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Archival Report

(*R,S*)-Ketamine Promotes Striatal Neurogenesis and Sensorimotor Recovery Through Improving Poststroke Depression–Mediated Decrease in Atrial Natriuretic Peptide

Yujing Zhang, Bing Xie, Yin Yuan, Ting Zhou, Ping Xiao, Yuming Wu, You Shang, Shiying Yuan, and Jiancheng Zhang

ABSTRACT

BACKGROUND: Poststroke social isolation could worsen poststroke depression and dampen neurogenesis. (*R,S*)-ketamine has antidepressant and neuroprotective effects; however, its roles and mechanisms in social isolation–mediated depressive-like behaviors and sensorimotor recovery remain unclear.

METHODS: Mice were subjected to transient middle cerebral artery occlusion, and then were pair-housed with ovariectomized female mice or were housed isolated (ISO) starting at 3 days postischemia. ISO mice received 2 weeks of (*R,S*)-ketamine treatment starting at 14 days postischemia. Primary ependymal epithelial cells and choroid plexus epithelial cells were cultured and treated with recombinant human atrial natriuretic peptide (ANP) protein.

RESULTS: The poststroke social isolation model was successfully established using middle cerebral artery occlusion combined with poststroke isolation, as demonstrated by a more prominent depression-like phenotype in ISO mice compared with pair-housed mice. (*R,S*)-ketamine reversed ISO-mediated depressive-like behaviors and increased ANP levels in the atrium. The depression-like phenotype was negatively correlated with ANP levels in both the atrium and plasma. Atrial GLP-1 and GLP-1 receptor signaling was essential to the promoting effects of (*R,S*)-ketamine on the synthesis and secretion of ANP from the atrium in ISO mice. (*R,S*)-ketamine also increased ANP and TGF-β1 levels in the choroid plexus of ISO mice. Recombinant human ANP increased TGF-β1 levels in both the primarily cultured ependymal epithelial cells and choroid plexus epithelial cells. Furthermore, (*R,S*)-ketamine increased TGF-β1 levels in the ischemic hemisphere and promoted striatal neurogenesis and sensorimotor recovery via ANP in ISO mice.

CONCLUSIONS: (*R,S*)-ketamine alleviated poststroke ISO-mediated depressive-like behaviors and thus promoted striatal neurogenesis and sensorimotor recovery via ANP.

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Poststroke depression is the most frequent neuropsychiatric disorder following ischemic stroke, affecting around 33% of stroke survivors (1,2). Poststroke depression could inhibit sensorimotor recovery, contribute to persistent disability, and increase mortality in stroke survivors (1–3). Although randomized controlled trials have demonstrated that antidepressant treatment could improve poststroke depression (1,4), the potential role of antidepressants for improving sensorimotor recovery remains poorly elucidated.

Biological and psychological factors have been shown to have important roles in poststroke depression (5). Stroke survivors are at risk of social isolation because of their limited functional mobility. Social isolation during stroke recovery could result in poorer outcomes in patients, while social networks improve sensorimotor recovery after stroke (6,7). Two weeks of social isolation following stroke, which is more clinically relevant as a target for therapeutic intervention, could lead to poststroke depression and contribute to decreased hippocampal neurogenesis and poor sensorimotor recovery (8,9). Pieces of evidence have shown that antidepressant therapy could foster long-term sensorimotor recovery after stroke (10–12), indicating that poststroke depression may be involved in the deterioration of poststroke neurogenesis and recovery. Therefore, social isolation–mediated inhibitory effects on poststroke striatal neurogenesis and recovery could be due to poststroke depression. However, the exact roles and mechanisms of poststroke depression in striatal neurogenesis and sensorimotor recovery need to be further clarified.

(*R,S*)-ketamine, a racemic mixture of the enantiomers (*R*)-ketamine and (*S*)-ketamine, has rapid and sustained efficacy as an antidepressant by improving core depressive symptoms (13–16). In addition to treating depression, (*R,S*)-ketamine

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could also protect against neuronal injury and neurological deficit in rodents after acute ischemic stroke (17,18). However, the roles and mechanisms of (*R,S*)-ketamine in poststroke social isolation–mediated depression and in decreased striatal neurogenesis in later phases of stroke remain unclear.

Ketamine injection could increase plasma atrial natriuretic peptide (ANP) in normal rats (19,20). Accumulating evidence has suggested that ANP, mainly synthesized in atrial myocytes (21), could exert anxiolytic and antidepression effects in animals and humans (22,23). Patients with depressive disorders exhibit lower plasma ANP levels than healthy individuals (22,24,25). Depressed patients have an attenuated N-terminal proANP response to acute physical stress (26). Exogenous injection of recombinant human ANP (rhANP) could reduce infarct volume (27), indicating that ANP has antidepressant and neuroprotective effects. However, whether ANP could be involved in the effects of (*R,S*)-ketamine on social isolation–mediated depression and striatal neurogenesis is unclear.

This study was to evaluate the roles and mechanisms of (*R,S*)-ketamine in striatal neurogenesis and sensorimotor recovery after poststroke social isolation–mediated depressive-like behaviors. The intrinsic interaction between ANP and depressive-like behaviors and its contributions to the effects of (*R,S*)-ketamine on poststroke striatal neurogenesis and sensorimotor recovery were also investigated.

METHODS AND MATERIALS

A detailed description of the methods can be found in Supplemental Methods and Materials.

Animals

Male C57BL/6J mice (8–10 weeks old) were obtained from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Mice were housed and maintained on a 12:12 light/dark cycle under specific pathogen-free conditions. Food and water were available ad libitum. All procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (publications no. 80-23), revised 1996. All animal protocols were approved by the committee of experimental animals of Tongji Medical College (Permission number: S2404).

Transient Middle Cerebral Artery Occlusion Procedure

Transient focal cerebral ischemia/reperfusion was induced by 1 hour of left middle cerebral artery occlusion (MCAO), as described in our previous studies (9,28–30).

Experimental Design and Treatment

The experiment was performed in three parts (Figure 1; Table S1). In part A, mice were subjected to MCAO or sham operation after housing with an ovariectomized female mouse (pair-housed [PH]) for 7 days in standard plastic cages (27 × 17 × 12.5 cm), and then they were randomly allocated to housing groups either isolated (ISO) or with their original partner starting 3 days after MCAO or sham operation. During pair housing before allocation, 4 pairs of mice were not compatible (indicated by fighting or weight loss) in pair housing and were excluded. (*R,S*)-ketamine hydrochloride (15 mg/kg; Fujian Gutian Pharmaceutical Co. Ltd., Fujian, China) or 0.9% saline was daily injected intraperitoneally for 14 consecutive days starting at 14 days postischemia (dpi). Exendin (9-39) (50 μg/kg; Bachem, Torrance, CA) or 0.9% saline was daily injected intravenously for 14 consecutive days starting at 14 dpi. The injection of exendin (9-39) was performed 15 minutes before (*R,S*)-ketamine injection. The dose of exendin (9-39) was selected according to a previous study (31). Blood glucose was measured using tail-tip blood in 3 random mice of each group to ensure no significant decline in blood glucose.

