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Steroid receptor RNA activator affects the development of poststroke depression by regulating the peroxisome proliferator-activated receptor γ signaling pathway

Baoying Jiang[a], Hongwei Wang[b] and Houchi Xu[c]

The long noncoding RNA, steroid receptor RNA activator (SRA), has been reported to be involved in the development of many types of disease in humans. The aim of this study was to evaluate whether SRA was associated with poststroke depression (PSD). A PSD rat model was established, and depression-like behaviors and sucrose consumption in rats with PSD were analyzed. Reverse transcription-quantitative PCR (RT-PCR), western blot and luciferase dual reporter assay analyses were performed to detect the expression of peroxisome proliferator-activated receptor γ (PPARγ) expression following SRA small interfering RNA (siRNA) treatment. Compared with the control, the horizontal and vertical movement scores and consumption of sucrose solution were decreased in the PSD, PSD + LV-SRA and PSD + pioglitazone groups at 7 days post-SRA-siRNA treatment, while they were increased in the PSD + LV-SRA and PSD + pioglitazone groups. Furthermore, SRA expression in the PSD, PSD + LV-SRA and PSD + pioglitazone groups was lowered compared with the control group at 7 days postinjection. SRA increased the reported luciferase activity, but pioglitazone had no effect on the luciferase activity induced by SRA. SRA upregulated PPARγ mRNA and protein expression, whereas SRA siRNA significantly downregulated its expression. No significant differences in characteristics were identified between rats with and without PSD. SRA was more highly expressed in rats with PSD than rats without PSD. Collectively, this study suggests that SRA is associated with PSD through PPARγ signaling, indicating a potential therapeutic target of SRA for controlling PSD. *NeuroReport* 31: 48–56 Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Haq *et al.* [1] demonstrated that poststroke depression (PSD) is an important and frequent neuropsychiatric disorder associated with stroke. Its prevalence ranges from 25 to 54% for poststroke patients, resulting in growing research in the cognitive impairment induced by PSD [1]. Li *et al.* [2] indicated that a large number of factors, including psychological, social and biological factors, can cause PSD. However, the detailed pathogenesis mechanisms of PSD remain uncharacterized. The main fields in PSD molecular pathogenesis research are neurotrophins, glutamate and inflammation [3–5].

Pioglitazone is a thiazolidinedione antidiabetic drug that acts as a peroxisome proliferator-activated receptor γ (PPARγ) agonist. PPARs are predominantly located in insulin-targeted tissues, including fat, muscle and the liver. They decrease insulin resistance and thus ameliorate the level of blood glucose [6]. Previous studies suggest [8] that low PPARγ expression levels have been identified in the brains of patients with depression [9]. The Pro12Ala polymorphism in PPARγ is associated with a high risk of depression, and the activation of PPARγ can improve depressive behaviors [10].

The transcripts of long non-coding RNA (lncRNA) are >200 nucleotides in length and have the same structure as mRNAs, including the polyadenylated 3′ tail and 5′ cap [11]. The total number of lncRNA loci reported in the human genome is >56 000, and novel lncRNAs continue to be identified [12]. lncRNAs were initially hypothesized to be chromatin regulators, but research has indicated their critical influence in many different cellular processes [13,14]. lncRNAs are also considered to be regulators of mammalian disease and development [14]. As one of the most highly expressed ncRNAs classes in the brain, lncRNAs have attracted an increasing amount of research attention regarding brain disorders and functions [15]. It was previously reported that lncRNAs may be associated with multiple psychiatric and neurodegenerative disorders. For example, the protein-coding transcript lncRNA, Disrupted

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in Schizophrenia 1 (DISC1) can overlap and antisense with DISC2, which is involved in the pathogenesis of autism spectrum disorder, schizophrenia and bipolar depression [16]. It was demonstrated that the expression of circulating lncRNAs in patients with major depressive disorder (MDD) is different from that of demographically matched controls. Three lncRNAs are differentially expressed in MDD, which may be regulatory factors via interactions with coding transcripts [17].

Steroid receptor RNA activator (SRA) is an lncRNA and activator of transcription of PPARγ that is functionally involved in the pathogenesis of PSD [8,18,23]. Pioglitazone is a well established activator of PPARγ and may serve a role in the treatment of depression [19]. Based on the previous studies regarding SRA, pioglitazone and PPARγ, it is hypothesized that SRA may attenuate the symptoms of depression following a stroke. In the present study, to prove the hypothesis, functional analysis was done in the in-vivo and in-vitro PSD models.

Materials and methods

Human subjects

From May 2016 to July 2018, a total of 60 patients were recruited from Rizhao People's Hospital, including 30 patients diagnosed with PSD, and 30 who had suffered a stroke without PSD. Patients with PSD at 3 months postadmission were diagnosed according to the criteria defined in the Diagnostic and Statistical Manual of Mental Disorders [20], with an activity of daily living scale >50 [21]. Blood was collected from all participants, and the serum was separated and stored at −80°C until required. All patients signed informed consent forms following an explanation of their participation in the study. The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of Rizhao People's Hospital. The information of the patients was shown in Supplemental Table 1, Supplemental digital content 1, *http://links.lww.com/WNR/A555*.

