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Effects of SPAK knockout on sensorimotor gating, novelty exploration, and brain area-dependent expressions of $NKCC_1$ and KCC_2 in a mouse model of schizophrenia

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ABSTRACT

SPAK (Sterile 20/SPS1-related proline/alanine-rich kinase) is a protein kinase belonging to the mitogen-activated protein kinase (MAPK) superfamily that has been found to be extensively distributed across the body. The SPAK downstream substrates $NKCC_1$ and KCC_2 in the central nervous system are important in the interpretation of developmental mental disorders. The present study aimed to clarify the role of SPAK–$NKCC_1/KCC_2$ using a rodent schizophrenia-like model. The mouse paradigm of isolation rearing (IR) was employed, as it simulates the sensorimotor gating abnormalities of schizophrenia. SPAK transgenic mice were used and were divided into four groups: social-wild type, social-$SPAK^{-/-}$, isolation-wild type, and isolation-$SPAK^{-/-}$. The prepulse inhibition (PPI) test and the novel object recognition test (NORT) were used to measure schizophrenia-associated dysfunctions in gating ability and the novelty recognition, respectively. Finally, the protein expressions of $NKCC_1/KCC_2$ in the prefrontal cortex and hippocampus were detected to determine correlations with the behavioral data. Our results demonstrated that SPAK-null mice had superior PPI and novelty recognition relative to wild type controls, with a concomitant increase in KCC_2 in the prefrontal cortex. IR disrupted PPI and NORT performances with an associated increase in KCC_2. Furthermore, rearing environment and gene manipulation had mutually interactive effects, as the IR-induced effects on PPI and NORT were reversed by SPAK knockout, and the increase in KCC_2 and the decreased in the $NKCC_1/KCC_2$ ratio in the prefrontal cortex induced by SPAK knockout were reversed by IR. Our data supported the gene-environment hypothesis and demonstrated the potential value of SPAK manipulation in future schizophrenia studies.

Contents

Abbreviations: IR, isolation rearing; KCC, K^+-Cl^-cotransporter; NKCC, $Na^+-K^+-2Cl^-$cotransporter; NORT, novel object recognition test; PPI, prepulse inhibition; SPAK, sterile 20/SPS1-related proline/alanine-rich kinase

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1. Introduction

SPAK (STE20/SPS1-related proline/alanine-rich kinase) was first discovered in pancreatic β cells (Johnston et al., 2000) and was later shown to be extensively distributed throughout the body, including in the peripheral and central nervous systems. To date, evidence has suggested that SPAK plays an important role in determining the direction of chloride flow via the regulation of two downstream cotransporter families, the Na^+–K^+–$2Cl^-$ cotransporter (NKCC) and the K^+–Cl^- cotransporter (KCC), (Flatman, 2007). In many regions of the brain during early development, the NKCC family facilitates the movement of chloride from outside to inside cells (by $NKCC_1$), whereas the KCC family performs the opposite function (by KCC_2) (Benarroch, 2013). Because the levels of these two cotransporters are age dependent (Wang et al., 2002), the ratio of $NKCC_1/KCC_2$ has been suggested to be useful in the interpretation of certain mental disorders with developmental origins (Hyde et al., 2011; Kalkman, 2011). For example, postmortem studies revealed that schizophrenia patients may have a relatively higher mRNA $NKCC_1$ level and $NKCC_1/KCC_2$ ratio in certain brain areas (Hyde et al., 2011).

Because the phosphorylation of SPAK activates $NKCC_1$ and inactivates KCC_2, the ratio of $NKCC_1/KCC_2$ is inversely controlled by SPAK (Flatman, 2007; Gagnon et al., 2006; Kahle et al., 2010). Therefore, manipulations of the SPAK gene may alter the $NKCC_1/KCC_2$ ratio and its downstream biological activities. Accordingly, SPAK-null mice are expected to have a lower $NKCC_1/KCC_2$ ratio in schizophrenia-related brain areas. It is necessary to examine whether SPAK knockout alters certain schizophrenia-relevant phenotypic characteristics.