In part B, mice were daily injected intravenously with rhANP protein (1.0 mg/kg; GL Biochem Ltd, Shanghai, China) or daily injected intraperitoneally with natriuretic peptide receptor A (NPR-A) selective antagonist A71915 (0.5 μg/g; Bachem) for 14 consecutive days starting at 14 dpi. A71915 was administrated 30 minutes before (*R,S*)-ketamine injection. The A71915 dose was chosen as previously reported (32).

In part C, primary ependymal epithelial cells (EECs) and choroid plexus epithelial cells (CPECs) were separated and purified as previously described (33,34). The cells were stimulated with rhANP (10^{-18}, 10^{-14}, or 10^{-8} M) for 6 hours, after which the cells and cell-free culture supernatant were collected.

Behavioral Tests

The depressive-like phenotype was assessed using the forced swim test (FST), tail suspension test (TST), and sucrose preference test (SPT), as previously reported (35,36). FST and TST were assessed at 15 and 28 dpi, and SPT was measured at 16 and 29 dpi.

The sensorimotor functional assessment was performed using the pole test, elevated body swing test (EBST), and rotarod test at 3, 4, 6, and 10 weeks after MCAO, according to our previous studies (28,29).

Enzyme-Linked Immunosorbent Assay

The mice were anesthetized with isoflurane and euthanized at 28 dpi, and blood was collected via cardiac puncture into EDTA-2 K-containing tubes and immediately centrifuged (3000*g*, 5 min at 4 °C) to obtain plasma. The plasma expression level of ANP (MAB3974; R&D Systems, Minneapolis, MN) was measured using commercial enzyme-linked immunosorbent assay kits, as per the manufacturer's instructions.

Protein Analysis

At 28 dpi, the mice were anesthetized with isoflurane and euthanized. The atrium, CP, and ischemic hemisphere were collected. The tissue samples, primary cultured cells, or cell culture supernatants were used for Western blotting, as we previously reported (9,28,29,35).

The paraffin sections of the heart and brain and the primary cultured cells were used for immunofluorescence staining, as we previously reported (9,28,37).

Statistical Analysis

Data were expressed as mean ± SEM. The samples analyzed were normally distributed with equal variances. Multiple comparisons were performed by one-way analysis of variance followed by post hoc Tukey's multiple comparison tests. Two

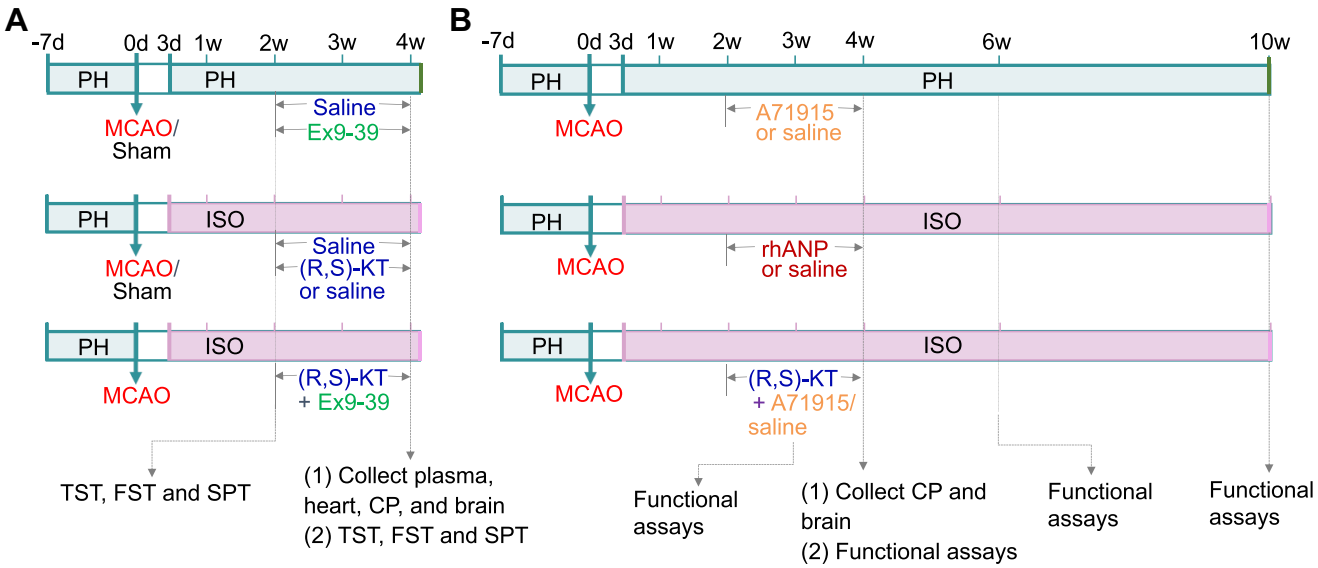

Figure 1. Experimental procedures and treatment schedule. Ischemic stroke was modeled with left MCAO for 60 minutes. **(A)** Adult mice were subjected to MCAO or sham operation after housing with an ovariectomized female mouse (PH) for 7 days and then they were randomly allocated to housing groups, either ISO or with their original partner starting 3 dpi or sham operation. (*R,S*)-ketamine (15 mg/kg, intraperitoneally), exendin (9-39) (intravenously), or 0.9% saline was daily injected for 14 consecutive days starting at 14 dpi. The injection of exendin (9-39) was performed 15 minutes before (*R,S*)-ketamine injection. The TST and FST were performed at 15 and 28 dpi, while the SPT was performed at 16 and 29 dpi to test depression-like phenotype. The sensorimotor functional assessment (pole test, elevated body swing test, and rotarod test) was performed at 3, 4, 6, and 10 weeks after MCAO. The plasma, heart, CP, and brain were collected at 28 dpi. **(B)** Adult male mice were daily injected with rhANP protein (intravenously), natriuretic peptide receptor A antagonist A71915 (intravenously), or 0.9% saline for 14 consecutive days starting at 14 dpi. A71915 was administrated 30 minutes before (*R,S*)-ketamine injection. CP, choroid plexus; dpi, days postischemia; Ex9-39, exendin (9-39); FST, forced swim test; ISO, isolated; MCAO, middle cerebral artery occlusion; PH, pair-housed; rhANP, recombinant human atrial natriuretic peptide; (R,S)-KT, (*R,S*)-ketamine; SPT, sucrose preference test; TST, tail suspension test.

groups were compared by unpaired Student's *t* test. Correlation was analyzed by Pearson correlation. $p < .05$ was considered to be statistically significant. Statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software, San Diego, CA).

RESULTS

(*R,S*)-Ketamine Increased the Synthesis and Secretion of ANP From the Atrium in Poststroke Socially Isolated Mice Through Improving Depressive-like Behaviors

Pair housing and isolation are widely used for evaluating social support and social isolation, respectively (7–9). Two weeks of social isolation following stroke could induce depressive-like behaviors (8,9). We found that social isolation starting at 3 dpi could also induce depressive-like behaviors, as demonstrated by an increased immobility time in FST and TST at 15 and 28 dpi and a decreased sucrose preference in SPT at 16 and 29 dpi in poststroke ISO mice compared with sham-operated mice (Figure 2A–C). Because pair housing is shown to be capable of improving depressive-like behaviors and promoting neurogenesis and sensorimotor recovery (8,9,38), poststroke pair housing in this study was used as a positive control.

