

Haploinsufficiency of *steroidogenic factor-1* in mice disrupts adrenal development leading to an impaired stress response

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Adrenal steroids are essential for homeostasis and survival during severe physiological stress. Analysis of a patient heterozygous for the *steroidogenic factor-1* (*SF-1*) gene suggested that reduced expression of this nuclear receptor leads to adrenal failure. We therefore examined *SF-1* heterozygous (+/–) mice as a potential model for delineating mechanisms underlying this disease. Here we show that *SF-1* +/- mice exhibit adrenal insufficiency resulting from profound defects in adrenal development and organization. However, compensatory mechanisms, such as cellular hypertrophy and increased expression of the rate-limiting steroidogenic protein StAR, help to maintain adrenal function at near normal capacity under basal conditions. In contrast, adrenal deficits in *SF-1* heterozygotes are revealed under stressful conditions, demonstrating that normal gene dosage of *SF-1* is required for mounting an adequate stress response. Our findings predict that natural variations leading to reduced *SF-1* function may underlie some forms of subclinical adrenal insufficiency, which become life threatening during traumatic stress.

Physiological adaptation to infection, injury, and starvation requires an endocrine stress response that is mediated by the hypothalamic-pituitary-adrenal (HPA) axis. In instances of adrenal crisis or adrenal insufficiency, the inability to mount an adequate response to acute physiological stress can lead to morbidity (1). In response to stress, adrenal secretion of glucocorticoids is initiated by the hypothalamic neuropeptide corticotropin releasing hormone (CRH), which stimulates pituitary release of adrenocorticotropic hormone (ACTH). Activation of ACTH receptors in the adrenal cortex promotes glucocorticoid synthesis and secretion; glucocorticoids then act on a wide range of target tissues (2). Human diseases associated with an impaired stress response arise from either primary defects in the adrenal or secondary defects at the level of the hypothalamus or pituitary. Primary adrenal insufficiency is most commonly due to bilateral adrenal gland destruction resulting from autoimmune and infectious diseases, such as AIDS and tuberculosis (1). Other forms of primary adrenal insufficiency result from inherited defects in cortisol biosynthesis and manifest as adrenal hyperplasia because of lack of adrenal feedback in the HPA axis (1). Finally, rarer forms of familial adrenal insufficiency are associated with mutations in members of the nuclear receptor superfamily. For example, mutations in the X-linked gene *Dax1* lead to abnormal adrenal development resulting in adrenal hypoplasia, as well as hypogonadotropic hypogonadism (3–5).

A similar, but less well-characterized form of adrenal insufficiency was revealed by the analysis of a single patient with a heterozygous mutation in the orphan nuclear receptor *steroidogenic factor-1* (*SF-1*). This individual was diagnosed with primary adrenal insufficiency as well as XY sex reversal, suggesting that *SF-1* functions in a dose-dependent manner in humans (6). A large number of *in vitro* studies have shown that *SF-1* regulates multiple genes, including those required for glucocorticoid

production (7) and male sexual differentiation (8). Indeed, *SF-1* null mice lack adrenals and gonads, pituitary gonadotropes, and the ventromedial nuclei of the hypothalamus and die shortly after birth because of adrenocortical insufficiency (9–12). However, because *SF-1* heterozygous (+/–) mice are viable, we asked whether these mice might serve as a genetic model of human adrenal insufficiency. Here, we show that, whereas *SF-1* +/- mice are externally indistinguishable from their wild-type littermates, they exhibit profound defects in adrenal development leading to adrenal insufficiency.

Materials and Methods

Animal Experiments and Hormone Measurements. *SF-1* wild type (+/+) and +/- mice (obtained from The Jackson Laboratory) were maintained on a C57BL/6J × FVB background. Similar adrenal growth defects and adrenocortical insufficiency were observed in *SF-1* +/- mice on a DBA/2J inbred background (obtained from Dr. K. Albrecht, The Jackson Laboratory, data not shown). Mice were kept on a 12-h light-dark cycle (lights on: 6 a.m. to 6 p.m.) and were given food and water ad libitum unless otherwise stated. Male mice 6–8 wk old were used for all experiments unless otherwise noted. For restraint experiments, blood samples were collected from the tail vein of *SF-1* +/+ and +/- mice at 1 and 30 min of restraint stress carried out in a 50-ml conical tube. For food deprivation experiments, *SF-1* +/+ and +/- mice were divided randomly into fed and fasted groups. After 48 h of ad libitum feeding or food deprivation, mice were euthanized by decapitation, and trunk blood and tissues were collected. Weight loss averaged 5.0 g for fasted +/+ mice and 5.6 g for fasted +/- mice. Thymus and spleen cells were harvested from fed and fasted mice, and 10⁶ cells per sample were stained with propidium iodide, anti-mouse CD4-phycoerythrin (Becton-Dickinson) and anti-mouse CD8-FITC (PharMingen), analyzed by flow cytometry using a FACScalibur (Becton-Dickinson), and CELLQUEST software. Lipopolysaccharide (*Escherichia coli* 0127:B8, Sigma) in 2% FCS was injected i.p. at a dose of 40 mg/kg at 8 a.m. in female *SF-1* +/+ and +/- mice. Mice were euthanized by decapitation 24 h later, and trunk blood was collected. Basal samples were collected at 8:00 a.m. and 5:00 p.m. within 1 min of disturbing the mouse's home cage.

