Effects of ovarian hormones on blood pressure regulation in middle-aged female rats lacking central serotonin

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Abstract

Hypertension is a significant problem for middle-aged men and post-menopausal women. Central serotonin (5-hydroxytryptamine; 5-HT) neurons project to pre-sympathetic and parasympathetic neurons that regulate autonomic drive to the heart and vasculature. However, whether defects in central 5-HT signaling contribute to hypertension in adult animals is unclear. Any effects of 5-HT on arterial blood pressure (ABP) could depend on sleep-wake state, as 5-HT neurons have maximal firing during wakefulness and progressively reduced firing during non-rapid eye movement (NREM) and REM sleep. Recently, we showed that 1-year-old rats lacking central 5-HT had increased ABP; this phenotype occurred only in males, and was most evident during REM. It may be that in females, ovarian hormones compensate for the loss of 5-HT, preventing a rise in ABP. We hypothesized that ovariectomy will lead to a greater increase in the ABP of female rats deficient in central 5-HT compared to female wild-type (+/+) controls. To test this, we will use 1-year-old female rats deficient in tryptophan hydroxylase 2 (TPH2-/-), an enzyme necessary for 5-HT synthesis, as well as +/+ controls. Using femoral catheters, ABP will be measured as the rats cycle between vigilance states (determined by behavioral observation). TPH2-/- and +/+ females will then be ovariectomized to deplete systemic ovarian hormones. Following a two-week recovery period, ABP will be measured again and compared to baseline values. It is expected that after ovariectomy, the ABP of female TPH2-/- rats will increase to a greater degree than female +/+. Our interpretation would be that ovarian hormones protect adult females from the hypertensive effects of central 5-HT deficiency.

Introduction

- We have an incomplete understanding of the neuronal mechanisms underlying hypertension, a significant problem in middle-aged men and post-menopausal women.
- 5-HT neurons project to pre-sympathetic and parasympathetic neurons that regulate autonomic drive to heart and vasculature, and are thus well positioned to influence the regulation of arterial blood pressure (ABP).
- The effect of 5-HT neurons on ABP may depend on state of vigilance, as their firing rate progressively decreases as animals move from wakefulness (QW) to non-rapid eye movement sleep (NREM) to REM sleep, when they are actually silent.
- Recent findings from our lab show that middle-aged (1 year old) male rats lacking 5-HT (tryptophan hydroxylase 2 knockout; TPH2-/-) have high ABP compared to +/+ controls, particularly during QW and REM sleep. This phenotype is absent in female TPH2-/- rats.

Ovarian hormones may be able to compensate for a loss of central 5-HT in order to maintain normal blood pressure in middle-aged females

