Dexmedetomidine inhibits LPS-induced inflammatory responses through peroxisome proliferator-activated receptor gamma (PPARγ) activation following binding to α_2 adrenoceptors

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ABSTRACT

Over the past decade, dexmedetomidine (DEX) has been found to possess an anti-inflammatory effect. However, the local anti-inflammatory mechanism of DEX has not been fully clarified. Some intracellular inflammatory pathways lead to negative feedback during the inflammatory process. The cyclooxygenase (COX) cascade synthesizes prostaglandins (PGs) and plays a key role in inflammation, but is known to also have anti-inflammatory properties through an alternative route of a PGD_2 metabolite, 15-deoxy-delta-12,14-prostaglandin J_2 (15d-PGJ_2), and its receptor, peroxisome proliferator-activated receptor gamma (PPARγ). Therefore, we hypothesized that DEX inhibits LPS-induced inflammatory responses through 15d-PGJ_2 and/or PPARγ activation, and evaluated the effects of DEX on these responses. The RAW264.7 mouse macrophage-like cells were pre-incubated with DEX, followed by the addition of LPS to induce inflammatory responses. Concentrations of TNFα, IL-6, PGE_2, and 15d-PGJ_2 in the supernatants of the cells were measured, and gene expressions of PPARγ and COX-2 were evaluated in the cells. Furthermore, we evaluated whether a selective α_2 adrenoceptor antagonist, yohimbine or a selective PPARγ antagonist, T0070907, reversed the effects of DEX on the LPS-induced inflammatory responses. DEX inhibited LPS-induced TNFα, IL-6, and PGE_2 productions and COX-2 mRNA expression,

and the effects of DEX were reversed by yohimbine. On the other hand, DEX significantly increased 15d-PGJ_2 production and PPARγ mRNA expression, and yohimbine reversed these DEX's effects. Furthermore, T0070907 reversed the anti-inflammatory effects of DEX on TNFα and IL-6 productions in the cells. These results suggest that DEX inhibits LPS-induced inflammatory responses through PPARγ activation following binding to α_2 adrenoceptors.

KEYWORDS:

Dexmedetomidine, α_2 adrenoceptors, PPARγ, 15d-PGJ_2, Anti-inflammation

1. Introduction

Dexmedetomidine (DEX), a selective α_2 adrenoceptor agonist, is known to have effects on sedation, analgesia, and the cardiovascular system via α_2 adrenoceptors in the central nervous system (Kamibayashi and Maze, 2000). Clinically, DEX has been used as an adjunct to anesthesia, analgesia, and intensive care unit sedation (Khan et al., 1999). Over the past decade, DEX has also been found to possess an anti-inflammatory effect through inhibiting inflammatory mediator production. There are some reports that DEX has the capacity to inhibit the overproduction of a variety of inflammatory molecules including TNFα, IL-1β, and IL-6 in several acute inflammatory animal models (Taniguchi et al., 2008; Gu et al., 2011). Furthermore, Xiang H et al. (2014) clearly indicated that the central α_2 agonist DEX suppresses systemic inflammation through a vagal- and alpha7 nicotinic acetylcholine receptor-dependent mechanism.

On the other hand, our recent studies demonstrated the inhibitory effect of locally injected DEX on carrageenan-induced edema, the accumulation of leukocytes, and TNFα and cyclooxygenase (COX) -2 expression at the injection site (Honda et al., 2015; Sukegawa et al., 2014). These findings suggest that DEX exhibits an anti-inflammatory effect against local acute inflammatory responses. However, the local anti-inflammatory mechanism of DEX has

not fully been clarified. DEX is known to induce a decrease of adenylate cyclase activity and intracellular 3,5-cyclic adenosine monophosphate (cAMP) following binding to α_2 adrenoceptors (Gertler et al., 2001), but its anti-inflammatory effect may be via a different route from adenylate cyclase activity and complex due to the involvement of various intracellular routes.