Plasma ACTH, corticosterone, leptin, and insulin were measured by using either in-house (ACTH) or commercially avail-

Abbreviations: *SF-1*, steroidogenic factor-1; HPA, hypothalamic-pituitary-adrenal; CRH, corticotropin releasing hormone; ACTH, adrenocorticotropic hormone; StAR, steroidogenic acute regulatory protein; PNMT, phenylethanolamine *N*-methyltransferase; TH, tyrosine hydroxylase.

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able (corticosterone, ICN; leptin and insulin, Linco Research Immunoassay, St. Charles, MO) radioimmunoassays. The glucose assay was performed on a Beckman LX-1 automated clinical chemistry analyzer in the University of California at San Francisco Clinical Laboratories. For measurement of adrenal catecholamine content, $+/+$ and $+/-$ adrenals were homogenized in 0.4 M perchloric acid. Supernatants were assayed for norepinephrine and epinephrine by liquid chromatography with electrochemical detection as described elsewhere (13).

Western and Northern Analyses. For Western analyses, adrenals were sonicated in 2% SDS, 100 mM DTT, and 60 mM Tris (pH 6.8), and protein concentration was measured with a Coomassie Plus Protein Assay (Pierce). Proteins (10 μ g) were separated by SDS/PAGE, transferred to nitrocellulose, blocked in 3% milk, and blotted overnight at 4°C with primary antibodies [rabbit anti-SF-1, 1:20,000; rabbit anti-steroidogenic acute regulatory protein (StAR), 1:7,000; rabbit anti-P450 side chain cleavage (P450 scc), 1:10,000; sheep anti-phenylethanolamine *N*-methyltransferase (PNMT), 1:7,000, Chemicon; rabbit anti-tyrosine hydroxylase (TH), 1:5000, Pel-Freez; and rabbit anti-actin, 1:2500, Sigma]. Membranes were washed, incubated with secondary antibody [sheep anti-rabbit horseradish peroxidase (HRP), 1:10,000 or donkey anti-sheep HRP, 1:10,000] for 2 h at room temperature. After washing, the blot was developed with a chemiluminescence kit (ECL, Amersham Pharmacia Biotech) and exposed to Kodak X-Omat film. For Northern analyses, total RNA (10 μ g) prepared from *SF-1* $+/+$ and $+/-$ adrenals was separated by formaldehyde-gel electrophoresis, transferred to nylon membranes, and hybridized overnight at 42°C with random-primed labeled DNA probes for fragments of the mouse *SF-1*, mouse *StAR*, mouse *MC2-R*, and rat *cyclophilin* cDNAs. Membranes were washed at high stringency (0.1 \times SSC, 0.1% SDS at 55°C) and exposed to Kodak X-Omat film.

Histological Analysis. Adrenals were fixed overnight in 4% paraformaldehyde at 4°C and cryoprotected in 30% sucrose in PBS. Adrenal sections were stained with hematoxylin and eosin (8 μ m) or oil red O (14 μ m). For immunocytochemistry, adrenal sections (14 μ m) were blocked for 30 min in 10% normal horse or normal goat serum, incubated overnight at 4°C with rabbit anti-TH (1:1000, Pel-Freez) or sheep anti-PNMT (1:1000, Chemicon), washed, incubated for 2 h at room temperature with goat anti-rabbit Alexa 488 (Molecular Probes) or donkey anti-sheep Cy3, and examined by fluorescence microscopy. Cellular hypertrophy was assessed by counting Hoechst-stained nuclei per 0.01 mm² in three sections per $+/+$ and $+/-$ adrenal ($n = 8$ adrenals per genotype). Fetal adrenal cross-sectional area was quantitated with National Institutes of Health IMAGE 1.61.

Statistical Analysis. Data are presented as means \pm SEM. Unpaired two-tailed *t* tests and ANOVA were used to determine statistical significance.

Results

SF-1 $+/-$ Mice Exhibit Decreased Endocrine and Physiological Responses to Stress. To determine whether *SF-1* gene dosage affects adrenal function, we subjected *SF-1* $+/+$ and $+/-$ mice to different stress paradigms and measured circulating levels of two HPA hormones, ACTH and corticosterone. Acute stress (restraint for 30 min) and chronic stress (food deprivation for 48 h) produced significantly lower plasma corticosterone levels in *SF-1* $+/-$ mice compared with $+/+$ mice (Fig. 1 *a* and *b*). Moreover, in response to inflammatory stress induced by injection of lipopolysaccharide, decreased corticosterone levels were also observed in *SF-1* heterozygotes ($+/+$, 113.0 ± 8.7 μ g/dl; $+/-$, 59.6 ± 1.4 μ g/dl; $P = 0.02$, $n = 4$ per group). Thus, in all three