Our results further showed that (*R,S*)-ketamine at a dose of 15 mg/kg, but not 10 mg/kg (Figure S1), decreased immobility time in FST and TST at 15 and 28 dpi and increased sucrose preference in SPT at 16 and 29 dpi in ISO mice compared with saline-treated ISO mice (Figure 2A–C; Figure S2), indicating that (*R,S*)-ketamine treatment could improve social isolation–induced depressive-like behaviors.

Because evidence has shown a close relationship between depression and ANP (22,24,25), we next studied the effects of (*R,S*)-ketamine–mediated inhibition of depressive-like behaviors on the synthesis and secretion of ANP from the atrium during stroke recovery. After 4 weeks of poststroke social intervention, poststroke ISO mice had a significantly decreased expression of ANP in both the atrium and plasma, as compared with sham-operated ISO mice or poststroke PH mice (Figure 2D–F). Poststroke social intervention had no significant effects on ANP levels in the atrium and plasma of sham-operated mice (Figure 2D–F). Although ANP is shown to be expressed in the striatum, cerebellum, prefrontal cortex, hypothalamus, hippocampus, amygdala, olfactory bulb, thalamus, and pituitary gland (39,40), we found that ANP levels in the ischemic hemisphere were too low to be detected by Western blotting after removing the CP (Figure S3). The atrium has been demonstrated to represent the major site of ANP synthesis (21). Therefore, our data suggest that poststroke social isolation could reduce atrial synthesis and secretion of ANP.

Intriguingly, (*R,S*)-ketamine significantly enhanced expression of ANP in the atrium and plasma of ISO mice compared with saline-treated ISO mice (Figure 2D–F). Of note, the promoting effects of (*R,S*)-ketamine on depressive-like behaviors

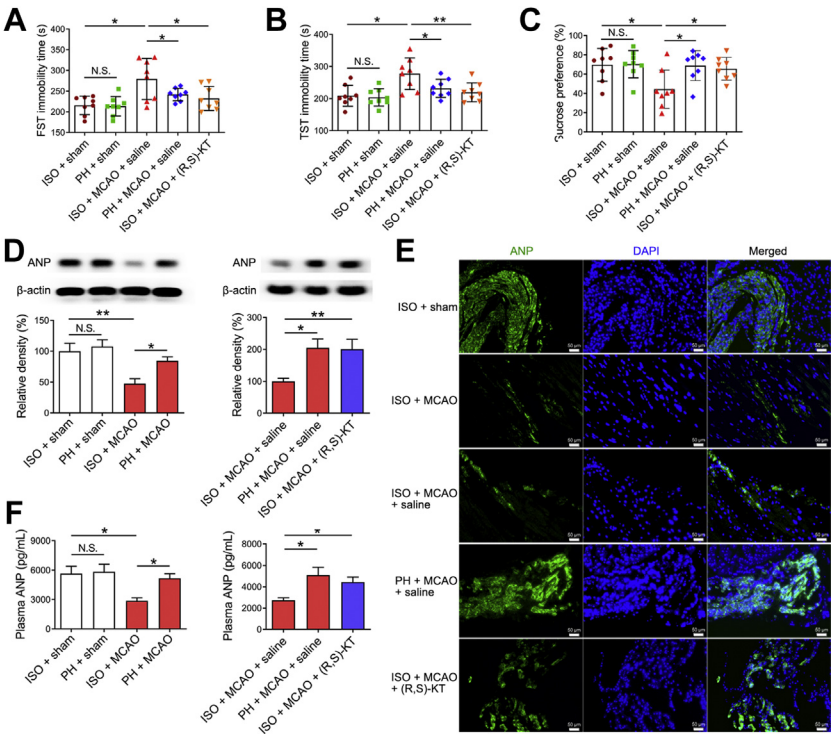

Figure 2. (*R,S*)-ketamine improved poststroke social isolation–induced depressive-like behaviors and decreased synthesis and secretion of ANP from the atrium. **(A)** The FST and **(B)** TST were performed at 28 days postischemia, while **(C)** the sucrose preference test was performed at 29 dpi in each group (n = 8/group) to test depression-like phenotype. The protein expression of ANP in the atrium of each group (n = 6–8/group) at 28 dpi was measured by **(D)** Western blotting and **(E)** immunofluorescence staining. Nuclei were stained with DAPI. Scale bar = 50 μm. **(F)** The plasma levels of ANP in each group (n = 8/group) at 28 dpi were measured by enzyme-linked immunosorbent assays. Data are shown as mean ± SEM; $^{*}p < .05$, $^{**}p < .01$. ANP, atrial natriuretic peptide; dpi, days postischemia; FST, forced swim test; ISO, isolated; MCAO, middle cerebral artery occlusion; N.S., not significant; PH, pair housed; (R,S)-KT, (*R,S*)-ketamine; TST, tail suspension test.

as well as the synthesis and secretion of ANP in ISO mice were comparable with that of PH mice (Figure 2A–C).

Atrial ANP was negatively correlated with immobility time in FST (Figure 3A) (r = −0.517) and immobility time in TST (Figure 3B) (r = −0.423). There was a negative correlation between plasma ANP and immobility time in TST (Figure 3E) (r = −0.567). Both atrial and plasma ANP were positively correlated with sucrose preference ratio in SPT (Figure 3C, F) (r = 0.442 and r = 0.594, respectively) among the three groups.

(*R,S*)-Ketamine Promoted the Atrial Synthesis of ANP via GLP-1 and GLP-1 Receptor Signaling

In light of findings suggesting that GLP-1 receptor (GLP-1R) could promote ANP secretion from the atrium after being activated by liraglutide or native GLP-1 (41), we sought to investigate whether (*R,S*)-ketamine could promote atrial synthesis of ANP through GLP-1 and GLP-1R signaling. As expected, the levels of GLP-1 and GLP-1R in atria of poststroke ISO mice were lower than those in the sham-operated ISO mice or poststroke PH mice (Figure 4A, C). The promoting effects of pair housing on ANP levels in the atrium were inhibited by antagonizing GLP-1R with exendin (9-39) (Figure 4E). Poststroke social intervention had no significant effects on GLP-1 or GLP-1R levels in the atrium of sham-operated mice (Figure 4A, C). However, one single dose of (*R,S*)-ketamine had no significant effects on atrial GLP-1 levels in ISO mice 24 hours after (*R,S*)-ketamine treatment (Figure S4).

Consistently, (*R,S*)-ketamine increased the levels of GLP-1 and GLP-1R in the atrium of poststroke ISO mice (Figure 4B, D). The promoting effects of (*R,S*)-ketamine on ANP expression in the atrium were also markedly compromised by exendin (9-39) (Figure 4F), indicating that (*R,S*)-ketamine could promote atrial synthesis of ANP through GLP-1 and GLP-1R signaling in poststroke ISO mice.