Animals

A total of 40 adult male Sprague–Dawley rats at age of 4 weeks were obtained from the Laboratory Animal Center of Zhejiang University (Hangzhou, China) and cared for in a specific pathogen-free environment. The rats weighed 250 ± 20 g, and were maintained at 25 ± 2°C, relative humidity 60 ± 5% with a 12-hour light/dark cycle. All rats were allowed ad libitum assess to water and food. The animal studies were approved by the Ethics Committee of Rizhao People's Hospital.

Experimental groups and model establishment

Open-field test behavior and consumption of sucrose solution were assessed. A total of 40 rats were randomly and equally divided into five groups including the control, PSD, PSD + green fluorescent protein (GFP) plasmids, PSD + GFP-SRA plasmids and PSD + pioglitazone groups. In all groups except the control group, an embolism was instigated via the occlusion of the right middle cerebral artery. Rats with neurological scores >1 and <4 were selected at 24 hours after operation according to the Longa 6-point scoring scale: 0, no injury; 1, left forelimb extension disorder; 2, circling to the left; 3, falling to the left when walking; 4, unconscious; 5, fatality. Subsequent to the middle cerebral artery occlusion, the rats were subjected to chronic unpredictable mild stress (CUMS) including wet litter, water deprivation, behavioral restriction, tail clamping, electric shock to the foot and forced ice-water swimming. CUMS began at 7 days after surgery and continued for 4 weeks. Following the completion of CUMS, lentiviral injections, as are subsequently described, were performed in the PSD + GFP, PSD + GFP-SRA and PSD + pioglitazone groups, but not in the control and PSD groups.

Movement analysis

A $100 \times 100 \times 50\,cm^3$ open box, with a base divided into 25 equal-area squares with black lines, was used to record the vertical and horizontal movement of the rats placed in the middle of the box for 3 minutes. The vertical movement score corresponded to the frequency of rearing, that is, clinging to the walls or removing both forelimbs from the ground more than once. Horizontal movement score corresponded to the frequency of crossing the box. The box was cleaned thoroughly prior to each test.

Sucrose solution consumption

Rats were restricted from water for 24 hours prior to the sucrose consumption assay. Rats then had access to 1% sucrose solution for 1 hour. Consumption was determined as grams of sucrose consumed during 1 hour/body weight (g) × 100.

Lentiviral injection for the overexpression of steroid receptor RNA activator

SRA cDNA was cloned into a lentiviral vector (pLV) containing GFP to generate pLV-GFP-SRA. pLV-GFP-SRA and pLV-GFP were injected into the corresponding groups of rats. Reverse transcription-quantitative PCR (RT-qPCR) was performed to determine the expression level of SRA in the rats in three independent tests.

Cell culture and transfection

In brief, U251 cells were obtained from the American Type Culture Collection (Manassas, Virginia, USA), and maintained in Dulbecco's modified Eagle's medium/F12 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) containing 10% fetal bovine serum (FBS), 100 µg/ml streptomycin and 100 U/ml penicillin (Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO_2. Cells were grown to 80% confluence and SRA siRNA (5′-CTCCCTTCTTACCACCACCA-3′) or a scrambled control (5′-AATTCTCCGAACGTGTCACGT-3′) synthesized by Genepharm, Inc. (Sunnyvale, California, USA) were transfected into U251 cells using Lipofectamine

2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The transfection was performed three times independently.

Reverse transcription-quantitative PCR

TRIzol (Thermo Fisher Scientific, Inc.) was used to extract total RNA, including lncRNA and mRNA, from serum and U251 cells as according to the manufacturer's protocol. For the RT-qPCR detection of SRA, a Taqman miRNA reverse transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was utilized to synthesize cDNA, and the ABI7900 Real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Inc.) was used with an miScript SYBR Green PCR kit (Qiagen GmbH, Hilden, Germany) to quantify the expression level, as according to the manufacturer's protocols.

In order to examine the expression of PPARγ, a Prime Script RT reagent kit (Takara Biotechnology, Inc., Otsu, Japan) was utilized to synthesize cDNA, then the ABI7900 Real-time PCR system was used with RT-qPCR Mixture reagent (Takara Biotechnology, Inc.) to quantify the expression of PPARγ. U6 and GAPDH served as internal controls. The $2^{-\Delta\Delta Cq}$ method was used to calculate the relative expressions of SRA and PPARγ [22]. All experiments were repeated three times. Primers were used as follows: PPARγ, 5′-TGTCGGTT TCAGAAGTGC CTTG-3′ (F), 5′-CCACAGACT CGGCACTC-3′ (R); SRA, 5′-CTCCCTTCTTAC CACCACC A-3′ (F), 5′-TGCAGATACA CAGGGAGCAG-3′ (R); GAPDH, 5′-AGCAGTCCCG TACA CTGGCAA AC-3′ (F), 5′-TCT GTGGTGATGTAAATGTCCTCT-3′ (R); U6, 5′-C TCGCTTCGG CAGCACA-3′ (F), 5′-AACGCTT CACGAATTTGCGT-3′ (R).