Some intracellular inflammatory pathways lead to negative feedback during the inflammatory process. COX catalyzes the synthesis of prostaglandins (PGs) from arachidonic acid. COX-2, a rate-limiting enzyme for PGs, is induced upon stimulation by inflammatory stimuli such as LPS, and plays a key role in inflammation (Herschman, 1996). COX-2 is a pro-inflammatory enzyme during the early phase of inflammation; in contrast, COX-2 has anti-inflammatory properties by generating an alternative set of anti-inflammatory PGs (Gilroy et al., 1999). The PGD_2 metabolite 15-deoxy-delta-12,14-prostaglandin J_2 (15d-PGJ_2) and its receptor, peroxisome proliferator-activated receptor gamma (PPARγ), have been reported to be involved in an anti-inflammatory pathway in the COX cascade (Buckley et al., 2014; Gilroy et al., 1999; Jiang et al., 1998; Ricote et al., 1998). Furthermore, our previous study suggested that midazolam, an intravenous benzodiazepine, inhibits the LPS-induced inflammatory response via 15d-PGJ_2 (Miyawaki et al., 2012).

Therefore, we hypothesized that DEX inhibits LPS-induced inflammatory responses through 15d-PGJ_2 and/or PPARγ activation, so we evaluated the effect of DEX on inflammatory responses, including TNFα and IL-6 productions and COX-2 expression, further 15d-PGJ_2 production and PPARγ expression in LPS-stimulated mouse macrophage-like cells, and also investigated the reversal effect of a selective antagonist of α_2 adrenoceptors or PPARγ on DEX's effect on inflammatory responses.

2. Materials and methods

2.1. Cell culture and treatments

RAW264.7 mouse macrophage-like cells (DS Pharma Biomedical, Osaka, Japan) were cultured in D-MEM (DS Pharma Biomedical, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine in 100-mm dishes. All cells were maintained at 37 ℃ and 5% CO_2 in a humidified atmosphere. The cells were harvested with 0.25% trypsin-EDTA, added to a 35-mm dish at 0.3 x 10^6 cells/dish (2 ml per dish) for ELISA or 1.0 x 10^6 cells/dish (2 ml per dish) for RT-PCR, and cultured overnight at 37 ℃ in fresh medium. The cells were pre-incubated with DEX for 15 min, followed by the addition of LPS. Selective antagonist for α_2 adrenoceptors or PPARγ and selective COX-2 inhibitor were added 1 h before LPS or DEX. After incubation for 6 h, we collected the supernatants or the cells to evaluate the effects of DEX.

2.2. Agents

Dexmedetomidine (Precedex®) was purchased from Maruishi Pharmaceutical Co. (Osaka, Japan). LPS (lipopolysaccharide from *Escherichia coli* O55:B5) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Yohimbine hydrochloride (yohimbine), a selective antagonist

of α_2 adrenoceptors, was purchased from Sigma-Aldrich (St. Louis, MO, USA) and diluted with ultra-pure water. The selective COX-2 inhibitor NS-398 was purchased from Cayman Chemical (Ann Arbor, MI, USA). The selective PPARγ antagonist T0070907 was purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of NS-398 and T0070907 were prepared in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) and dissolved before use in external media to a final concentration containing no more than 0.1% DMSO. The selective PPARγ agonist thiazolidinedione (2,4-thiazolidinedione) was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) and diluted with ultra-pure water.

2.3. Evaluation of TNFα and IL-6 productions in the cells

The cells were incubated with LPS at 10 ng/ml to induce inflammatory responses. We elucidated the effects of DEX at concentrations of 1, 10, and 50 μM. After the incubation, the supernatants of the cells were collected, and TNFα and IL-6 concentrations were measured using specific ELISA kits (Thermo Fisher Scientific, Waltham, MA, USA). Furthermore, we evaluated whether yohimbine at a concentration of 100 μM reversed the effect of DEX at 10 μM on TNFα and IL-6 productions.