Background

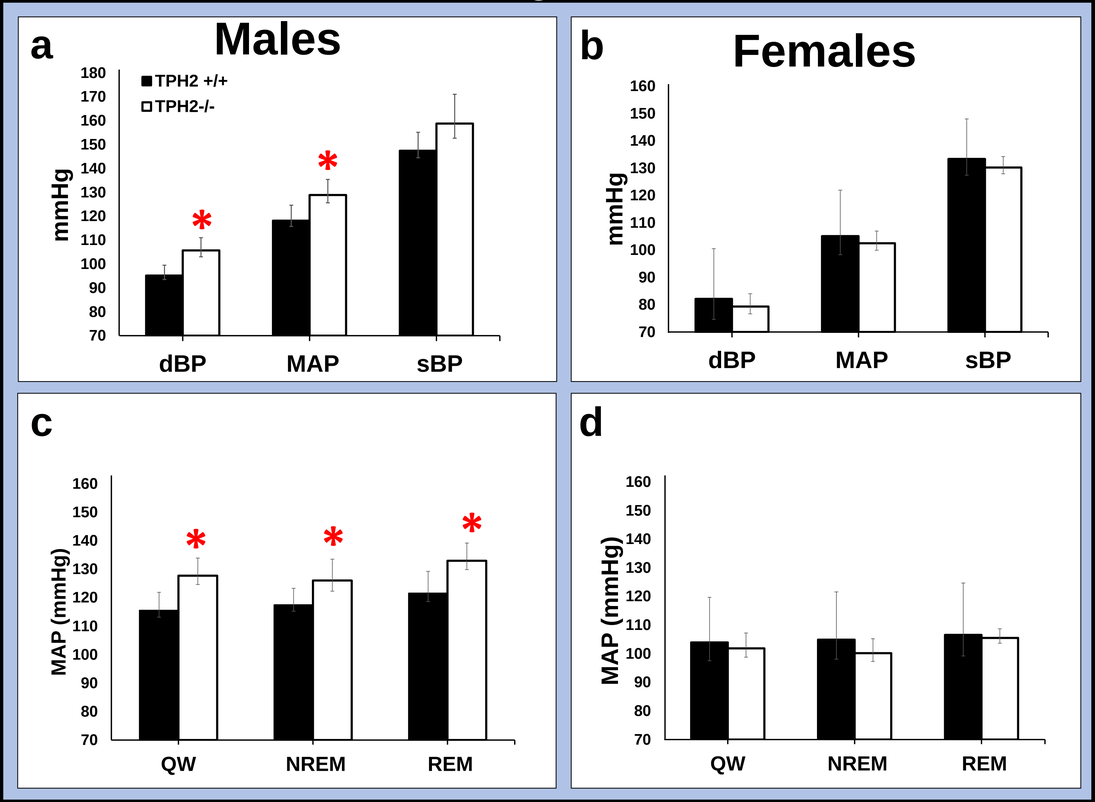

Figure 1: Middle-aged male TPH2-/- rats have high ABP compared to +/+ controls ***(a, c)*** Compared to middle-aged male +/+ rats (black bars, n=8), middle-aged male TPH2-/- rats deficient in CNS 5-HT (white bars, n=5) had a significantly elevated mean arterial pressure (MAP) across all states of vigilance due to marginally increased systolic blood pressure (sBP) and a significantly increased diastolic blood pressure (dBP). For males of both genotypes, MAP, sBP, and dBP were highest in REM. In females, 5-HT deficient TPH2-/- females (white bars, n=4) had the same sBP, dBP and MAP as female +/+ controls ***(b)***, irrespective of state of vigilance***(d)***.

Hypothesis

We hypothesize that ovariectomy will lead to a greater increase in the ABP of female rats deficient in central serotonin compared to female wild-type controls

Methods

Animals and General Procedures

We will use middle-aged (12-13 months old) female rats lacking tryptophan hydroxylase 2 (TPH2-/-), the enzyme catalyzing the rate-limiting step of 5-HT biosynthesis, as well as wild-type (+/+) controls. ABP will be measured prior to and following ovariectomy using femoral arterial catheters as the rats cycle between vigilance states. Whole-body plethysmography will be used to monitor respiratory variables (Figure 2a). Vigilance state will be determined visually, using previously validated behavioral and physiological criteria. We will compare the change in ABP (pre- vs. post-ovariectomy) for TPH2-/- and +/+ rats.

Surgery

Under ~2.5% isoflurane, a catheter will be placed in the left femoral artery, and tunneled up the dorsum through the subcutaneous space, allowing catheter access through a nuchal incision. Bilateral ovariectomy will be performed under ~2.5% isoflurane through a single flank incision. Ovaries will be isolated from uterine horns and surgically excised.

Experimental Protocol

For each group, we will allow 2 recovery days following the implantation of catheters. Rats will then be allowed to cycle between vigilance states for 4 hours. Rats will then undergo an ovariectomy to deplete systemic ovarian hormones. To date we have measured ABP (pre-surgery) and ovariectomized 4 +/+ and 3 TPH2-/- rats.

The rats will be given 2-3 weeks to recover from surgery, and to ensure near complete depletion of ovarian hormones. ABP will again be measured, and for each rat we will determine the change in ABP variables from pre- to post-ovariectomy (Figure 2b). After the study, an ELISA assay for ovarian metabolites will be taken from the feces to ensure adequate depletion of ovarian hormones.

Data Analyses

ABP and respiratory variables will be measured offline using LabChart 8.0 software (ADInstruments).

Data and Statistical Analyses

sBP, dBP and MAP will be analyzed from each animal across multiple episodes of QW, NREM, and REM. Average ABP data will be determined for each animal in each state. Significant effects of 5-HT deficiency and ovariectomy, as well as potential interactions, on ABP will be determined using a two-factor analysis of variance (2FA; factor 1: genotype; factor 2: presence or absence of ovaries).