Luciferase assay

A luciferase assay was performed to determine the effect of pioglitazone on SRA expression. An SRA promoter segment was amplified and inserted into a GL3 plasmid upstream of firefly luciferase. Sequencing was performed to confirm the successful transfection of the SRA promoter. U251 cells seeded into a 48-well plate were transfected with pLV-GFP or pLV-GFP-SRA using Lipofectamine 2000 (Introgen, Austin, Texas, USA). Then a dual-luciferase reporter assay system (Promega Corporation, Madison, Wisconsin, USA) was utilized to determine the firefly and *Renilla* luciferase activity at 48 hours posttransfection. The experiment was repeated 3 times.

Western blot assay

The total protein was extracted from U251 cells and tissue samples using Lysis Buffer (Beyotime Institute of Biotechnology, Haimen, China), and an Enhanced BCA Protein Assay kit (Beyotime Institute of Biotechnology) was utilized to measure protein concentration according to the manufacturer's instructions. SDS-PAGE was performed to separate 20 μg proteins using 8–12% polyacrylamide gels, and proteins were transferred to polyvinylidene fluoride membranes (Merck KGaA, Darmstadt, Germany). The membranes were blocked in 5% skimmed milk for 2 hours at room temperature, then primary antibodies monoclonal anti-human PPARγ (dilution, 1:5000; Abcam, Cambridge, UK) or β-actin (dilution, 1:12 000; Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) were added for 12 hours at 4°C. A secondary horseradish peroxidase (HRP)-conjugated mouse antigoat antibody was incubated with the membranes (dilution, 1:3000; Santa Cruz Biotechnology, Inc., USA) at 37°C for 1 hour. Chemiluminescence HRP substrate (Merck KGaA) was used to visualize the protein bands and data were analyzed using Image J software version 1.44 (National Institutes of Health, Bethesda, Maryland, USA). All western blots were performed three times.

Statistical analysis

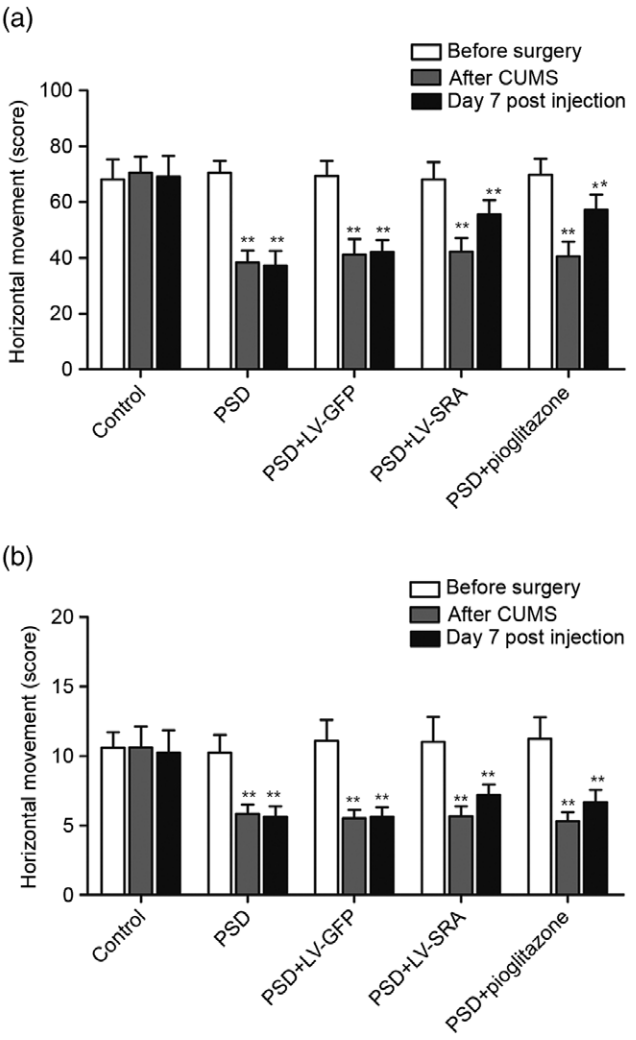
Statistical Product and Service Solutions (SPSS) software program version 12.0 (SPSS, Inc., Chicago, Illinois, USA) was used to perform all statistical analyses and all data are presented as the mean ± SD. A two-tailed χ^2, Mann–Whitney or Student's *t*-test, a one-way analysis of variance with Tukey's post-hoc test or Fisher's exact test were used to determine the significance of differences between various groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Lentivirus-induced steroid receptor RNA activator expression induces depression-like movement characteristics in poststroke depression model rats