(*R,S*)-Ketamine Increased the Synthesis and Secretion of TGF-β1 From the CP via ANP and NPR-A Signaling

ANP can regulate cerebrospinal fluid production and intracranial pressure by combining with NPR-A expressed in EECs and CPECs (42). We found that high levels of ANP were observed in EECs and CPECs of the lateral ventricle, third ventricle, fourth ventricle, and aqueduct of midbrain in (*R,S*)-ketamine–treated ISO mice at 28 dpi (Figure S5A). Furthermore, the results of coimmunoprecipitation and immunofluorescence performed in (*R,S*)-ketamine–treated ISO mice at 28 dpi identified that ANP could bind to NPR-A in EECs and CPECs (Figure S5B, C).

Similarly, (*R,S*)-ketamine increased ANP expression in the CP of ISO mice at 28 dpi (Figure 4G). The stimulative effects of pair housing on the expression of ANP in the CP were attenuated by exendin (9-39) at 28 dpi (Figure 4H). Moreover, exendin (9-39) also abrogated the promoting effects of (*R,S*)-ketamine on ANP expression in the CP of ISO mice at 28 dpi (Figure 4I). Our data suggested that (*R,S*)-ketamine could promote the circulation of cardiac-derived ANP into the CP.

EECs and CPECs are important sources of various neurotrophic factors and growth factors (43–45). The synthesis and

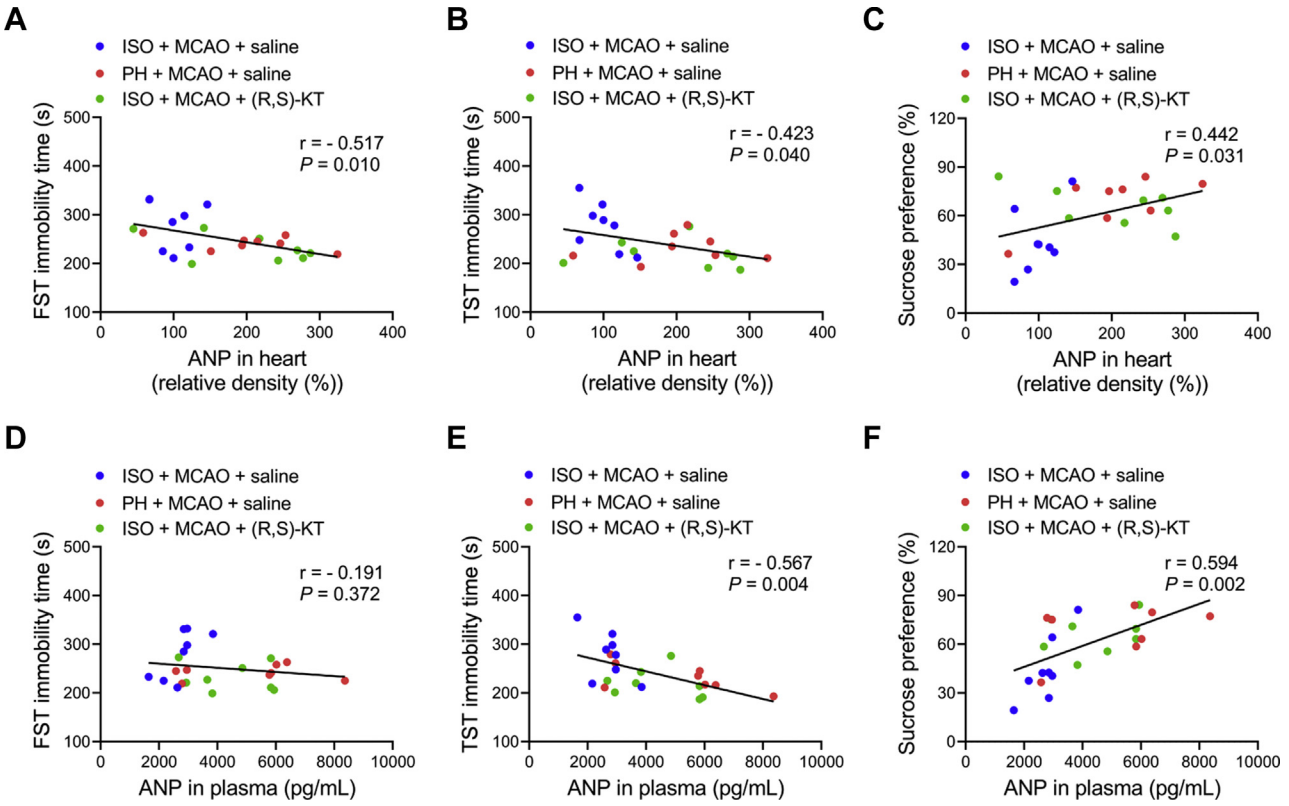

Figure 3. Pearson correlation analysis of the depression-like phenotype and ANP levels in the atrium and plasma at 28 days postischemia. There was a negative correlation between the ANP levels in the atrium and **(A)** FST immobility time ($r = -0.517$, $p = .010$) and **(B)** TST immobility time ($r = -0.423$, $p = .040$). **(C)** The ANP levels in the atrium were positively correlated with sucrose preference in the sucrose preference test ($r = 0.442$, $p = .031$). **(D)** No significant correlation between the ANP levels in the plasma and FST immobility time was detected ($r = -0.191$, $p = .372$). **(E)** The ANP levels in the plasma negatively correlated with TST immobility time ($r = -0.567$, $p = .004$) and **(F)** positively correlated with the sucrose preference in the sucrose preference test ($r = 0.594$, $p = .002$). ANP, atrial natriuretic peptide; FST, forced swim test; ISO, isolated; MCAO, middle cerebral artery occlusion; PH, pair housed; (R,S)-KT, (*R,S*)-ketamine; TST, tail suspension test.

secretion of TGF-β1 by EECs and CPECs in the human brain are markedly decreased in patients with major depressive disorder (46). Furthermore, ANP could stimulate TGF-β1 expression in vitro (47). We found that TGF-β1 levels were lower in the CP of ISO mice compared with PH mice at 28 dpi (Figure 5A). (*R,S*)-ketamine also increased TGF-β1 levels in ISO mice compared with those in saline-treated ISO mice (Figure 5B). As expected, rhANP treatment increased TGF-β1 levels in ISO mice, while blocking NPR-A with A71915 decreased TGF-β1 levels in PH mice at 28 dpi (Figure 5C; Figure S6). However, one single dose of (*R,S*)-ketamine had no significant effects on TGF-β1 levels in the CP of ISO mice 24 hours after (*R,S*)-ketamine treatment (Figure S7A).

