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Dexmedetomidine Exerts an Anti-inflammatory Effect *via* α_2 Adrenoceptors to Prevent Lipopolysaccharide-induced Cognitive Decline in Mice

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Administration of lipopolysaccharide to young and middle-aged mice is associated with neuroinflammation and cognitive impairment
- Dexmedetomidine has been shown to decrease neuroinflammation in mice

What This Article Tells Us That Is New

- Administration of dexmedetomidine to mice treated with lipopolysaccharide decreased neuroinflammation and cognitive impairment in both young and aged mice
- The effects of dexmedetomidine on neuroinflammation and cognitive impairment in mice treated with lipopolysaccharide are likely mediated by α_2 adrenoceptor–mediated anti-inflammatory pathways

ABSTRACT

Background: Clinical studies have shown that dexmedetomidine ameliorates cognitive decline in both the postoperative and critical care settings. This study determined the mechanism(s) for the benefit provided by dexmedetomidine in a medical illness in mice induced by lipopolysaccharide.

Methods: Cognitive decline, peripheral and hippocampal inflammation, blood–brain barrier permeability, and inflammation resolution were assessed in male mice. Dexmedetomidine was administered in the presence of lipopolysaccharide and in combination with blockers. Cultured macrophages (RAW 264.7; BV-2) were exposed to lipopolysaccharide ± dexmedetomidine ± yohimbine; tumor necrosis factor α release into the medium and monocyte NFκB activity was determined.

Results: *In vivo*, lipopolysaccharide-induced cognitive decline and inflammation (mean ± SD) were reversed by dexmedetomidine (freezing time, 55.68 ± 12.31 *vs.* 35.40 ± 17.66%, $P = 0.0286$, n = 14; plasma interleukin [IL]-1β: 30.53 ± 9.53 *vs.* 75.68 ± 11.04 pg/ml, $P < 0.0001$; hippocampal IL-1β: 3.66 ± 1.88 *vs.* 28.73 ± 5.20 pg/mg, $P < 0.0001$; n = 8), which was prevented by α_2 adrenoceptor antagonists. Similar results were found in 12-month-old mice. Lipopolysaccharide also increased blood–brain barrier leakage, inflammation-resolution orchestrator, and proresolving and proinflammatory mediators; each lipopolysaccharide effect was attenuated by dexmedetomidine, and yohimbine prevented dexmedetomidine's attenuating effect. *In vitro*, lipopolysaccharide-induced tumor necrosis factor α release (RAW 264.7: 6,308.00 ± 213.60 *vs.* 7,767.00 ± 358.10 pg/ml, $P < 0.0001$; BV-2: 1,075.00 ± 40.41 *vs.* 1,280.00 ± 100.30 pg/ml, $P = 0.0003$) and NFκB–p65 activity (nuclear translocation [RAW 264.7: 1.23 ± 0.31 *vs.* 2.36 ± 0.23, $P = 0.0031$; BV-2: 1.08 ± 0.26 *vs.* 1.78 ± 0.14, $P = 0.0116$]; phosphorylation [RAW 264.7: 1.22 ± 0.40 *vs.* 1.94 ± 0.23, $P = 0.0493$; BV-2: 1.04 ± 0.36 *vs.* 2.04 ± 0.17, $P = 0.0025$]) were reversed by dexmedetomidine, which was prevented by yohimbine.

Conclusions: Preclinical studies suggest that the cognitive benefit provided by dexmedetomidine in mice administered lipopolysaccharide is mediated through α_2 adrenoceptor–mediated anti-inflammatory pathways.

(ANESTHESIOLOGY 2020; 133:393–407)

The National Academy of Medicine's (Washington, D.C.) treatise on cognitive aging states that "the most treasured ability is to stay sharp—to think clearly, remember accurately, and make decisions with careful thought."[1] Although the onset of cognitive decline from neurodegenerative conditions, such as Alzheimer's disease, is a major neurocognitive dysfunction concern in both patients and their caregivers,[2] apprehensions have recently escalated over hospital-acquired cognitive decline after either surgical interventions or bouts of severe acute medical illness requiring admission to critical care settings. In surgical patients, the advent of postoperative neurocognitive disorders,[3] encompassing conditions that span a spectrum from delirium to dementia, threatens both life and functional independence.[4] Also, in the setting of medical illness cognitive

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dysfunction, comprising the gamut from acute delirium to chronic dementia, has been observed[5]; sepsis-associated encephalopathy is the most prevalent cognitive disorder in the serious medical illness category.[6]

Because of improvements in perioperative and critical care, more severely ill surgical and medical patients are surviving, which may portend an epidemic of hospital-acquired cognitive decline. Successful interventional strategies to prevent, attenuate, and reverse hospital-acquired cognitive decline will require identification of potential therapeutic targets from a thorough understanding of the pathophysiologic processes that produce these forms of cognitive decline. Validated preclinical models have identified therapeutic targets that appear to differ depending on whether cognitive decline is initiated by a pathogen-associated molecular pattern, for example in sepsis, or from a damage-associated molecular pattern after the aseptic trauma of surgery.[7] Whether initiated by damage-associated molecular patterns or pathogen-associated molecular patterns, the engagement of the innate immune response is a pivotal pathophysiologic factor, and strategies to modulate or resolve the resulting inflammation, including neuroinflammation, are currently being contemplated.[8,9]

Dexmedetomidine, an α_2 adrenoceptor agonist that also has activity for the imidazoline receptor[10] as well as at the α_1 adrenergic receptor,[11] is efficacious in preventing delirium in the setting of severe medical illness[12] as well as in the postoperative setting,[13] although the latter has been disputed.[14] Recently, we reported on the mechanism for the purported efficacy of dexmedetomidine in a preclinical model of postoperative cognitive decline in which dexmedetomidine's imidazoline receptor–mediated vagomimetic action promoted the neural and humoral resolution of both damage-associated molecular pattern– and surgery-induced systemic and neuroinflammation.[15] Because signaling and cell types differ in pathogen-associated molecular pattern– and damage-associated molecular pattern–initiated inflammation,[16,17] we investigated the efficacy and possible mechanism(s) for dexmedetomidine's amelioration of lipopolysaccharide-associated cognitive decline in mice. Because of the additional risk that age may impose in this preclinical model,[18] we also investigated dexmedetomidine's beneficial action in lipopolysaccharide-associated cognitive decline in older adult mice. We posit that dexmedetomidine's ameliorative effect on lipopolysaccharide-associated cognitive decline is effected by an α_2 adrenoceptor–mediated anti-inflammatory action.

Materials and Methods

Animals

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco, and were performed in accordance with the National Institutes of Health guidelines. For all the experiments, 12- to 14-week male C57BL/6J mice (Jackson Laboratory, USA) were used. Twelve-month-old male C57BL/6J mice were also used for cognitive and inflammation assessment. The mice were group-housed (five per cage) with 12-h light/dark cycles in an air-conditioned environment. The mice were given *ad libitum* access to standard rodent chow and water. The animals were randomly allocated to groups before interventions. Behavioral tests were conducted between 9:00 AM and 3:00 PM, and all of other experiments were conducted between 9:00 AM and 5:00 PM. The method of euthanasia was carbon dioxide inhalation.

Lipopolysaccharide Treatment and Drug Administration

The control mice received 0.9% sterile saline. Lipopolysaccharides derived from *Escherichia coli* (O111:B4, Sigma–Aldrich, USA) were prepared in 0.9% sterile saline and administered intraperitoneally at 1 mg/kg to induce cognitive dysfunction, as we reported previously.[19] Dexmedetomidine (Sigma–Aldrich) was prepared in 0.9% sterile saline and administered intraperitoneally at 50 μg/kg every 2 h for three doses immediately after lipopolysaccharide administration, a dose based on our previous findings, which successfully attenuated systemic and neuroinflammatory response to damage-associated molecular pattern–induced cognitive decline.[15] Autoclaved mouse igloos and Enviro-Dri were provided to the mice that had received dexmedetomidine to maintain normal body temperature. Sterile hydrogels were provided to the animals for easy access to water after sedation (fig. 1).

