Iptakalim confers an antidepressant effect in a chronic mild stress model of depression through regulating neuro-inflammation and neurogenesis

Ming Lu, Jing-Zhe Yang, Fan Geng, Jian-Hua Ding and Gang Hu
Jiangsu Key Laboratory of Neurodegeneration, Department of Pharmacology, Nanjing Medical University, Nanjing, Jiangsu, China

Abstract
Depression is a serious mental disorder in the world, but the underlying mechanisms remain unclear and the effective cures are scarce. Iptakalim (Ipt), an ATP-sensitive potassium (K-ATP) channel opener that can cross the blood-brain barrier freely, has been demonstrated to inhibit neuro-inflammation and enhance adult hippocampal neurogenesis. But it is unknown whether Ipt is beneficial to therapy of depression by modulating neurogenesis and neuro-inflammation. This study aimed to determine the potential antidepressant efficacy of Ipt in a chronic mild stress (CMS) mouse model of depression. We showed that treatment with Ipt (10 mg/kg/day, i.p) for 4 wk restored the decrease of sucrose preference and shortened the immobile time in forced swimming tests (FST) and tail suspension tests (TST) in CMS model mice. We further found that Ipt reversed the CMS-induced reduction of the adult hippocampal neurogenesis and improved cerebral insulin signalling in the CMS mice. Furthermore, Ipt negatively regulated nod-like receptor protein 3 (NLRP3) expression and, in turn, inhibited microglia-mediated neuro-inflammation by suppressing the activation of NLRP3-inflammasome/caspase-1/interleukin 1β axis in the hippocampus of CMS mice. Taken together, our findings demonstrate that Ipt plays a potential antidepressant role in CMS model mice through regulating neuro-inflammation and neurogenesis, which will provide potential for Ipt in terms of opening up novel therapeutic avenues for depression.

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Introduction
Depression is a serious mental disorder with a very high prevalence in the general population, and it will be the second leading illness in the world by 2020 (Masi and Brovedani, 2011). Although there are existing antidepressants that target serotonin and/or norepinephrine systems, not all depressed patients respond to these drugs and only some of drug-responsive patients achieve full remission of symptoms (Cassano and Fava, 2004; Lu et al., 2006). The in-depth understanding of further mechanisms underlying the disease is thus required for developing novel remedies for depression. A major current finding is that stress or depression decreases hippocampal neurogenesis whereas chronic treatment with antidepressant drugs increases hippocampal neurogenesis (Kheirbek et al., 2012). Therefore, impairment of adult hippocampal neurogenesis has been proposed as a crucial biological and cellular pathogenesis of depression. Enhancing adult hippocampal neurogenesis is thus considered to be an effective strategy for the development of novel antidepressants.

In addition to the decreased neurogenesis, epidemiological studies show that patients with major depression are associated with a higher risk of metabolic syndrome and activated inflammatory pathways (Dunbar et al., 2008). Increasing evidence suggests that inflammation contributes to the reduction of adult neurogenesis and stress responses (Dutta et al., 2013). Even though antidepressant drugs such as fluoxetine and paroxetine can reduce the inflammatory cytokines in the brain and blood (Hwang et al., 2008), little is known about the key trigger of inflammation and neurogenesis in the pathophysiology of depression. Interleukin-1 (IL-1) is a central regulator of stress responses and IL-1β is associated with modulation of neuro-endocrine systems, particularly the hypothalamic pituitary-adrenal axis (HPA) (Goshen and Yirmiya, 2009). IL-1β has been shown to inhibit neurogenesis in adult hippocampal progenitor cells in response to stress challenge (Kuzumaki et al., 2010). Moreover, recent studies indicate that IL-1β gene is associated with resistance to depression treatment (Baune et al., 2010). IL-1β in the brain is mostly produced by a protein complex called NLRP3 (Nucleotide-binding oligomerization domain (NOD)-containing protein-like receptors) inflammasome (Schröder et al., 2010). NLRP3 inflammasome functions...
as a sensor for metabolic stress and plays an important role in various metabolic diseases, including diabetes, Alzheimer’s disease and atherosclerosis (Schröder and Tschopp, 2010). Therefore, inflammasome may be a promising target for the treatment of these diseases. Although inflammation, especially that associated with IL-1β, is related to the pathophysiology of depression, it remains unknown whether NLRP3 inflammasome is associated with the pathogenesis of depression.