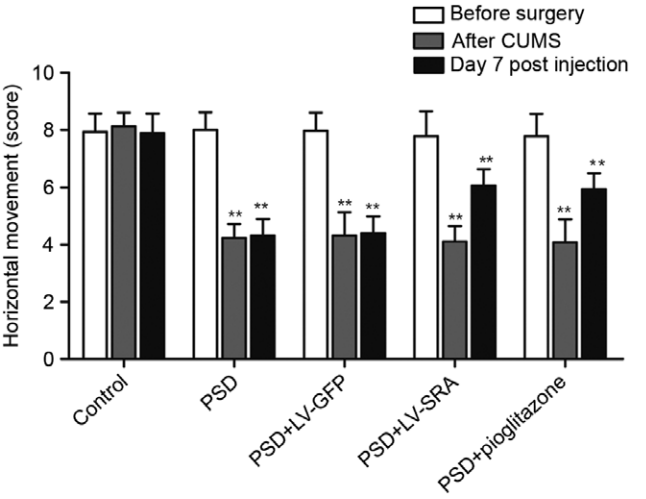
The horizontal and vertical movement scores were determined for the five treatment groups (control, PSD, PSD + LV-GFP, PSD + LV-SRA and PSD + pioglitazone) at three different time points (prior to surgery, post-CUMS and 7 days after injection). It was demonstrated that there were no differences between the horizontal (Fig. 1a) and vertical (Fig. 1b) movement scores among the five groups prior to surgery ($P > 0.05$). However, horizontal and vertical movement scores were lowered in the PSD, PSD + LV-GFP, PSD + LV-SRA and PSD + pioglitazone groups compared with the control group post-CUMS [$t(\mathrm{df} = 7) = 12.3$, $P < 0.01$; $t(\mathrm{df} = 7) = 11.3$, $P < 0.01$; $t(\mathrm{df} = 7) = 12.2$, $P < 0.01$; $t(\mathrm{df} = 7) = 12.3$, $P < 0.01$]. At 7 days postinjection, horizontal and vertical movement scores were higher in the PSD + LV-SRA and PSD+ pioglitazone groups than those in PSD + LV-GFP group [$t(\mathrm{df} = 7) = 7.7$, $P < 0.01$; $t(\mathrm{df} = 7) = 8.8$, $P < 0.01$]. Furthermore, there was no difference in the horizontal and vertical movement scores between the PSD group and PSD + LV-GFP group ($P > 0.05$). Above results indicated that SRA and pioglitazone affected the severity of PSD as demonstrated by horizontal and vertical movement scores.

Fig. 1



Effect of SRA expression on depression-associated behaviors in PSD rats. (a) The PSD, PSD + LV-GFP, PSD + LV-SRA and PSD + pioglitazone groups exhibited lower horizontal movement scores than the control group; the PSD + LV-SRA and PSD + pioglitazone groups exhibited higher horizontal movement scores than the PSD + LV-GFP group. (b) PSD, PSD + LV-GFP, PSD + LV-SRA and PSD + pioglitazone groups exhibited lower vertical movement scores than the control group; the PSD + LV-SRA and PSD + pioglitazone groups exhibited higher vertical movement scores compared with the PSD + LV-GFP group. CUMS, chronic unpredictable mild stress; LV-GFP, lentiviral-green fluorescent protein; PSD, poststroke depression; SRA, steroid receptor RNA activator. $^{*}P<0.05$ and $^{**}P<0.01$ vs the control.

Fig. 2



Influence of SRA expression on the consumption of sucrose solution in PSD rats. The PSD, PSD + LV-GFP, PSD + LV-SRA and PSD + pioglitazone groups demonstrated a lower consumption of sucrose solution than the control group; PSD + LV-SRA and PSD + pioglitazone groups exhibited a higher consumption of sucrose solution compared with the PSD + LV-GFP group. CUMS, chronic unpredictable mild stress; LV-GFP, lentiviral-green fluorescent protein; PSD, poststroke depression; SRA, steroid receptor RNA activator. $^{*}P<0.05$ and $^{**}P<0.01$ vs the control.