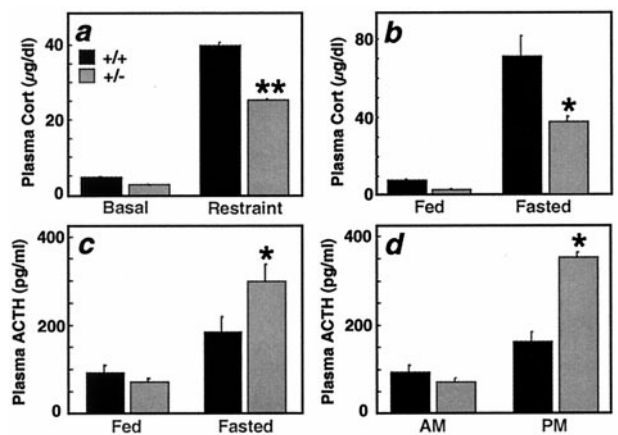


Fig. 1. Impaired stress response and altered circadian hormone secretion in *SF-1* $+/-$ mice. (a) Plasma corticosterone (Cort) levels in *SF-1* $+/+$ and $+/-$ mice after 1 min (basal) and 30 min (restraint) of restraint stress ($n = 11$ or 12 per group). (b and c) Plasma corticosterone (b) and ACTH (c) levels in fed and fasted *SF-1* $+/+$ and $+/-$ mice ($n = 4$ per group). (d) Plasma ACTH levels in *SF-1* $+/+$ and $+/-$ mice at 8 a.m. ($n = 4$ per group) and 5 p.m. ($n = 8$ per group). Plasma corticosterone levels tended to be decreased in *SF-1* $+/-$ mice at 8 a.m. ($+/+$, 4.8 ± 0.4 μ g/dl; $+/-$, 2.8 ± 0.2 μ g/dl; $n = 11$ or 12 per group, $P = 0.085$) and at 5 p.m. ($+/+$, 11.5 ± 1.0 μ g/dl; $+/-$, 6.8 ± 0.4 μ g/dl; $n = 8$ per group, $P = 0.075$), although these results fall short of statistical significance. *, $P \leq 0.05$ vs. wild type; **, $P \leq 0.01$ vs. wild type.

experimental paradigms, loss of one *SF-1* allele results in a blunted adrenal response to stress.

To assess whether a normal hypothalamic-pituitary hormone response to stress is maintained in *SF-1* $+/-$ mice, plasma ACTH was measured under basal and stress conditions. In response to chronic stress, decreased corticosterone levels in *SF-1* $+/-$ mice were accompanied by higher ACTH levels, indicating that normal feed-forward actions of the hypothalamus were intact (Fig. 1c). Interestingly, evening ACTH levels were also higher in $+/-$ mice without the expected increase in evening corticosterone levels, which tended to be decreased in $+/-$ compared with $+/+$ mice (Fig. 1d and data in legend). Morning levels of ACTH were equivalent in $+/+$ and $+/-$ mice, whereas morning corticosterone levels tended to be decreased in $+/-$ mice (data in Fig. 1 legend). Taken together, these data demonstrate that the HPA axis responds appropriately to lower corticosterone output by *SF-1* $+/-$ adrenals at night and during stress, implying that deficits in the stress response reside in the adrenal gland and not in the pituitary or hypothalamus.

Adrenal glucocorticoids mediate physiological responses to stress through their actions on multiple target tissues. During food deprivation, glucocorticoids maintain circulating glucose levels by activating lipolysis of fat stores and hepatic gluconeogenesis (14). We found that fasted $+/-$ mice lost significantly less fat mass from perirenal and subcutaneous white adipose tissues compared with fasted $+/+$ mice (Fig. 2a and data not shown). Consistent with increased fat stores observed in fasted *SF-1* $+/-$ mice, leptin levels were also elevated (Fig. 2b). Moreover, fasting plasma glucose levels were decreased in heterozygous mice compared with their wild-type littermates (Fig. 2c). Thus, glucocorticoid effects on energy balance are impaired in *SF-1* heterozygotes.

Glucocorticoids are potent suppressors of the immune response and at high levels cause thymic atrophy (15–17). This latter phenomenon depends on a functional glucocorticoid receptor (GR) and involves apoptosis of immature, double-positive thymocytes ($CD4^+CD8^+$) (17, 18). We used FACS analysis to quantify the population of $CD4^+CD8^+$ T cells in fed

