

Neuroprotective effects of dopamine D2 receptor agonist on neuroinflammatory injury in olfactory bulb neurons in vitro and in vivo in a mouse model of allergic rhinitis

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ABSTRACT

Available evidence indicates that dopamine D2 receptor modulates the neurotoxic effects induced by glutamate. However, neurotoxicity mediated by AMPA-subtype glutamate receptor has rarely been studied in the olfactory bulb. This study mainly explores the neuroprotective effects of dopamine D2 receptor agonist on AMPA receptor-mediated neurotoxicity in the olfactory bulb in a mouse model of allergic rhinitis (AR) with olfactory dysfunction (OD). In our study, we found that AR with OD was closely associated with increased surface expression of the AMPA receptor GluR1, reduced surface expression of GluR2, and apoptosis damage in the olfactory bulb in vivo. Quinpirole (a dopamine D2 receptor agonist) improved olfactory function in mice, ameliorated apoptosis injury in the olfactory bulb but not in the olfactory mucosa, and inhibited the internalization of GluR2-containing AMPA receptor in vitro and in vivo. In addition, phosphorylation plays a crucial role in the regulation of AMPA receptor trafficking. Our results showed that quinpirole reduced the phosphorylation of GluR1 S845 and GluR2 S880 in olfactory bulb neurons in vitro, but it had no obvious effect on GluR1 S831. Therefore, dopamine D2 receptor agonist may inhibit the phosphorylation of GluR1 S845 and GluR2 S880, thereby reducing AMPA receptor-mediated neurotoxicity and alleviating neurotoxic injury to the olfactory bulb caused by AR.

1. Introduction

Allergic rhinitis (AR) is a common clinical otorhinolaryngology disease. It has a high incidence in the population, and the current incidence is increasing yearly (Wang et al., 2016). In particular, it is one of the clinical causes of olfactory dysfunction (OD). Currently, various epidemiological studies tend to believe that in the population, the incidence of allergic rhinitis is approximately 10 %–30 % (Mims, 2014). Boris A Stuck et al. summarized that the incidence of OD in AR was 10 %–88 %, and it increased with the progression of the disease (Stuck and Hummel, 2015). The underlying mechanism of AR-induced OD is not yet clear, though it is generally believed to be related to nasal edema or the direct toxic effects of inflammatory mediators on olfactory receptor neurons (Coward et al., 1993; Guss et al., 2009). However, the olfactory bulb is the superior conduction structure of the olfactory system, and its pathological changes deserve more attention.

The olfactory bulb is a transfer station for the transmission of

olfactory signals to the brain. It processes the information of olfactory receptor neurons and further transmits that information to the higher olfactory cortex (Corthell et al., 2012). Jie Ma et al. proposed that the AMPA receptor is the main glutamate receptor subtype in the olfactory bulb, which mainly mediates rapid excitatory synaptic transmission, and is a necessary condition for olfactory signal transduction and olfactory formation (Ma and Lowe, 2007). The AMPA receptor consists of a combination of GluR1–GluR4 subunits, of which GluR2 on the membrane forms specific Q/R and R/G structures, and determines the selective opening of ion channels (Isaac et al., 2007). In transgenic mice with reduced GluR2 or unedited GluR2 in the forebrain and olfactory bulb, olfactory recognition and learning ability were enhanced, which indicates that the calcium permeability of the AMPA receptor in the olfactory bulb can affect olfactory function (Shimshek et al., 2005). Furthermore, the absence or lack of editing of GluR2 makes the AMPA receptor permeable to Ca^{2+} , and intracellular Ca^{2+} becomes overloaded, triggering neurotoxic reactions such as oxidative stress and cell

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apoptosis. This process plays an important role in the process of brain damage in many inflammatory diseases, such as peripheral inflammatory pain, autoimmune encephalopathy, traumatic brain injury, and inflammatory bowel disease (Khatri et al., 2018; Nyuyki and Pittman, 2015; Riazzi et al., 2015; Su et al., 2015). However, the excitotoxic effects mediated by AMPA receptor are rarely studied in AR-induced OD.

The dopamine D2 receptor belongs to a superfamily of seven transmembrane domain receptors in a single polypeptide, and they exert biological effects through the intracellular Gi/Go-protein coupled signal cascade. Evidence shows that dopamine D2 receptor modulates glutamate-induced neurotoxicity (Garside et al., 1996; Sawada et al., 1998). Dopamine D2 receptor agonists have shown a protective effect on the hippocampus and substantia nigra striatum after ischemia (Hall et al., 1996; Liu et al., 1995). Systemic administration of the glutamatergic agonist alginic acid resulted in the death of hippocampal cells in dopamine D2 receptor knockout mice but not wild-type mice (Bozzi and Borrelli, 2002; Bozzi et al., 2000). In the striatum, stimulation of dopamine D2 receptor leads to a decrease in the glutamate response, which is largely achieved through negative regulation of the AMPA receptor (Cepeda et al., 2001; Hernandez-Echeagaray et al., 2004). Therefore, we speculate that dopamine D2 receptor can improve olfactory function by reducing the excitotoxicity mediated by the AMPA receptor.

2. Materials and methods

2.1. A mouse model of allergic rhinitis with olfactory dysfunction

The 6–8 week old C57BL/6 mice were provided by the Animal Experiment Center of Renmin Hospital of Wuhan University. All experimental procedures were approved by the Animal Ethics Committee of Renmin Hospital of Wuhan University (License No. WDRM 20190419). The method of mouse modeling was performed according to a published article (Lv et al., 2021). In brief, 0.1 mg of ovalbumin (OVA, Sigma, Germany) and 5 mg of aluminum hydroxide (Al(OH)₃) were mixed with 300 µL of saline to form a suspension, which was intraperitoneally injected once a week for 3 consecutive weeks. At the end of the sensitization stage, 0.2 mg of OVA dissolved in 20 µL of saline was dropped into the nasal cavities of AR mice (n = 20), 10 µL on each side, for 2 weeks. The control group (n = 10) was treated with saline instead of OVA, and the other methods and procedures remained unchanged. The frequencies of sneezing and rubbing were recorded immediately after the last challenge for 10 min. After modeling, AR mice with OD were screened by the buried food pellet test.