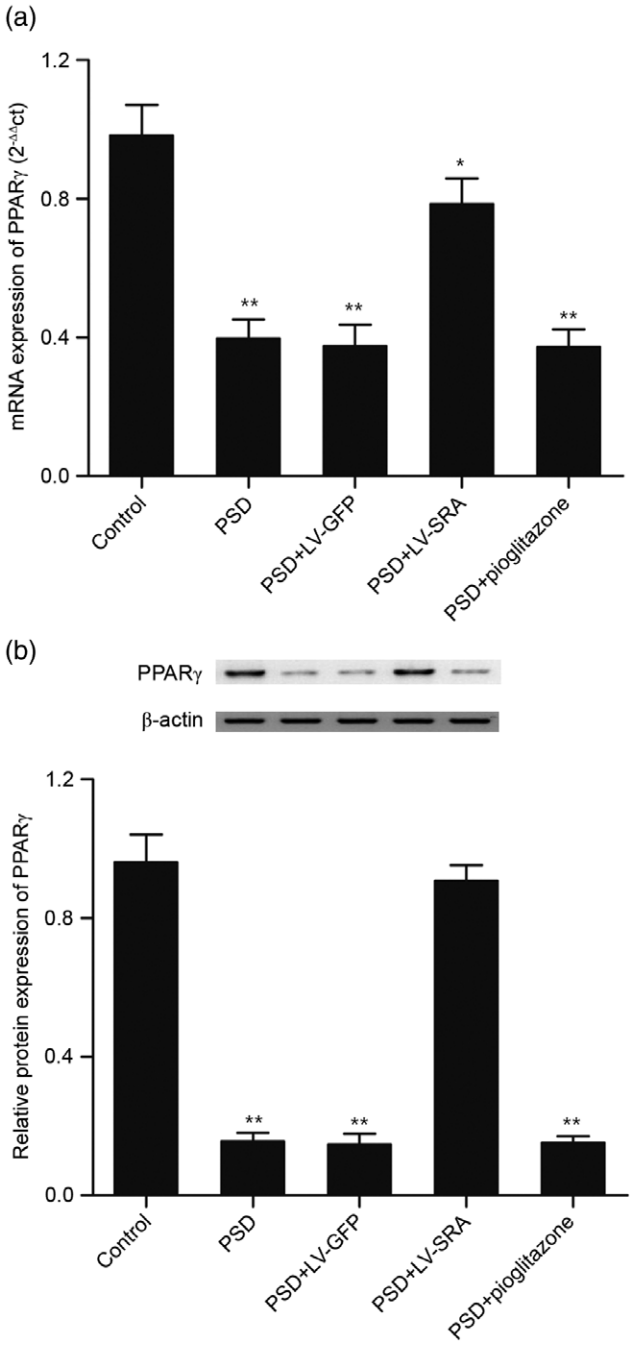
Lentivirus-induced steroid receptor RNA activator expression increases the consumption of sucrose solution in poststroke depression model rats

As illustrated in Fig. 2, there was no difference in the consumption of sucrose solution among the five groups prior to surgery ($P>0.05$). The consumption of sucrose solution was decreased heavily in the PSD, PSD + LV-GFP, PSD + LV-SRA and PSD + pioglitazone groups compared with the control group post-CUMS [$t(\mathrm{df}=7)=13.9$, $P<0.01$; $t(\mathrm{df}=7)=10.8$, $P<0.01$; $t(\mathrm{df}=7)=13.7$, $P<0.01$; $t(\mathrm{df}=7)=10.7$, $P<0.01$]. At 7 days postinjection, consumption of sucrose solution was upregulated in the PSD + LV-SRA and PSD + pioglitazone groups compared with the PSD + LV-GFP group [$t(\mathrm{df}=7)=3.7$, $P=0.002$; $t(\mathrm{df}=7)=3.6$, $P=0.003$]. There was no difference between the PSD group and PSD + LV-GFP group ($P>0.05$). It was suggested that SRA and pioglitazone affected PSD through the influence on sucrose consumption.

Steroid receptor RNA activator changes peroxisome proliferator-activated receptor γ mRNA and protein expressions in poststroke depression model rats

Following treatment of LV-GFP, LV-SRA and pioglitazone in PSD model rats, the expression levels of PPARγ mRNA (Fig. 3a) and protein (Fig. 3b) were detected by RT-PCR and Western blot, respectively. It was found that the expressions of PPARγ were all significantly decreased in the PSD, PSD + LV-GFP and PSD + pioglitazone groups compared with the control group at 7 days post-injection [$t(\mathrm{df}=2)=10.2$, $P=0.001$; $t(\mathrm{df}=2)=9.9$, $P=0.001$; $t(\mathrm{df}=2)=10.3$, $P=0.001$]. But the levels of PPARγ were higher in the PSD + LV-SRA group compared with the PSD + LV-GFP group [$t(\mathrm{df}=2)=6.7$, $P=0.003$; $t(\mathrm{df}=2)=14.3$, $P<0.01$], indicating SRA promoted PPARγ expression at both mRNA and protein levels. Moreover, there was no difference in PPARγ mRNA and protein expression between the PSD, PSD + LV-GFP and PSD + pioglitazone groups ($P>0.05$). The data revealed that SRA effectively treated depression via PPARγ activation.