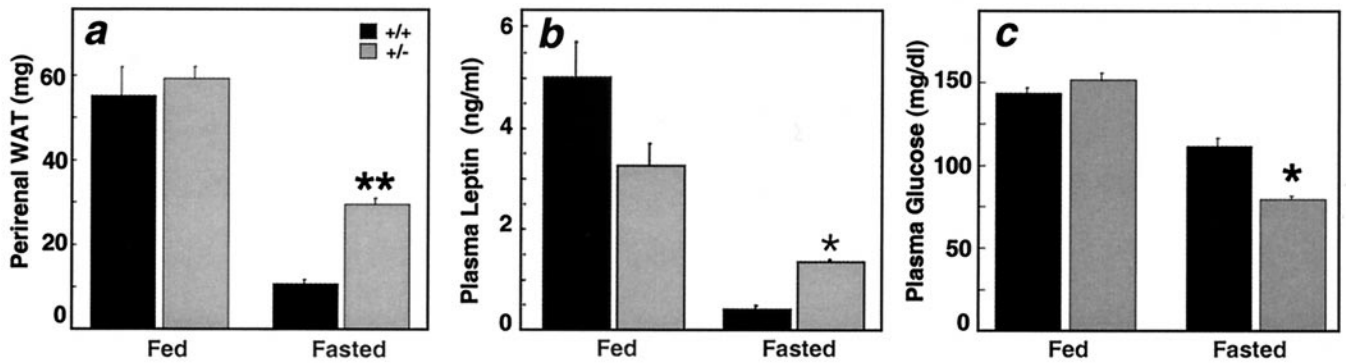


Fig. 2. Physiological consequences of decreased corticosterone secretion in fasted *SF-1* $+/-$ mice. (a) Perirenal white adipose tissue (WAT) weight in fed and fasted *SF-1* $+/+$ and $+/-$ mice. Similar results were obtained for subcutaneous WAT (data not shown). (b) Plasma leptin levels reflected fat mass in *SF-1* $+/+$ and $+/-$ mice. (c) Plasma glucose levels in fed and fasted *SF-1* $+/+$ and $+/-$ mice. For all panels, $n = 4$ per group. Plasma insulin levels were significantly decreased in fasted mice compared with fed mice, but did not differ between genotype (data not shown). *, $P \leq 0.05$ vs. wild type; **, $P \leq 0.01$ vs. wild type.

and fasted *SF-1* $+/+$ and $+/-$ mice. As expected, there was a significant decrease in the number of $CD4^+CD8^+$ cells in wild-type mice after fasting (Fig. 3a and b); however, fasted *SF-1* heterozygotes exhibited little or no loss of double positive thymocytes (Fig. 3b Right). Thus, whereas *SF-1* $+/-$ mice are capable of mounting a glucocorticoid response to stress, albeit at lower levels than wild-type mice, this response is insufficient to cause apoptosis of immature T cells. Collectively, our findings that *SF-1* heterozygous mice display attenuated glucocorticoid-mediated stress responses raise the possibility that these mice would exhibit decreased survival outside of the laboratory setting.

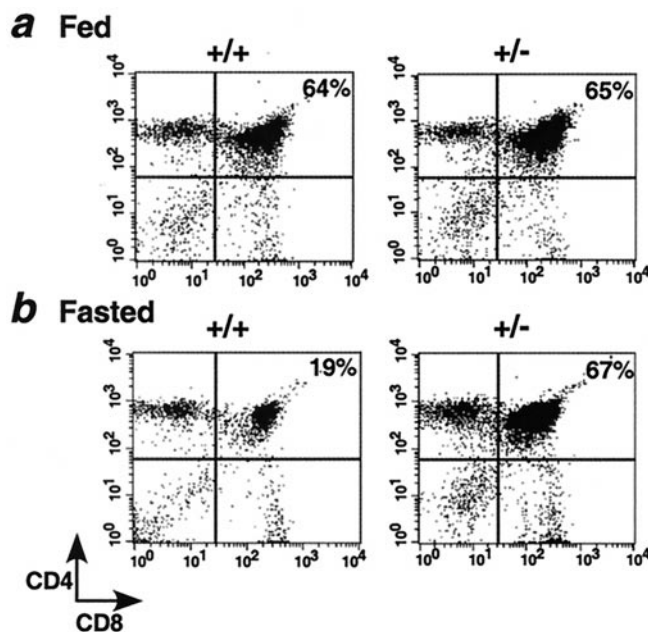


Fig. 3. Glucocorticoid-induced thymocyte cell death is absent in *SF-1* $+/-$ mice. FACS analysis of thymocytes from fed (a) and fasted (b) *SF-1* $+/+$ and $+/-$ mice using CD4-PE and CD8-FITC ($n = 4$ mice per group). A FACS analysis from one mouse of each group is shown with the percentage of $CD4^+CD8^+$ cells per total cells analyzed shown in the upper right corner of each graph. Data shown for the $+/+$ fasted mouse reflects the strongest response. The average number of $CD4^+CD8^+$ cells per total cells analyzed did not differ between fed *SF-1* $+/+$ and $+/-$ mice ($+/+$, $68.6 \pm 2.0\%$; $+/-$, $71.3 \pm 1.6\%$), but was significantly different between fasted groups ($+/+$, $33.1 \pm 3.6\%$; $+/-$, $61.6 \pm 2.0\%$; $P = 0.013$).

Loss of One *SF-1* Allele Results in Adrenal Hypoplasia and Altered Gene Expression.