ATP-sensitive potassium (K-ATP) channels, extensively expressed in most tissues, provide a unique link between the metabolic state and electrical activity in response to cytoplasmic nucleotide levels and are identified as a critical component in maintaining the body’s homeostasis during stress (Nichols, 2006). Iptakalim (Ipt) is a novel K-ATP channel opener, which is a lipophilic para-amino compound with low molecular weight and crosses the blood–brain barrier freely (Zhou et al., 2007). Our previous studies have demonstrated that Ipt not only exerts a protective effect against neuronal necrosis and apoptosis in the early stages of acute cerebral ischemia, but also plays a key role in regulating neuro-inflammation. Furthermore, we demonstrated that Ipt potentiates the adult mouse hippocampal neurogenesis via opening the Kir6.1-composed K-ATP channels (Yang et al., 2012). Therefore, we suppose that Ipt may exert its therapeutic effect on depression through inhibiting NLRP3 inflammasome and, in turn, maintaining adult neurogenesis under stress. The chronic mild stress (CMS) paradigm is widely considered as a model of depression with predictive and etiological validity. In this study, we established a CMS model to investigate the potential antidepressant-like effects of Ipt on depression. Our findings indicate that Ipt improves depressive symptoms via inhibiting inflammation and enhancing neurogenesis under stress. This provides a potential for Ipt in terms of opening up novel therapeutic avenues for depression.

Materials and method

Animals and drugs

The experiments were carried out with eight-week old C57BL/6J male mice in the early stages of unpredictable chronic mild stress, obtained from the Animal Resource Centre of the Faculty of Medicine, Nanjing Medical University. Mice were maintained before and during unpredictable chronic mild stress states in small individual cages (30 cm × 19 cm × 13 cm), with free access to standard chow and water in a room with an ambient temperature of 24 ± 1 °C and a 12:12 h light/dark cycle. A non-stressed group (n = 15), housed at 4–5 mice per cage (65 cm × 25 cm × 15 cm), was kept in the same room under identical controlled conditions, but not submitted to the stress protocol. All procedures were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Iptakalim hydrochloride (Ipt), with a purity of 99.36%, was synthesized and provided by the Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences of China. Fluoxetine (Flx), with a purity of 99.9%, was obtained from Eli Lilly, Indianapolis, Indiana. Both Ipt and Flx were dissolved in saline.

Chronic mild stress and drug administration

A chronic mild stress (CMS) model was achieved as described previously (Yang et al., 2012). Briefly, individually housed C57BL/6J male mice (n = 16–20 for each group) were allowed to acclimate for 1 wk and then were subjected to 3 wks of stressors, which were mild and unpredictable in nature, duration and frequency. Stressors included inversion of day/night light cycle, soiled cage bedding, 45° tilted cages, restraint, overnight food and water deprivation and pairing with another stressed animal, which were introduced over a period of 3 wks and repeated thereafter. Sucrose preference was monitored consistently throughout the course of experiments as described below. In CMS-treated mice, Ipt (10 mg/kg per day, i.p) or Flx (10 mg/kg per day, i.p) was administered for 28 d, starting 3 wk after the beginning CMS. Behavioural tests were performed (between 08:00 and 12:00) 3 d before the last Ipt or Flx administration. The mice were sacrificed immediately after the last stressor and drug administration at the end of 7-wk experiment.

Sucrose preference test

Mice were given the choice to drink from two bottles for 12 h: one contained a sucrose solution (1%) and the other contained only tap water. To prevent possible effects of side-preference in drinking behaviour, the positions of the bottles in the cage were switched after 6 h. The animals were deprived of water for 8 h before the test. The consumption of tap water, sucrose solution and total intake of liquids was estimated simultaneously in the control and experimental groups by weighing the bottles. The preference for sucrose was measured as a percentage of the consumed sucrose solution relative to the total amount of liquid intake.

Physical state and body weight

Before and during unpredictable CMS, body weights were recorded every Monday and the physical state was recorded on the last day of CMS procedure. Observers carried out physical state assessments unaware of treatments, by evaluating the coat in the head or neck, dorsal, ventral and on the fore paws, hind paws and tail (0–4 points). A score of 1 for a coat in a good state, or 0 for a dirty coat was given for each of these areas.
Tail suspension test

The tail suspension test (TST) is one of the most widely used models for assessing antidepressant-like activity in mice. In this experiment, mice were individually suspended by the distal portion of their tails with adhesive tape for a period of 6 min (30 cm from the floor) in a visually isolated area. The time of immobility of the tail-suspended mice during the last 4 min was measured with TailSuspScan™ (Clever Sys Inc., USA).