Fig. 3



SRA expression in PSD rats. (a) PPARγ mRNA expression was downregulated in the PSD, PSD + LV-GFP, PSD + LV-SRA and PSD + pioglitazone groups compared with the control group at 7 days post-injection. However, PPARγ mRNA expression was upregulated in the PSD + LV-SRA group compared with the PSD + LV-GFP group. (b) PPARγ protein level was downregulated in the PSD, PSD + LV-GFP, PSD + LV-SRA and PSD + pioglitazone groups compared with the control group at 7 days post-injection. However, the PPARγ protein level was upregulated in the PSD + LV-SRA group compared with the PSD + LV-GFP group. CUMS, chronic unpredictable mild stress; LV-GFP, lentiviral-green fluorescent protein; PPARγ, peroxisome proliferator-activated receptor; PSD, poststroke depression; SRA, steroid receptor RNA activator. $^{*}P<0.05$ and $^{**}P<0.01$ vs the control.

Pioglitazone does not affect steroid receptor RNA activator expression

A luciferase assay was performed to assess the effect of pioglitazone on SRA expression. As demonstrated in Fig. 4, the luciferase activity of SRA was significantly increased in SRA overexpressed cells compared with the cells transfected with an empty vector [$t(\mathrm{df}=2)=13.9$, $P<0.01$] (Fig. 4a). Cells treated with pioglitazone displayed a comparable luciferase activity to cells untreated with pioglitazone (Fig. 4b), suggesting that pioglitazone had no effect on SRA expression.

Steroid receptor RNA activator regulates peroxisome proliferator-activated receptor γ expression in U251 cells

RT-qPCR and western blot analyses were performed to examine the interaction between SRA and PPARγ. As demonstrated in Fig. 5, PPARγ mRNA and protein expression were upregulated in U251 cells subsequent to transfection with SRA overexpression plasmids compared with those transfected with control plasmids [$t(\mathrm{df}=2)=9.6$, $P<0.01$] (Fig. 5a), In contrast, SRA knockdown obviously downregulated PPARγ expression in comparison with a scrambled control [$t(\mathrm{df}=2)=11.3$, $P<0.01$] (Fig. 5b). This suggested that SRA positively regulated PPARγ expression.

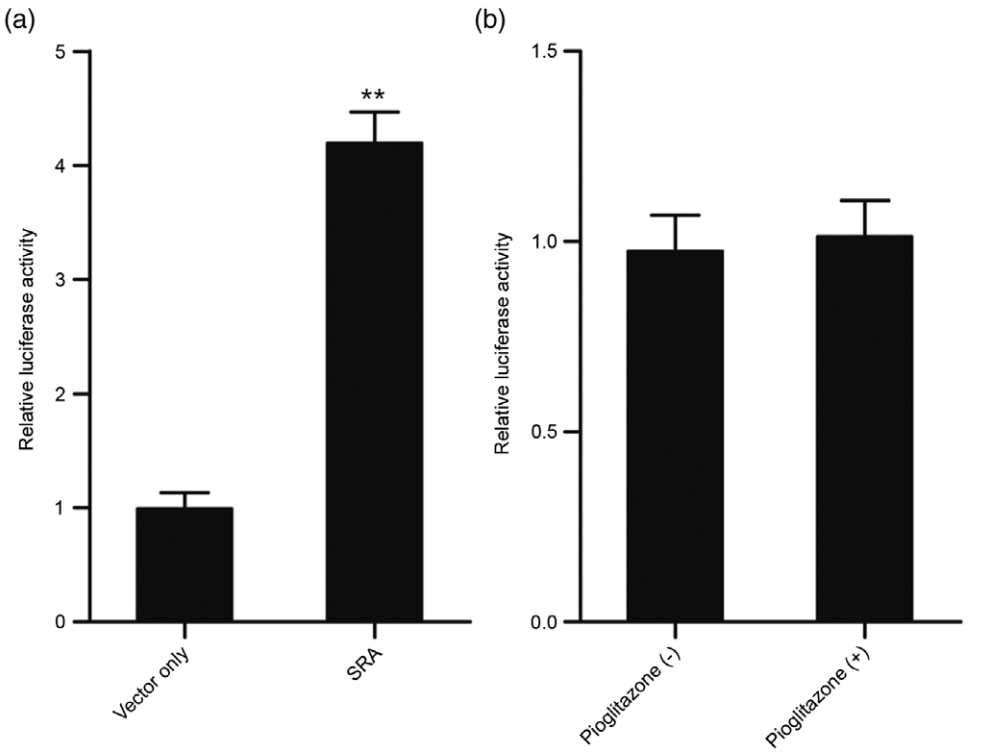
Characteristics of the participants

A total of 54 participants were recruited in the present study, including 32 stroke patients diagnosed with PSD and 22 without PSD. The demographic and clinicopathological characteristics of the participants, including age, sex, hypertension, diabetes mellitus and coronary heart disease status, smoking and drinking history, and the levels of total cholesterol, triglyceride, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were provided in Supplemental Table 1, Supplemental digital content 1, *http://links.lww.com/WNR/A555*. None of the patient characteristics listed in Supplemental Table 1 differed significantly between PSD (+) and PSD (−). As illustrated in Fig. 6, SRA was highly expressed in patients with PSD (+) compared with patients without PSD (−) [$t(\mathrm{df}=52)=5.7$, $P<0.01$], indicating that SRA was associated with PSD.