2.2. Olfaction test

Olfactory function was examined by the buried food pellet test (BFPT) (Talaga et al., 2017). The diet of the mice was restricted one day in advance, without restricting water. A clean cage (45 cm × 24 cm × 20 cm) was prepared, and clean padding was spread evenly on the bottom of the cage, about 3 cm thick. Four grams of food pellet was randomly buried under the bedding, approximately 0.5 cm deep. Then, the mouse was placed in the center of the cage. When the mouse found the food, this time the mouse took to find the food pellets was recorded for statistical analysis. If the mouse did not find the food pellet within 300 s, it was considered olfactory dysfunction. Another olfactory test was also used. As described in previous studies (Langley et al., 2021; Ngwa et al., 2014), the bedding removed from a cage containing pregnant female mice was introduced into the cage of the male mice used in the experiment. The number and position of each cushion remained the same. The total amount of time the mice spent sniffing the female bedding was measured during a five-minute test.

2.3. Drug treatment and experimental grouping

AR mice with OD were identified and randomly divided into the following groups: quinpirole (3 mg/kg, Sigma, Germany) was dissolved in 20 µL of saline and administered nasally every day for 3, 6, 9, and 12 days (n = 6 for each group), and the control group (n = 6) was given 20 µL of saline. The dosing pattern and dosage of quinpirole were based on previous reports (Zhang et al., 2015). In our preliminary experiments, both nasal administration and intraperitoneal injection improved olfactory function, but quinpirole administered nasally at the same drug dose achieved drug effects more effectively and earlier. Consistent with this, several studies have confirmed the advantage of dopamine transfer to the brain along the olfactory pathway (Dahlin et al., 2000, 2001). During this period, the mice were challenged with OVA every other day. Olfactory function was evaluated 24 h after the final administration, and the mice were sacrificed for the following experiments (Fig. 2A).

2.4. HE staining

After the mice were sacrificed, the heads of the mice were isolated and the scalps were removed. The entire mice head was then immersed in formalin buffer for 24–48 h. After fixation, the mouseheads were immersed in decalcification solution (Servicebio, China) for 2 weeks at room temperature. After decalcification was completed, the samples were dehydrated with gradient ethanol and embedded in paraffin. The sections of each specimen were selected for hematoxylin-eosin staining. The number of eosinophils in the nasal mucosa was counted in five randomly selected high-power fields (×400).

2.5. Immunofluorescence

The mice were sacrificed 24 h after the last stimulation, and the olfactory bulb was fixed with 4% paraformaldehyde for 48 h. After removing the blocking solution, 0.1 % Triton-X was used to break the membrane, CD11b primary antibody (1:500, ab184308, Abcam, UK) was added, and the sections were incubated overnight at 4 °C. However, when GluR1 (1:200, sc-55509, Santa Cruz, USA) and GluR2 (1:2000, ab206293, Abcam, UK) were detected, the slices were not treated for permeability by Triton-X. On the next day, the corresponding fluorescent secondary antibody (ab150080, Abcam, UK) was added dropwise at room temperature and incubated for 1 h. After washing with PBS, DAPI staining was performed. The slices were sealed with anti-fluorescence quenching agent and observed under a fluorescence microscope.

2.6. ELISA

Twenty-four hours after the last intranasal challenge, blood was obtained after the removal of the eyeball. After centrifugation for 20 min at 3000 rpm, the serum was stored at –80 °C for use. OVA-specific IgE in serum was detected using a Mouse OVA-specific IgE ELISA Kit (Bioswamp, China) based on the manufacturer's instructions.

2.7. Coculture of olfactory bulb neurons and microglia

The use of the Transwell cocultivation system and magnetic bead sorting maintained the in vivo state of the microglia to a greater extent. As previously reported (Holt et al., 2019; Nikodemova and Watters, 2012), microglia were isolated from the olfactory bulbs after successful modeling. The olfactory bulbs were enzymatically digested using a Neural Tissue Dissociation Kit (Miltenyi Biotec, Germany), and filtered through 70 µm cell strainers to prepare a single cell suspension. The microglia were sorted with CD11b magnetic beads (Miltenyi Biotec, Germany). After resuspension, loading, and washing, the positive cells on the sorting column were collected. Furthermore, olfactory bulb neurons were isolated within 3 days after birth, and digested with trypsin-EDTA (0.25 %) at 37 °C for 15 min after removing the meninges.

and blood vessels. The cells were resuspended in DMEM/F12 medium containing 10 % FBS (Gibco, USA) and 2% B-27 Supplement (Thermo Fisher, USA), inoculated into a polylysine-coated culture plate, and cultured in a humidified CO₂ (5%) incubator at 37 °C. After 7 days of culture, they were co-cultivated with purified microglia for 24 h, and were simultaneously treated with quinpirole (20 μmol/L).

2.8. TUNEL staining

TUNEL staining was carried out according to the manufacturer's instructions (Roche, Germany). The cells or paraffin sections were incubated with 50 μl of TUNEL reaction solution at 37 °C for 60 min in the dark. DAPI was used to stain the nucleus. The sections were observed and photographed under a fluorescence microscope (Olympus, Japan). In 6 nonrepeating high-power (×400) fields, the number of TUNEL-positive cells as a percentage of the number of DAPI-positive cells was used to calculate the positivity rate.