2.4. Evaluation of PGE_2 and 15d-PGJ_2 productions in the cells

The cells were incubated with LPS at 10 ng/ml and LPS plus DEX at 10 μM. After the incubation, the supernatants of the cells were collected, and concentrations of PGE_2 and 15d-PGJ_2 were measured using specific ELISA kits (MYBioSource, San Diego, CA, USA). Furthermore, we evaluated whether yohimbine at a concentration of 100 μM reversed the effect of DEX at 10 μM on PGE_2 and 15d-PGJ_2 productions.

2.5. Evaluation of inhibitory effects of DEX on TNFα and IL-6 via PPARγ in the cells

To elucidate the role of PPARγ, four groups of cells were allocated to be incubated with LPS at 10 ng/ml, LPS plus DEX at 10 μM, LPS plus DEX plus T0070907 at 100 μM, and LPS plus T0070907. After the incubation, the supernatants of the cells were collected, and TNFα and IL-6 concentrations were measured using specific ELISA kits (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Evaluation of COX-2 and PPARγ mRNA expression by reverse-transcription and polymerase chain reaction (RT-PCR)

To evaluate the effect of DEX and yohimbine or NS-398 on mRNA expression of COX-2 and PPARγ, the cells were incubated with LPS at 10 ng/ml, DEX at 10 μM, LPS plus DEX, and LPS plus DEX plus yohimbine at 100 μM or LPS plus DEX plus NS-398 at 1 μM. After the incubation, total RNA was extracted from the cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. Elimination of genomic DNA and reverse transcription were performed with QuantiTect (Qiagen, Hilden, Germany). The primers for mouse COX-2 were 5-GGAAAAGGTTCTTCTACGGAG-3 and 5-GGGTAGATCACTTCTACCTGAGTG-3. The primers for mouse PPARγ mRNA were 5-CATGGCCATTGAGTGCCGAGT-3 and 5-ACATCCCCACAGCAAGGCAC-3. Quantitative real-time PCR for COX-2 and PPARγ mRNA was performed using Miniopticon (Bio-rad, Hercules, CA, USA) and TB Green® Premix Ex Taq^{TM} Ⅱ (Takara, Tokyo, Japan), The reactions were incubated at 95 ℃ for 30 s, followed by a cycling protocol consisting of two stages: 10 s at 95 ℃, 15 s at 58 ℃ and 10 s at 72 ℃. After 40 cycles of PCR, melting curve analysis was routinely performed. Individual targets for each sample were quantified by determining the cycle threshold and comparison with the control sample (only medium, delta-delta Ct method). The relative amount of the target mRNA was normalized with the housekeeping gene beta-actin.

2.7. Evaluation of inhibitory effect of the combination of DEX and a selective PPARγ agonist on TNFα production in the cells

To evaluate the inhibitory effect of the combination of DEX and a selective PPARγ agonist thiazolidinedione, the cells were incubated with LPS at 10 ng/ml, LPS plus DEX at 10 μM, LPS plus thiazolidinedione at 10 μM, and LPS plus DEX plus thiazolidinedione. After the incubation, the supernatants of the cells were collected, and TNFα concentration was measured using the specific ELISA kit (Thermo Fisher Scientific, Waltham, MA, USA).

2.8. Statistical analysis

Differences among the values were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test or Tukey's multiple comparisons test using statistical analysis software (GraphPad Prism ver.7.0b, San Diego, CA, USA). The data are presented as the mean ± S.D.. $P < 0.05$ was regarded as significant.

3. Results

3.1. Effects of DEX on TNFα and IL-6 productions and COX-2 mRNA expression

The effects of DEX on the LPS-induced pro-inflammatory productions and gene expression in the cells were evaluated by ELISA and RT-PCR, respectively. In the incubated cells, LPS at 10 ng/ml significantly increased TNFα and IL-6 productions and COX-2 mRNA expression. The cells, pre-incubated with DEX at 1, 10, and 50 μM, showed significant inhibitions of TNFα and IL-6 productions and COX-2 mRNA expression in dose-dependent manners (Fig. 1). Furthermore, these effects of DEX at 10 μM were inhibited by a selective antagonist of the α_2 adrenoceptor, yohimbine, at 100 μM (Fig. 2).