Forced swimming test

The forced swimming test (FST) employed was essentially similar to that described elsewhere. Briefly, mice were individually placed in a transparent 21 glass cylinder (19 cm tall) filled with water at 23 °C, to a depth of 13 cm, and left there for 6 min. A mouse was judged to be immobile when it floated in an upright position, and made only small movements to keep its head above water. The duration of immobility was recorded during the last 4 min of the 6-min testing period by TailSuspScan™ (Clever Sys Inc., USA).

Bromodeoxyuridine labelling

For analysis of cell proliferation, 2 h after the last injection of the 4-week period of Flx treatment, 6 mice of each group were given bromodeoxyuridine (BrdU) i.p. (4×50 mg/kg every 2 h). The mice were then killed and transcardially perfused (0.1 M cold PBS for 5 min followed by 4% cold paraformaldehyde) 24 h after the last BrdU administration. Other mice were sacrificed 24 h after the last drug or saline injection. The hippocampus were quickly isolated and frozen at −70 °C.

Immunostaining and cell quantification of staining

For immunohistochemistry and immunofluorescence, free-floating sections were immunolabelled according to previous procedures (Kong et al., 2009). For BrdU immunohistochemistry, sections were incubated in anti-mouse BrdU (1:2500; Millipore, USA) for MAC-1 immunohistochemistry, sections were incubated in anti-rat MAC-1; and for immunofluorescence, sections were incubated mouse anti-Tuj1 (1:800; CST, USA) or rabbit anti-Dcx (1:50; Abcam, UK) over night at 4 °C. After washes, secondary antibodies were applied for 1 hr. The sections were then washed with PBS, wet-mounted and later dried in the dark.

All the positive cells were counted using the Optical Fractionator method with Microbrightfield Stereo Investigator software (Stereo Investigator software, Microbrightfield, USA) on a Z-series of sections of 240 μM apart. BrdU+ counts were limited to the hippocampal granular cell layer and adjacent hilar SGZ margin using an overlay grid of 100×100 mm.

Preparation of nuclear extracts

Brain tissues were lysed with 30 vertical strokes by a glass homogenizer. Nuclei were collected by centrifugation, and supernatant was saved as the cytoplasmic fraction. Pellets were re-suspended in nuclear extraction buffer (in mM: 20 HEPES, 1.5 MgCl2, 500 NaCl, 0.5 EDTA, 0.2 PMSF, 1 DTT; 200 μM/ml protease inhibitor and 25% glycerol; pH 7.9 at 48 °C) followed by sonication in 10 s bursts, and 20 min incubation. After clarification by centrifugation, supernatant was retained as the nuclear fraction. All procedures were done at 4 °C using precooled tubes. Samples were separated by 8% SDS-PAGE and nuclear marker H3 (1:1000, CST, USA) was used to evaluate nuclear fractionation.

Western blotting analysis

Protein lysates were prepared from the right lobes of hippocampus using KeyGene (USA) and protein concentrations were quantified by BCA method [Biyuntian (China)]. 40–100 μg of protein samples were separated on SDS-PAGE gels and then transferred to PVDF membranes (Millipore). The membranes were washed for 5 min with T-BST and subsequently blocked in T-BST (10 or 5% non-fat milk) for 1 h followed by incubation with primary antibodies at 4 °C overnight. The primary antibodies used were rabbit anti-pro-IL-1β/IL-1β or pro-Caspapse-1/ Caspase-1 (1:600, USA); rabbit anti-NLRP-3; rabbit anti-pIRS (Ser-636) (1:500, SAB); rabbit anti-pAkt (Tyr-636) (1:800, SAB); rabbit anti-pGSK3β (Tyr-216) (1:1000, SAB); rabbit anti-p65 or mouse anti-H3 (1:1000, CST, USA). Next, after washing three times with T-BST, the membranes were incubated with secondary antibodies for 1 hr at room temperature. Then, after washing, the signals were detected by enhanced chemiluminescence (ECL) Western blotting detection reagents (Pierce, USA). The membranes were scanned and analysed using an Omega 16ic Chemiluminescence Imaging System (Ultra-Lum, USA).