2.9. Western blot

Total protein was extracted by cell lysis. The tissues and cells were added with RIPA lysate, lysed on ice for 30 min, and then centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected and stored at –80 °C. The surface proteins were extracted according to the instructions of the Cell Fractionation Kit (89842, Thermo Fisher, USA). The protein concentration was measured by a BCA kit (Absin, China), 40 μg of protein was taken according to the protein concentration, and 5× loading buffer was added and boiled for 5 min. After electrophoresis, the protein was transferred to PVDF membrane. The protein was sealed with 5% skimmed milk powder on a shaker for 1.5 h, and thoroughly rinsed with TBST. The following primary antibodies were incubated at 4 °C in a shaker overnight. Primary antibodies included: GluR1 (1:1000, ab183797, Abcam, UK), GluR2 (1:2000, ab206293, Abcam, UK), GluR1 p-S845 (1:1000, ab76321, Abcam, UK), GluR1 p-S831 (1:1000, ab109464, Abcam, UK), GluR2 p-S880 (1:1000, ab52180, Abcam, UK),

β3-tubulin (1:1000, ab78078, Abcam, UK) and Na-K-ATPase (1:1000, 3010S, CST, USA). The secondary antibody (Servicebio, China) was added after thorough rinsing with TBST and incubated for 1.5 h at room temperature. Rinse thoroughly with TBST and visualized with a gel imaging system (Bio-Rad, USA). Repeat the above steps with β3-tubulin and Na-K-ATPase as internal reference. ImageJ software was used to analyze the images, and the relative expression level of target protein was indicated by the ratio of target protein to internal reference.

2.10. Statistical analysis

All results are represented as the mean ± SEM. The data and graphs were analyzed by GraphPad Prism 8.0 (GraphPad Software, USA). Two groups were compared using the Student's t test. Multiple groups were analyzed by one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. A p value < 0.05 was considered significant.

3. Results

3.1. Mice model of allergic rhinitis with olfactory dysfunction were successfully established

The buried food pellet test was used to evaluate the olfactory function of the AR group (20) and the control group (10). Among them, 10 mice in the control group could find the buried food pellets within 300 s, with an average time of (47.80 ± 27.32)s. In the allergic rhinitis (AR) group, 9 mice found food pellets within 300 s, with an average time of (75.33 ± 52.32)s, and the other 11 mice could not find the buried food pellets within 300 s, with an average time of (367.36 ± 32.12)s (Fig. 1A). AR mice with olfactory dysfunction (OD) accounted for 55 % of all mice in the AR group. Similarly, another method of olfactory detection suggested that the time for AR mice with OD to sniff the female's bedding was significantly shorter (Fig. 1B). The OVA-specific IgE in serum increased in AR mice with OD (Fig. 1C). Then, AR mice with OD began to show typical AR manifestations after 3 days of challenge,

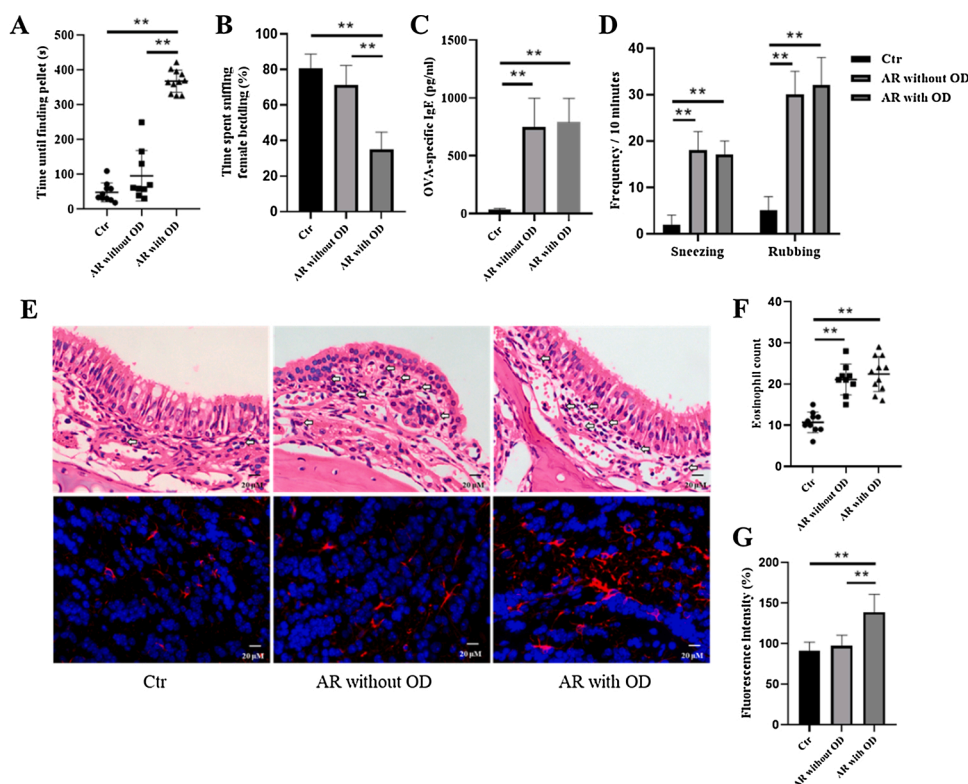


Fig. 1. Mice model of allergic rhinitis with olfactory dysfunction was successfully established. (A) The time for mice to find the food pellets was recorded in each group by the buried food pellet test. (B) The time for mice to sniff the female's bedding was recorded in each group. (C) OVA-specific IgE in serum was measured by ELISA. (D) Symptoms of sneezing and nose rubbing were counted in 10 min after the final challenge. (E) The picture of HE staining of nasal mucosa and immunofluorescence of CD11b. Eosinophils were labeled by arrows. (F) The eosinophil count in nasal mucosa of mice. (G) Fluorescence intensity of CD11b in the olfactory bulb. Ctr (n = 10), AR without OD (n = 9), AR with OD (n = 11). * P < 0.05, ** P < 0.01. Ctr, the control group; AR without OD, the group of allergic rhinitis without olfactory dysfunction; AR with OD, the group of allergic rhinitis with olfactory dysfunction.

