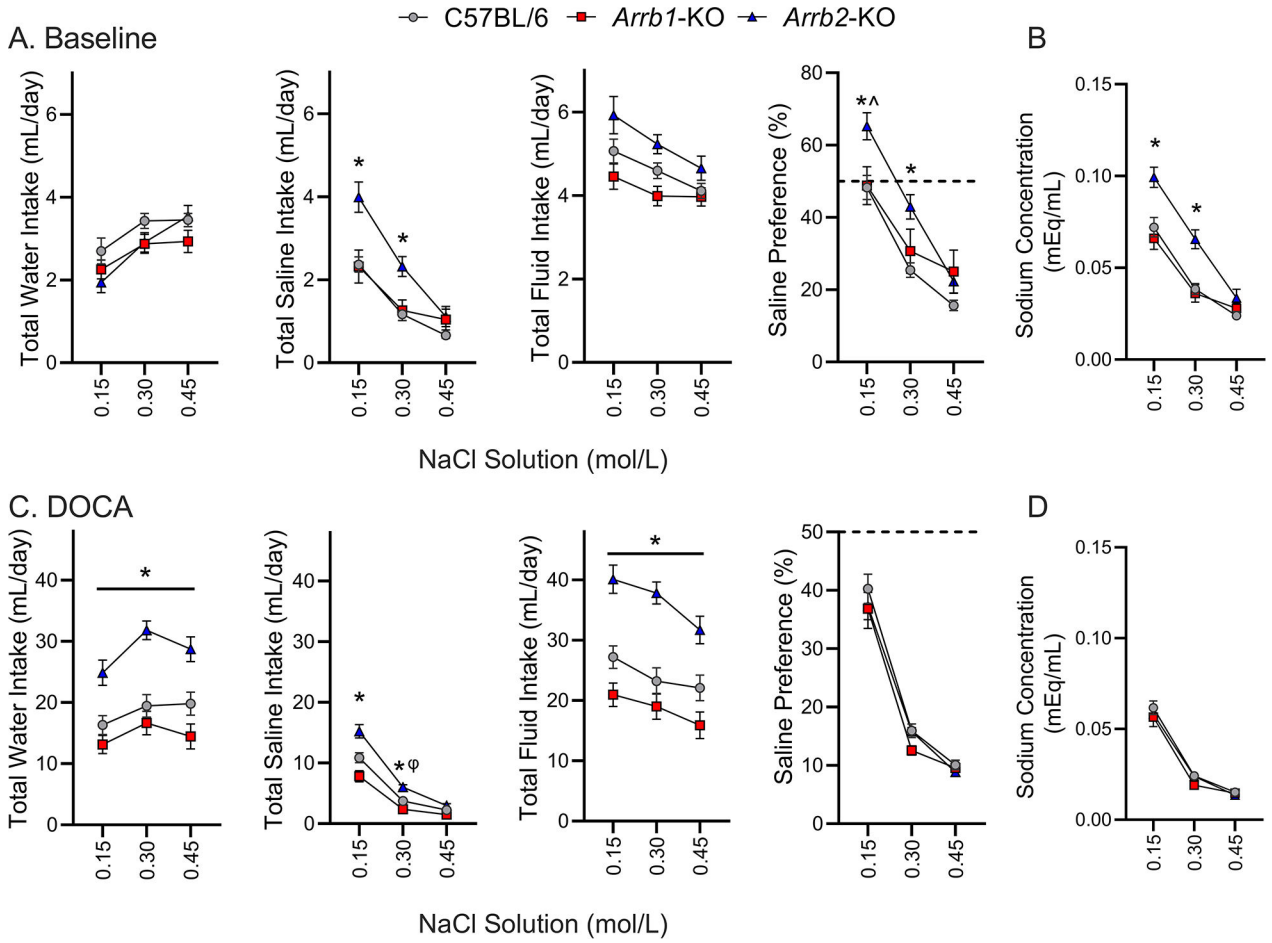

Figure 3. Summary of Drinking Responses to 0.15 M, 0.30 M, and 0.45 M Saline.