Subsequently, we found that rhANP at a dose of 10^{-8} M increased TGF-β1 levels in the cell lysate and cell-free culture supernatant of primarily cultured EECs and CPECs 6 hours after stimulation with rhANP (Figure 5D–G). Additionally, in the cultured CPECs, rhANP at smaller doses (10^{-18} and 10^{-14} M) could also increase TGF-β1 levels in the cell lysate and cell-free culture supernatant (Figure 5F, G). Our data indicated that (*R,S*)-ketamine could increase the synthesis and secretion of TGF-β1 via the ANP/NPR-A system in the CP.

(*R,S*)-Ketamine Improved Poststroke Social Isolation–Mediated Decrease in Striatal Neurogenesis and Sensorimotor Recovery via ANP

Because the neurotrophic factors and growth factors secreted from the CP could be released into brain parenchyma through the cerebrospinal fluid and exert important effects (43–45), we next examined whether the increased TGF-β1 levels in the CP induced by ANP are accompanied by increased TGF-β1 levels in the brain parenchyma. As expected, TGF-β1 levels in the ischemic hemisphere were decreased in ISO mice, as compared with those in PH mice, rhANP-treated ISO mice, or (*R,S*)-ketamine–treated ISO mice at 28 dpi (Figure 6A–C). In contrast, A71915 decreased TGF-β1 levels in the ischemic hemisphere in PH mice at 28 dpi (Figure 6B). However, one single dose of (*R,S*)-ketamine had no significant effects on TGF-β1 levels in the ischemic hemisphere of ISO mice 24 hours after (*R,S*)-ketamine treatment (Figure S7B).

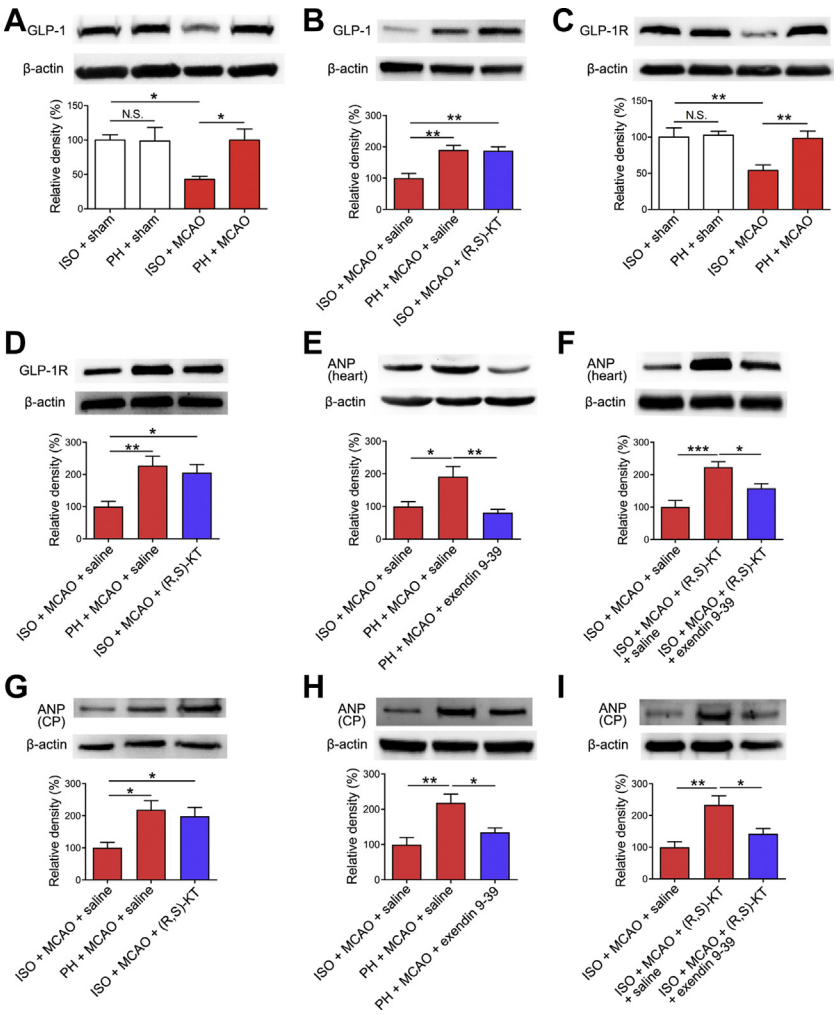

Figure 4. (*R,S*)-ketamine improved poststroke social isolation–induced decrease in the atrial synthesis of ANP from the atrium via GLP-1 and GLP-1R signaling. **(A, B)** The protein expression of GLP-1 in the atrial of each group at 28 dpi was measured by Western blotting. **(C, D)** Western blotting analysis of the protein levels of GLP-1R in the atrial of each group at 28 dpi. **(E, F)** The protein levels of ANP in the atrial of each group at 28 dpi were measured. **(G–I)** Western blotting analysis of the protein levels of ANP in the CP of each group at 28 dpi. Data are shown as mean ± SEM, n = 6–8/group; *$p < .05$, **$p < .01$. ANP, atrial natriuretic peptide; CP, choroid plexus; dpi, days postischemia; GLP-1R, GLP-1 receptor; ISO, isolated; MCAO, middle cerebral artery occlusion; N.S., not significant; PH, pair-housed; (R,S)-KT, (*R,S*)-ketamine.

Increased TGF-β1 levels in the ischemic hemispheres can promote striatal neurogenesis in the striatum and enhance sensorimotor recovery in mice after ischemic stroke (48). We hypothesized that ANP may promote striatal neurogenesis and sensorimotor recovery by increasing TGF-β1 levels in the ischemic hemispheres. As expected, rhANP increased the number of $BrdU^+/DCX^+$ cells in the ischemic striatum of ISO mice at 28 dpi (Figure 6D, E). In contrast, the number of $BrdU^+/DCX^+$ cells in the ischemic striatum was decreased in PH mice after the blockade of NPR-A with A71915 (Figure 6D, E). No significant differences were detected in the number of $BrdU^+/DCX^+$ cells in the ischemic subventricular zone (SVZ) of ISO mice among the four groups at 28 dpi (Figure 6D, E). The assessment of sensorimotor function further showed that rhANP decreased the times to turn and to reach the floor in the pole test, reduced behavioral asymmetries in EBST, and increased the times until drop in the rotarod test in ISO mice at 21 and 28 dpi (Figure 6F). However, the sensorimotor deficits in the pole test, EBST, and rotarod test were worse in PH mice after the blockade of NPR-A with A71915 at 21 and 28 dpi (Figure 6F).

Finally, we sought to determine whether (*R,S*)-ketamine could promote striatal neurogenesis and sensorimotor recovery via ANP. We found that (*R,S*)-ketamine increased the number of $BrdU^+/DCX^+$ cells in the ischemic striatum of ISO mice at 28 dpi (Figure 7A, B). No significant differences were detected in the number of $BrdU^+/DCX^+$ cells in the ischemic SVZ of ISO mice among the three groups at 28 dpi (Figure 7A, B). The sensorimotor deficits in the pole test, EBST, and rotarod test were better in (*R,S*)-ketamine–treated ISO mice at 21 and 28 dpi (Figure 7C). The improving effects of (*R,S*)-ketamine on the striatal neurogenesis and sensorimotor deficits in the pole test, EBST, and rotarod test were markedly compromised in ISO mice after blockade of NPR-A with A71915 (Figure 7A–C).