Atipamezole (Antisedan, Zoetis, USA), an α_2 adrenergic and imidazoline receptor antagonist, was dissolved in 0.9% sterile saline and administered intraperitoneally at 3 mg/kg, a dose that effectively blocks the α_2 adrenergic and imidazole properties of dexmedetomidine.[15] Yohimbine (Sigma–Aldrich), an α_2 adrenergic receptor antagonist with no activity at the imidazoline receptor, was dissolved in 0.9% sterile saline and administered intraperitoneally at 1.5 mg/kg.[15] Prazosin (Selleckchem, USA), an α_1 adrenergic receptor antagonist, was dissolved in 5% dimethyl sulfoxide in saline and administered intraperitoneally at 1 mg/kg.[20] The assessors were blinded to the interventions that the reagents received.

Cognitive Assessment (Primary Outcome)

Trace fear conditioning was used to assess learning and memory in rodents as previously described.[15,21,22] Briefly, the trace fear conditioning paradigm composed of a training session that associates a conditional stimulus (tone) with an aversive, unconditional stimulus (foot shock) to introduce aversive memory and a contextual test session 3 days later to test the subjects' ability to recall the memory for the context in which the mice previously received the shock.

Training. A conditioning chamber equipped with an infrared video camera (Med Associates Inc., USA) was used throughout the training and testing sessions. The mice were

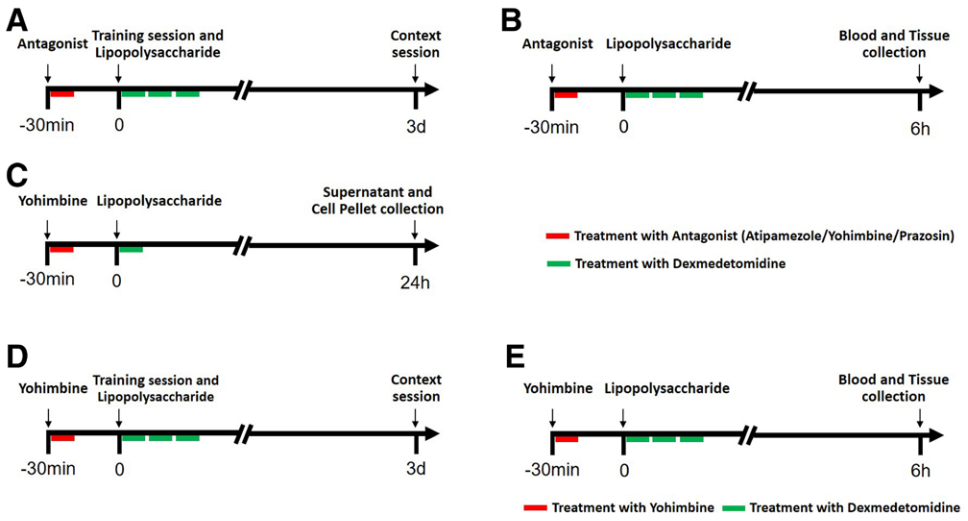

Fig. 1. Study design. (*A*) A cohort of 12- to 14-week-old mice were randomly allocated to 10 groups (n = 14/group) and were pretreated intraperitoneally with antagonists (atipamezole/yohimbine/prazosin). Thirty minutes later, mice were trained in the trace fear conditioning paradigm. After the training session, lipopolysaccharide or vehicle was administered intraperitoneally. Dexmedetomidine was administered every 2 h for a total of three doses. Three days after lipopolysaccharide, testing was performed in the trace fear conditioning. (*B*) Another cohort of 12- to 14-week-old mice were randomly allocated to 6 groups (n = 8/group) and were pretreated intraperitoneally with antagonists (atipamezole/yohimbine/prazosin), and 30 min later lipopolysaccharide was administered. Dexmedetomidine was administered every 2 h for a total of three times. Blood and tissue were collected 6 h later. (*C*) RAW 264.7 and BV-2 cells were allocated to 4 groups (n = 6/group) and were pretreated with yohimbine, and 30 min later were treated with dexmedetomidine and lipopolysaccharide. The supernatant and cell pellet were collected 24 h later. (*D*) A cohort of 12-month-old mice were randomly allocated to 4 groups (n = 12/group) for behavioral assessment and underwent the same procedures as for younger mice (those shown in *A*). (*E*) Another cohort of 12-month-old mice were randomly allocated to 4 groups (n = 8/group) for inflammation assessment and underwent the same procedures as for younger mice (those shown in *B*).

placed in the conditioning chamber for 100 s and followed by a tone (75 to 80 dB) for 20 s. After a further 20 s, an unconditional stimulus (a 2-s foot shock of 0.75 mA) was administered to introduce aversive memory. The procedure was repeated with an intertrial interval of 100 s.

Testing. The animals were reintroduced to the same context for testing 3 days after the training session. During the testing session, neither foot shock nor tone was delivered. The behavior of the animals was recorded and analyzed by the Video Freeze software (Med Associates Inc.). The duration of freezing behavior, indicated by the lack of movement, was calculated and subjected to statistical analysis.

Tissue Harvesting and Sample Preparation

Six hours after lipopolysaccharide administration, blood was collected from the inferior vena cava under terminal isoflurane anesthesia in heparin-coated syringes. Immediately after blood collection, the mice were perfused with ice-cold phosphate-buffered saline. Thereafter, the hippocampus or whole-brain, spleen, and kidney were rapidly dissected on an ice-cold Petri dish and stored at −80°C for subsequent analysis. Plasma was collected by centrifugation of the blood at 2,000 × g for 20 min at 4°C and stored at −80°C for later analysis.

Systemic Inflammatory Response (Secondary Outcome)

Plasma interleukin 1β was assayed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA) according to the manufacturer's instructions.

Hippocampal Inflammatory Response (Secondary Outcome)

The hippocampus was homogenized in cell lysis buffer (Cell Signaling Technology, USA) with protease and phosphatase inhibitors (Thermo Fisher Scientific, USA) and phenylmethanesulfonyl fluoride (Cell Signaling Technology). Protein concentration was measured with a Pierce BCA protein assay kit (Thermo Fisher Scientific). Interleukins 6 and 1β were assayed using commercially available ELISA kits (R&D Systems) according to the manufacturer's instructions.

Blood–Brain Barrier Leakage (Secondary Outcome)

Plasma proteins such as albumin are poorly transported across an intact blood–brain barrier. Blood–brain barrier leakage was assayed by albumin expression in the whole brain and hippocampus using immunoblotting as we reported previously.[15]

Circulating Leukotriene B4 and Lipoxin A4 (Secondary Outcome)

Plasma leukotriene B4 and lipoxin A4 were assayed using commercially available ELISA kits, according to the manufacturer's instructions (Biomatik, USA).

Macrophage Culture

The murine RAW 264.7 cell line (American Type Culture Collection, USA) and microglial BV-2 cell line (provided by Dr. Jonathan Pan from University of California, San Francisco) were used to study the effect of dexmedetomidine on macrophage and microglia function after stimulation with lipopolysaccharide. The cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin and grown at 37°C in a 5% CO_2 and 95% humidified incubator. For experiments, the cells were exposed to combinations of vehicle, lipopolysaccharide (100 ng/ml), dexmedetomidine (1 μM) + lipopolysaccharide, and yohimbine (50 μM) + dexmedetomidine + lipopolysaccharide. Yohimbine was administered 30 min before lipopolysaccharide, and dexmedetomidine was administered immediately after lipopolysaccharide as previously reported.[15,23] The supernatant and cell pellet were collected at 24 h after lipopolysaccharide exposure.

Cytokine Measurement (Secondary Outcome)

Tumor necrosis factor α in RAW 264.7 and BV-2 cell culture supernatant were assayed using commercially available ELISA kits (R&D Systems) according to the manufacturer's instructions.

Nuclear and Cytoplasmic Extraction (Secondary Outcome)

Cytoplasmic and nuclear extracts from RAW 264.7 and BV-2 cells were separated and collected respectively using a nuclear extraction kit (Novus Biologicals, USA) according to the manufacturer's instructions.

Western Blotting

The brain, hippocampus, spleen, kidney tissues, and cell pellet were lysed in radio immunoprecipitation assay lysis or extraction buffer (Thermo Fisher Scientific) containing protease and phosphatase inhibitors (Thermo Fisher Scientific) and phenylmethanesulfonyl fluoride (Cell Signaling Technology). The lysate was placed on ice for 15 min and centrifuged at 14,000 rpm for 15 min at 4°C. Protein concentration was measured by a Pierce BCA protein assay kit (Thermo Fisher Scientific).