and the frequencies of sneezing and rubbing increased significantly after 2 weeks (Fig. 1D). In addition, eosinophils were increased in AR mice with OD (Fig. 1F). Consistent with our previous report, abnormal activation of microglia might be the key cause of OD (Fig. 1G). The above results demonstrate that the mouse model of AR with OD was successfully established.

3.2. Dopamine D2 receptor agonist ameliorates apoptosis injury in the olfactory bulb but not in the olfactory mucosa, and improves olfactory function

To explore the neuroprotective effects of dopamine D2 receptor, we administered quinpirole (Quin, a dopamine D2 receptor agonist) intranasally to observe olfactory function and damage to the olfactory mucosa and olfactory bulb. Six days after quinpirole was given, the time for the mice to find the food pellets began to decrease, with the most significant decrease on day 12 (Fig. 2B). Similarly, the time for mice to sniff female's bedding increased most significantly on day 12 (Fig. 2C). Therefore, apoptotic damage in the olfactory mucosa and olfactory bulb was detected on day 12. TUNEL staining results showed that the olfactory mucosa showed more obvious apoptotic damage than the control group. Similarly, the olfactory bulb of AR mice with OD showed significantly more apoptotic cells than the control group, with most apoptotic cells were located in the glomerular layer of the olfactory bulb. Twelve days after the administration of quinpirole, the apoptotic cells in the olfactory bulb decreased significantly, but not in the olfactory mucosa (Fig. 2D–G). These results suggest that dopamine D2 receptor agonist attenuates apoptosis injury in the olfactory bulb but not in the olfactory mucosa and improves olfactory function.

3.3. Dopamine D2 receptor agonist inhibits the internalization of GluR2-containing AMPA receptors in the olfactory bulb

GluR2 is the molecular switch that determines Ca^{2+} permeability. In the process of neurotoxic injury, the internalization of GluR2 in the postsynaptic membrane is enhanced, resulting in a decrease in the content of GluR2 on the membrane, and an increase in GluR2-deficient AMPA receptors on the membrane, thereby mediating the rapid influx of Ca^{2+} , leading to a series of neurotoxic cascades (Isaac et al., 2007). We found that the surface expression of the AMPA receptor GluR1 in the olfactory bulb of AR mice with OD increased, and the surface expression of GluR2 decreased (Fig. 3A, B). Moreover, the fluorescence intensity of GluR1 on the membrane of the olfactory bulb of AR mice with OD increased, while the fluorescence intensity of GluR2 decreased (Fig. 3C, D). In order to further explore the mechanism by which dopamine D2 receptor improves olfactory bulb damage, we examined the changes in AMPA receptor after administration of a dopamine D2 receptor agonist. The western blotting results showed that quinpirole reversed the increase of GluR1 surface protein and the decrease of GluR2 surface protein on days 6, 9, and 12 (Fig. 4A–D). The results suggest that GluR2-containing AMPA receptors on the membrane are decreased in the olfactory bulb of AR mice with OD, and quinpirole may inhibit the internalization of GluR2-containing AMPA receptors to improve neurotoxic damage to the olfactory bulb.

3.4. Dopamine D2 receptor agonist ameliorates olfactory bulb neuron apoptosis *in vitro*

To simulate the neuronal environment *in vivo*, we used magnetic bead sorting to extract microglia from the olfactory bulbs of AR mice with OD, and cocultured them with olfactory bulb neurons. We found that the microglia in the olfactory bulb of AR mice with OD induced significantly more apoptotic olfactory bulb neurons (Fig. 5A, B), significantly increased GluR1 surface expression and decreased GluR2 surface expression in olfactory bulb neurons. Quinpirole significantly reversed the effect of microglia sorted from AR with OD on olfactory

