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Lack of the serotonin transporter does not prevent mineralocorticoid hypertension in rat and mouse

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Abstract

We hypothesized that lack of a functional serotonin transporter (SERT) would increase basal blood pressure and enhance the development of deoxycorticosterone acetate (DOCA)-salt hypertension compared to wild type (WT) controls. Mean arterial blood pressure was measured in WT and SERT knockout (KO) mice and rat models through radiotelemetry. Basal blood pressures were not different between respective WT and KO. Moreover, blood pressure elevated similarly (~50 mm Hg) in all strains given DOCA and salt. Thus, the lack of functional SERT did not prevent development of DOCA-salt induced hypertension or modify basal blood pressure significantly.

Keywords

5-HT; serotonin transporter; systemic hypertension

1. Introduction

Serotonin (5-HT, 5-hydroxytryptamine) was discovered and characterized over 60 years ago by the Italian scientist Erspamer. The circulatory system is exposed to 5-HT primarily through aggregation of platelets, which take up and store a near millimolar concentration of 5-HT through the actions of the serotonin transporter (SERT). Tissues also take up and concentrate 5-HT via SERT (Ni *et al.*, 2004; Ni and Watts, 2006). 5-HT acts as both a dilator and constrictor of the arterial circulation (Watts, 2005).

Several findings suggest that 5-HT contributes to systemic hypertension. These include that 1) plasma levels (free) of 5-HT are higher in multiple forms of human hypertension including preeclampsia and essential hypertension (Brenner *et al.*, 2007; Carrasco *et al.*, 1998; Fetkovska *et al.*, 1990); 2) SERT inhibitors such as fluoxetine cause an increase in blood pressure (Amsterdam *et al.*, 1999; Lazartigues *et al.*, 2000); 3) the ability of arteries to take up 5-HT through SERT is impaired in hypertension (Fetkovska *et al.*, 1990; Ni *et al.*, 2006); and 4) arteries from hypertensive humans and experimental models are hyperresponsive to 5-HT (for review, see Watts 2005). The necessity of SERT in pulmonary hypertension is well established

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(MacLean, 2007). We thus hypothesized that removal of SERT would exacerbate the development of systemic hypertension. SERT knockout mice and, more recently, knockout rats have become available to test this hypothesis (Homborg *et al.*, 2007; Kim *et al.*, 2005). In these experiments, we are also able to examine the question of whether long-term inhibition of SERT modified basal blood pressure. The animals used in this study were adult (over 12 weeks old) and thus have developed without the benefit of SERT function.

The model of hypertension chosen in this study was the deoxycorticosterone (DOCA) salt model. Reasons for choosing this model include an identified dependence of this model on serotonergic receptor stimulation (Watts and Fink, 1999) and established supersensitivity of arteries to 5-HT (Watts, 2005). Mineralocorticoid dependence grows increasingly recognized as a potential cause of essential hypertension, and thus this is a valuable model to study (Funder 2007).

2. Materials and Methods

2.1 Experimental Protocol

Male WT and KO mice (22–30 grams; C57Bl6 background) were provided by Dr. Dennis Murphy (NIMH, Bethesda, MD, USA). Male WT and KO rats (250–300 grams; Wistar background) were provided by Dr. Edwin Cuppen (Hubrecht Laboratory, Utrecht, The Netherlands). Animals were anesthetized with isoflurane, and telemeters implanted (Data Sciences International, St. Paul, MN USA: mice: PA-C10 in the aorta *via* the carotid artery; rat: PA-C40 *via* the femoral artery). Rodents were allowed 3–4 days to recover post-operatively, and 7 days of baseline measurements were made. At this time, animals underwent uninephrectomy (flank incision, left side) and implantation of DOCA (50 mg/kg pellet for mice, 200 mg/kg for rat). All animals were placed on 1.0% NaCl + 0.2% KCl water for the duration of the experiment. Mean arterial pressure and heart rate were monitored remotely using a radiotelemetry data acquisition program (Dataquest ART 4.1, Data Sciences International, St. Paul, MN, USA) on a Dell computer. Hemodynamic measurements were sampled for 10 seconds every 10 minutes for the duration of the experiment. Data are reported as 24-hour averages.

2.2 Data analysis

For blood pressure data analysis, within group differences were assessed by a one-way repeated measures ANOVA with post-hoc multiple comparisons using Dunnett's procedure (GraphPad Instat 3). Between group differences were assessed by a two-way mixed design ANOVA and post-hoc testing at each time point was performed using Bonferroni's procedure to correct for multiple comparisons (GraphPad Prism 4). In all cases, a P-value of <0.05 was considered significant. All results are presented as means $\pm$ S.E.M.