3.2. Effects of DEX on PGE_2 and 15d-PGJ_2 productions

The effects of DEX on the LPS-induced pro-inflammatory PG, PGE_2 and 15d-PGJ_2 productions in the cells were evaluated by ELISA. In the incubated cells, LPS at 10 ng/ml significantly increased PGE_2 production. In the cells pre-incubated with DEX at 10 μM significantly inhibited LPS-induced PGE_2. On the other hand, LPS at 10 ng/ml increased 15d-PGJ_2 production compared with control. DEX at 10 μM itself didn't increase 15d-PGJ_2

production, but the combination of LPS and DEX significantly increase it. Furthermore, these effects of DEX were reversed by yohimbine at 100 μM (Fig. 3).

3.3. Effects of DEX on PPARγ mRNA expression

The effect of DEX on the PPARγ mRNA expression in the cells was evaluated by RT-PCR. PPARγ mRNA expression was slightly increased by LPS at 10 ng/ml compared with control. DEX at 10 μM significantly increased PPARγ mRNA expression in the presence of LPS, but didn't increase it in the absence of LPS. The effect of DEX in the presence of LPS was reversed by yohimbine at 100 μM (Fig. 4).

3.4. Effects of a selective COX-2 inhibitor on the action of DEX to PPARγ mRNA expression

We investigated whether a selective COX-2 inhibitor, NS-398, reverses the DEX-induced PPARγ mRNA expression in the cells. DEX at 10 μM significantly increased the PPARγ mRNA expression in the presence of LPS, while NS-398 at 1 μM reversed the DEX-induced increase of PPARγ mRNA expression (Fig. 5).

3.5. Effects of a selective antagonist of PPARγ on the action of DEX to TNFα and IL-6 productions

We investigated whether DEX would exert anti-inflammatory effects via PPARγ in the cells incubated with LPS. We used a selective PPARγ antagonist, T0070907, to evaluate the effects on LPS-induced pro-inflammatory cytokine productions in the cells. As well as previous results, DEX at 10 μM significantly inhibited LPS-induced TNFα and IL-6 productions in the cells. On the other hand, treatment with T0070907 at 100 μM significantly counteracted the inhibitory effects of DEX at 10 μM on TNFα and IL-6 productions, whereas T0070907 alone had no effects on LPS-induced pro-inflammatory cytokine productions (Fig. 6).

3.6. Effects of thiazolidinedione and the combination of DEX and a PPARγ agonist on LPS-induced TNFα production

We investigated whether the combination of DEX and a PPARγ agonist, thiazolidinedione, enhance each anti-inflammatory effect of DEX and thiazolidinedione in the cells. In the cells, thiazolidinedione at 10 μM significantly inhibited LPS-induced TNFα production like DEX

at 10 μM. Furthermore, the combination of DEX and thiazolidinedione enhanced the inhibitory effect of each on LPS-induced TNFα production (Fig. 7).

4. Discussion

The present study has shown that DEX inhibits LPS-induced inflammatory responses in mouse macrophage-like RAW264.7 cells in a dose-dependent manner through PPARγ activation following binding to α_2 adrenoceptors, and the effect of DEX on PPARγ mRNA expression was reversed by yohimbine, a selective antagonist of α_2 adrenoceptors. Furthermore, we confirmed the distribution of subtype of α_2 adrenoceptors, α_{2A} adrenoceptors, on the RAW264.7 cells, evaluating the gene expression (data not shown). These results indicated that the inhibitory effects of DEX on inflammatory responses were induced via α_2 adrenoceptors in the cells. The finding is consistent with previous reports that DEX has anti-inflammatory effects via α_2 adrenoceptors in RAW264.7 cells (Chang et al., 2013; Lai et al., 2009).