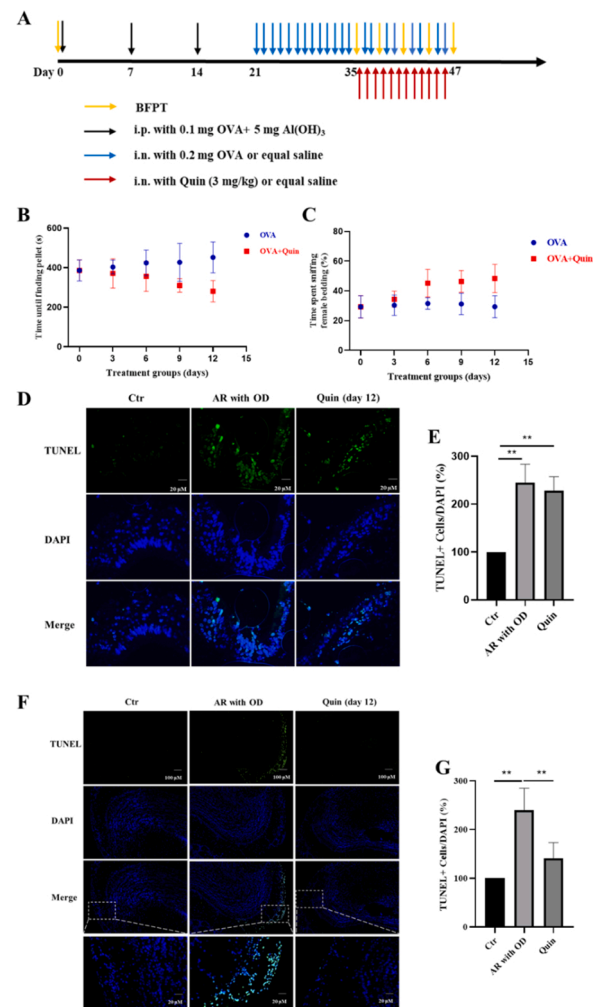


Fig. 2. Dopamine D2 receptor agonist attenuates olfactory bulb damage and improves olfactory function. (A) Protocol for the mice model of AR with OD. (B) The time for mice to find buried pellets after treatment with quinpirole (Quin, 3 mg/kg) or equal saline on days 3, 6, 9 and 12 ($n = 6$ for each group). (C) The time for mice to sniff the female's bedding after treatment with quinpirole or equal saline on days 3, 6, 9 and 12 ($n = 6$ for each group). (D, E) The apoptotic cells of olfactory mucosa were detected by TUNEL staining in the control group, AR with OD group and the group of treatment with Quin on day 12. (F, G) The apoptotic cells of olfactory bulb were detected by TUNEL staining in the control group, AR with OD group and the group of treatment with Quin on day 12. Ctr ($n = 10$), AR with OD ($n = 11$), Quin ($n = 6$). * $P < 0.05$, ** $P < 0.01$. i.p., intraperitoneal; i.n., intranasal; BFPT, the buried food pellet test; Ctr, the control group; AR with OD, the group of allergic rhinitis with olfactory dysfunction; Quin, the group of treatment with quinpirole.

bulb neurons, decreased the surface expression of GluR1, and increased the surface expression of GluR2 in olfactory bulb neurons (Fig. 6A–D). These results suggest that dopamine D2 receptor agonist may ameliorate olfactory bulb neuron apoptosis, related to the internalization of the GluR2-deficient AMPA receptor.

3.5. Dopamine D2 receptor agonist inhibits phosphorylation at the S831 and S880 sites of the AMPA receptor in olfactory bulb neurons

Phosphorylation regulation is an important regulatory mechanism for AMPA receptor trafficking. The externalization of GluR1 is mainly regulated by the phosphorylation of S845 and S831 (Hakansson et al., 2006; Roche et al., 1996). In addition, the internalization of GluR2 is mainly regulated by PKC-mediated phosphorylation of S880 (States et al., 2008). We found that microglia in the olfactory bulb of AR mice

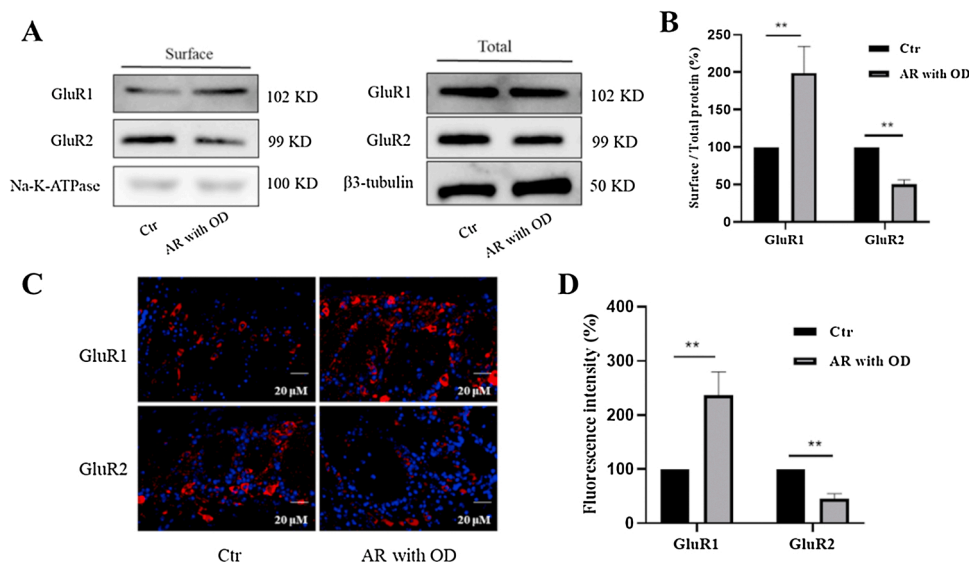


Fig. 3. The internalization of GluR2-containing AMPA receptor in the olfactory bulb of AR mice with OD. (A, B) The surface and total protein expression of GluR1 and GluR2 were detected by western blotting. (C, D) The expression of GluR1 and GluR2 were detected by immunofluorescence. Ctr (n = 10), AR with OD (n = 11) * $P < 0.05$, ** $P < 0.01$. Ctr, the control group; AR with OD, the group of allergic rhinitis with olfactory dysfunction.