Real time transcriptase-polymerase chain reaction

Total RNA was extracted from brain tissues using Trizol reagent (Invitrogen Life Technologies, USA) followed by treatment with RNase-free DNaseI (Invitrogen Life Technologies, USA). Reverse transcription was performed with the One-Step RNA-PCR Kit (Takara), according to the manufacturer’s protocol. PCR primers were as follows: GAPDH (sense 5′-CAAAAGGTGTCATCTCC-3′; anti-sense 5′-CCCCAGCATCAAAGGTG-3′), TNF-α (sense 5′-CATCTTCTCAAAATTCGAGTGACAA-3′; anti-sense 5′-TGGGAGTAGACAAGGTACAACCC-3′), IL-6 (sense 5′-ATCCAGTTGCGCTTCTGGGACTGA-3′; anti-sense 5′-TGGAGATGAGCAAGTTGGAATCCC-3′), IFN-γ (sense 5′-TCATTTTGTACATCATGATGCCTCT-3′; anti-sense 5′-CATTTGTACATCATGATGCCTCT-3′). Quantitative real-time PCR was performed on a 7300 Real-Time PCR
System using the SYBR Green PCR Master Mix. After the addition of primers, and template DNA to the master, PCR thermal cycle parameters were as follows: 95°C for 10 min, 40 cycles of 60°C for 60 s, and 95°C for 15 s, and a melting curve from 60 to 95°C to ensure amplification of a single product. Relative expression of genes of interest was calculated and expressed as 2−ΔΔCT, in which 2−ΔΔCT=[(CT target gene−CT endogenous control) test sample−(CT target gene−CT endogenous control) control sample].

**Measurements of plasma corticosterone levels**

Plasma corticosterone levels were measured using Cayman Enzyme Immunoassay kits (No. 500651; Ann Arbor, USA) according to manufacturer’s recommendations. Plasma samples (100 μl) were extracted 3 times with 4 volumes (400 μl) of dichloromethane. After extraction, samples were reconstituted in buffer dilutions optimized for the standard curve (1:40 dilution). Final corticosterone values were adjusted for individual sample recovery. Values are expressed as ng/ml of starting plasma.

**Statistical analysis**

All values are reported as mean±S.E.M. The significance of the difference between controls and samples treated with various drugs was determined by one-way ANOVA test followed by the least significant difference (LSD) for post-hoc comparisons using SPSS 17.0 for Windows (SPSS Inc., USA). The data of physical state scales in a depression model were analysed by Wilcoxon-Mann-Whitney non-parametric test. The level of statistical significance is defined as p<0.05 difference.

**Results**

**Iptakalim improves depression-related behaviours in CMS model mice**

In order to examine whether Ipt has a potential antidepressant effect, we used CMS model mice, which can induce depression-like behaviours. Mice were first exposed to the CMS procedure for 3 wk, followed by 4 wk of Ipt or Flx treatment during which CMS exposure continued as shown in Fig. 1a. CMS mice exhibited a decreased body weight and sucrose preference at the second week of the CMS procedure (F=6.858, p=0.001), which was ameliorated by treatment with Ipt or Flx for 4 wk (Fig. 1b, c, F=10.089, p=0.012). CMS procedure markedly degraded physical condition compared to non-CMS mice (p<0.01). Either Ipt or Flx treatment significantly ameliorated CMS procedure-induced physical degradation (Fig. 1d). Forced swimming tests (FST) and tail suspension tests (TST) were performed to assess depression-related behaviours. Following 3 wk of CMS, the mice displayed a significant increase in immobility time during FST and TST (F=8.815, p=0.02). Ipt recovered CMS-induced increase of immobility time in the TST and FST (F=15.799, p=0.012, Fig. 1 e, f). Furthermore, the CMS procedure resulted in a 2.5-fold elevation in plasma corticosterone level in mice (F=26.33, p=0.005). However, either Ipt or Flx administration could reverse this elevation (Fig. 1g). These results indicate Ipt possesses the potential anti-depressive effect on a CMS mouse model of depression.