DISCUSSION

Poststroke depression can be effectively induced in mice via a variety of approaches, for example, photothrombosis-induced ischemia in the left anterior cortical layers in combination with social isolation (49) and endothelin-1 microinjection in the left medial prefrontal cortex to generate a unilateral focal ischemic lesion (50). Both of the above two methods for establishing a model of poststroke depression could avoid sensorimotor impairment caused by massive cortical and striatal ischemia but

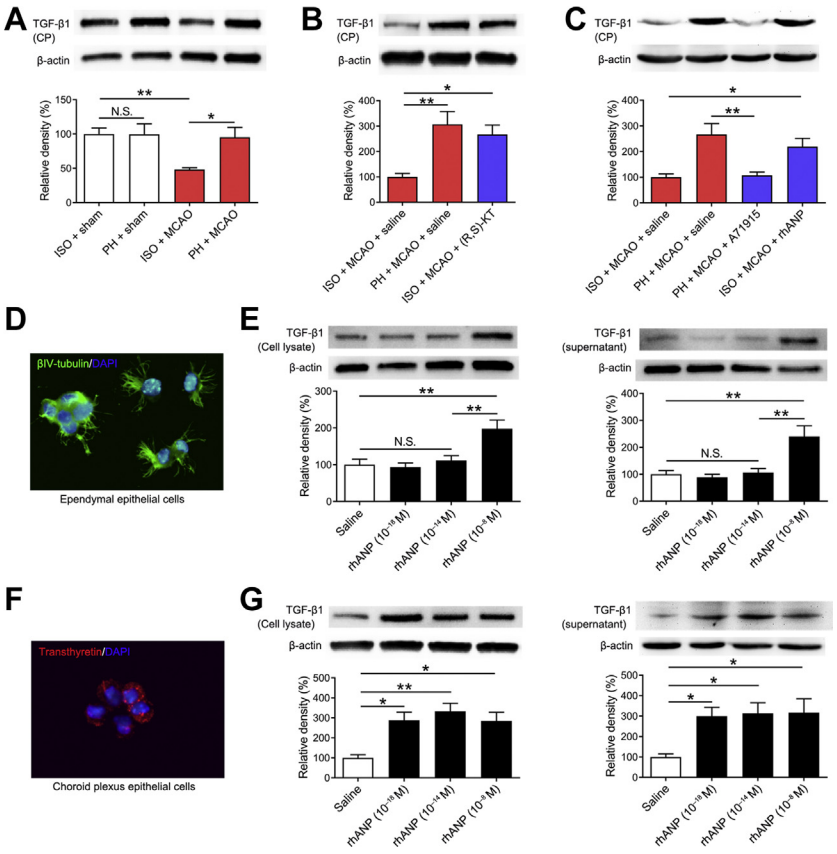

Figure 5. (*R,S*)-ketamine promoted the synthesis and secretion of TGF-β1 from the CP via ANP. **(A–C)** Western blotting analysis of the protein levels of TGF-β1 in the CP of each group at 28 days post-ischemia. **(D)** Immunofluorescence staining of βIV-tubulin in the primarily cultured ependymal epithelial cells. Nuclei were stained with DAPI. Scale bar = 20 μm. **(E)** The protein expression of TGF-β1 in the cell lysate and cell-free culture supernatant of ependymal epithelial cells after treatment with rhANP at doses of 10^{-18}, 10^{-14}, or 10^{-8} M was measured by Western blotting. **(F)** Immunofluorescence staining of transthyretin in the primarily cultured CP epithelial cells. Nuclei were stained with DAPI. Scale bar = 20 μm. **(G)** Western blotting analysis of the protein levels of TGF-β1 in the cell lysate and cell-free culture supernatant of CP epithelial cells after the treatment with rhANP (10^{-18}, 10^{-14}, or 10^{-8} M). Data are shown as mean ± SEM, n = 6–8/group. Cell culture experiments were repeated three times; $^{*}p < .05$, $^{**}p < .01$. ANP, atrial natriuretic peptide; CP, choroid plexus; ISO, isolated; MCAO, middle cerebral artery occlusion; N.S., not significant; PH, pair-housed; rhANP, recombinant human ANP; (R,S)-KT, (*R,S*)-ketamine.

could be limited to the poor clinical relevance as a target for therapeutic intervention. Previous studies have shown that left hemisphere lesions tend to be associated with a higher incidence of depression, and the location of subcortical lesions had a greater influence on depression (51–53). In this study, transient left MCAO was performed to cause left-hemispheric cortical and subcortical lesions. Our finding has broadened our understanding that depressive-like behaviors could be caused by either social isolation immediately after MCAO (8,9) or social isolation starting 3 days after MCAO. Considering the published literature showing that infarct size at the acute phase could influence poststroke neurogenesis and sensorimotor recovery (54–56) and immediate poststroke isolation could lead to a significant increase in infarct size and mortality at 3 dpi, while poststroke isolation starting 3 days after MCAO has no obvious effects on the infarct size at 8 dpi (38), the social isolation in our study was thus begun 3 days after MCAO (Figure S8). A recent study showed that 8 weeks of social isolation could induce depression-like phenotype in mice regardless of sex (57). However, we did not observe significant inductive effects of 4 weeks of social isolation on depression-like phenotype in male mice, which may be due to unaltered neuronal remodeling and downstream neuroplasticity in the hippocampal CA1 after a relatively short period of social isolation (57).

Cell proliferation in the central nervous system occurs throughout adulthood in two main neurogenic niches that contain neural stem cells: the SVZ of the lateral ventricles and the dentate gyrus subgranular zone (58,59). The SVZ is one of the most important regions for neurogenesis in the adult mammalian brain and harbors a large population of neural stem/precursor cells (59–61). Ventricular SVZ-derived neuroblasts use the vascular scaffold to assist their migration toward the poststroke peri-infarct cortex and striatum and differentiate into mature neurons (62,63), which are critical for neuroregeneration and spontaneous sensorimotor recovery (64). According to our previous studies (28–30), adult neurogenesis in the striatum was detected with BrdU and DCX double marker. In addition to its previously demonstrated neuroregenerative properties on acute ischemic stroke (17,18), our data found that (*R,S*)-ketamine could also promote striatal neurogenesis in the ischemic hemisphere and enhance sensorimotor recovery in ISO mice via ANP. The ANP antagonist A71915 abrogated the promoting effects of (*R,S*)-ketamine on poststroke striatal neurogenesis and sensorimotor recovery in ISO mice. However, rhANP or (*R,S*)-ketamine did not increase the number of $BrdU^{+}/DCX^{+}$ cells in the ischemic SVZ of poststroke ISO mice at 28 dpi, indicating that the promoting effects of rhANP or (*R,S*)-ketamine on poststroke neurogenesis and sensorimotor recovery could be due to increasing the migration of newborn neuroblasts from the SVZ to the ischemic striatum, which is essential to the spontaneous recovery of sensorimotor functions within the first few months (64,65).