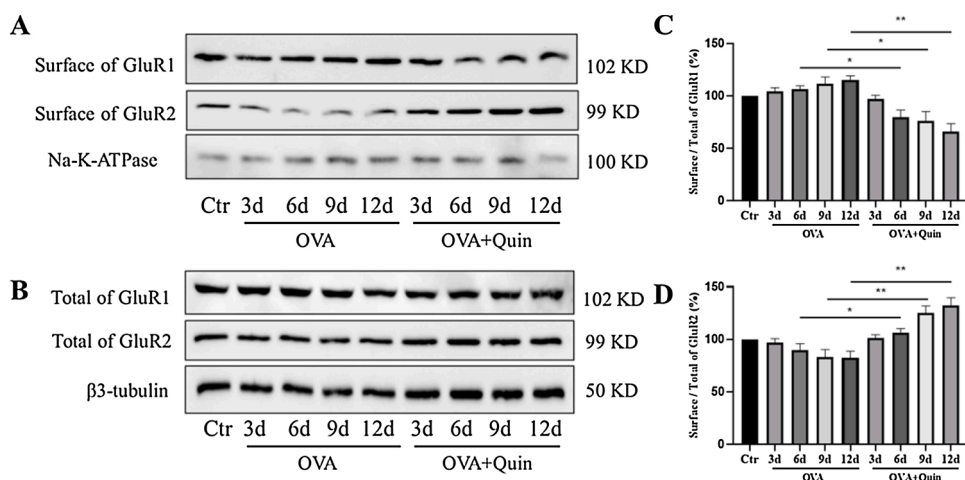


Fig. 4. Dopamine D2 receptor agonist inhibits the internalization of GluR2-containing AMPA receptor in the olfactory bulb. (A–D) The surface and total protein expression of GluR1 and GluR2 were detected by western blotting after treatment with Quin (3 mg/kg) or equal saline on days 3, 6, 9 and 12 (n = 6 for each group). * $P < 0.05$, ** $P < 0.01$. Ctr, the control group; OVA, the group of treatment with OVA; OVA + Quin, the group of treatment with OVA and quinpirole.

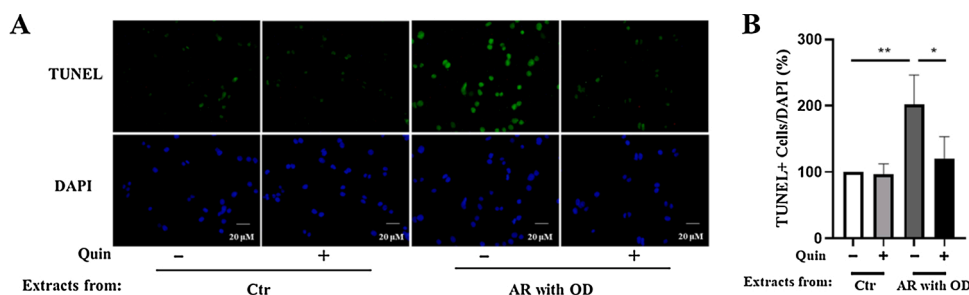


Fig. 5. Dopamine D2 receptor agonist ameliorates olfactory bulb neurons apoptosis in vitro. (A, B) The coculture system of microglia and olfactory bulb neurons by treatment with Quin (20 μ M/L), and apoptotic cells of olfactory bulb neurons were detected by TUNEL staining after 24 h. n = 5 for each group. * $P < 0.05$, ** $P < 0.01$. Ctr, the control group; AR with OD, the group of allergic rhinitis with olfactory dysfunction.

with OD significantly activated the phosphorylation of AMPA receptor residues S845, S831 and S880 in olfactory bulb neurons. Quinpirole significantly reversed the phosphorylation of AMPA receptor residues

S845 and S880 in olfactory bulb neurons by microglia sorted from AR mice with OD, but had no obvious effect on the expression of p-S831 (Fig. 7A–D). These findings suggest that dopamine D2 receptor agonist

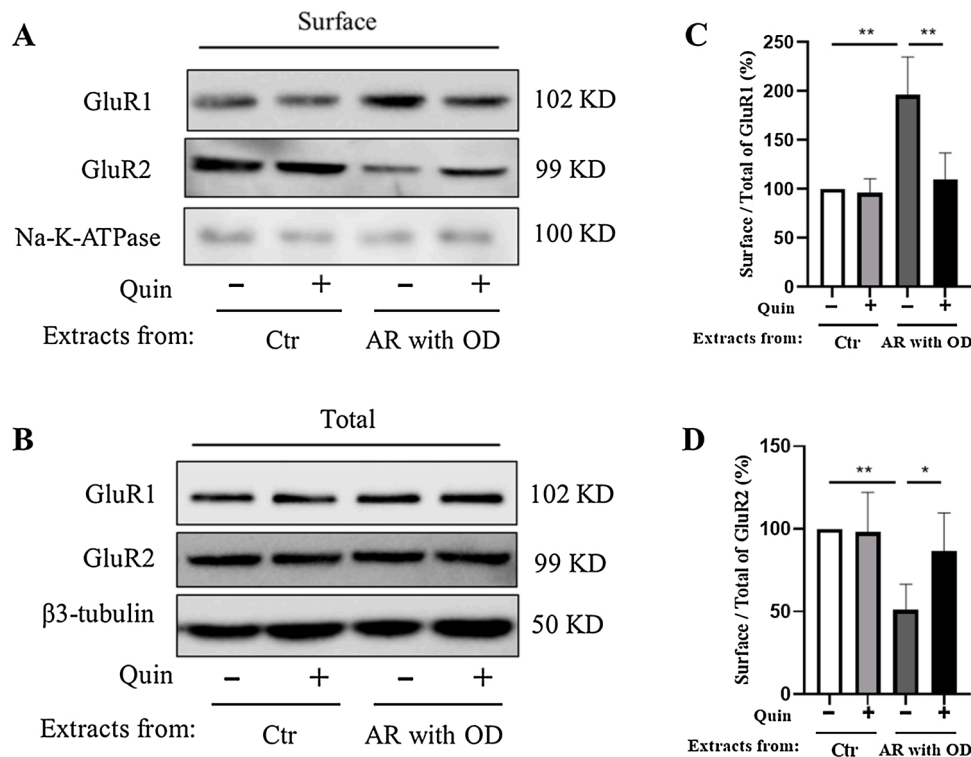


Fig. 6. Dopamine D2 receptor agonist inhibits the internalization of GluR2-containing AMPA receptor in olfactory bulb neurons. (A–D) The coculture system of microglia and olfactory bulb neurons by treatment with Quin (20 μ mol/L), and the surface and total protein expression of GluR1 and GluR2 were detected by western blotting after 24 h. $n = 5$ for each group. * $P < 0.05$, ** $P < 0.01$. Ctrl, the control group; AR with OD, the group of allergic rhinitis with olfactory dysfunction.

regulates AMPA receptor trafficking through the S831 and S880 sites in olfactory bulb neurons.