Per lane, 20 μg of protein was loaded onto a 12% sodium dodecyl sulfate–polyacrylamide gel (Bio-Rad, USA) for electrophoresis, and the analytes were transferred onto a 0.2-μm nitrocellulose membrane (Bio-Rad). Thereafter, membranes were incubated with Odyssey blocking buffer (LICOR Biosciences, USA) for 1 h at room temperature and followed by an overnight incubation of primary antibodies: rabbit anti-albumin, netrin-1, glyceraldehyde-3-phosphate dehydrogenase, NFκB–p65, and histone H3 (Abcam, USA) and phospho–NFκB–p65 and β-Actin (Cell Signaling Technology) at 4°C. After the incubation of IRDye 800CW–labeled goat anti-rabbit IgG (LICOR Biosciences) for 1 h at room temperature, blots were captured with a LICOR Odyssey imaging system and analyzed by Image Studio software (LICOR Biosciences). Glyceraldehyde-3-phosphate dehydrogenase and β-Actin were separately used to normalize the intensities of the corresponding bands in total and cytoplasmic protein; histone H3 was used to normalize the intensities of the corresponding bands in nuclear protein.

Statistical Analysis

Statistical analyses were performed using the Prism 7.0 (GraphPad Software, USA), and the data are expressed as means ± SD. Normality was tested with the D'Agostino and Pearson normality test. A one-way (between-subjects) ANOVA was used for statistical comparisons followed by Tukey *post hoc* test. Statistical significance was set at $P < 0.05$. The sample size was estimated based on our previous experience.[15,21]

Results

For the *in vivo* experiment, n refers to the number of mice that were used. For the *in vitro* experiment, n refers to the number of cell-plated wells that were used.

Dexmedetomidine Prevents Lipopolysaccharide-induced Cognitive Decline through an α_2 Adrenoceptor-dependent Mechanism

Lipopolysaccharide significantly decreased freezing time (%) compared with the control group (35.40 ± 17.66% *vs.* 56.29 ± 14.65%, $P = 0.0209$; n = 14/group; fig. 2). Although dexmedetomidine alone was no different from the control group, administration of dexmedetomidine prevented lipopolysaccharide-induced cognitive decline (55.68 ± 12.31% *vs.* 35.40 ± 17.66%, $P = 0.0286$). Each of the two α_2 adrenoceptor antagonists, atipamezole and yohimbine with and without I receptor activity, respectively, prevented dexmedetomidine-induced reversal of lipopolysaccharide-induced cognitive decline (atipamezole: 33.83 ± 17.48% *vs.* 55.68 ± 12.31%, $P = 0.0124$; yohimbine: 33.56 ± 19.00% *vs.* 55.68 ± 12.31%, $P = 0.0106$). Prazosin, the α_1 adrenoceptor antagonist, did not prevent dexmedetomidine-induced reversal of lipopolysaccharide-induced cognitive decline. One of the mice in the yohimbine + lipopolysaccharide + dexmedetomidine group did not survive.

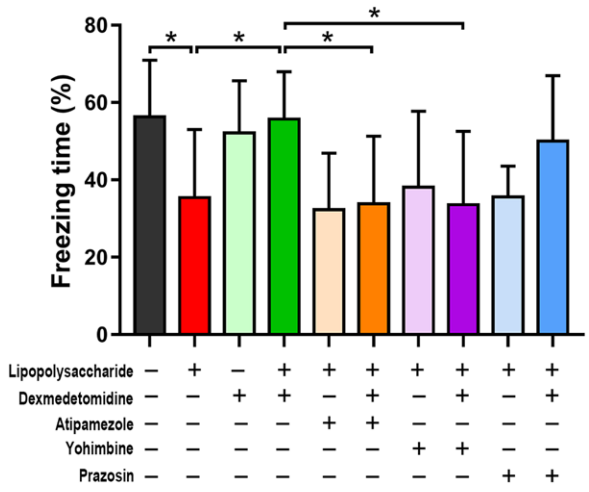

Fig. 2. Dexmedetomidine prevent lipopolysaccharide-induced decrease in freezing behavior in an α_2 receptor–dependent manner. Ten groups of randomly assigned mice (n = 14/group) were administered one of a series of antagonists (atipamezole/yohimbine/prazosin) before lipopolysaccharide and subjected to trace fear conditioning training with or without dexmedetomidine administration. Testing for freezing behavior in the same context was undertaken 3 days later. One of the mice in yohimbine + lipopolysaccharide + dexmedetomidine group did not survive. Freezing time data are expressed as means ± SD and were analyzed by one-way ANOVA and Tukey *post hoc* test. *$P < 0.05$ for comparisons shown.

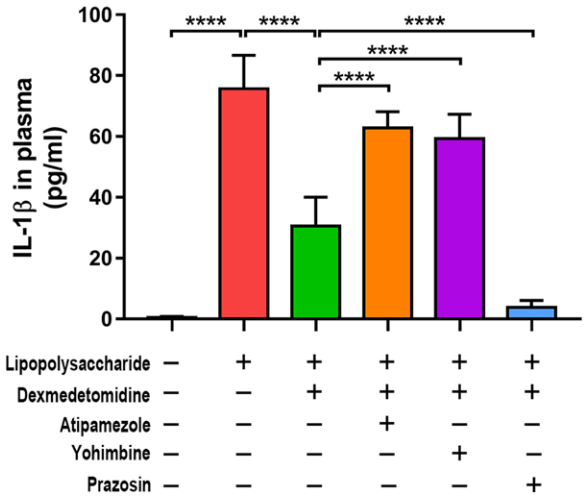

Fig. 3. Dexmedetomidine prevents lipopolysaccharide-induced peripheral inflammation in an α_2 receptor-dependent manner. Six groups of randomly assigned mice (n = 8/group) were administered saline vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, atipamezole + lipopolysaccharide + dexmedetomidine, yohimbine + lipopolysaccharide + dexmedetomidine, or prazosin + lipopolysaccharide + dexmedetomidine. Six hours after lipopolysaccharide, the mice were euthanized, and the blood was harvested and assayed by enzyme-linked immunosorbent assay for circulating interleukin (IL)–1β. The data are expressed as means ± SD and analyzed by one-way ANOVA and Tukey *post hoc* test. ****$P < 0.0001$ for comparisons shown.

Dexmedetomidine Prevents Lipopolysaccharide-induced Peripheral Inflammation through an α_2 Adrenoceptor-dependent Mechanism

Six hours after lipopolysaccharide injection, plasma interleukin 1β was significantly increased (75.68 ± 11.04 pg/ml *vs.* 0.54 ± 0.30 pg/ml, $P < 0.0001$; n = 8/group; fig. 3). Administration of dexmedetomidine reduced the plasma level of interleukin 1β (30.53 ± 9.53 pg/ml *vs.* 75.68 ± 11.04 pg/ml, $P < 0.0001$). Both of the α_2 adrenoceptor antagonists, atipamezole (which also has I receptor activity) and yohimbine, abolished dexmedetomidine's attenuation of the lipopolysaccharide-induced interleukin 1β upregulation (atipamezole: 62.76 ± 5.40 pg/ml *vs.* 30.53 ± 9.53 pg/ml, $P < 0.0001$; yohimbine: 59.25 ± 8.08 pg/ml *vs.* 30.53 ± 9.53 pg/ml, $P < 0.0001$). Conversely, the α_1 adrenoceptor antagonist, prazosin, did not block but promoted dexmedetomidine's anti-inflammatory effect on lipopolysaccharide-induced interleukin 1β upregulation (3.85 ± 2.25 pg/ml *vs.* 30.53 ± 9.53 pg/ml, $P < 0.0001$).

Dexmedetomidine Prevents Lipopolysaccharide-induced Hippocampal Inflammation through an α_2 Adrenoceptor-dependent Mechanism

Six hours after lipopolysaccharide injection, hippocampal interleukin 1β (fig. 4A) and interleukin 6 (fig. 4B) were significantly increased (interleukin 1β: 28.73 ± 5.20 pg/mg *vs.* 2.21 ± 0.93 pg/mg, $P < 0.0001$; interleukin 6: 23.46 ± 2.76 pg/mg *vs.* 1.00 ± 0.42 pg/mg, $P < 0.0001$; n = 8/group). Administration of dexmedetomidine reduced the hippocampal level of both proinflammatory cytokines (interleukin 1β: 3.66 ± 1.88 pg/mg *vs.* 28.73 ± 5.20 pg/mg, $P < 0.0001$; interleukin 6: 7.66 ± 3.33 pg/mg *vs.* 23.46 ± 2.76 pg/mg, $P < 0.0001$). Both of the two α_2 adrenoceptor antagonists, atipamezole and yohimbine, abolished dexmedetomidine's anti-inflammatory response of lipopolysaccharide-stimulated interleukin 1β (atipamezole: 24.93 ± 7.67 pg/mg *vs.* 3.66 ± 1.88 pg/mg, $P < 0.0001$; yohimbine: 20.68 ± 9.34 pg/mg *vs.* 3.66 ± 1.88 pg/mg, $P < 0.0001$) and interleukin 6 (atipamezole: 23.84 ± 5.70 pg/mg *vs.* 7.66 ± 3.33 pg/mg, $P < 0.0001$; yohimbine: 20.34 ± 7.31 pg/mg *vs.* 7.66 ± 3.33 pg/mg, $P < 0.0001$). The α_1 adrenoceptor antagonist, prazosin, did not abolish dexmedetomidine's anti-inflammatory effect on lipopolysaccharide-stimulated hippocampal proinflammatory cytokines.