Given the established role of *SF-1* in adrenal organogenesis, adrenocortical insufficiency in *SF-1* $+/-$ mice could arise from decreased adrenal mass, which consequently would limit their capacity to secrete glucocorticoids. Indeed, we found that *SF-1* $+/-$ adrenals were significantly smaller than $+/+$ adrenals in adult male and female mice (Fig. 4a and data in legend). Marked adrenal hypoplasia was noted as early as embryonic (E) day 15.5 and persisted at E18.5 (data in Fig. 4 legend). Histological inspection of the adrenal cortex in adult mice revealed normal zonation, but marked cellular hypertrophy within the zona fasciculata (corticosterone-producing cell layer) of *SF-1* heterozygotes. Moreover, we observed striking dilation of the cortical vasculature as evident by the large empty spaces present in the *SF-1* $+/-$ adrenal cortex (Fig. 4b). Given the trophic effects of ACTH on adrenal growth, it is likely that these gross changes in adrenal cellular morphology are due to chronically elevated evening ACTH (refer back to Fig. 1d) (19).

SF-1 regulates the expression of multiple genes involved in steroidogenesis. Consequently, decreased corticosterone output by *SF-1* $+/-$ adrenals might result not only from reduced adrenal size, but also from decreased expression of *SF-1* target genes, such as the steroidogenic acute regulatory protein (*StAR*), the steroid hydroxylases (including *P450scc*) (7), the ACTH receptor (*MC2-R*) (20), and *Dax1* (21). As expected, *SF-1* mRNA and protein levels were reduced in *SF-1* $+/-$ adrenals. In contrast, basal mRNA and protein levels of *StAR* were significantly elevated in *SF-1* $+/-$ adrenals and nearly equivalent to wild-type levels after chronic stress (Fig. 4c and d). Similarly, *MC2-R* mRNA expression was elevated in *SF-1* $+/-$ adrenals, whereas *Dax1* mRNA expression appeared unchanged (Fig. 4c and data not shown). *P450scc* protein levels were also unchanged (Fig. 4d). We noted equivalent oil red O staining of lipids in *SF-1* $+/+$ and $+/-$ adrenals, suggesting that defects in cholesterol transport (22, 23) are unlikely to contribute to the *SF-1* heterozygote adrenocortical insufficiency (refer to Fig. 5a). We posit that *SF-1* $+/-$ mice partially compensate for their adrenal insufficiency through mechanisms such as cortical cellular hypertrophy and increased expression of the rate-limiting steroidogenic protein *StAR*. Such adaptive mechanisms likely result from elevated evening ACTH levels and permit *SF-1* $+/-$ adrenals to function at near normal capacity during basal conditions (19, 24).

***SF-1* $+/-$ Adrenal Medullae Are Hypoplastic and Disorganized.** Finally, we asked whether loss of one allele of *SF-1*, which is expressed in the cortex, might affect the neural crest-derived

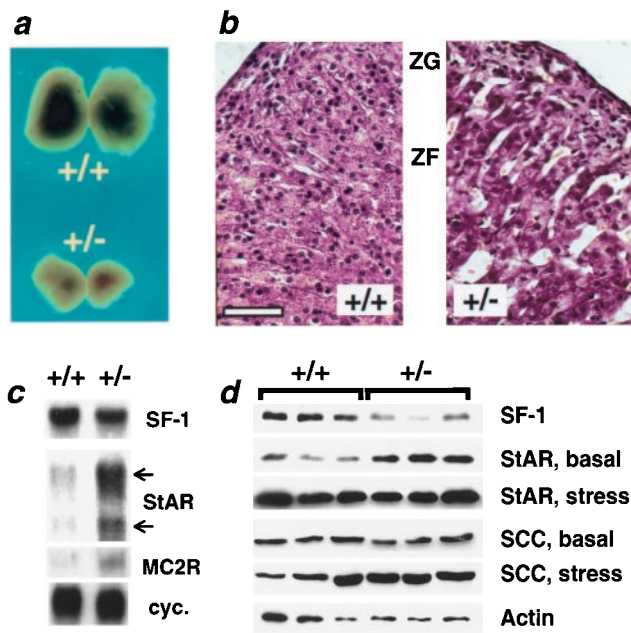


Fig. 4. Altered histology and gene expression in *SF-1* $+/-$ adrenal cortex. (a) Adult *SF-1* $+/+$ adrenals are significantly larger than $+/-$ adrenals (total adrenal weight for males: $+/+$, 3.3 ± 0.06 mg; $+/-$, 1.4 ± 0.02 mg; $P < 0.01$, $n = 8$ per group; females: $+/+$, 6.5 ± 0.06 mg; $+/-$, 2.3 ± 0.08 mg; $P < 0.01$, $n = 7$ per group). Body weight is equivalent in *SF-1* $+/+$ and $+/-$ mice (data not shown). Adrenal cross-sectional area is 2–3 times smaller in *SF-1* $+/-$ embryos compared with $+/+$ embryos at both E15.5 ($+/+$, 0.148 ± 0.001 mm²; $+/-$, 0.043 ± 0.001 mm²; $P \leq 0.01$) and E18.5 ($+/+$, 0.216 ± 0.004 mm²; $+/-$, 0.090 ± 0.001 mm²; $P \leq 0.01$). (b) Histological analysis of *SF-1* $+/+$ and $+/-$ adrenals. *SF-1* $+/-$ adrenals display cortical cell hypertrophy (cells per 0.01 mm²: $+/+$, 90.1 ± 0.9 ; $+/-$, 66.1 ± 0.7 ; $P \leq 0.01$), dilated adrenocortical sinusoids, and a hypoplastic zona fasciculata (ZF) adjacent to the zona glomerulosa (ZG). Bar = 50 μ M. (c) Northern blot analyses of *SF-1*, *StAR*, and *MC2R* mRNA expression in *SF-1* $+/+$ and $+/-$ adrenals. Whereas *SF-1* $+/-$ adrenals express lower levels of *SF-1* mRNA, they express higher levels of both the 3.4- and 1.6-kb *StAR* transcripts and the 1.7-kb *MC2R* transcript. *Cyclophilin* mRNA levels (*cyc*) are shown for loading comparison. (d) Western blot analyses of SF-1, StAR, SCC, and actin protein expression in adrenals from basal (fed) or stressed (fasted) *SF-1* $+/+$ and $+/-$ mice.

