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Review

Central nervous system-specific knockout of steroidogenic factor 1

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ABSTRACT

Steroidogenic factor 1 (SF-1) is a nuclear receptor that plays important roles in the hypothalamus–pituitary–steroidogenic organ axis. Global knockout studies in mice revealed the essential *in vivo* roles of SF-1 in the ventromedial hypothalamic (VMH) nucleus, adrenal glands, and gonads. One limitation of global SF-1 knockout mice is their early postnatal death from adrenocortical insufficiency. To overcome limitations of the global knockout mice and to delineate the roles of SF-1 in the brain, we used Cre/loxP recombination technology to genetically ablate SF-1 specifically in the central nervous system (CNS). Mice with CNS-specific knockout of SF-1 mediated by nestin-Cre showed increased anxiety-like behavior, revealing a crucial role of SF-1 in a complex behavioral phenotype. Our studies with CNS-specific SF-1 KO mice also defined roles of SF-1 in regulating the VMH expression of target genes implicated in anxiety and energy homeostasis. Therefore, this review will focus on our recent studies defining the functional roles of SF-1 in the VMH linked to anxiety and energy homeostasis.

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1. Introduction

Steroidogenic factor 1 (SF-1; officially named NR5A1) was originally identified and characterized as an essential regulator of the enzymes that make steroid hormones in steroidogenic cell lines. SF-1 is a member of the nuclear hormone receptor family of transcriptional regulators based on its sequence and functional analysis (Lala et al., 1992). Subsequently, several groups have reported that SF-1 is a key regulator in establishment and function of endocrine organ axis (reviewed in Parker et al., 2002).

To understand *in vivo* roles of SF-1, targeted gene disruption in embryonic stem cells was used to generate mice with global knockout of SF-1. Analyses of these global SF-1 KO (SF-$1^{-/-}$) mice confirmed its pivotal roles in endocrine development in the ventromedial hypothalamus (VMH), adrenal glands, and gonads (Luo et al., 1994; Sadovsky et al., 1995; Shinoda et al., 1995). In the brain, SF-1 expression commences at approximately embryonic day 9 (E9) in the ventral neural tube that forms the hypothalamic primordium and subsequently becomes the VMH. The only site of SF-1 expression in the adult mouse brain is the VMH, a region closely linked to physiological effects, including the regulation of sexual behavior, mood regulation, energy homeostasis, thermogenesis, and cardiovascular function (McClellan et al., 2006). Histologically, the VMH can be subdivided into major three regions: dorsomedial (dm), central (c), and ventrolateral (vl) VMH; these subdivisions have been characterized with respect to their various functions (reviewed in McClellan et al., 2006).

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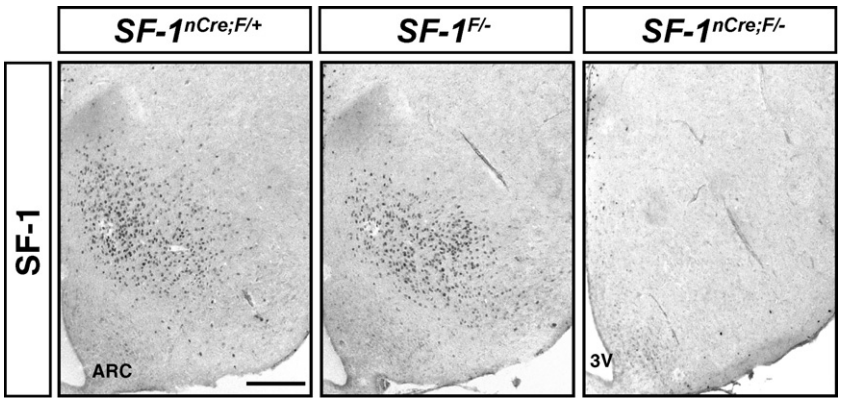

Fig. 1. Effect of CNS-specific SF-1 inactivation on SF-1 immunoactivity in the VMH. Immunohistochemical analysis showing SF-1 expression in mice of the indicated genotypes. The CNS-specific SF-1 KO (*SF-1*$^{nCre;F/-}$) mice show markedly decreased expression of SF-1 in the VMH compared to WT (*SF-1*$^{nCre;F/+}$) and heterozygous (*SF-1*$^{F/-}$) mice. These data were originally published in Kim et al. (2008). ARC, arcuate nucleus; 3V, third ventricle. Scale bar, 200 μm.