Dexmedetomidine Reverses Lipopolysaccharide-induced Leakage of the Blood–Brain Barrier through an α_2 Adrenoceptor-dependent Mechanism

Accompanying the lipopolysaccharide-induced inflammation (figs. 3 and 4), the blood–brain barrier was

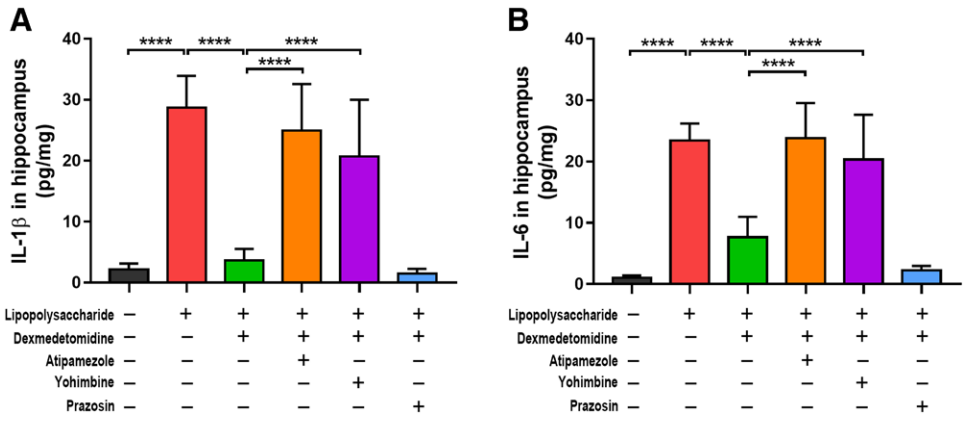

Fig. 4. Dexmedetomidine prevents lipopolysaccharide-induced hippocampal inflammation in an α_2 adrenoceptor-dependent manner. Six groups of randomly assigned mice (n = 8/group) were administered saline vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, atipamezole + lipopolysaccharide + dexmedetomidine, yohimbine + lipopolysaccharide + dexmedetomidine, or prazosin + lipopolysaccharide + dexmedetomidine. Six hours after lipopolysaccharide, the mice were euthanized, and the hippocampus was harvested and assayed by enzyme-linked immunosorbent assay for interleukin (IL)–1β (*A*) and IL-6 (*B*). Data are expressed as means ± SD and analyzed by one-way ANOVA and Tukey *post hoc* test. *****P* < 0.0001 for comparisons shown.

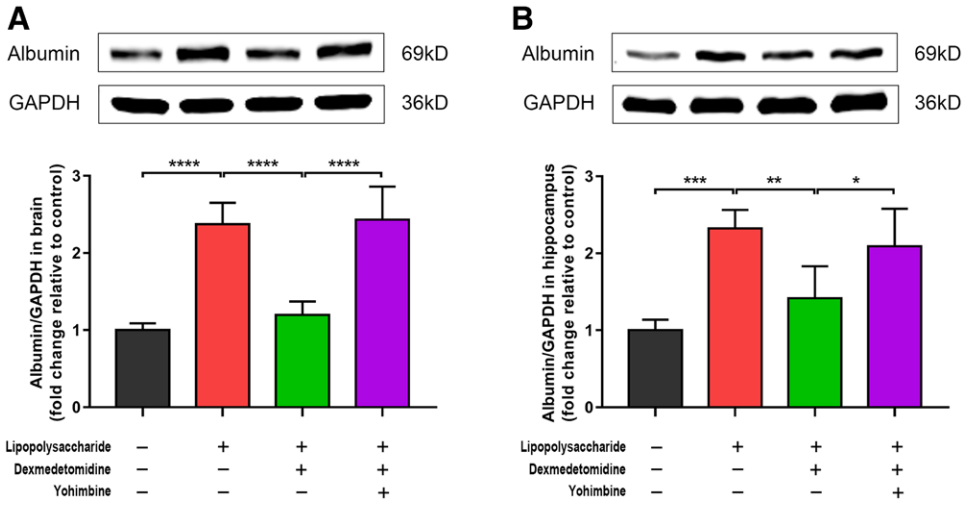

Fig. 5. Dexmedetomidine reverses lipopolysaccharide-induced leakage of blood–brain barrier. Four groups of randomly assigned mice (n = 5/group) were administered saline vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, or yohimbine + lipopolysaccharide + dexmedetomidine. Six hours later, mice were euthanized, and whole brain (*A*) and hippocampus (*B*) were harvested for expression of albumin by immunoblotting. Data are expressed as means ± SD relative to control and were analyzed by one-way ANOVA and Tukey *post hoc* test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001 for comparisons shown. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

disrupted, as evidenced by a significant upregulation of glyceraldehyde-3-phosphate dehydrogenase–normalized albumin expression in the brain (2.37 ± 0.28 *vs.* 1.00 ± 0.09, $P < 0.0001$; n = 5/group; fig. 5A) and hippocampus (2.32 ± 0.24 *vs.* 1.00 ± 0.14, $P = 0.0001$; n = 5/group; fig. 5B) assessed by immunoblotting. Administration of dexmedetomidine reversed lipopolysaccharide-induced upregulation of albumin expression (brain: 1.20 ± 0.18 *vs.* 2.37 ± 0.28, $P < 0.0001$; hippocampus: 1.42 ± 0.41 *vs.* 2.32 ± 0.24, $P = 0.0041$), and this reversal was prevented by the non-imidazoline α_2 adrenoceptor antagonist, yohimbine (brain: 2.44 ± 0.43 *vs.* 1.20 ± 0.18, $P < 0.0001$; hippocampus: 2.09 ± 0.49 *vs.* 1.42 ± 0.41, $P = 0.0341$).

Dexmedetomidine Reverses Lipopolysaccharide-induced Upregulation of Netrin-1 Expression

Accompanying the peripheral and hippocampal inflammation induced by lipopolysaccharide (figs. 3 and 4), glyceraldehyde-3-phosphate dehydrogenase–normalized netrin-1 expression was significantly increased in the spleen (2.49 ± 0.21 *vs.* 1.00 ± 0.12, $P < 0.0001$; n = 5/group; fig. 6A) and kidney (2.91 ± 0.45 *vs.* 1.00 ± 0.12, $P < 0.0001$; n = 5/group; fig. 6B), two organs that are vagally innervated. Administration of dexmedetomidine reversed lipopolysaccharide-induced netrin-1 upregulation (spleen: 1.12 ± 0.23 *vs.* 2.49 ± 0.21, $P < 0.0001$; kidney: 1.74 ± 0.40 *vs.* 2.91 ± 0.45, $P = 0.0007$). This reversal was prevented by α_2 adrenoceptor antagonist, yohimbine (spleen: 2.37 ± 0.26 *vs.* 1.12 ± 0.23, $P < 0.0001$; kidney: 2.67 ± 0.42 *vs.* 1.74 ± 0.40, $P = 0.0052$).