component of the adrenal, the medulla. Oil red O staining was used to distinguish the adrenal cortex from the unstained medulla and the female X zone (Fig. 5a). In *SF-1* $+/-$ males, the medulla is located in an eccentric position with respect to the cortex, suggesting that medullary precursors fail to infiltrate the cortical blastema during development, and therefore are not surrounded by the cortex in the adult. In contrast, the unstained portion of the *SF-1* $+/-$ female adrenal is concentric to the cortex (Fig. 5a). However, further histological analysis revealed that this central tissue consists primarily of the X zone and few chromaffin cells, unlike the wild-type female adrenal in which both the X zone and medulla are observed (Fig. 5b).

The adrenal medulla participates in the stress response through synthesis and release of the catecholamines epinephrine and norepinephrine. We found significantly reduced adrenal catecholamine content in *SF-1* $+/-$ mice compared with $+/+$ mice, with equivalent epinephrine to norepinephrine ratios (Fig. 5c and data in legend). The enzyme TH catalyzes the initial step in catecholamine synthesis and marks all adrenal chromaffin cells. In a subset of chromaffin cells, epinephrine synthesis is carried out by the enzyme PNMT, which is regulated at the transcriptional level by glucocorticoids (17, 25, 26). In male and female $+/+$ adrenals, TH-immunoreactive cells were found throughout the large, central medulla (Fig. 6a and data not

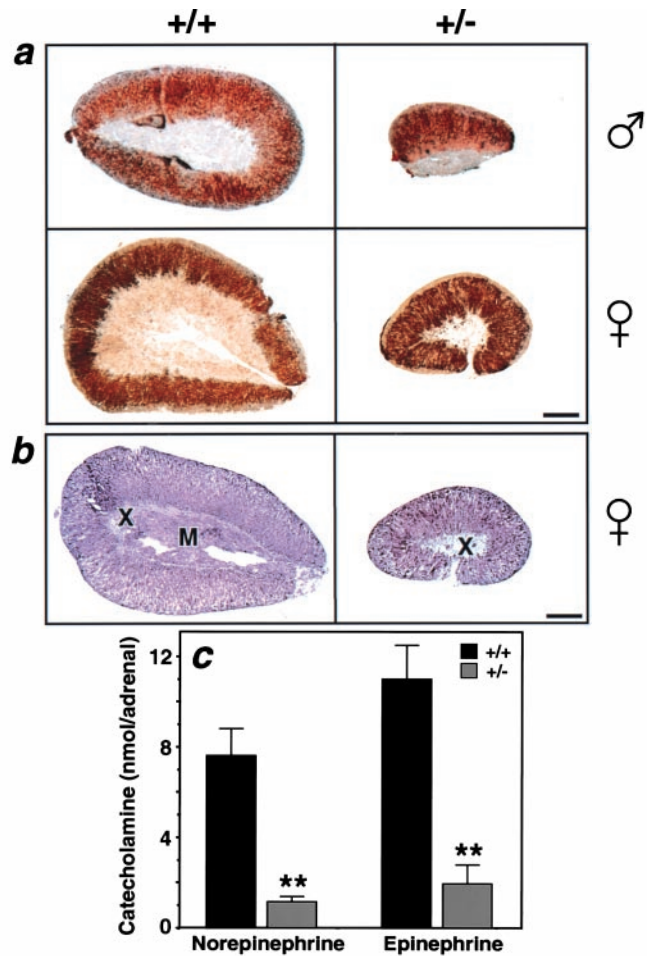


Fig. 5. Altered adrenal medulla structure and function in *SF-1* $+/-$ mice. (a) Oil red O staining of male and female *SF-1* $+/+$ and $+/-$ adrenals reveals decreased adrenal cortex and medulla size in $+/-$ adrenals. An eccentric medulla (unstained) is observed in male $+/-$ adrenals, whereas a small central medulla with few chromaffin cells is observed in female $+/-$ adrenals. Bar = 250 μ M. (b) Histological analysis reveals an X zone (X) interior to the cortex and surrounding the medulla (M) in female $+/+$ adrenals. In female $+/-$ adrenals, the central portion of the gland is composed largely of the X zone, with few medullary cells. Bar = 250 μ M. (c) Male *SF-1* $+/+$ and $+/-$ adrenal norepinephrine and epinephrine content ($n = 6$ adrenals per group). The contribution of epinephrine to total adrenal catecholamine content was similar in *SF-1* $+/+$ and $+/-$ mice (60% vs. 55%). **, $P \leq 0.01$ vs. wild type.