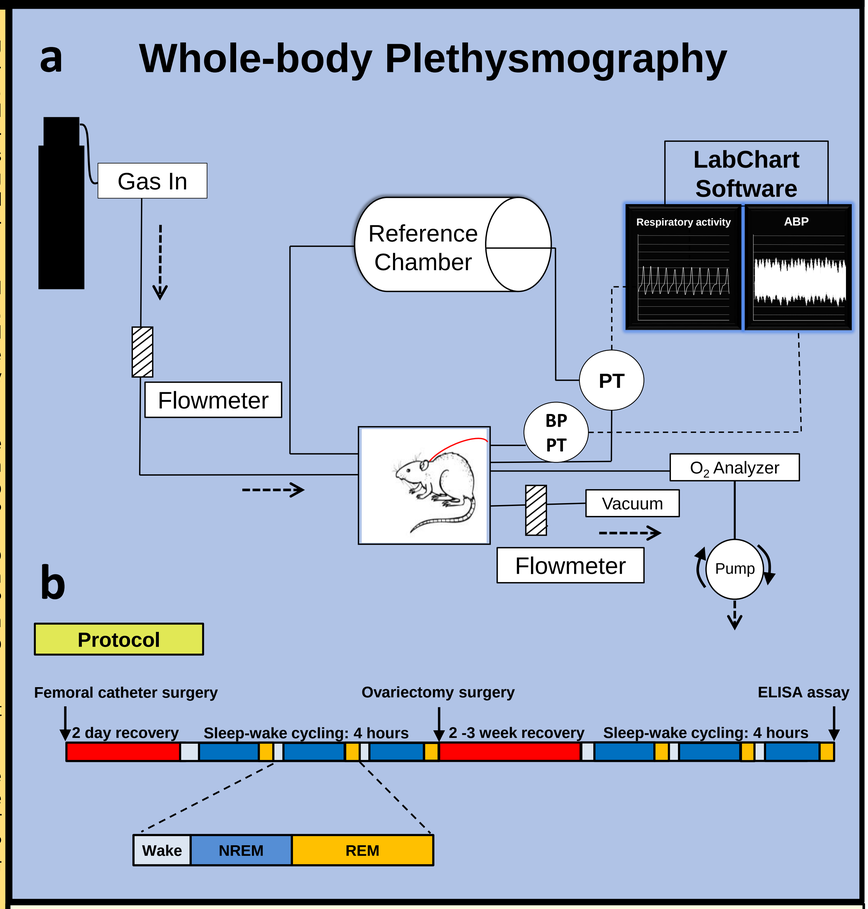

Figure 2: ***(a)*** Schematic of plethysmography setup we will use to monitor ABP and respiratory variables. A blood pressure transducer (BP-PT) will be used to measure ABP**.** All values were recorded in LabChart software for data analysis. ***(b)*** Experimental protocol.

Expected and Alternative Outcomes, Limitations

- We expect that following ovariectomy, the ABP of female TPH2-/- rats will increase more than +/+ controls. Post-ovariectomy, the ABP of female TPH2-/- will be similar to male TPH2-/-, providing evidence that ovarian hormones protect females from the deleterious effects of central 5-HT deficiency on ABP regulation.
- Alternative Outcome: It may be that rather than a lack of estrogen, the presence of testosterone contributes to the high ABP of male TPH2-/-
- Limitation: We will not be able to resolve the relative effects of estrogen and progesterone on the ABP of +/+ and TPH2-/- rats.

Future Directions

- If the ABP of female TPH2-/- is unaffected by ovariectomy, we will measure the ABP of +/+ and TPH2-/- males before and after castration. Rather than a protective effect of female sex hormones, it may be that in the absence of 5-HT, a hypertensive effect of testosterone emerges. Thus, we would expect to see that, following castration, ABP would fall more in TPH2-/- males compared to +/+ males.
- Hypertension is a known contributor to heart failure. Our plan is to analyze the structure and function of the hearts of male TPH2-/- and +/+ rats. We expect to see evidence of cardiac remodeling in TPH2-/- rats (hypertrophy) and possibly reduced ejection fraction.

References

- Magnusson JL, and Cummings KJ. A loss of central serotonin in middle-aged rats leads to increased blood pressure solely in males. Poster presented at: Experimental Biology Conference 2017. 2017 April 22-26; Chicago, IL.

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