Dexmedetomidine Reverses Lipopolysaccharide-induced Upregulation of Circulating Leukotriene B4 and Lipoxin A4 Expression

Interestingly, both of the proinflammatory lipid mediators, leukotriene B4 (532.20 ± 128.70 pg/ml *vs.* 24.44 ± 4.47 pg/ml, $P < 0.0001$; n = 8/group; fig. 7A), and the specific proresolving lipid mediator, lipoxin A4 (0.05 ± 0.05 ng/ml *vs.* 23.63 ± 2.14 ng/ml, $P < 0.0001$; n = 8/group; fig. 7B) in the peripheral circulation, were significantly increased by lipopolysaccharide. Dexmedetomidine reversed lipopolysaccharide-induced upregulation of each of leukotriene B4 (367.10 ± 105.60 pg/ml *vs.* 532.20 ± 128.70 pg/ml, $P = 0.0400$) and lipoxin A4 (12.34 ± 3.88 ng/ml *vs.* 23.63 ± 2.14 ng/ml, $P < 0.0001$). This reversal was abolished by both α_2 adrenoceptor antagonists, atipamezole (leukotriene B4: 552.20 ± 110.50 pg/ml *vs.* 367.10 ± 105.60 pg/ml, $P = 0.0153$; lipoxin A4: 21.35 ± 2.97 ng/ml *vs.* 12.34 ± 3.88 ng/ml, $P < 0.0001$) and yohimbine (leukotriene B4: 539.70 ± 120.90 pg/ml *vs.* 367.10 ± 105.60 pg/ml, $P = 0.0281$; lipoxin A4: 16.51 ± 2.20 ng/ml *vs.* 12.34 ± 3.88 ng/ml, $P = 0.0191$). Prazosin, the α_1 adrenoceptor antagonist, did not reverse dexmedetomidine's suppression of either leukotriene B4 or of lipoxin A4.

Dexmedetomidine Prevents Lipopolysaccharide-induced Cytokine Release through an α_2 Receptor-dependent Mechanism

Corroborating the *in vivo* lipopolysaccharide-induced systemic and hippocampal inflammation, cumulative *in vitro* tumor necrosis factor α release from both RAW 264.7 (fig. 8A) and BV-2 (fig. 8B) cell culture supernatant was significantly increased 24 h after lipopolysaccharide exposure (RAW 264.7: 7,767.00 ± 358.10 pg/ml *vs.* 67.40 ± 6.33 pg/ml, $P < 0.0001$; BV-2: 1,280.00 ± 100.30 pg/ml *vs.* 12.82 ± 1.36 pg/ml, $P < 0.0001$; n = 6/group). Dexmedetomidine reduced the tumor necrosis factor α release in both cell types (RAW 264.7: 6,308.00 ± 213.6 pg/ml *vs.* 7,767.00 ± 358.10 pg/ml, $P < 0.0001$; BV-2: 1,075.00 ± 40.41 pg/ml *vs.* 1,280.00 ± 100.30 pg/ml, $P = 0.0003$). The α_2 adrenoceptor antagonist, yohimbine, reversed dexmedetomidine's suppression of lipopolysaccharide-induced tumor necrosis factor α release (RAW 264.7: 7,424.00 ± 759.40 pg/ml *vs.* 6,308.00 ±

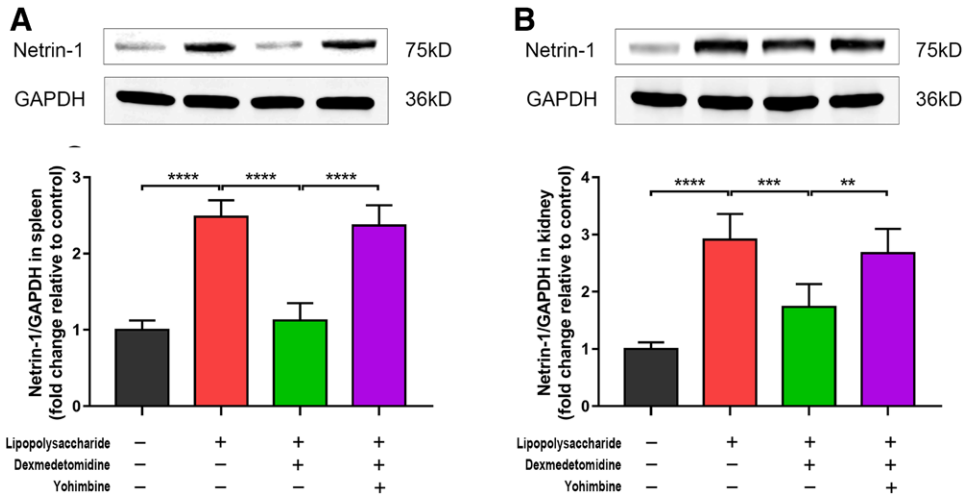

Fig. 6. Dexmedetomidine reverses lipopolysaccharide-induced upregulation of netrin-1 expression. Four groups of randomly assigned mice (n = 5/group) were administered saline vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, or yohimbine + lipopolysaccharide + dexmedetomidine. Six hours later, the mice were euthanized, and the spleen (*A*) and kidney (*B*) were harvested for expression of netrin-1 by immunoblotting. The data are expressed as means ± SD relative to control and were analyzed by one-way ANOVA and Tukey *post hoc* test. **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ for comparisons shown. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

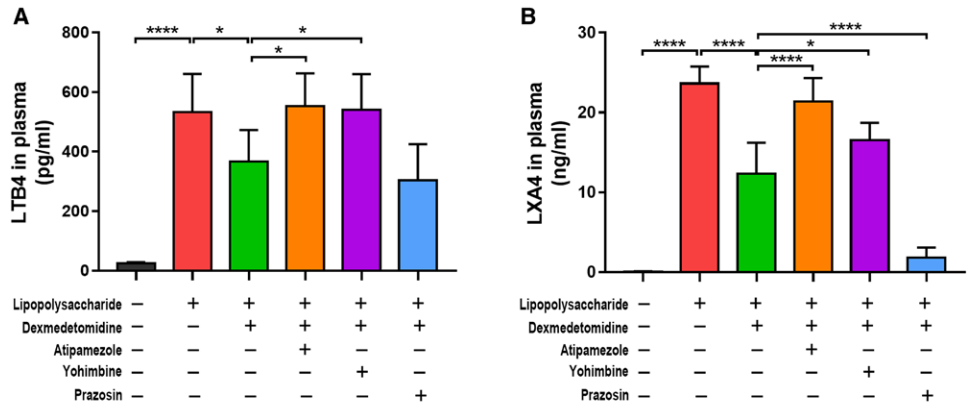

Fig. 7. Dexmedetomidine downregulates the circulating proinflammatory mediator leukotriene B4 (LTB4) and the circulating proresolving mediator lipoxin A4 (LXA4). Six groups of randomly assigned mice (n = 8/group) were administered saline vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, atipamezole + lipopolysaccharide + dexmedetomidine, yohimbine + lipopolysaccharide + dexmedetomidine, or prazosin + lipopolysaccharide + dexmedetomidine. Six hours later, the mice were euthanized, and the blood was harvested and assayed by enzyme-linked immunosorbent assay for plasma leukotriene B4 (*A*) and lipoxin A4 (*B*). The data are expressed as means ± SD and were analyzed by one-way ANOVA and Tukey *post hoc* test. *$P < 0.05$; ****$P < 0.0001$ for comparisons shown.

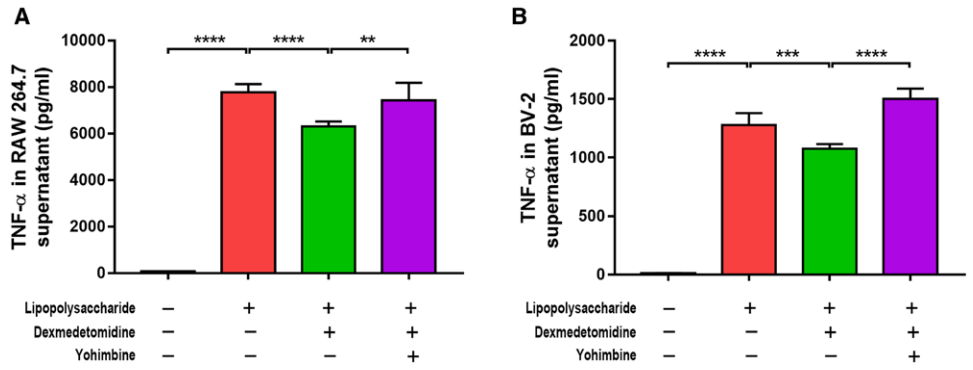

Fig. 8. Dexmedetomidine prevents lipopolysaccharide-induced tumor necrosis factor (TNF)–α release in an α_2 receptor–dependent manner. Four groups of randomly assigned cells (n = 6/group) were exposed to medium (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, or yohimbine + lipopolysaccharide + dexmedetomidine. Twenty-four hours after lipopolysaccharide, RAW 264.7 (*A*) and BV-2 (*B*) cell culture supernatant were collected and assayed by enzyme-linked immunosorbent assay for TNF-α. The data are expressed as means ± SD and were analyzed by one-way ANOVA and Tukey *post hoc* test. **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ for comparisons shown.

