Early life stress sensitizes the renal and systemic sympathetic system in rats

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Loria AS, Brands MW, Pollock DM, Pollock JS. Early life stress sensitizes the renal and systemic sympathetic system in rats. Am J Physiol Renal Physiol 305: F390–F395, 2013. First published May 15, 2013; doi:10.1152/ajprenal.00008.2013.—We hypothesized that maternal separation (MS), an early life stress model, induces a sensitization of the sympathetic system. To test this hypothesis, we evaluated the renal and systemic sympathetic system in 12- to 14-wk-old male control or MS rats with the following parameters: 1) effect of renal denervation on conscious renal filtration capacity, 2) norepinephrine (NE) content in key organs involved in blood pressure control, and 3) acute systemic pressor responses to adrenergic stimulation or ganglion blockade. MS was performed by separating pups from their mothers for 3 h/day from day 2 to 14; controls were nonhandled litters. Glomerular filtration rate (GFR) was examined in renal denervated (DnX; within 2 wk) or sham rats using 125I-iothalamate plasma clearance. MS-DnX rats showed significantly increased GFR compared with MS-SHAM rats (3.8 ± 0.4 vs. 2.4 ± 0.2 ml/min, respectively, P < 0.05), whereas DnX had no effect in controls, indicating that renal nerves regulate GFR in MS rats. NE content was significantly increased in organ tissues from MS rats (P < 0.05, n = 6–8), suggesting a sensitization of the renal and systemic sympathetic system. Conscious MS rats displayed a significantly greater increase in mean arterial pressure (MAP) in response to NE (2 μg/kg ip) and a greater reduction in MAP in response to mecamylamine (2 mg/kg ip, P < 0.05, n = 4) monitored by telemetry, indicating that MS rats exhibit exaggerated responses to sympathetic stimulation. In conclusion, these data indicate that MS sensitizes the renal and systemic sympathetic system ultimately impairing blood pressure regulation.

early life stress; denervation; glomerular filtration rate; maternal separation

DEVELOPMENTAL PROGRAMMING is now recognized as an important determinant of adult chronic diseases such as diabetes, obesity, and hypertension (1, 4, 44, 47). This phenomenon has been defined as the adaptation to a specific insult during a critical timeframe in life that induces permanent changes in the adult phenotype. Compelling studies showing a relationship between a diverse number of adverse factors in the early life environment and the increased susceptibility to develop cardiovascular disease later in life have provided consistent supporting evidence (7, 47).

Maternal separation (MS) is a rodent model that mimics the effects of early life stress (ELS) in humans (22, 24, 39). Systematic separation from the dams during this period induces higher anxiety and an exaggerated response to behavioral stress during adulthood as shown in several reports (11, 30, 36, 39). A number of investigations demonstrate that MS induces exaggerated responses to acute stress from the hypothalamic-pituitary-adrenal axis and sympathetic nervous system (SNS) hyperactivity (11, 24, 28), although only a few studies have assessed pressor responses (26, 45). Previously, we observed that MS does not manifest a change in baseline metabolic parameters, blood pressure, and heart rate but it sensitizes rats to a chronic prohypertensive stimulus (26).

It is well-established that the SNS is important in the acute and chronic regulation of arterial blood pressure. The role of the SNS in the development of hypertension has been described in several experimental models such as spontaneously hypertensive rats and Dahl salt-sensitive rats (6, 43). In terms of fetal programming, Samuelsson et al. (37, 38) showed that the hypertension observed in weanlings from obese dams is from sympathetic origin. Additionally, kidneys play a critical role in the regulation of fluid homeostasis and chronic blood pressure control (8, 15). Using a model of intrauterine growth restriction (IUGR), Alexander et al. (35) showed that the renal nerves play a causative role in the early onset of IUGR-induced hypertension, although established hypertension involves interaction of other regulatory systems in addition to the renal nerves. In humans, the use of renal denervation (DnX) as a therapy for resistant hypertension is becoming a possible lifelong treatment (9, 12, 20, 40), although the mechanism(s) leading to overactivation of the renal nerves in humans with resistant hypertension are unknown.

Many investigations have shown that alterations in normal renal development are associated with changes in renal structure/function and subsequently result in hypertension (14, 31, 34, 47). However, there is a gap in the literature regarding the impact of behavioral stress early in life and the mechanisms underlying the programming of the adult renal phenotype. Recently, we showed that creatinine clearance, a parameter of renal function, is significantly reduced in MS rats (27). Thus, reduced renal filtration capacity is a potential mechanism by which MS impairs chronic blood pressure control.