Discussion

lncRNAs are noncoding transcripts and have been demonstrated as key regulators in various biological processes, including cellular differentiation, embryogenesis and stem cell pluripotency. However, the function of lncRNAs in adipogenesis remains unclear [11]. SRA is an lncRNA that may function as an RNA coactivator for the expression of steroid receptor-dependent genes [24]. It has been demonstrated in our previous work [25] and other investigations [26] that SRA also serves as an RNA coactivator for myogenic differentiation factor and nonsteroid nuclear receptors. An alternative transcript, encoding the

Fig. 4

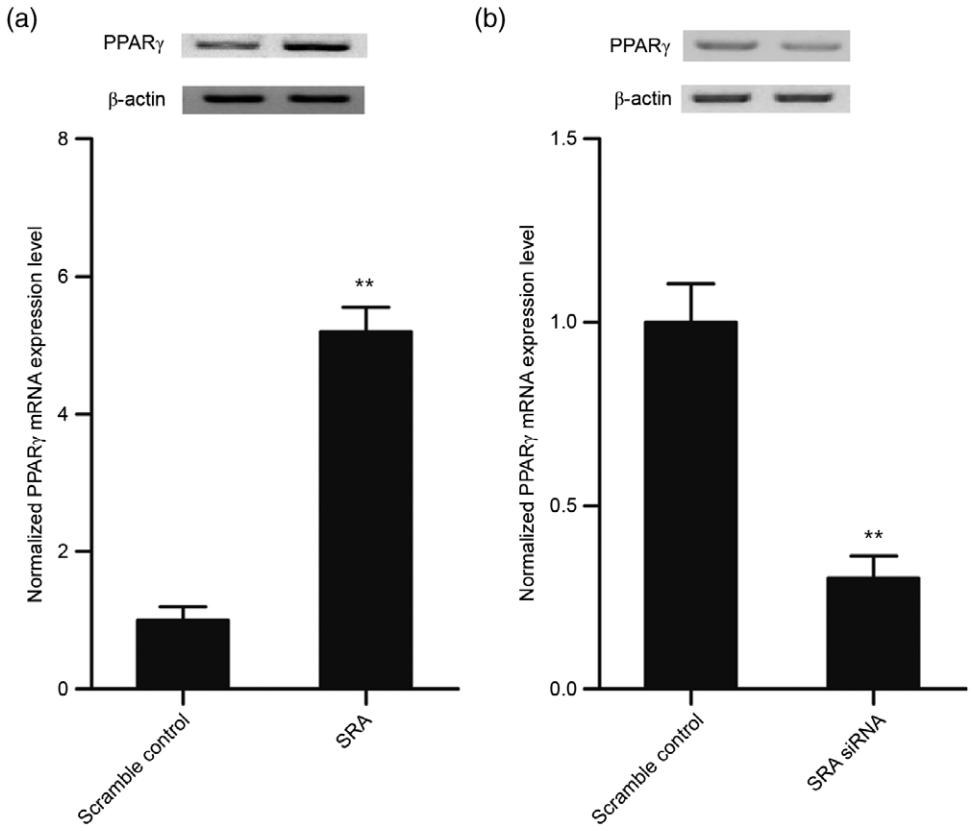


Pioglitazone has no significant effect on SRA expression. (a) Luciferase activity induced by SRA was significantly increased in SRA overexpressing cells compared with control cells. (b) Cells treated with pioglitazone exhibited a similar luciferase activity compared to the control cells. SRA, steroid receptor RNA activator. $^{*}P<0.05$ and $^{**}P<0.01$ vs the control.

steroid receptor RNA activator protein (SRAP), may be also produced by the *Sra1* gene. However, the function of SRAP remains unclear [27]. Although the previous findings indicated that SRA has an important influence on steroidogenesis, myogenesis, cardiomyopathy and tumorigenesis, the current understanding of the in-vivo biology of SRA is limited due to the lack of a loss-of-function mouse model [18,26]. The previous study has reported the association of SRA and PPARc in 3T3-L1 adipocytes *in vitro*, and its function in enhancing the transcriptional activity of PPARc [28]. Another study suggested that adipocyte differentiation can be enhanced by SRAs, the expression of CCAAT/enhancer-binding protein alpha, PPARc and other adipocyte genes can be upregulated by SRAs, and the phosphorylation of Forkhead box protein O1 and Akt in response to insulin and glucose uptake can be increased by SRAs [18]. In the present study, an animal model of PSD was established, which exhibited a significantly altered SRA expression compared with the control. When treated mice with pioglitazone or SRA, lower horizontal and vertical movement scores were discovered at 7 days postinjection. Furthermore, The PSD + LV-SRA and PSD + pioglitazone groups demonstrated higher horizontal and vertical movement scores and sucrose consumption than the PSD + LV-GFP group.