Summary of combined data of male and female C57BL/6, *Arrb1*-KO and *Arrb2*-KO mice subjected to the 2-bottle choice paradigm at Baseline (A and B) and after 2 weeks of DOCA (C and D). Total water intake, saline intake, fluid intake (calculated as total water intake plus total saline intake), saline preference (calculated as the percentage of total saline intake over total fluid intake) and sodium concentration are shown. Data are expressed as mean±SEM. Data were analyzed by 2-way ANOVA with Tukey multiple comparisons procedure. **P*<0.05 *Arrb2*-KO vs. C57BL/6; ᵠ*P*<0.05 *Arrb1*-KO vs. C57BL/6J; ^*P*<0.05 one sample *t*-test compared to 50%. C57BL/6 (n=21), *Arrb1*-KO (n=19), and *Arrb2*-KO (n=24).

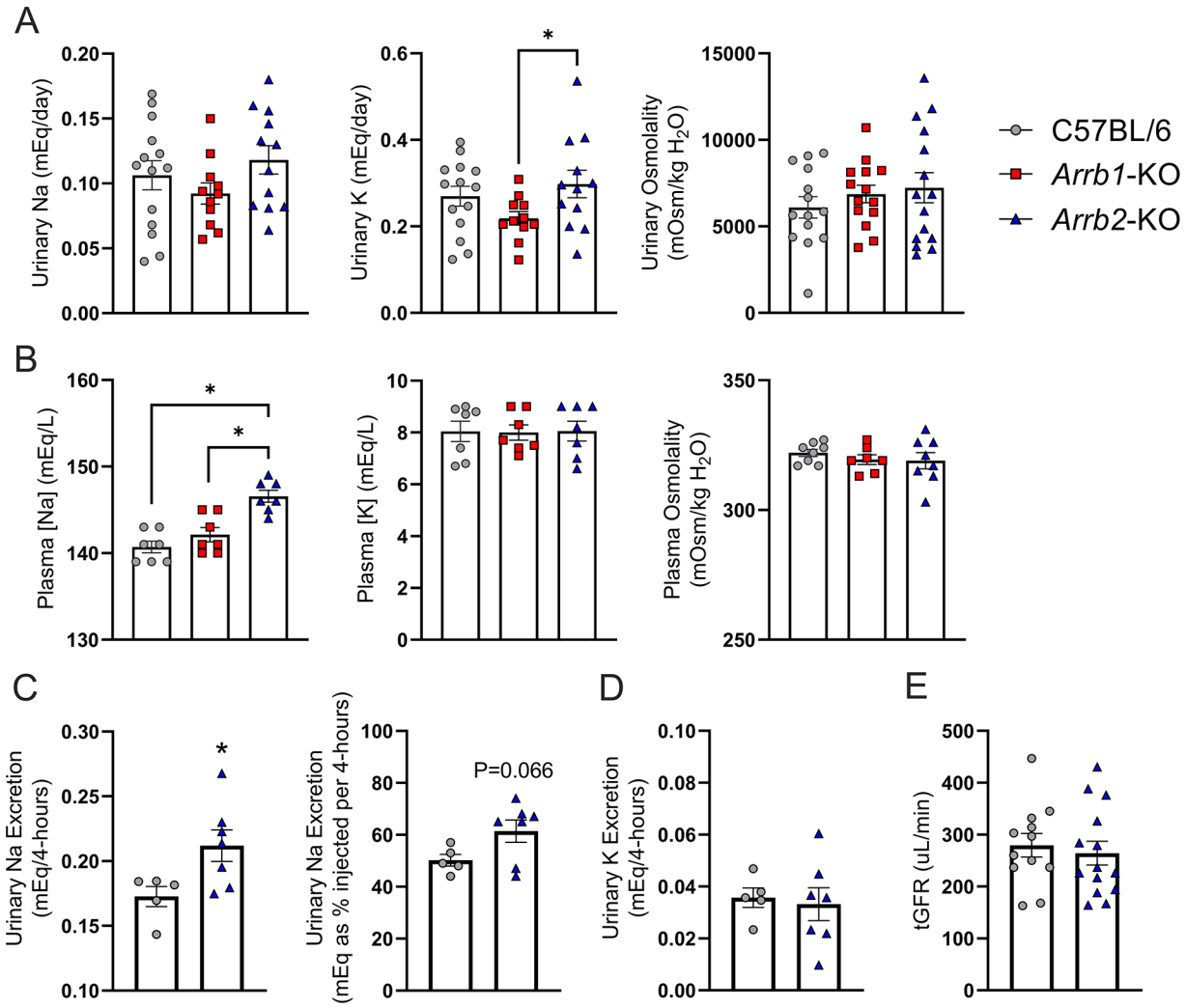

Figure 4. Plasma and Urine Electrolytes.