We hypothesized that MS sensitizes the renal and systemic sympathetic system. The aims of the current study were to test this hypothesis by evaluating three parameters. First, we examined whether renal nerves play a role in the MS-induced loss of renal filtration capacity in conscious rats. Second, we determined the norepinephrine (NE) content to test the status of a primary mediator of sympathetic postganglionic fibers in kidney, heart, spleen, adrenal, and large arteries. Finally, we elucidated the acute systemic pressor responses to adrenergic stimulation and ganglion blockade in telemetry-instrumented, conscious rats.

METHODS

MS protocol. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved and monitored by the Georgia Regents University Institutional Animal Care and Use Committee.
With the use of Wistar-Kyoto breeders, pups were separated from their mothers and littersmates for 3 h a day in the morning and identified as “MS” at postnatal days 2 to 14 of life (26). Nonhandled littersmates served as control group. Each experimental group was comprised of adult male rats (12–14 wk old) from at least three different litters.

**Chronic catheter implantation and DnX.** Chronic in-dwelling catheters were placed in the femoral vein and abdominal aorta artery (3, 5, 21). Sterile catheters were implanted in the femoral vein and abdominal and blood pressure and heart rate were monitored daily. Urinary volume was assessed during 24 h and expressed in milliliters per day. Urinary sodium and potassium were measured in 24-h urine collections (Easy Lyte, Medica).

**Acute mean arterial pressure responsiveness.** Rats were implanted with telemetry transmitters at 10 wk of age (Data Sciences, St. Paul, MN) as previously described (26). Mean arterial pressure (MAP) and heart rate (HR) were continuously recorded throughout the study using the Datquest ART Acquisition program (Data Sciences International, St. Paul, MN). After recovery (10 days) and a baseline period (7 days), separate groups of rats were subjected to intraperitoneal injections of mecamylamine (2 mg/kg) or NE (2 μg/kg). Telemetry data were collected every 10 min for 30 s and averaged for the mecamylamine experiment and baseline blood pressure, and every 30 s for 4 s for the NE experiment.

**Statistical analysis.** Statistical comparisons of MAP and GFR in SHAM and DnX rats were made by two-way ANOVA followed by a Tukey post hoc test. For statistical comparisons of MAP, HR, and NE levels between control and MS, unpaired Student’s t-test was utilized. All statistical analyses were conducted using Prism 5.01 (GraphPad Software, 2007). A value of \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Effect of DnX on MAP and GFR.** MAP directly measured from the abdominal aorta was not different in control-SHAM or MS-SHAM rats similar to our previous reports using telemetry-implanted rats (26) (Fig. 1A). Renal DnX significantly reduced MAP in both control and MS groups (Fig. 1A). GFR was significantly reduced in MS-SHAM rats compared with control-SHAM rats as previously reported using creatinine clearance (27) (Fig. 1B). DnX did not affect GFR notably in control rats but significantly increased GFR in MS rats (Fig. 1B).
Metabolic variables in renal denervated rats. Renal DnX did not significantly affect food and water intake, urine volume, or sodium excretion in control or MS rats (Table 1).

Plasma and tissue NE content. In MS rats, the renal content of NE was significantly increased in cortical, outer medullary, and inner medullary regions compared with control rats (Fig. 2A). Other organs regulated in part by the SNS such as left ventricle, spleen, and adrenals also showed significantly increased NE content in the MS group compared with control (Fig. 2B). NE content in isolated large vessels from the kidney, thoracic aorta, and abdominal aorta was not different in control and MS rats (Fig. 2C). Plasma levels of NE in control and MS rats were not significantly different (211.0 ± 24.4 and 222.9 ± 25.2 pg/ml, respectively).

Acute hemodynamic response to NE and mecamylamine. Baseline MAP and HR monitored in conscious telemetry-instrumented rats were not different between control and MS groups as previously reported (26). Acute administration of NE (2 μg/kg ip), a nonselective adrenergic agonist, significantly increased MAP and decreased HR in control and MS rats, while acute saline injection did not change either hemodynamic parameter (Fig. 3). The acute increase in MAP to NE was significantly exaggerated (Fig. 3A) and the HR response displayed an enhanced biphasic response (Fig. 3B) in MS rats compared with control rats.

Acute administration of the ganglion blocker, mecamylamine (2 mg/kg ip), lowered blood pressure in both control and MS rats. However, the drop in MAP was exaggerated in MS rats (Fig. 4). Mecamylamine increased HR similarly in control and MS rats after 30 min (382 ± 12 and 353 ± 12 beats/min, respectively).