**Iptakalim alleviates the decreased neural stem cell proliferation and differentiation in the dentate gyrus in CMS model mice**

The cell in the dentate gyrus of mouse hippocampus was assessed by 5-bromodeoxyuridine (BrdU) labelling so as to examine whether Ipt regulates neural stem cell (NSC) proliferation. As shown in Fig. 2a, the number of BrdU positive cells in cluster at SGZ was significantly decreased in CMS mice compared with that of the control group (F=13.089, p=0.003). Treatment with Ipt or Flx recovered the impaired proliferation of NSC to the levels of non-CMS mice (Fig. 2b).

Since the process of adult hippocampal neurogenesis encompasses the proliferation, survival and differentiation into adult hippocampal neuronal progenitors (Kheirbek et al., 2012), we examined whether Ipt could affect the fate of newborn cells. Doublecortin (DCX) and β-tubulin III (Tuj-1) are used as the markers of neural progenitor cells (NPCs) and newborn neurons, respectively (Ming and Song, 2011). As shown in Fig. 2c, DCX+ cells were randomly expressed in the SGZ of hippocampus, and Tuj-1+ cells were distributed in the DG (Fig. 2d). Both the numbers of DCX+ cells and Tuj-1+ cells were significantly decreased in mouse SGZ after CMS procedure (p<0.05), which was also significantly ameliorated by treatment with Ipt or Flx (Fig. 2e, f).

**Iptakalim inhibits neuro-inflammation in the hippocampus of CMS model mice**

Since inflammation is responsible for the neurogenesis impairment, we further examined the role of Ipt in the neuro-inflammatory response in the context of depression. As shown in Fig. 3a, CMS mice exhibited a higher expression of MAC-1 (CD11b) in the microglia, indicating that the CMS procedure led to over-activation of microglia in the DG of hippocampus (p=0.002). As expected, this activation was significantly ameliorated by Ipt or Flx treatment (Fig. 3b, F=110.613, p=0.034).

We further evaluated the effects of Ipt on the production of pro- and anti-inflammatory cytokines in the hippocampus of mice with CMS procedure. CMS procedure resulted in a significant increase of IL-6 and TNF-α compared with that of the control group (F=12.231, p<0.01, Fig. 3d, e), which was significantly abrogated by Ipt or Flx treatment (Fig. 3d, e). In contrast, IL-10 generation was decreased after chronic mild stress...
Ipt treatment reversed the decrease of IL-10 in CMS mice (Fig. 3c). Moreover, Ipt treatment inhibited the nuclear translocation of p65, which was known to be involved in the inflammatory response, in the hippocampus induced by CMS ($F=0.757$, $p<0.01$, Fig. 3f).

**NLRP3 inflammasome contributes to the neuro-inflammation in depression progression**

The nod-like receptor protein 3 (NLRP3) inflammasome in the brain is mainly expressed in glia cells and contains NLRP3, the adaptor protein apoptosis-associated speck-like protein (ASC) and the proinflammatory caspase (caspase-1) (Schroder et al., 2010). The NLRP3 inflammasome is responsible for the maturation of IL-1β. Here, we found that the CMS procedure significantly increased IL-1β production in the mouse hippocampus compared with control mice ($F=0.621$, $p<0.01$, Fig. 4a). Notably, this increased IL-1β secretion was inhibited by treatment with Ipt or Flx ($F=0.812$, $p<0.05$, Fig. 4a). Consistently, Ipt treatment also significantly inhibited the increase of serum IL-1β levels induced by the CMS procedure ($F=0.315$, $p<0.01$, Fig. 4b). Furthermore, Ipt treatment

(F=9.034, $p<0.01$). Ipt treatment reversed the decrease of IL-10 in CMS mice (Fig. 3c). Moreover, Ipt treatment inhibited the nuclear translocation of p65, which was known to be involved in the inflammatory response, in the hippocampus induced by CMS ($F=0.757$, $p<0.01$, Fig. 3f).

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significantly inhibited NLRP3 up-regulation and caspase-1 activation in the hippocampus of CMS mice ($F=3.721, p<0.05$, Fig. 4c, d).