3. Results

3.1 Blood Pressure in SERT KO animals; effect of DOCA-salt

Figure 1 depicts the results of these experiments. Three days of basal, control blood pressures are shown. Both mouse (fig. 1, **top**) and rat (fig. 1, **bottom**) SERT KO had a normal blood pressure compared to the WT, as estimated by comparing blood pressures during these control days. After DOCA and salt were introduced (at arrow), blood pressure in all groups elevated rapidly with mice achieving a plateau in elevated blood pressure prior to rats. When respective WT were compared to KO, there were no differences in the rate of blood pressure increase or absolute magnitude of blood pressure elevation in the mouse (~45 mm Hg) and the rat (~55 mm Hg). DOCA-salt-induced changes in heart rate were also not different between WT and KO of either species (data not shown).

4. Discussion

These *in vivo* studies suggest that removal of total body SERT does not change the ability of DOCA-salt to elicit a hypertension in either the rat or the mouse. While DOCA-salt hypertension is but one form of hypertension, these findings are consistent with an earlier report that the SERT KO rat became hypertensive when given an inhibitor of nitric oxide synthase (Homberg *et al.*, 2006). Our studies were designed specifically to investigate the measure of blood pressure and thus used the more sensitive technique of radiotelemetry that measures mean arterial blood pressure in the conscious state, as opposed to solely systolic blood pressure. Importantly, we have previously established that the isolated arteries of the SERT KO rat remain responsive to 5-HT. In fact, the potency of 5-HT in contracting the aorta from the SERT KO rat is increased, while the efficacy of 5-HT is reduced (Linder *et al.*, 2008). Additionally, the aorta of the SERT KO mouse contracts normally to 5-HT (Ni *et al.*, 2005). Thus, any differences observed are unlikely to contribute to by a lack of response to 5-HT.

The findings of this study are important for several reasons. First, these studies directly compare two different species that lack SERT. Second, the independence of systemic hypertension from SERT function contrasts sharply with the strong dependence of pulmonary hypertension on SERT (McLean, 2007), highlighting the significant functional differences between systemic and pulmonary circulations. Third, these data suggest that SERT deficiency does not profoundly influence normal blood pressure, nor does it modify development of hypertension (mineralocorticoid-based), a finding of potential relevance to humans with SERT genetic variants that reduce SERT expression and function (Murphy and Lesch, 2008). We have not measured blood pressure as the SERT KO animal ages, and thus cannot exclude the possibility that blood pressure variability and/or development of hypertension with age could occur differently in the SERT KO *vs* the wild type animal.

One difficulty in interpreting these experiments is in considering how 5-HT and SERT independently modify blood pressure. Both models have a virtually undetectable level of blood 5-HT, and this is expected given the dependence of the platelet on SERT for carrying 5-HT (Homberg *et al.*, 2007; Kim *et al.*, 2005). However, 5-HT is still synthesized, evidenced by a normal level of 5-HT in the raphe of at least the SERT KO rat (Homberg *et al.*, 2007). We speculate that the 5-HT made is trapped at sites of synthesis and cannot move into the blood platelet without a functional SERT, resulting in the nearly undetectable amount of 5-HT in platelet free plasma and platelet rich plasma. These SERT KO animals contrast with the tryptophan hydroxylase 1 KO mouse that has a functional SERT but possesses no peripheral 5-HT synthesis (Walther *et al.*, 2003a). Future experiments will be done to delineate the potentially separate roles of 5-HT and SERT. This is important because it is unclear whether 5-HT function inside the cell is as important or different from function outside the cell (Ni *et al.*, 2004). Small proteins, such as Rho, can be covalently modified by 5-HT and become constitutively active (Walther *et al.*, 2003b). Thus, intracellular 5-HT may very well modify smooth muscle tone. Another potential limitation to our studies is the idea that genetic ablation of SERT stimulates compensatory mechanisms redundant to the function of SERT, thereby masking a physiological effect SERT may have. In this regard, upregulation of the protein expression and function of the organic cation transporter has been observed in the SERT KO mouse (Chen *et al.*, 2001), suggesting that adaptations are made in the mouse for removal of SERT. The SERT KO rat is too new a tool about which to have information of compensatory changes.

In summary, removal of SERT function from birth does not influence the development or absolute magnitude of elevated blood pressure in the adult rat or mouse in response to mineralocorticoid and salt. SERT removal also does not modify the normal daily blood pressure of the mouse and rat.

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Figure 1.

Mean arterial blood pressure of mice (top) and rats (bottom) in which SERT is either present (WT) or absent (KO). The arrow marks the point at which DOCA and salt were introduced. Points represent means $\pm$ SEM for number of animals in parentheses. * signify statistical difference ($p < 0.05$) from control period.