213.60 pg/ml, $P = 0.0013$; BV-2: 1,501.00 ± 90.18 pg/ml *vs.* 1,075.00 ± 40.41 pg/ml, $P < 0.0001$).

Dexmedetomidine Reverses Lipopolysaccharide-induced NFκB–p65 Phosphorylation and Nuclear Translocation of RAW 264.7 and BV-2 Cells

In correspondence with lipopolysaccharide-induced tumor necrosis factor α release in cell culture supernatant, NFκB–p65 nuclear translocation in both RAW 264.7 (fig. 9A) and BV-2 (fig. 9B) cells was significantly increased 24 h after lipopolysaccharide exposure (RAW 264.7: 2.36 ± 0.23 *vs.* 1.00 ± 0.05, $P = 0.0007$; BV-2: 1.78 ± 0.14 *vs.* 1.00 ± 0.05, $P = 0.0055$; n = 4/group). Dexmedetomidine reduced the NFκB–p65 nuclear translocation in both cell types (RAW 264.7: 1.23 ± 0.31 *vs.* 2.36 ± 0.23, $P = 0.0031$; BV-2: 1.08 ± 0.26 *vs.* 1.78 ± 0.14, $P = 0.0116$). The α_2 adrenoceptor antagonist, yohimbine, abolished dexmedetomidine's anti-inflammatory response of NFκB–p65 (RAW 264.7: 2.11 ± 0.59 *vs.* 1.23 ± 0.31, $P = 0.0181$; BV-2: 1.68 ± 0.43 *vs.* 1.08 ± 0.26, $P = 0.0319$).

Similarly, NFκB–p65 phosphorylation in both RAW 264.7 (fig. 10A) and BV-2 (fig. 10B) cells was significantly

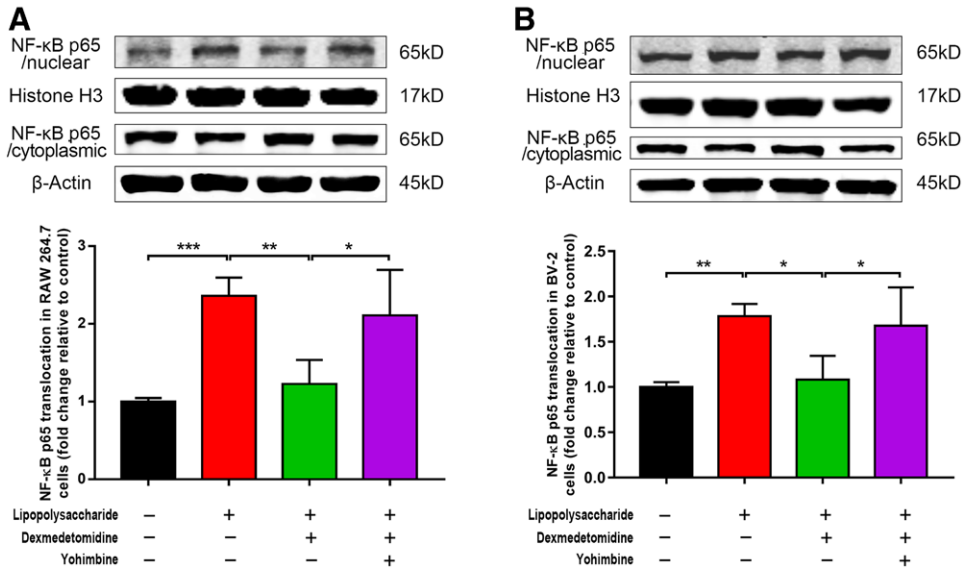

Fig. 9. Dexmedetomidine reverses lipopolysaccharide-induced NFκB–p65 nuclear translocation in an α_2 receptor–dependent manner. Four groups of randomly assigned cells (n = 4/group) were treated medium vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, or yohimbine + lipopolysaccharide + dexmedetomidine. Twenty-four hours later, RAW 264.7 (*A*) and BV-2 (*B*) cell pellet were collected respectively, and the cytoplasmic and nuclear extracts were obtained separately for expression of NFκB–p65 nuclear translocation by immunoblotting. The data are expressed as means ± SD relative to control and were analyzed by one-way ANOVA and Tukey *post hoc* test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ for comparisons shown.

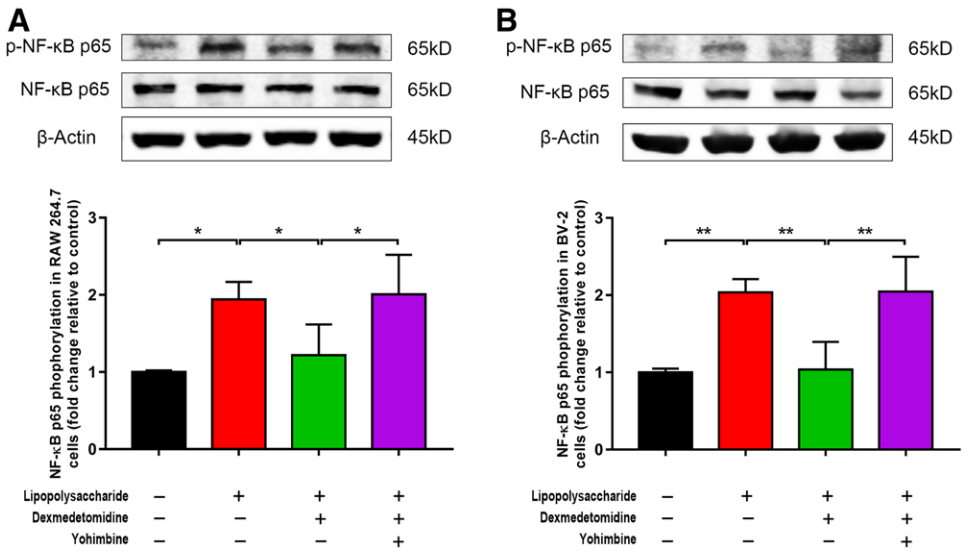

Fig. 10. Dexmedetomidine reverses lipopolysaccharide-induced NFκB–p65 phosphorylation in an α_2 receptor–dependent manner. Four groups of randomly assigned cells (n = 4/group) were treated medium vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, or yohimbine + lipopolysaccharide + dexmedetomidine. Twenty-four hours later, RAW 264.7 (*A*) and BV-2 (*B*) cell pellet were collected respectively, and the cell lysates were obtained for expression of NFκB–p65 phosphorylation by immunoblotting. The data are expressed as means ± SD relative to control and were analyzed by one-way ANOVA and Tukey *post hoc* test. *$P < 0.05$; **$P < 0.01$ for comparisons shown.

increased 24 h after lipopolysaccharide exposure (RAW 264.7: 1.94 ± 0.23 *vs.* 1.00 ± 0.02, $P = 0.0103$; BV-2: 2.04 ± 0.17 *vs.* 1.00 ± 0.05, $P = 0.0019$; n = 4/group). Dexmedetomidine reduced the NFκB–p65 phosphorylation in both cell types (RAW 264.7: 1.22 ± 0.40 *vs.* 1.94 ± 0.23, $P = 0.0493$; BV-2: 1.04 ± 0.36 *vs.* 2.04 ± 0.17, $P = 0.0025$). The α_2 adrenoceptor antagonist, yohimbine, abolished dexmedetomidine's anti-inflammatory response of NFκB–p65 (RAW 264.7: 2.01 ± 0.51 *vs.* 1.22 ± 0.40, $P = 0.0313$; BV-2: 2.05 ± 0.45 *vs.* 1.04 ± 0.36, $P = 0.0023$).

Dexmedetomidine Prevents Lipopolysaccharide-induced Cognitive Decline through an α_2 Adrenoceptor-dependent Mechanism in Older Mice Too

In 12-month-old mice, lipopolysaccharide significantly decreased freezing time (%) compared with the control group (40.86 ± 14.11% *vs.* 60.64 ± 15.78%, $P = 0.0028$; n = 12/group; fig. 11). Administration of dexmedetomidine prevented lipopolysaccharide-induced cognitive decline (60.38 ± 11.08% *vs.* 40.86 ± 14.11%, $P = 0.0032$). The α_2 adrenoceptor antagonist, yohimbine, prevented dexmedetomidine-induced reversal of lipopolysaccharide-induced cognitive decline (39.65 ± 9.79% *vs.* 60.38 ± 11.08%, $P = 0.0016$).