Mice were placed in metabolic cages for 24-hour urine collection. A and B) Urine Na, K and osmolality (A); and plasma Na, K and osmolality (B) were measured in *Arrb1*-KO, *Arrb2*-KO and wildtype controls. C-E) Capacity to excrete Na was assessed in *Arrb2*-KO mice and wildtype controls. C) Urine Na excretion 4-hours post intraperitoneal injection of isotonic saline solution. D) Urine K excretion 4-hours post intraperitoneal injection of isotonic saline solution. E)Transcutaneous measurement of glomerular filtration (tGFR) rate was assessed in *Arrb2*-KO mice and wildtype controls. Data are expressed as mean±SEM. Data were analyzed by *t*-test. **P*<0.05 compared with C57BL/6 or *Arrb1*-KO. C57BL/6 (n=7–14), *Arrb1*-KO (n=7–11), and *Arrb2*-KO (n=7–15).

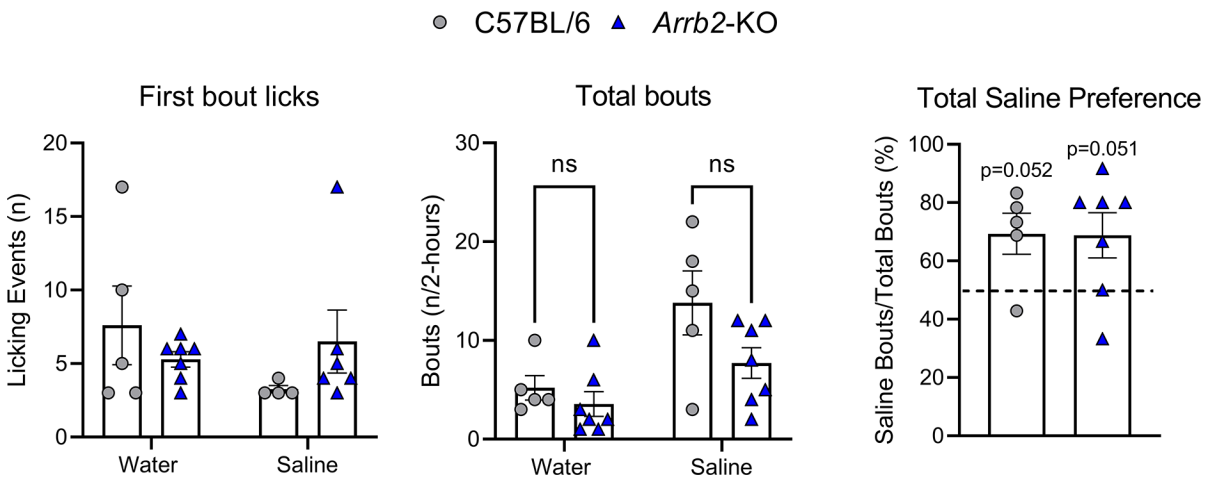

Figure 5. Saline Intake in a Brief-Access Paradigm.