MicroRNAs have emerged as key protagonists in the pathophysiology of depression (Serafini et al., 2013). Among the large number of miRs, miR-16 has been regarded as a modulator of serotonin transporters (SERT), which is implicated in the pathogenesis of depression (Baudry et al., 2010). Meanwhile, miR-7 was demonstrated to negatively regulate the expression of NLRP3 in our previous study (not published). Therefore, we detected the expression of miR-16 and miR-7 in mouse hippocampus in both CMS and non-CMS mice. Interestingly, we found that CMS procedure significantly inhibited the expression of miR-7 in the mouse hippocampus ($F=6.229, p<0.01$) and both Ipt and Flx treatment reversed the decrease of miR-7 induced by the CMS procedure (Fig. 4f). Moreover, we found that the expression of miR-16 was up-regulated in CMS model mice (Fig. 4c, $F=1.068, p<0.01$) and Flx treatment inhibited the increase of miR-16 in the hippocampus, consistent with a previous report (Baudry et al., 2010). Ipt failed to influence the expression of miR-16 in CMS mice. These findings illustrate that Ipt plays a critical role in inflammation via post-transcriptional controlling. Furthermore, the therapeutic effect of Ipt on depression is independent of the serotonin neurotransmitter system.

Iptakalim restores insulin signalling in the hippocampus of CMS model mice

It has been considered that inflammation interferes with insulin/insulin-like growth factor 1 (IGF-1) action and neurogenesis is associated with insulin signalling (Dandona et al., 2004; Hu et al., 2013). Insulin can induce serine phosphorylation of insulin receptor substrate-1 (IRS-1) and the subsequent activation of PI3K-Akt (Dandona et al., 2004). Western blotting analysis showed that serine-636 phosphorylation of IRS-1 was increased in the hippocampus of CMS mice compared with that of control mice. Either Ipt or Flx treatment reversed the increased phosphorylation of IRS-1 induced by the CMS procedure ($F=17.291, p<0.05$, Fig. 5a). Furthermore, treatment with Ipt or Flx reversed the inhibition of Akt phosphorylation in the hippocampus of CMS mice ($F=51.368, p<0.05$, Fig. 5b), which in turn suppressed the CMS procedure-induced activation of GSK-3β (Tyr-473) ($F=9.386, p<0.05$, Fig. 5c).

Discussion

Depression is a serious mental disorder accompanied by neurogenesis impairment and inflammation (Zhang et al., 2013). In the present study, we demonstrated that Ipt treatment ameliorated depression-like behaviours, alleviated neurogenesis inhibition and attenuated microglial activation-mediated inflammation in CMS model mice. Moreover, Ipt increased miR-7 expression and inhibited NLRP3-inflammasome activation, which may contribute to the suppression of neuro-inflammation and enhancement of adult hippocampal neurogenesis in CMS mice. These findings indicate that Ipt exerts potential antidepressant roles in CMS mice, which give us an insight into the potential of Ipt in therapeutic implications for depression.
The integration of adult-born neurons into the circuitry of hippocampal neurogenesis is required for learning and memory as well as emotional regulation (Deng et al., 2010). Optimal function of the hippocampal formation is critical for modulation of the hypothalamic-pituitary axis (HPA) and regulation of the stress response (Sahay and Hen, 2007). Blocking adult hippocampal neurogenesis results in the depression-like behaviours such as cognitive decline and anxiety (Saxe et al., 2007). Furthermore, adult hippocampal neurogenesis is required for the behavioural effects of antidepressants. We also confirmed that NSCs, NPCs and newborn neurons were decreased in the CMS mice. Either Ipt or Flx treatment reversed the loss of adult hippocampal neurogenesis in the CMS mouse model. Our previous studies demonstrated that Ipt could enhance adult hippocampal neurogenesis via opening Kir6.1-composed K-ATP channels (Yang et al., 2012). Since K-ATP channels are identified as critical components in maintaining the body’s homeostasis during the adaptive reaction to stress, it is reasonable that K-ATP channels govern the regulation of NSC proliferation and differentiation under stress. Adult hippocampal neurogenesis buffers stress responses and depressive behaviours (Snyder et al., 2011; Soskin et al., 2012). Therefore, opening of K-ATP channels by Ipt relieves the depressive behaviours during chronic stress including the decrement of sucrose preference and increment of immobility time in the TST and FST. The NSCs in the hippocampus are modulated by microglia under stress conditions (Ekdahl et al., 2009). We found that microglia was significantly activated in the hippocampus of the CMS mice and the proinflammatory cytokines IL-6, IL-1β and TNF-α were increased in the hippocampus. These results indicate that Ipt exerts an antidepressant effect by inhibiting neuro-inflammation. Peripherally released inflammatory cytokines can access the brain and engage in the regulation of adult hippocampal neurogenesis (Gelfo et al., 2012). Among