Dexmedetomidine Prevents Lipopolysaccharide-induced Peripheral Inflammation through an α_2 Adrenoceptor-dependent Mechanism in Older Mice

In 12-month-old mice, 6 h after lipopolysaccharide injection, plasma interleukin 1β was significantly increased (49.28 ± 12.10 pg/ml *vs.* 1.56 ± 0.72 pg/ml, $P < 0.0001$; n = 8/group; fig. 12). Administration of dexmedetomidine reduced the plasma level of interleukin 1β (18.54 ± 4.84 pg/ml *vs.* 49.28 ± 12.10 pg/ml, $P < 0.0001$. The α_2 adrenoceptor antagonist, yohimbine, abolished dexmedetomidine's attenuation of the lipopolysaccharide-induced interleukin 1β upregulation (36.56 ± 14.90 pg/ml *vs.* 18.54 ± 4.84 pg/ml, $P = 0.0057$).

Dexmedetomidine Prevents Lipopolysaccharide-induced Hippocampal Inflammation through an α_2 Adrenoceptor-dependent Mechanism in Older Mice

In 12-month-old mice, 6 h after lipopolysaccharide injection, hippocampal interleukin 1β was significantly increased (115.70 ± 41.27 pg/mg *vs.* 0.96 ± 0.43 pg/mg, $P < 0.0001$; n = 8/group; fig. 13). Administration of dexmedetomidine reduced the hippocampal level of interleukin 1β (25.31 ± 18.43 pg/mg *vs.* 115.70 ± 41.27 pg/mg, $P < 0.0001$). The α_2 adrenoceptor antagonist, yohimbine, abolished dexmedetomidine's anti-inflammatory response

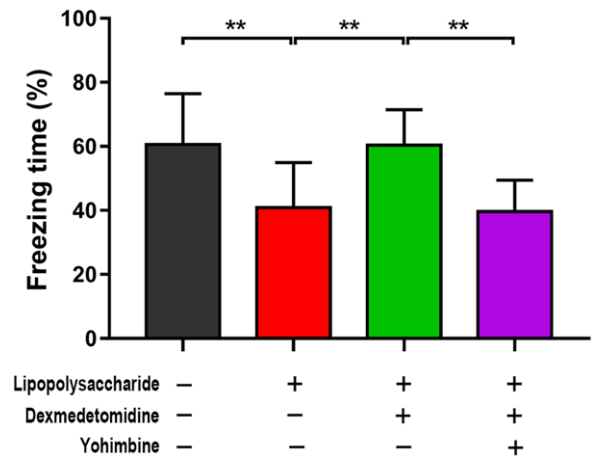

Fig. 11. Dexmedetomidine prevent lipopolysaccharide-induced decrease in freezing behavior in an α_2 receptor–dependent manner in older mice. Four groups of randomly assigned 12-month-old mice (n = 12/group) were administered saline vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, or yohimbine + lipopolysaccharide + dexmedetomidine and subjected to trace fear conditioning training. Testing for freezing behavior in the same context was undertaken 3 days later. Freezing time data are expressed as means ± SD and were analyzed by one-way ANOVA and Tukey *post hoc* test. $^{**}P < 0.01$ for comparisons shown.

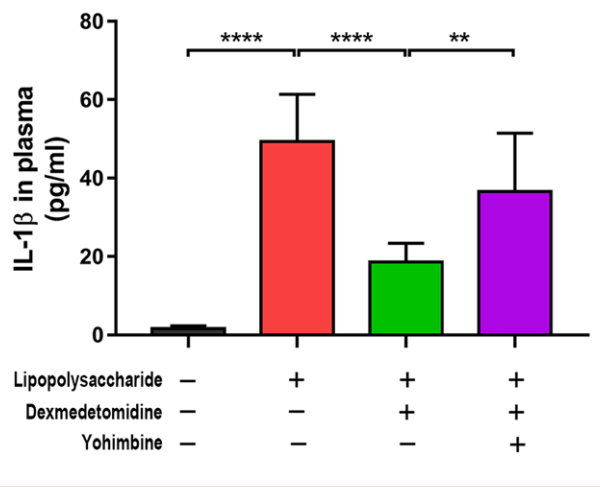

Fig. 12. Dexmedetomidine prevents lipopolysaccharide-induced peripheral inflammation in an α_2 receptor–dependent manner in older mice. Four groups of randomly assigned 12-month-old mice (n = 8/group) were administered saline vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, or yohimbine + lipopolysaccharide + dexmedetomidine. Six hours after lipopolysaccharide, the mice were euthanized, and the blood was harvested and assayed by enzyme-linked immunosorbent assay for circulating IL-1β. The data are expressed as means ± SD and analyzed by one-way ANOVA and Tukey *post hoc* test. $^{**}P < 0.01$; $^{****}P < 0.0001$ for comparisons shown. IL, interleukin.

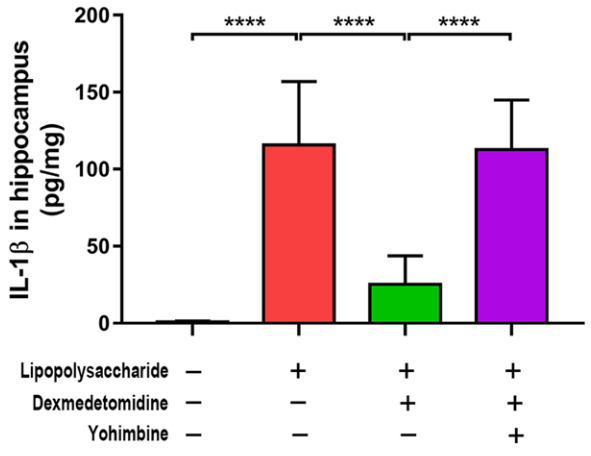

Fig. 13. Dexmedetomidine prevents lipopolysaccharide-induced hippocampal inflammation in an α_2 adrenoceptor–dependent manner in older mice. Four groups of randomly assigned 12-month-old mice (n = 8/group) were administered saline vehicle (control), lipopolysaccharide, lipopolysaccharide + dexmedetomidine, or yohimbine + lipopolysaccharide + dexmedetomidine. Six hours after lipopolysaccharide, the mice were euthanized, and the hippocampus was harvested and assayed by enzyme-linked immunosorbent assay for IL-1β. The data are expressed as means ± SD and analyzed by one-way ANOVA and Tukey *post hoc* test. ****$P < 0.0001$ for comparisons shown. IL, interleukin.

of lipopolysaccharide-stimulated interleukin 1β (112.70 ± 32.20 pg/mg *vs.* 25.31 ± 18.43 pg/mg, $P < 0.0001$).

Discussion

Recapitulation of Principal Findings

In 12- to 14-week-old mice, dexmedetomidine, an α_2 adrenoceptor agonist, prevented cognitive decline (fig. 2) after intraperitoneally administered lipopolysaccharide; associated with the improvement in cognitive function, there was a reduction in both systemic- (fig. 3) and neuroinflammation (fig. 4). Lipopolysaccharide-induced disruption of the blood–brain barrier was attenuated by dexmedetomidine (fig. 5). Additionally, elements of inflammation-resolution pathway, including lipopolysaccharide-induced upregulation of netrin-1 (fig. 6), lipoxin A4, and leukotriene B4 (fig. 7), were each attenuated by dexmedetomidine. Additionally, we have explored the effects of dexmedetomidine and the non-imidazole α_2 adrenoceptor antagonist after lipopolysaccharide was administered to older (12 months old) mice. Here too, dexmedetomidine prevented lipopolysaccharide-induced cognitive decline (fig. 11), systemic inflammation (fig. 12), and neuroinflammation (fig. 13) through an α_2 adrenoceptor mechanism. In cultured monocytes representing either tissue macrophages (RAW 264.7) or microglia (BV-2), lipopolysaccharide-induced (1) cytokine release (fig. 8) and (2) activation of NFκB (figs. 9 and 10), the transcription factor regulating proinflammatory cytokine synthesis and release, were inhibited by dexmedetomidine. Dexmedetomidine attenuated each proinflammatory response (figs. 2–13) by activating α_2 adrenoceptors with no involvement of imidazoline receptors.