Voluntary saline intake was assessed in Arrb2-KO mice and C57BL/6. Animals were subjected to the two-bottle paradigm in lickometer-equipped cages. Mice were presented with 2 burettes, one containing water and one containing 0.15 M saline. Licking events from each burette were recorded for 2 hours. Number of licking events during the first bout (first minute) were totaled during the 2-hour experiment. Data are expressed as mean±SEM. One point was identified as an outlier in each of the C57 and *Arrb2*-KO (Saline) by Grubb's test and were excluded from the analysis. Data were analyzed by one sample *t*-test. C57BL/6 (n=5) and *Arrb2*-KO males (n=7).

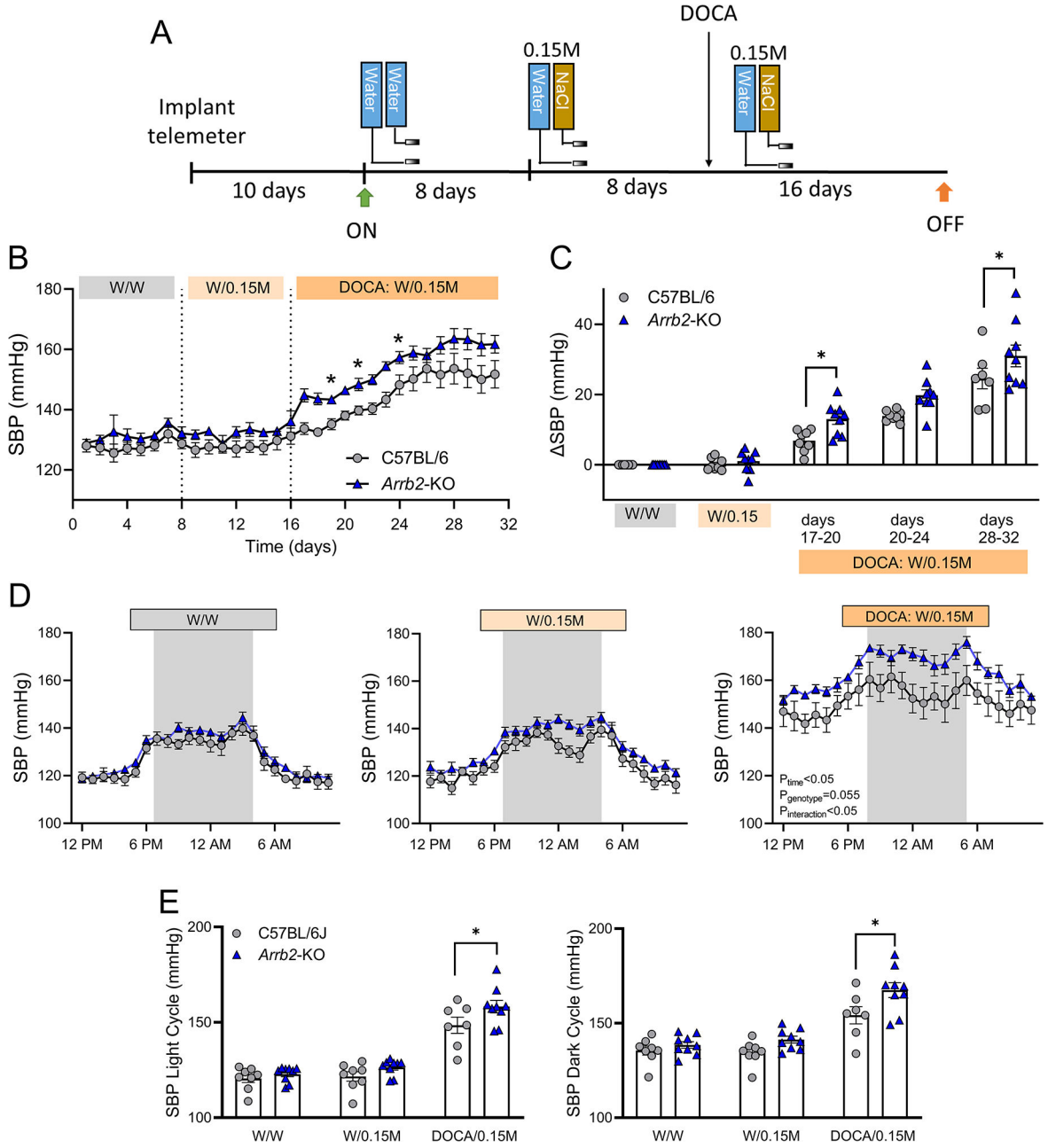

Figure 6. Ablation of β-Arrestin2 Exacerbates DOCA-salt Hypertension.