**DISCUSSION**

This study supports the hypothesis that ELS, utilizing the rat model of MS, sensitizes the renal and systemic sympathetic system. At the kidney level, we found that MS induces decreased GFR that is mediated by renal nerve activation. MS rats also display an exaggerated pressor response to adrenergic stimulation and a greater reduction in blood pressure to ganglion blockade providing evidence for increased basal sympathetic tone modulating baseline blood pressure. Furthermore, NE content was significantly greater in renal tissue, as well as left ventricle, spleen, and adrenal, from MS rats compared with control rats. However, NE levels were not significantly different in the large conduit vessels, or plasma from control and MS rats. We propose that sensitization of the renal and systemic sympathetic system may provide one of the mechanism(s) for exaggerated responses to secondary hypertensive stimuli observed in this model of ELS.

Classic sympathetic activation, seen in many hypertensive models including fetal programming models, is linked to heightened basal blood pressure, increases in HR, sodium retention, as well as increased plasma and tissue catecholamine levels. In this model of postnatal chronic behavioral stress, adult MS rats display many cardiovascular parameters within

**Table 1. Metabolic variables in SHAM and DnX rats**

<table>
<thead>
<tr>
<th>Metabolic variable</th>
<th>Control SHAM (n = 4)</th>
<th>Control DnX (n = 6)</th>
<th>MS-SHAM (n = 4)</th>
<th>MS-DnX (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>376 ± 8</td>
<td>369 ± 6</td>
<td>370 ± 7</td>
<td>371 ± 6</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>21.0 ± 1.1</td>
<td>18.7 ± 1.8</td>
<td>21.2 ± 0.5</td>
<td>21.4 ± 0.3</td>
</tr>
<tr>
<td>Water intake, ml/day</td>
<td>40.1 ± 3.0</td>
<td>43.7 ± 4.3</td>
<td>42.2 ± 4.3</td>
<td>44.5 ± 1.7</td>
</tr>
<tr>
<td>Urine flow rate, ml/day</td>
<td>27.3 ± 3.4</td>
<td>24.9 ± 5.8</td>
<td>26.1 ± 2.2</td>
<td>26.7 ± 2.4</td>
</tr>
<tr>
<td>Na excretion, meq/day</td>
<td>1.67 ± 0.06</td>
<td>1.34 ± 0.17</td>
<td>1.73 ± 0.11</td>
<td>1.45 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. MS, maternal separation; DnX, denervated.
vascular responses to secondary environmental challenges in system may be a critical mediator of the detrimental cardio-
ated responses that led us to conclude that the sympathetic
sion or blockade of the sympathetic system produced exagger-
sical sense, in vivo studies showed that acute systemic activa-
basally do not demonstrate sympathetic activation in the clas-
"primed" in the MS model of ELS. Even though MS rats
This observation may signify that the sympathetic system is
regions of the kidney, left ventricle, spleen, and adrenal tissues.
Nevertheless, we demonstrated increased NE content in all
in large vessels was similar in both control and MS rats, which
consistent with our previous findings showing that phenyl-
phrine-induced constriction of thoracic aorta (using ex vivo
wire myography) was not different in control and MS rats (25).
Moreover, if sympathetic-dependent changes in GFR are
presumed to depend predominantly on afferent arteriolar resis-
vascular resistance (RVR) may be exaggerated in the MS rats. In an attempt to assess the RVR parameter in this study, we determined the ratio between MAP and GFR. MAP/
GFR ratio was ~30% greater in MS than in control rats at
baseline. However, we found that denervation reduced this
ratio ~50% in the MS rats compared with ~15% in control
rats, suggesting that attenuation in RVR due to denervation
might be greater in MS rats. Although this ratio is not a
substitute for direct measurements of RVR, the calculation
gives us confidence to pursue these measurements in conscious
rats to probe the hypothesis further.

Resistance vessels provide a powerful mechanism to regu-
late regional blood flow (23, 46). The arterioles, which consti-
tute the majority of resistance vessels, especially within the
kidney, are the major contributors to total peripheral resis-
tance (17) and are regulated mainly by autonomic mechanisms (32). The vasculature of MS rats may have specific alterations either in the nerve innervations density, adrenergic receptor number, subtype, or affinity that could result in greater sensitivity to second stressors that trigger the sympathetic response. However, whether resistance vessels of MS rats are sensitized to

The catecholamines, epinephrine and NE, are the primary
SNS mediators (13, 41). Epinephrine is produced exclusively
by the adrenal medulla, whereas a large amount of NE is
produced by sympathetic postganglionic fibers. Thus, the ef-
effects of NE are largely mediated by the SNS primarily through
activation of α- and β1-adrenergic receptors located near
postganglionic sympathetic fiber terminals (10). Increased tis-
ue NE content may be secondary to a greater sympathetic
nerve activity (SNA) in many organs, although measurement
of tissue NE content may not directly reflect the autonomic
sympathetic fiber outflow to their effector organ. This may add
a limitation to the physiological relevance of this finding in MS
rats. Nevertheless, we would propose that normalization of GFR after denervation provides substantial support for the
concept that MS induces a sensitization of the renal sympa-
thetic system. Direct measurements of SNA in each organ will
be the focus of future experiments to decipher the mechanistic
role of the increased tissue NE.