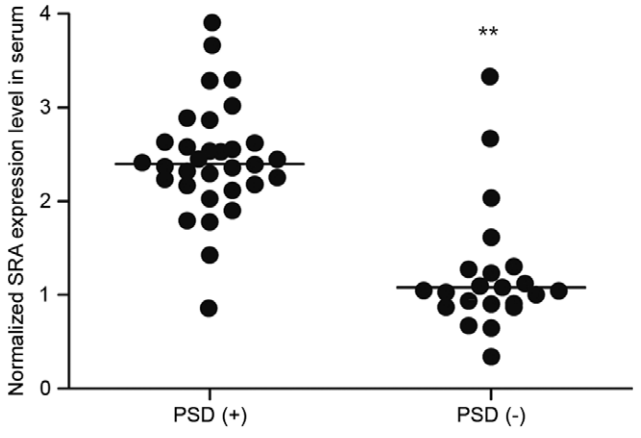
Multiple inflammatory biomarkers, including tumor necrosis factor-α (TNF-α), highly-sensitive C-reactive protein (hs-CRP) and interleukin-6 (IL-6) are generally increased in patients who have bipolar disorde [29]. It has also been indicated that inflammatory cytokines inhibit neurogenesis via the activation of nuclear factor κB (NF-κB), a pathway also associated with hepatic insulin resistance [30]. Inflammatory processes may influence the maintenance and development of depressive episodes [31]. The ability of PPARγ agonists to reduce inflammation and enhance the sensitivity to insulin has been reported [32]. A total of three PPAR isotypes, PPARα, PPARγ and PPARδ, have been identified [33]. PPARγ is a major regulator of adipocyte differentiation and highly expressed in adipose tissue [34]. The expression of PPARγ has also been detected in several other types of tissues such as the lung, ovary, breast, thyroid, prostate and colon [35]. Anti-inflammatory activity was among the first nondiabetic functions associated with PPARγ and its ligands [36]. It has been demonstrated that the PPARγ agonists troglitazone, rosiglitazone and 15d-PGJ2 inhibit the expression of proinflammatory genes, including matrix metalloproteinase 9, scavenger receptor A and nitric oxide synthase in murine macrophages, and IL-6, TNF-α and IL-1β in human monocytes [37]. The

Fig. 5



SRA induces PPARγ production. (a) Overexpression of SRA increased the PPARγ mRNA and protein levels. (b) SRA siRNA decreased the PPARγ mRNA and protein levels. PPARγ, peroxisome proliferator-activated receptor; siRNA, small interfering RNA; SRA, steroid receptor RNA activator. $^{*}P<0.05$ and $^{**}P<0.01$ vs the control.

Fig. 6



SRA is highly expressed in patients with PSD. PSD, poststroke depression; SRA, steroid receptor RNA activator. $^{*}P<0.05$ and $^{**}P<0.01$ vs the control.

anti-inflammatory activities of PPARγ are associated with its ability to antagonize the transcriptional regulation of adaptor protein complex 1, signal transducer and activator of transcription and NF-κB [37]. PPARγ can reduce the function of NF-κB directly through interfering with the transcription-activating capacity of the NF-κB complex, or indirectly, via regulating the proteins suppressing the NF-κB activation or via competing for the proteins that are critical for NF-κB function [38]. In the present study, the effect of SRA on the transcription of PPAR was assessed using RT-qPCR, Western blot and a luciferase reporter assay, and it was demonstrated that SRA transfection could substantially upregulate the transcriptional ability of the PPAR promoter.

Depressive symptoms are proved to be attenuated by pioglitazone. It was demonstrated by Kemp *et al.* [39] that pioglitazone might have an important influence in the therapy of patients with PSD. A significant improvement in insulin resistance and an antidepressant

response were described in another investigation. The improvements in insulin sensitivity and antidepressive effects were also reported among 23 patients with severe depression who received pioglitazone therapy [39]. It was also demonstrated that patients receiving metformin treatment had higher Hamilton depression rating scale scores than the patients who received pioglitazone therapy, independent of the insulin-sensitizing activity of pioglitazone [7]. Various conclusions also have been obtained regarding whether pioglitazone's antidepressive effect is produced by the improvement of insulin resistance. A number of researchers and their teams have reported that immobility time can be reduced by pioglitazone in forced swimming tests of depression in animal models, which cannot be interpreted as a result of insulin-sensitization effects [40].

In conclusion, this is the first report to indicate that lncRNA SRA directly regulates the transcriptional activation of PPARγ and affects PSD through the coactivation of PPAR, which may affect the expression of inflammatory genes and the associated signal transduction. The identification of these roles for SRA in PSD provides a novel insight into the mechanisms underlying PSD physiology and pathophysiology.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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