4. Discussion

In the process of neurotoxic injury, the internalization of GluR2 in the postsynaptic membrane is enhanced, resulting in an increase in GluR2-deficient AMPA receptors (especially GluR1-containing AMPA receptors) on the membrane, thereby mediating the rapid influx of Ca^{2+} , triggering a series of neurotoxic cascades (Khatri et al., 2018; Nyuyki and Pittman, 2015; Riazzi et al., 2015; Su et al., 2015). Our study found that the expression of GluR1 surface protein in AR mice with OD was upregulated, and the expression of GluR2 surface protein was significantly decreased, which was related to neuronal apoptosis and olfactory damage caused by the inflammatory response. Consistent with this result, in transgenic mice with reduced GluR2 or unedited GluR2 in the forebrain and olfactory bulb, olfactory discrimination and learning capabilities were enhanced, but olfactory memory was impaired (Ma and Lowe, 2007; Shimshek et al., 2005). Upregulating the amount of GluR2-deficient AMPA receptors on dendritic synapses selectively increased Ca^{2+} entry (Wachowiak and Shipley, 2006). Therefore, the AMPA receptor may be an effective target to reverse the damage to olfactory bulb neurons.

Our previous research found that neuroinflammation induced by glial cells of the olfactory bulb, activates the upregulation of TLR4/NF- κ B signaling, TNF- α , IL-1 β and IL-6, which is an important mechanism of AR with OD, and the activation of microglia is more obvious (Lv et al., 2021). Numerous studies have shown that microglia release the proinflammatory cytokines TNF- α and IL-1 β to participate in the regulation of AMPAR trafficking (Beattie et al., 2002). In a cervical spinal cord contusion model, in vivo nanoinjection of TNF- α into the ventral horn of the spinal cord resulted in increased GluR1 and decreased GluR2 at extrasynaptic and synaptic plasma membrane sites (Ferguson et al., 2008). In subsequent studies, the expression of the GluR1 subunit was

increased in human NT2-N neurons exposed to TNF- α , leading to an increased susceptibility to kainate-induced necrosis (Yu et al., 2002). Similar findings were observed in hippocampal and lumbar motor neurons, in which TNF- α or IL-1 β increased the surface expression of GluR1-containing AMPA receptors, accompanied by a significant increase in AMPAR-mediated excitatory postsynaptic currents (Beattie et al., 2002; Han and Whelan, 2010). In addition, this research suggests that the microglia in the olfactory bulb of AR mice with OD significantly increased GluR2-deficient AMPA receptor expression on the membrane. Therefore, neuroinflammation induced by microglia facilitates the internalization of GluR2-containing AMPA receptors in the olfactory bulb, which may be a mechanism of AR with OD.

Dopamine D2 receptor exerts biological effects through the intracellular Gi/Go-protein coupled signal cascade. The activation of dopamine D2 receptor negatively regulates adenylate cyclase, which reduces the level of cAMP and hinders the phosphorylation or dephosphorylation of downstream signals. Dopamine D2 receptor inhibits the PKA/DARPP-32 cascade, thereby weakening the response of the AMPA receptor (Hakansson et al., 2006). Dopamine D2 receptor activation can promote the formation of direct protein-protein coupling between the intracellular domain of dopamine D2 receptor and N-ethylmaleimide sensitizing factor (NSF), downregulate the expression of AMPA receptor on the cell membrane, and weaken the excitotoxicity mediated by the AMPA receptor (Zou et al., 2005). Dopamine D2 receptor can also activate the phosphatidylinositol 3 kinase (PI3K) pathway to attenuate the AMPA receptor response (Zou et al., 2005). However, inhibition of PKA is unlikely to explain this effect of dopamine D2 receptor activation (Sun et al., 2005). In addition to adenylate cyclase, dopamine D2 receptor also affects many signaling molecules, including ion channels, mitogen-activated protein (MAP) kinase and phospholipase (Neve et al., 2004). This research suggests that a dopamine D2 receptor agonist reversed the increase of GluR2-deficient AMPA receptors on the membrane in vivo or in vitro, thereby improving the apoptosis of olfactory bulb neurons.