Animal Models of Sepsis

Several preclinical models of the dysregulated response to infection have been investigated to identify modifiable pathophysiologic targets of sepsis; however, none fully captures the complex biology of the clinical condition of sepsis.[24] lipopolysaccharide binds to Toll-like receptor 4, a pattern recognition receptor on many cell types including immunocytes, to initiate the NFκB-mediated inflammation that accompanies endotoxemia.[25] However, lipopolysaccharide is a single component of the complex pathogen-associated molecular patterns derived from Gram-negative bacteria, and experiments with lipopolysaccharide do not provide information of either sepsis from polymicrobial or Gram-positive organisms. The peak inflammatory response to lipopolysaccharide occurs earlier with faster resolution than is typically seen in human sepsis.[26] Although more physiologically relevant models such as the cecal ligation and puncture model are widely used, it still has limitations of animal mortality variance, surgical outcome heterogeneity, and individual differences. Mindful of these experimental considerations, we opted to use lipopolysaccharide because of our previous experience with this reagent.[19] Although severe sepsis occurs most commonly at the extremes of life, it can also occur in young and middle-aged adults.[27] Therefore, we used both young and middle-aged mice in this study.

Properties of Dexmedetomidine That May Benefit Cognitive Decline

The pleiotropic properties of dexmedetomidine are predicated on ubiquitously distributed α_2 adrenoceptors that mediate several responses that can change outcome in encephalopathic conditions that accompany serious medical illness.[28] Inflammation is pivotally involved in many neuropsychiatric disorders, including the cognitive decline accompanying sepsis[29] and after surgery.[30] Because α_2 adrenoceptors are present on circulating and tissue immunocytes,[31] dexmedetomidine affects the innate immune response to stressors.[32] Furthermore, because of the imidazole-ringed structure of dexmedetomidine, it has activity at the imidazoline receptor[33] capable of mediating a cholinergic-induced resolution of the innate immune response.[15,34] Dexmedetomidine is a neuroprotectant both clinically[35] and preclinically[36]; therefore, dexmedetomidine may mitigate cognitive decline through its neuroprotective properties.

Use of Dexmedetomidine for Cognitive Decline Associated with Medical Illness

Delirium-reducing effects of dexmedetomidine in the setting of severe medical illness have been reported.[12,37] Further, dexmedetomidine may confer a mortality benefit for medical intensive care unit patients who have sepsis.[38]

Others have reported on the neurologic benefits of dexmedetomidine *versus* lipopolysaccharide. Rats exposed to lipopolysaccharide (10 mg/kg) developed astrocytic toxicity and neuroinflammation that was ameliorated by dexmedetomidine, although the receptor mechanism mediating this effect was not definitively established.[39] Neuroinflammation, NFκB upregulation, and learning disability (Y-maze test) in rats administered 0.1 mg/kg of lipopolysaccharide was prevented by pretreatment with dexmedetomidine, although the mediating receptor mechanism was not probed.[40] Mice administered 0.33 mg/kg lipopolysaccharide developed neuroinflammation and sickness behavior (anorexia, social withdrawal) that was prevented by dexmedetomidine, although neither the receptor nor the molecular mechanisms were defined.[41] Rats administered 1 mg/kg of lipopolysaccharide developed neuroinflammation and upregulation of several microRNAs that were attenuated by pretreatment with dexmedetomidine; the mechanism was not defined.[42] The neuroapoptosis and neuroinflammation induced by 10 mg/kg lipopolysaccharide in mice was prevented by pretreatment of dexmedetomidine by upregulating antiapoptotic (Bcl2) and downregulating proapoptotic (Bax) factors, although the receptor mechanism was not addressed.[43]

The post–α_2 adrenoceptor signaling pathway for dexmedetomidine's attenuation of the Toll-like receptor 4–regulated NFκB activity has not been identified in neurologic injury settings; however, in ventilator-induced lung injury and ischemic-reperfusion injury in both the liver and the heart, dexmedetomidine's activation of α_2 adrenoceptors downregulates Toll-like receptor 4 expression.

Limitations and Anomalies

There are several limitations of this study. First, our model of severe medical illness involved the administration of lipopolysaccharide, which causes a rapid onset of an acute illness that typically resolves at doses of up to 1 mg/kg in rodents. However, physiologic derangements, such as a hyperdynamic circulatory state and/or hypovolemia, that accompany sepsis/septic shock in patients are not seen in this lipopolysaccharide model. Second, in this initial investigation, we have only studied an early time point (6 h) at which to define the putative mechanisms for the cognitive improvement (3 days) that was noted. This time point was selected because this is the peak of the inflammatory response.[26] However, the timing of the cognitive assessment (3 days) does not capture a possible long-lived cognitive decline. Third, the inhibitory effect of dexmedetomidine on lipopolysaccharide-upregulated NFκB activity (figs. 9 and 10) and proinflammatory cytokine release (fig. 8) was studied *in vitro*. However, we feel justified in using these cell types because they reflect the sources for the peripheral (RAW 264.7 cells)[17] and central (BV-2 cells)[22] proinflammatory cytokines that are noted *in vivo*. Fourth, sepsis is most common at extremes of life. Although we have included both young (12 weeks old) and middle-aged (12 months old) mice, these studies do not capture age-related changes that are present at the extremes of age. Therefore, it remains to be tested whether the ameliorative effects of dexmedetomidine in this model are as prominent in very young and old mice. Also, studies were confined to male mice and may not necessarily be extrapolatable to female mice. Finally, lipopolysaccharide does not replicate the sepsis that occurs in humans.

Regarding anomalous findings, dexmedetomidine's inhibitory effect on lipopolysaccharide-induced upregulation of netrin-1 (fig. 6) and of both the proresolving lipid mediator lipoxin A4 and the proinflammatory mediator leukotriene B4 (fig. 7) cannot be reconciled with our current understanding of the roles that these mediators play in inflammation. Netrin-1 is at the juncture between the neural and humoral inflammation resolution pathways[44]; an increase in vagal activity will upregulate netrin-1, which in turn increases lipoxin A4, the humoral resolution mediator, and decreases leukotriene B4 because they are biotransformed from the same precursor, leukotriene A4.[45] For example, in the setting of postoperative cognitive decline, we observed that dexmedetomidine enhanced netrin-1 through its imidazoline receptor–mediated vagotonic action and that this was associated with the observed increase in lipoxin A4 and decrease in leukotriene B4.[15] We speculate that at this early time point (6 h), the resolution pathways have not fully adapted to the profound inflammatory response to lipopolysaccharide, resulting in this anomalous finding. Another apparent anomalous finding concerns the results obtained with prazosin. Because prazosin, a selective blocker of the α_1 adrenoceptor, did not reverse dexmedetomidine's attenuation of lipopolysaccharide-induced inflammation and cognitive decline, we can conclude that dexmedetomidine's benefit is not mediated by α_1 adrenoceptor. However, administration of prazosin resulted in an *enhancement* of dexmedetomidine's ameliorative action on lipopolysaccharide-induced peripheral inflammation (fig. 3). The most likely explanation is that α_1 adrenoceptor stimulation functionally antagonizes α_2 adrenoceptor stimulation and that by removing any dexmedetomidine stimulation of the α_1 adrenoceptor with prazosin, there will be enhancement of dexmedetomidine's α_2 receptor–mediated properties as we reported earlier.[11,46]

Conclusions

The data collected at an early time point after lipopolysaccharide administration demonstrate that dexmedetomidine's

efficacy in preventing lipopolysaccharide-associated cognitive decline is due to suppression of the inflammatory response by inhibiting NFκB through a pathway mediated by α_2 adrenoceptors. The mechanisms involved in achieving a favorable cognitive outcome with dexmedetomidine appear to differ depending on whether the context is postoperative cognitive decline (imidazoline receptor–mediated resolution of inflammation)[15] or lipopolysaccharide-induced illness (α_2 adrenoceptor–mediated anti-inflammatory action; current study). Future studies need to address the appropriate timing of dexmedetomidine administration to optimize care.

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Competing Interests

Dr. Maze co-patented the sedative use of dexmedetomidine in 1987. Stanford University reassigned its rights to the patent in 1988 for $250,000, which was provided to the Maze Laboratory. For at least the last 10 years, Dr. Maze has received no support of any kind related to the commercial exploitation of dexmedetomidine. Dr. Maze is a co-founder and shareholder of NeuroproteXeon (Buffalo, New York), a company involved in trialing xenon for use as a neuroprotectant. The other authors declare no competing interests.

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