ANP is a heart-derived secretory peptide. Although it has been shown that ANP could be expressed in the striatum,

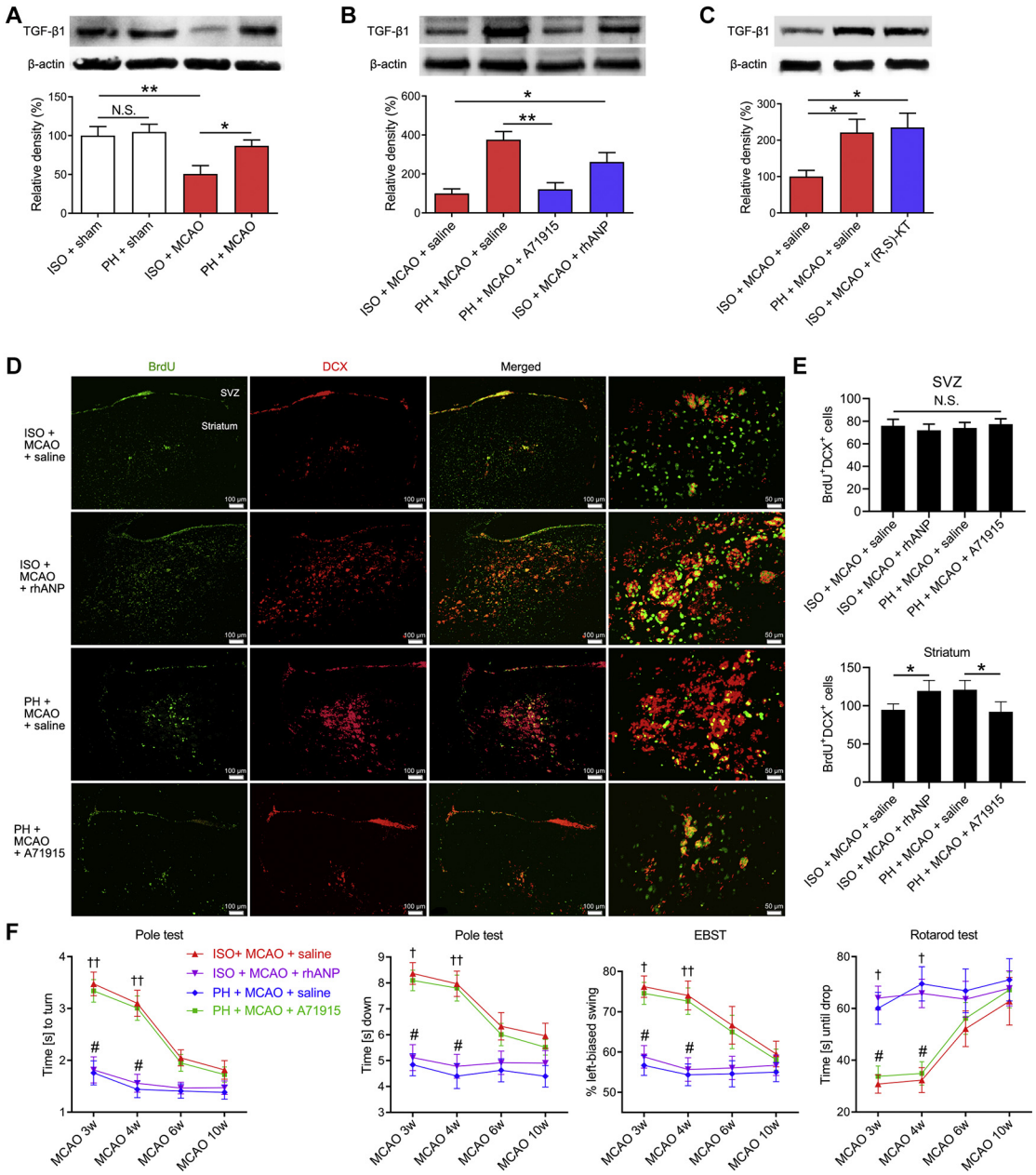

Figure 6. TGF-β1 might be involved in the promoting effects of ANP on poststroke striatal neurogenesis and sensorimotor recovery. **(A–C)** Western blotting analysis of the protein levels of TGF-β1 in the ischemic hemisphere of each group (n = 6–8/group) at 28 days postischemia. **(D)** Representative images of cells double labeled for BrdU and DCX in the ischemic striatum of each group (n = 6/group). Scale bar = 100 μm (the first three columns) or 50 μm (the last column). **(E)** Quantitative determination of BrdU and DCX double-labeled cells in the ischemic striatum of each group. **(F)** The sensorimotor functional assessment was performed at 3, 4, 6, and 10 weeks after ischemia, including the pole test, EBST, and rotarod test (n = 12/group). Data are shown as mean ± SEM; $^{*}p < .05$, $^{**}p < .01$; $^{\#}p < .05$, significantly different between poststroke ISO mice treated with rhANP and poststroke ISO mice treated with 0.9% saline; $^{\dagger}p < .05$ and $^{\dagger\dagger}p < .01$, significantly different between poststroke PH mice treated with A71915 and poststroke PH mice treated with 0.9% saline. EBST, elevated body swing test; ISO, isolated; MCAO, middle cerebral artery occlusion; N.S., not significant; PH, pair-housed; rhANP, recombinant human atrial natriuretic peptide; (R,S)-KT, (*R,S*)-ketamine; SVZ, subventricular zone.

cerebellum, prefrontal cortex, hypothalamus, hippocampus, amygdala, olfactory bulb, thalamus, and pituitary gland (39,40), the expression of ANP was too low to be detected by Western blotting in the ischemic hemisphere of each group after removing the CP in our study, and the stimulative effects of (*R,S*)-ketamine on ANP levels in the CP were markedly compromised by treatment with GLP-1R antagonist exendin (9-39). Therefore, increased expression of ANP in the CP was due to the increase of circulating ANP secreted from the atrium. Studies have shown that EECs and CPECs can secrete