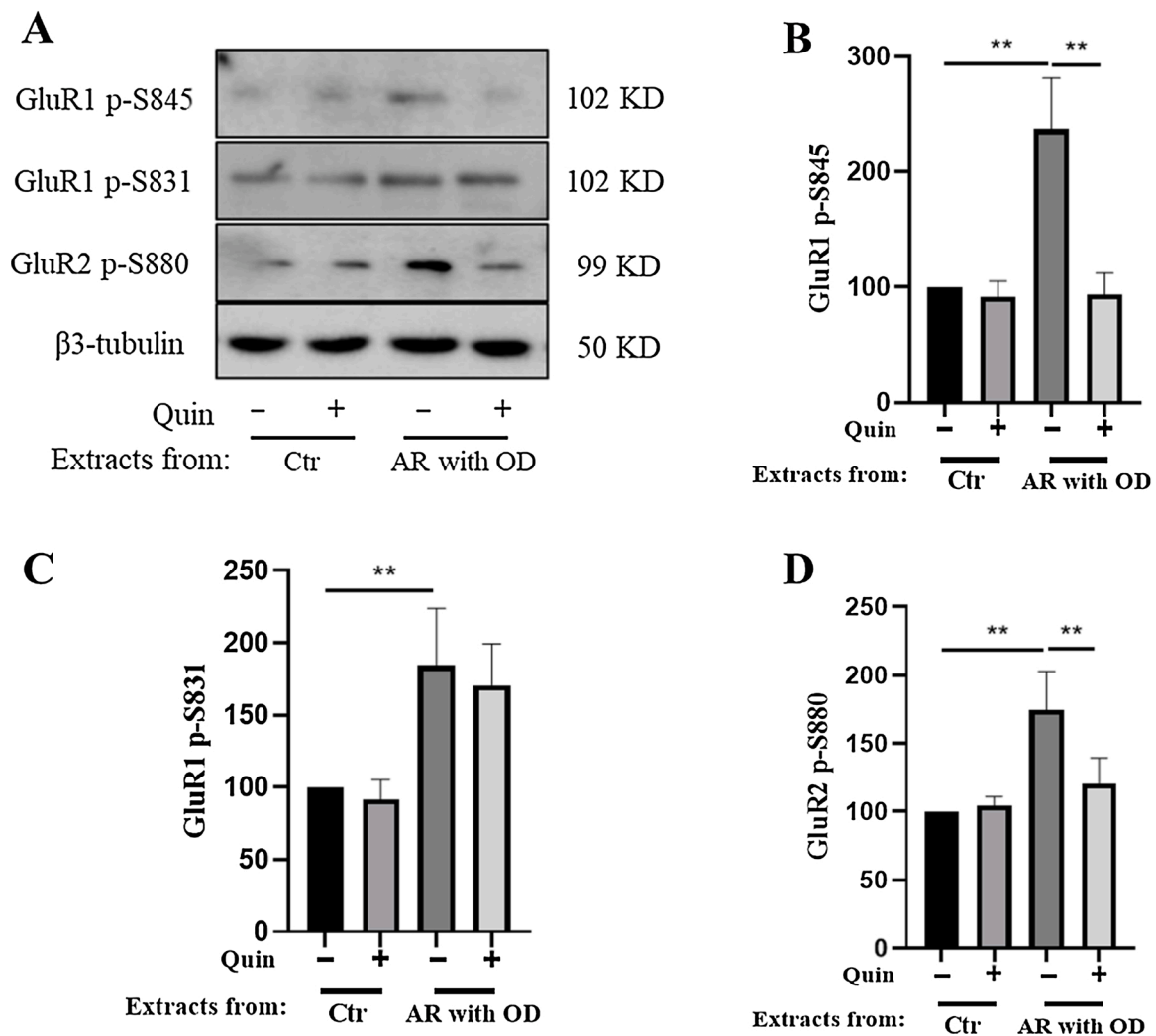


Fig. 7. Dopamine D2 receptor agonist inhibits phosphorylation at S831 and S880 sites of AMPA receptor in olfactory bulb neurons. (A–D) The coculture system of microglia and olfactory bulb neurons by treatment with Quin (20 μ mol/L), and the protein expression of GluR1 p-S845, GluR1 p-S831 and GluR2 p-S880 were detected by western blotting after 24 h. $n = 5$ for each group. * $P < 0.05$, ** $P < 0.01$. Ctr, the control group; AR with OD, the group of allergic rhinitis with olfactory dysfunction.

Protein phosphorylation plays a crucial role in the regulation of AMPA receptor trafficking. The externalization of GluR1 is mainly regulated by the phosphorylation of S845 and S831. Phosphorylation of the S845 site by PKA can increase the opening probability of the AMPA receptor, and also affect the number of GluR1-containing AMPA receptor on the membrane and change the expression of long-term potentiation (LTP) (Roche et al., 1996). PKC and CaMKII catalyze the phosphorylation of S831 of GluR1, thereby increasing the conductance of AMPA receptor channels (Hakansson et al., 2006). The internalization of GluR2 is mainly regulated by PKC phosphorylation of S880, which reduces the expression of GluR2 on the membrane (States et al., 2008). This research showed that dopamine D2 receptor agonist inhibited the AMPA receptor through S831 and S880, and had nothing to do with S831. Consistent with our results, overexpression of mutant GluR1 S831A in neurons did not affect the current of AMPA receptor in the basal state, nor does it affect the increase in AMPA receptor currents caused by CaMKII activation (Hayashi et al., 2000). Moreover, neither LTP nor long-term depression (LTD) were changed in the mutant GluR1 S831A knockout mice (Lee et al., 2010), all of which suggest that phosphorylation at the GluR1 S831 site does not play a role in AMPA receptor trafficking.

In conclusion, allergic rhinitis with olfactory dysfunction was closely associated with the internalization of the GluR2-containing AMPA

receptor, and accompanied apoptosis damage. Quinpirole, a dopamine D2 receptor agonist, may increase the expression of GluR2-containing AMPA receptors on the membrane, and alleviate the olfactory bulb damage caused by AR. Our findings suggest associations among dopamine D2 receptor, AMPA receptor and neuronal damage in the olfactory bulb, providing a novel target for the treatment of olfactory dysfunction induced by allergic rhinitis.

CRediT authorship contribution statement

Peiqiang Liu: Investigation, Formal analysis, Data curation, Writing – original draft. **Danxue Qin:** Methodology, Formal analysis, Data curation. **Hao Lv:** Methodology, Resources. **Wenjun Fan:** Methodology, Resources. **Zezhang Tao:** Conceptualization, Supervision. **Yu Xu:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflicts of interests.

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