Fig. 3. Ipt inhibited the inflammation induced by CMS in the hippocampus. (a) Ipt alleviated the activation of microglia in DG induced by CMS. Representative image showed MAC-1-positive cells in the DG. (b) Quantification of MAC-1+ cells in DG. Activated microglia were increased after CMS while Ipt and Flx treatment decreased the number of MAC-1+ cells in DG. CMS procedure significantly increased the relative mRNA level of IL-6 in hippocampus of stressed mice. (c) Ipt restored the relative mRNA level of IL-10 in hippocampus of stressed mice. Ipt and Flx prevented the increase of IL-6 in mouse hippocampus. (d) Ipt and Flx decreased the relative mRNA level of TNF-α in the hippocampus of stressed mice. (f) CMS-induced increase of p65 nuclear translocation in mice hippocampus was significantly inhibited by Ipt treatment. n=4, **p<0.01 vs. Control; #p<0.05 vs. CMS; ##p<0.01 vs. CMS. Scale bar: 100 μm.

Antidepressant role of Iptakalim

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these factors, IL-1 is an important cytokine involved in the modulation of neuroendocrine systems, particularly the HPA. Recent studies showed that IL-1β is involved in neurobehavioural alterations (Goshen and Yirmiya, 2009). The mutation of IL-1β is controlled by the NLRP3-inflammasome (Schroder and Tschopp, 2010). NLRP3 is activated by numerous endogenous ‘danger signals’ and environmental irritants (Ogura et al., 2006). Our findings indicated that CMS treatment led to NLRP3 up-regulation and caspase-1 activation in mouse hippocampus, suggesting that NLRP3-inflammasome/caspase-1/IL-1β axis is associated with the pathogenesis of neurobehavioural alterations.
of depression. Ipt treatment inhibited the activation of NLRP3 inflammasome, accompanied by the decrease of IL-1β levels in the hippocampal and serum. We further found that Ipt alleviated the inhibition of miR-7 in the hippocampus, but did not affect the expression of miR-16 in CMS model mice. MicroRNAs have emerged as key protagonists in depression and miR-16 acts as a post-transcriptional repressor of the SERT (Baudry et al., 2010) and we detected miR-16 levels in the hippocampus of CMS mice. As a result, CMS mice displayed a marked elevation of miR-16 in the hippocampus and Flx inhibited the expression of miR-16, suggesting that Flx regulates the expression of miR-16 and SERT in the treatment of depression. Differentially, Ipt failed to affect the level of miR-16, but enhanced the expression of miR-7. Since we have proved that nlrp3 is a target gene of miR-7 (not published), Ipt may inhibit neuro-inflammation in the hippocampus of CMS mice through elevating miR-7 levels, subsequently down-regulating NLRP3 expression and suppressing inflammasome activation.

K-ATP channels function as a critical component in maintaining the body’s energy metabolic homeostasis during the adaptive reaction to stress (Zingman et al., 2002). Inflammation has been considered to cause dysregulation of insulin signal transduction, which is responsible for adult neurogenesis (Dandona et al., 2004; Hurtado-Chong et al., 2009; Hu et al., 2013). Furthermore, many metabolic diseases, such as obesity and diabetes raise the risk of depression (McElroy et al., 2004). Therefore, we speculated that Ipt could modulate the insulin signal pathways in the brain via inhibiting the inflammatory response induced by the CMS procedure. The results showed that in CMS mice, enhanced inflammation was accompanied by the impairment of insulin signalling. Our findings are in agreement with a previous report that depression is associated with the individual components of metabolic dysfunction (McElroy et al., 2004). Therefore, Ipt may exert its role in promoting neurogenesis through improving insulin signalling.

In conclusion, our study demonstrates that NLRP3-inflammasome is associated with the pathogenesis of stress-induced depression and that Ipt exerts a potential therapeutic effect on major depression. Ipt ameliorates depressive symptoms through inhibiting NLRP3-inflammasome and improving insulin signalling, enhancing the adult hippocampal neurogenesis under stress. These findings not only give us an insight into the further pathogenesis of depression, but also provide a potential foe Ipt in terms of opening up novel therapeutic avenues for the treatment of depression.

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Conflict of Interest

None.

References