shown). In female $+/-$ adrenals, we observed a small number of centrally located TH-positive chromaffin cells, confirming that the central portion of the female $+/-$ adrenal consists largely of the X zone, whereas TH- and PNMT-immunoreactive cells were observed in an eccentric location in male *SF-1* $+/-$ adrenals (Fig. 6a and b). Finally, TH and PNMT protein expression levels were found to be markedly lower in *SF-1* $+/-$ adrenals compared with $+/+$ adrenals (Fig. 6c). Taken together, our data show that loss of one *SF-1* allele has dramatic consequences not only for adrenal cortex function, but also for the growth and function of the medulla.

Discussion

Our results in mice support the initial limited findings by Jameson and colleagues suggesting that *SF-1* gene dosage contributes to human endocrine disorders (6). In mice, *SF-1* haploinsufficiency results in an adrenal developmental defect that leads to an impaired stress response. *SF-1* $+/-$ adrenals com-

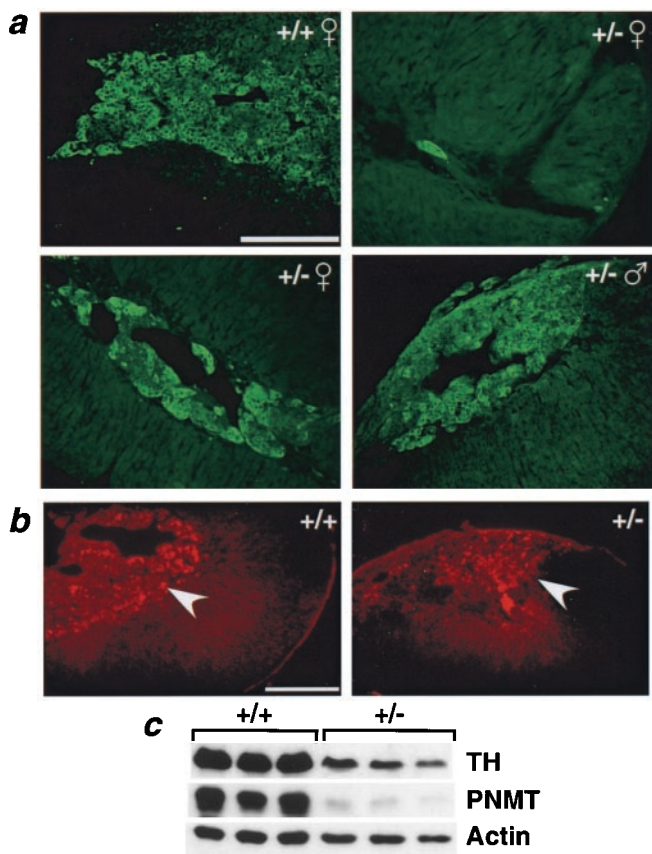


Fig. 6. Altered histology and gene expression in *SF-1*^{+/-} adrenal medulla. (a) TH-immunoreactivity in female and male *SF-1*^{+/+} and *SF-1*^{+/-} adrenal medullae. Two examples of female *SF-1*^{+/-} adrenals are included to demonstrate the extremes of phenotypic variability observed in female *SF-1*^{+/-} medulla size. Bar = 250 μ M. (b) Both *SF-1*^{+/+} and *SF-1*^{+/-} adrenals express PNMT. Arrowheads point to PNMT immunoreactivity in the adrenal medulla. Bar = 250 μ M. (c) Western blot analyses of TH, PNMT, and actin protein expression in *SF-1*^{+/+} and *SF-1*^{+/-} adrenals.

compensate for their small size by activating key genes in steroidogenesis and by hypertrophy of cortical cells. Despite these compensatory mechanisms, stress-induced corticosterone secretion in *SF-1*^{+/-} mice is impaired greatly, illustrating the importance of organ size for maintenance of normal stress physiology. Furthermore, *SF-1*^{+/-} mice provide a new model of adrenal insufficiency, one which is precluded in *SF-1* null mice because of early lethality and complete adrenal agenesis.

SF-1 heterozygotes exhibited marked reduction in both fetal and adult adrenal size, suggesting that the urogenital primordium, from which the adrenals, ovaries, and testes develop, is smaller as a result of decreased SF-1 levels. Indeed, *SF-1* heterozygotes also have smaller testes (85.6 mg vs. 100.2 mg, $P < 0.05$) and ovaries (3.9 mg vs. 6.5 mg, $P < 0.01$) compared with wild-type mice. The reduced size of adrenals and gonads in *SF-1*^{+/-} mice and the complete agenesis of these organs in *SF-1* null mice illustrate that SF-1 acts in a dose-dependent manner. It will be of interest to determine whether other SF-1-expressing cell groups, such as the ventromedial nuclei of the hypothalamus and pituitary gonadotropes, are similarly affected by *SF-1* haploinsufficiency. Both the molecular signals that activate SF-1 and the identity of SF-1 target genes in early adrenal growth are currently unknown. However, it is unlikely that signals from the HPA axis are required for adrenal organogenesis because deletions of the hypothalamic neuropeptide *CRH* and its receptor *CRH-R1* have no effect on fetal adrenal size as seen here (27, 28).