The improvement in the renal filtration capacity with DnX in
MS rats may be due to an MS-induced attenuation of the renal
SNA. Moreover, if sympathetically-dependent changes in GFR
were observed in response to a saline injection. *P < 0.05, n = 3.

Fig. 3. Acute bolus of a nonselective adrenergic agonist (NE, 2 mg/kg ip) in
telemetry-implanted rats. A: MAP showed a greater MAP in MS rats. B: heart
rate (HR) was similar in MS and control rats. C: no significant changes in MAP
were observed in response to a saline injection. *P < 0.05, n = 3.

the normal range at basal conditions—for example, blood
pressure, HR, plasma levels of glucose, insulin, and vasoactive
peptides such as catecholamines, angiotensin II, angiotensin
1–7, and endothelin-1 (25, 26). We found that the NE content
in large vessels was similar in both control and MS rats, which
consistent with our previous findings showing that phenyl-
epinephrine-induced constriction of thoracic aorta (using ex vivo
wire myography) was not different in control and MS rats (25).
Nevertheless, we demonstrated increased NE content in all
regions of the kidney, left ventricle, spleen, and adrenal tissues.
This observation may signify that the sympathetic system is
"primed" in the MS model of ELS. Even though MS rats
basally do not demonstrate sympathetic activation in the clas-
sical sense, in vivo studies showed that acute systemic activa-
tion or blockade of the sympathetic system produced exagger-
ated responses that led us to conclude that the sympathetic
system may be a critical mediator of the detrimental cardio-
vascular responses to secondary environmental challenges in
adult life.

Fig. 4. Acute bolus of a ganglion blockade (2 mg/kg ip) induced a greater drop
in MAP in MS telemetry-implanted rats compared with control rats. *P <
0.05, n = 4.
neural or pharmacologic adrenergic stimulation and play a role in enhancing the blood pressure response needs further investigation. Although future studies will require measurement of renal blood flow in conscious rats to determine accurately renal vasculature resistance, these renal and systemic hemodynamic data further support the concept that the impaired GFR displayed by MS rats may be secondary to an exacerbated sympathetic outflow to the kidney. Further study of the adrenergic receptor subtypes in the renal vasculature may provide mechanistic pathways to understand the difference between the chronic and acute responses observed in MS rats.

Sympathetic nerve fibers innervate all organs that are involved in peripheral control of cardiovascular function such as the heart, the peripheral vasculature, and the kidneys. Renal nerves contribute to basal control of blood pressure in both control and MS rats as demonstrated by the DnX intervention. Other reports in normotensive rats and humans found similar results (16, 29), thus the results obtained in this study were not surprising. In particular, DnX has been used in a large number of hypertensive experimental animal models (18, 19, 33). In humans, the SNS plays a crucial role in the development and progression of hypertension (9). The absence of an effective treatment using antihypertensive drugs in patients who are resistant to conventional pharmacological treatment led to the use of renal nerve ablation by selective catheter-based renal sympathetic denervation to attenuate renal sympathetic nerve activity. Outcomes from at least two clinical trials demonstrated efficacy and safety of this intervention (12, 20). In this regard, the results provided in a near future by the randomized controlled study Simplicity HTN-3 (NCT01418261 at http://clinicaltrials.gov) will be crucial for the approval of the therapy in this model of ELS. Therefore, the renal nerves may play a significant role in the mechanism by which ELS increases the risk to chronic disease in the adult life.

In humans, enhanced stress-induced responses predict future cardiovascular events (7). Although, it is unknown whether exaggerated responses to stimulation of the SNS are a predictor of cardiovascular disease and renal dysfunction in humans with childhood stressors. Autonomic and renal dysfunctions are common chronic conditions that frequently coexist in the same individual, and both are associated with significant morbidity and mortality. The understanding of the impact of ELS in the cardiovascular and renal sympathetic outcomes will contribute to design-personalized therapies to achieve blood pressure control in adulthood.

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REFERENCES


AUTHOR CONTRIBUTIONS