The present study examined the role of the SPAK–$NKCC_1/KCC_2$ pathway in a rodent paradigm that models schizophrenia-like abnormalities. Isolation rearing (IR) is a paradigm known to cause behavioral deficits in rodents that are similar to the sensorimotor gating dysfunction observed in schizophrenia patients. Animals in the IR paradigm are socially deprived from the time of weaning, and they may exhibit strengthened locomotor activity and impaired prepulse inhibition (PPI) in startle response in adulthood. These phenomena are effectively applied to both rats (for review, see Hall, 1998) and mice (Dai et al., 2004; Koda et al., 2008). Previous evidence from our own and other laboratories demonstrated that IR-induced PPI impairment is irreversible and age-dependent (Cilia et al., 2001; Liu et al., 2011); therefore, it is a suitable tool for investigating mental dysfunctions of developmental origins. Because SPAK-null mice may have a lower $NKCC_1/KCC_2$ ratio, these mice are expected to exhibit less IR-induced PPI dysfunction. In addition to PPI, which represents a pre-attentional gating function, IR mice also exhibit more cognitive abnormalities, as observed in the novel object recognition test (NORT) (Niwa et al., 2011). The test examines the degree of the tendency to explore a novel or unfamiliar object and has been used successfully to explore the memory impairment in rodents (Park et al., 2014). Thus, NORT was used in the present study to determine whether it is sensitive to the SPAK manipulation. Finally, the protein expressions of $NKCC_1/KCC_2$ in the prefrontal cortex and hippocampus were measured to determine correlations with the behavioral data.

In summary, the first aim of the present study was to test the hypothesis that SPAK knockout mice have a lower $NKCC_1/KCC_2$ ratio and superior PPI and NORT performances when compared to wild type controls. The secondary aim of our study was to examine whether these effects are changed when animals are raised with the IR paradigm, which negatively affects PPI and NORT performances. The results of the present study may help indicate the value of SPAK–$NKCC_1/KCC_2$ manipulation in exploring the interactions between genetic makeup and postnatal social environments.

2. Materials and methods

2.1. Animals

All male $SPAK^{-/-}$ homozygous mice (HOM) and the wild type littermates (WT) used in the present study were age-matched and were produced from $SPAK^{+/-}$ heterozygous breeding pairs with the C57BL/6 genetic background. The $SPAK^{+/-}$ heterozygous mice were obtained from the laboratory of Dr Yang (the co-author of the present study) at the National Defense Medical Center (for details of how to generate the $SPAK^{+/-}$ heterozygous mice, see Yang et al., 2010). After birth, mice from the same litter were fostered by their mother (7 or 8 pups per female) until weanling at postnatal day 21. Tail genomic DNA was applied for each mouse for genotyping by PCR (Yang et al., 2010). Mice were then pseudo-randomly assigned (balanced across litters) to social or IR groups. Socially reared mice were housed in a group of five mice, and IR mice were singly housed after weanling. At 10 weeks of age, all mice (4 groups: WT-social, $SPAK^{-/-}$-social, WT-isolated, and $SPAK^{-/-}$-isolated) were entered into a longitudinal experimental protocol that included tests of locomotor activity (N = 11/each group), prepulse inhibition (N = 8/each group), and novel object recognition (N = 8/each group). The tests were performed separately with an interval of at least 7 days between any two tests. At the end of the study, the brains of the mice were removed for neurochemical studies (N = 4 or 5/each group). Animals were housed at a constant cage temperature (22 ± 1 °C) and humidity (40%–70%), and were kept under regular light–dark conditions (light on at 07:00 AM and off at 7:00 PM) with food and water available ad libitum, except during behavioral testing. The study was approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center.

2.2. Locomotor activity

Total distance of a mouse traveled in a plexiglass chamber ($30 \times 30 \times 30$ cm^3) was measured in darkness, except for infrared lightening. The mouse was placed at the center of the arena, and the test was video recorded (LocoScan, CleverSys, VA, USA). After each test, the chamber was cleaned thoroughly with 80% ethanol.