We therefore conclude that SF-1 functions independently of the HPA axis in adrenal development.

That *SF-1*^{+/-} adrenals contain an eccentric or poorly formed medulla is a novel phenotype and one which has not been reported to date for natural or genetically engineered strains of mice.^{††} One might speculate that adrenal insufficiency or cortical hypoplasia accounts for the eccentric position and small size of the medulla observed in *SF-1*^{+/-} mice. However, this phenotype is not recapitulated in other genetic models of adrenocortical insufficiency and reduced cortex size, such as *GR*, *CRH*, or *CRH-R1* null mice (25, 27, 29, 30). Little is currently known about genetic pathways that regulate migration and infiltration of neural crest-derived medullary precursors into the developing adrenal cortex and their subsequent proliferation and differentiation into chromaffin cells. Recently, a careful analysis of *GR* null mice has questioned the hypothesis that glucocorticoid signaling is essential for chromaffin cell migration and differentiation (25). Here, our findings establish that full expression of SF-1 in the cortex is critical for organization and growth of the medulla and further suggest that this nuclear receptor regulates genes involved in cortical-medullary interactions during organogenesis. Interestingly, we noted a sexual dimorphism in the location of the medulla in *SF-1* heterozygotes; whether this is due to sexually dimorphic expression of some SF-1 antagonist or cofactor is unclear at this time.

SF-1^{+/-} adrenals contain dramatically lower levels of both norepinephrine and epinephrine. This global loss of catecholamines is in contrast to *CRH* null mice where TH expression and norepinephrine secretion are maintained at or above wild-type levels, whereas PNMT expression and epinephrine secretion are selectively reduced because of low glucocorticoid levels (26). Lower circulating glucocorticoid levels in *SF-1*^{+/-} mice do not appear to contribute to a selective loss of PNMT expression and epinephrine synthesis. Although medullary hypoplasia in *SF-1*^{+/-} mice most likely accounts for reduced TH and PNMT expression and decreased catecholamine content, we are unable to exclude additional defects in medullary development or innervation that may contribute to this phenotype. Finally, it will be of interest to determine whether low adrenal catecholamines would impair energy homeostasis during food deprivation or compromise the ability of *SF-1* heterozygotes to thermoregulate as observed for *TH* null mice, which lack both adrenal and sympathetic catecholamines (31). Hormone replacement studies will be necessary to assess the respective contributions of adrenal glucocorticoids and catecholamines in mediating physiological stress responses in *SF-1*^{+/-} mice.

Whereas our data clearly show that reduced SF-1 levels contribute to disease by disrupting adrenal development, it is difficult to assess how *SF-1* heterozygosity impacts adult adrenal function. Indeed, one might predict that expression of SF-1 target genes, such as *StAR*, *MC2-R*, and *P450scc* would be decreased in *SF-1*^{+/-} adrenals. Instead, we found either no change (*P450SCC*) or marked up-regulation (*StAR* and *MC2-R*) of these targets in *SF-1*^{+/-} adrenals, as well as in *SF-1*^{+/-} ovaries (M.L.B. and H.A.I., unpublished observations on *StAR* expression). For these SF-1 target genes, additional regulatory mechanisms aside from *SF-1* dosage must exist, such as up-regulation of other transcription factors, enhanced activity of SF-1 by posttranslational modification (32), or increased availability of a putative SF-1 ligand. Another SF-1 target gene with interesting implications in adrenal development and disease is *Dax1* (33). *Dax1* interacts with SF-1 and, at least *in vitro*, represses SF-1 activity (34–36). Whereas we do not observe gross changes in *Dax1* expression in *SF-1*^{+/-} adrenals, an

^{††}The Jackson Laboratory, <http://jaxmice.jax.org/index.shtml> (2000).

altered SF-1:Dax1 ratio might be predicted to affect SF-1 target gene expression in either the adult or fetal adrenal. For example, the adrenal hypoplasia observed in *SF-1* +/- mice may result from a decreased SF-1:Dax1 ratio. However, this hypothesis is confounded by the relatively normal adrenal structure observed in *Dax1* null mice (37). Continued efforts using compound heterozygotes or conditional alleles of *SF-1* may help to resolve how interactions between SF-1 and Dax1 regulate adrenal gene expression.

Our findings demonstrate that, without the full complement of SF-1, development of both the adrenal cortex and medulla is profoundly altered, leading to major deficits in the stress response. Furthermore, the heretofore unrecognized haploinsufficiency of *SF-1* in mice now provides a genetic model to clarify the *in vivo* roles of this nuclear receptor in adrenal biology and further delineate molecular mechanisms of stress disorders.

Note Added in Proof. Biason-Lauber and Schoenle (38) have reported a novel heterozygous loss of function mutation in SF-1 that is associated with adrenal insufficiency but apparently normal ovarian function in an XX patient.

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