Studies of mice with global knockout of SF-1 have revealed its critical roles in establishment of the VMH cytoarchitecture. However, the *in vivo* regulatory roles of SF-1 in the VMH are less well defined. Therefore, to directly address the *in vivo* function of SF-1 and circumvent the limitations of global SF-1 KO—including possible secondary effects of pituitary, adrenal and gonadal insufficiency—we studied physiological and biochemical roles of SF-1 in the VMH using CNS-specific SF-1 KO mice. Based on our recent findings, this review will discuss the functional roles of SF-1 in the brain, focusing particularly on physiological and biochemical impact of SF-1 in the VMH.

2. Generation of CNS-specific SF-1 KO mouse

To define specific functional roles of SF-1 in the VMH, we generated CNS-specific SF-1 KO mice by using the Cre-loxP technology. Conditional (F) SF-1 allele (Zhao et al., 2001) combined with SF-1 null allele (*SF-1*$^{+/-}$) (Luo et al., 1994) carrying a nestin-Cre (nCre) transgene that directs Cre expression to the CNS (Tronche et al., 1999). Our study showed that the expression of SF-1 outside of VMH such as anterior pituitary, adrenal cortex, and gonads in CNS-specific SF-1 KO (*SF-1*$^{nCre;F/-}$) mice was comparable with WT counterparts. In addition, levels of hormones such as corticosterone, testosterone, and estradiol were indistinguishable from those in wild-type mice (Zhao et al., 2008). In contrast, the VMH nuclear organization was not discernable and SF-1 immunoactivity was markedly decreased (Fig. 1); these findings are highly reminiscent of the global SF-1 KO mice (Ikeda et al., 1995; Shinoda et al., 1995). In addition, temporal examination revealed that SF-1 inactivation by the nestin-Cre transgene predominantly occurred by E13.5, with complete silencing by E18.5 (Zhao et al., 2008).

3. CNS-specific SF-1 KO mice have increased anxiety-like behavior

One obvious behavioral phenotype in the CNS-specific SF-1 KO mice was increased anxiety-like behavior compared to their wild-type counterparts. This was seen in open behavior in cages and in multiple standardized paradigms using described methods, including the light/dark test, elevated plus maze, open field test, locomotor activity in a novel environment, and marble burying test (Zhao et al., 2008). The anxiety-like behavior was not sexually dimorphic, since both male and female CNS-specific SF-1 KO mice showed significantly increased anxiety-like behavior relative to WT mice of the same sex.

In agreement with previous findings (Dielenberg et al., 2001; Canteras, 2002), the VMH has been characterized as part of a medial hypothalamic defensive system based on robust neuronal activations in the VMH in response to a potential predator. The neuronal activation in the VMH in response to potential predators may be important when considering anxiety-like behavior, because predators may be associated with anxiety in rodent models. In accordance with this concept (Fig. 2), we found that CNS-specific SF-1 KO mice had decreased expression or altered distribution in the mediobasal hypothalamus of several genes implicated in anxiety-like behavior, including brain-derived neurotrophic factor (BDNF), the type 2 receptor for corticotrophin releasing hormone receptor 2 (Crhr2), and urocortin 3 (Ucn3) (Zhao et al., 2008). These findings suggest that SF-1 expression in the mouse VMH neurons is closely connected with the neural circuitry of anxiety-like behavior and raise the possibility that SF-1 may play similar roles in VMH neurons of human beings.

4. Identification of novel SF-1-reponsive genes related to anxiety-like behavior

A number of target genes of SF-1 have been identified and characterized in pituitary gonadotropes, the adrenal cortex, and gonads (Parker et al., 2002). In the CNS, the expression of SF-1 is tightly restricted to the VMH, where its physiological roles and target genes have long been elusive. A recent report considerably expanded the list of SF-1 targets in the VMH, including cell adhesion molecules such as Amigo2, Cdh4, Sema3a, Slit3, and Netrin3 and other genes that are highly expressed in the VMH, such as Fezf1, Nptx2, Nkx2-2, and A2bp1 (Kurrasch et al., 2007).

Of the anxiety-related genes studied (Fig. 2), BDNF has been characterized as a direct target gene of SF-1 (Tran et al., 2006). We next explored promoter sequences of β form of Crhr2, the isoform that is expressed in the VMH, to examine whether it also might be a SF-1 target gene in the VMH. Sequence inspection revealed two sequences matching binding requirements for SF-1 (5′-TGACCT-3′ at site −1761 and 5′-TTACCT-3′ at site +6). Both electrophoretic mobility shift assays with oligonucleotides containing each sequence and transient cotransfection experiments implicated the element at +6 in the β form of Crhr2 as a potential SF-1 regulatory site, arguing that Crhr2 is a direct target of SF-1 in the VMH.

5. Regulation of energy homeostasis in SF-1 neurons of the VMH

The VMH has long been implicated as an important brain region for the regulation of appetite and energy homeosta-