A) *Arrb2*-KO mice and wildtype controls were instrumented with radiotelemeters. Subsequently, mice were subjected to 3 separate conditions and blood pressure (BP) and heart rate (HR) were continuously recorded. First, animals were presented with 2 burettes of water. Second, animals were presented with water vs. 0.15M saline. Third, animals were implanted with DOCA pellets and given water vs. 0.15 M saline. B and C) The daily average systolic blood pressure (SBP, B) and averaged per condition (C) are plotted. D) Hourly SBP average during water, saline and DOCA conditions. E) Average SBP during water vs. water, water vs. 0.15M saline and DOCA conditions are shown separated into the light and dark (shadowed region) cycles. Data are expressed as mean±SEM. Data were analyzed by 2-way

ANOVA with Tukey multiple comparisons procedure (B-D) or Sidak multiple comparisons procedure (E). *P<0.05 compared to C57BL/6. C57BL/6 (n=8), and *Arrb2*-KO (n=9).

Table 1:

Body Composition, Fluid Compartmentalization, and Na Stores

	C57BL/6J		*Arrb2*-KO		ANOVA p-values		
Endpoint	**Male n=4**	**Female n=5**	**Male n=6**	**Female n=3**	**G**	**S**	**GxS**
Age (wk)	9.3±0.0	9.2±0.0	11.5±0.9	9.7±0.0	0.06	0.19	0.20
Body mass (g)	25.7±2.2	20.6±1.0	27.2±1.1	20.1±0.3	0.72	<0.01	0.48
Body fat (%)	19.6±0.7	20.1±0.5	22.2±1.9	17.9±0.6	0.88	0.20	0.11
Total body water (mL)	15.1±1.4	12.1±0.5	15.4±0.4	12.1±0.3	0.83	<0.01	0.87
Hydration (% body mass)	58.9±0.5	58.5±0.3	57.0±1.4	60.1±0.4	0.88	0.19	0.11
Extracellular Fluid (mL)	6.1±0.6	5.0±0.2	6.0±0.2	5.1±0.2	0.99	<0.01	0.70
Intracellular Fluid (mL)	9.0±0.8	7.1±0.4	9.4±0.3	7.0±0.1	0.74	<0.01	0.59
Extracellular Fluid (% TBW)	40.6±1.0	41.6±0.9	39.1±0.6	42.5±0.5	0.72	0.02	0.19
Total body Na (mEq)	1.24±0.11	1.12±0.06	1.26±0.02	1.09±0.05	0.96	0.04	0.72
Total body Na (mEq/g)	0.048±0.001	0.054±0.002	0.047±0.002	0.054±0.002	0.64	<0.01	0.61
Osm. Active Na (mEq)	0.93±0.09	0.75±0.03	0.91±0.03	0.77±0.02	0.97	<0.01	0.79
Osm. Active Na (mEq/g)	0.036±0.001	0.036±0.001	0.034±0.001	0.038±0.001	0.76	0.03	0.08
Osm. Active Na (% total Na)	74.4±2.1	67.7±2.6	72.5±2.1	70.3±2.2	0.88	0.09	0.37
Osm. Inactive Na (mEq)	0.32±0.04	0.37±0.05	0.35±0.03	0.33±0.03	0.90	0.74	0.37
Osm. Inactive Na (mEq/g)	0.012±0.001	0.018±0.002	0.013±0.001	0.016±0.002	0.78	0.02	0.56

Data presented as mean±sem, and analyzed by 2-way ANOVA.