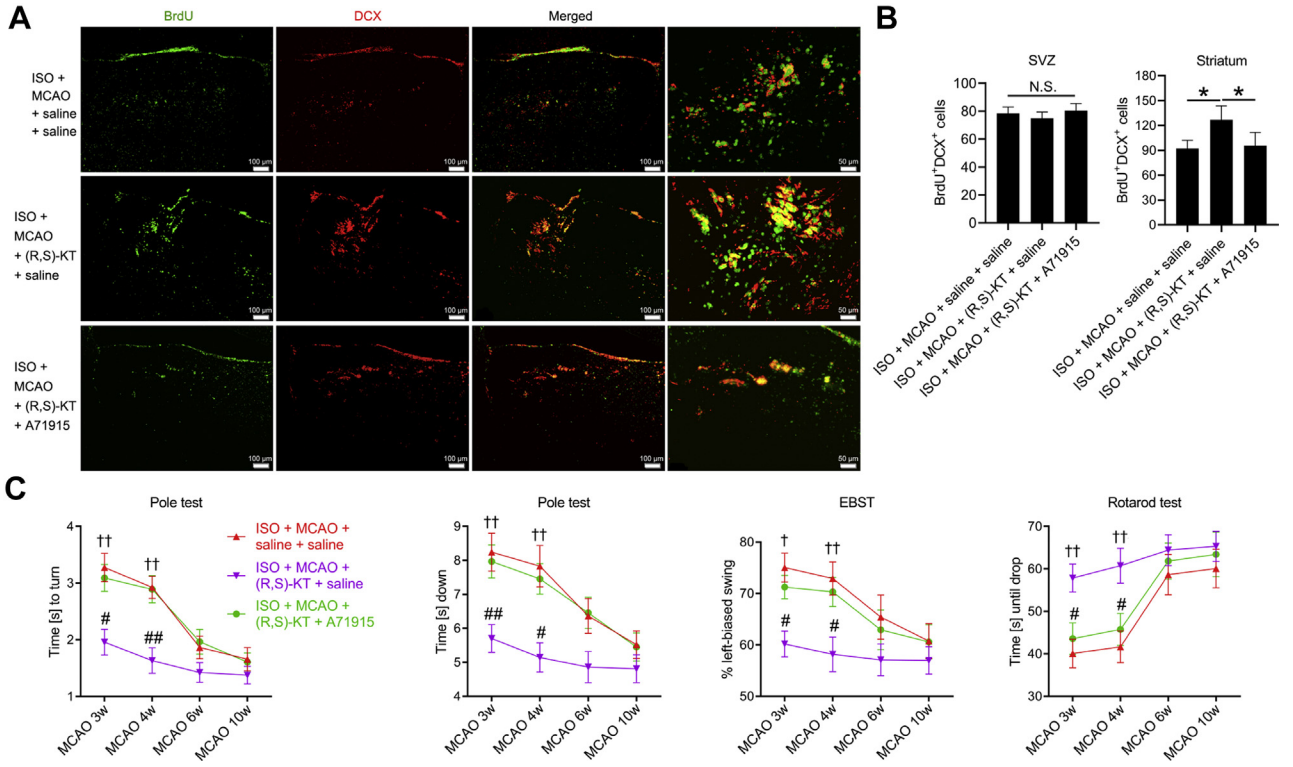

Figure 7. (*R,S*)-ketamine improved poststroke social isolation–induced decrease in striatal neurogenesis and sensorimotor recovery via atrial natriuretic peptide. **(A)** Immunofluorescence imaging for $BrdU^+$ cells coexpressing DCX (red) in the ischemic striatum of each group (n = 6/group) at 28 days poststischemia. Scale bar = 100 μm (the first three columns) or 50 μm (the last column). **(B)** Quantitative comparison of BrdU/DCX double-labeled cells in the ischemic striatum of each group at 28 days postischemia. **(C)** The sensorimotor functional assessment was performed at 3, 4, 6, and 10 weeks after ischemia, including the pole test, EBST, and rotarod test (n = 12/group). Data are shown as mean ± SEM; $^*p < .05$; $^\dagger p < .05$ and $^{\dagger\dagger}p < .01$, significantly different between poststroke ISO mice treated with (R,S)-KT plus 0.9% saline and poststroke ISO mice treated with 0.9% saline; $^\#p < .05$ and $^{\#\#} < .01$, significantly different between poststroke ISO mice treated with (R,S)-KT plus A71915 and poststroke ISO mice treated with (R,S)-KT plus 0.9% saline. EBST, elevated body swing test; ISO, isolated; MCAO, middle cerebral artery occlusion; N.S., not significant; (R,S)-KT, (*R,S*)-ketamine; SVZ, subventricular zone.

various neurotrophic factors and growth factors, which could be released into the cerebrospinal fluid and exert effects during brain development and in various CNS disorders, including traumatic brain injury and ischemia (43–45). ANP is shown to be able to reduce cerebrospinal fluid production and lower intracranial pressure through binding to its main receptors NPR-A in EECs and CPECs (42). EECs and CPECs in the human brain are shown to be capable of producing and secreting TGF-β, and the synthesis and secretion of TGF-β are markedly reduced in patients with major depressive disorder (46). In addition, ANP could stimulate the expression of TGF-β in cultured murine mesangial cells (47). Our in vivo and in vitro data revealed a novel mechanism by which ANP promoted the synthesis and secretion of TGF-β1 in the CP after ischemic stroke. Our previous study showed that intracerebroventricular or intranasal administration of recombinant TGF-β1 could restore reduced hippocampal neurogenesis and memory function induced by social isolation (9). The elevation of TGF-β levels or overexpression of TGF-β type I receptor in the ischemic hemispheres has also been confirmed to promote neurogenesis in the striatum and enhance sensorimotor recovery in rodents after ischemic stroke (48,66). In our study, (*R,S*)-ketamine increased TGF-β1 protein expression in the ischemic hemisphere of ISO mice, indicating that (*R,S*)-ketamine might promote striatal neurogenesis and sensorimotor recovery through ANP-mediated increase in the secretion of TGF-β1 from the CP. One study has demonstrated that one single dose of (*R*)-ketamine could alleviate depression-like behaviors in a mouse stress model of depression via cerebral TGF-β1 (67). However, our data found that one single dose of (*R,S*)-ketamine could rapidly improve depressive-like behaviors without significant effects on TGF-β1 levels in the ischemic hemisphere of ISO mice, indicating a different mechanism mediating the rapid antidepressive-like actions of (*R,S*)-ketamine in the social isolation–induced depression model. In addition, evidence has shown that 7 weeks of social isolation following stroke could decrease the levels of BDNF (brain-derived neurotrophic factor) in the ischemic cerebral hemisphere (8). Decreased BDNF levels have been shown to be critical for the pathogenesis of depression (68,69) and play important roles in poststroke neurogenesis and recovery after social isolation (38). Therefore, BDNF may have important roles in the improving effects of (*R,S*)-ketamine on social isolation–induced depressive-like behaviors and compromised neurogenesis in the striatum after social isolation, which needs to be further investigated.

In conclusion, our findings showed that (*R,S*)-ketamine treatment during stroke recovery could improve poststroke social isolation–induced depressive-like behaviors and thus subsequently increased the secretion of ANP from the atrium via GLP-1 and GLP-1R signaling. Increase in the circulating ANP increased the secretion of TGF-β1 from the CP to the ischemic hemisphere and promoted striatal neurogenesis and sensorimotor recovery in mice with depression (Figure S9). To our knowledge, this is the first study to investigate the potential roles and mechanisms of (*R,S*)-ketamine on social isolation–induced compromise of striatal neurogenesis and sensorimotor recovery. Our study could provide potential new therapeutic strategies for neurorehabilitation therapy for stroke patients with depression.

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ARTICLE INFORMATION

From the Department of Critical Care Medicine and the Institute of Anesthesia and Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

YZ, BX, YY, and TZ contributed equally to this work.

Address correspondence to Jiancheng Zhang, M.D., Ph.D., at zhjcheng1@126.com, or Shiying Yuan, M.D., Ph.D., at yuan_shiying@163.com.

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