It has been well known that DEX inhibited COX-2 gene expression and PGE_2 production in LPS-stimulated cells (Lai et al., 2009; Sun et al., 2019). Therefore, the mechanism for the anti-inflammatory action of DEX was hypothesized to be involved in a COX cascade. 15d-PGJ_2, a PGD_2 metabolite, is known to be a key factor on an anti-inflammatory pathway in the COX cascade as an endogenous agonist of PPARγ, and the increase of 15d-PGJ_2 can result in increasing PPARγ mRNA expression and activating PPARγ (Forman et al., 1995; Murphy

and Holder, 2000). Furthermore, 15d-PGJ_2 was known to work as an intracellular signaling mediator, which retains the low expression level of COX-2 by a negative feedback loop meditated through PPARγ in macrophages (Inoue et al., 2000). These findings are consistent with the results of the present study showing that DEX increased both 15d-PGJ_2 production and PPARγ mRNA expression and a selective COX-2 inhibitor reversed the DEX-induced increase PPARγ mRNA expression. Furthermore, we demonstrated that a selective PPARγ agonist, thiazolidinedione, also had the inhibitory effect on TNFα production in the cells and the combination of DEX and thiazolidinedione enhanced each inhibitory effect. These results suggest that DEX directly or indirectly increases 15d-PGJ_2 production and enhances PPARγ activation in the process of the anti-inflammatory pathway of the COX cascade. The finding suggests that this combination might be clinically useful for anti-inflammatory therapy.

PPARγ is connected to the nuclear membrane, and a ligand-dependent transcription factor belonging to the family of nuclear receptors that includes estrogen receptors, thyroid hormone receptors, and glucocorticoid receptors (Tyagi et al., 2011; Clarke et al., 1999). PPARγ was first identified regarding its role in lipid and glucose regulation (Okuno et al., 1998). Over time, it became more and more evident that PPARγ is also a key regulator of inflammatory and immune system (Chinetti et al., 2003; Luconi et al., 2010). PPARγ is

expressed on numerous cells of the immune system, including monocytes/macrophages, dendritic cells, T and B lymphocytes, and platelets (Clark et al., 2000; Harris and Phipps, 2001; Padilla et al., 2002; Ray et al., 2008; Szatmari et al., 2004; Welch et al., 2003). It has been shown that PPARγ agonists act as negative regulators of monocytes and macrophages and dose-dependently inhibit the production of pro-inflammatory cytokines, such as TNFα, IL-1β, and IL-6, in human monocytes (Jiang et al., 1998). These findings are consistent with the results of the present study.

According to the PPARγ pathway for anti-inflammatory action, it has already been well-established that PPARγ ligands inhibit certain pro-inflammatory responses. 15d-PGJ_2, an endogenous agonist of PPARγ, is known to bind to PPARγ in the cytoplasm. Subsequently, it forms a heterodimer with the retinoid X receptors (RXR), which then migrates into the nucleus and control the expression of genes that have PPAR response elements. As a result, the pro-inflammatory responses of a number of transcription factors, such as activator protein 1 (AP-1), signal transducers and activators of transcription 1 (STAT1), and nuclear factor-κB (NF-κB) are inhibited in macrophages, monocytes, and epithelial cells (Jiang et al., 1998; Ricote et al., 1998; Welch et al., 2003). DEX increases 15d-PGJ_2 and activates PPARγ, and DEX may consequently regulate the expression of these pro-inflammatory genes.

Furthermore, it was reported that DEX inhibited the inflammatory reaction in lung tissues of septic rats by suppressing the TLR-4 /MyD88/ NF-κB pathway (Wu et al., 2013). On the other hand, PPARγ has also been reported the critical roles of PPARγ signaling in regulating macrophage M1/M2 activation (Bouhlel et al., 2007). Furthermore, Zhou et al. (2020) reported that DEX preconditioning inhibited intrahepatic pro-inflammatory innate immune activation by promoting macrophage M2 activation in a PPARγ/STAT3 dependent manner. These findings support the present study that DEX inhibits inflammatory responses via PPARγ activation.

PPARγ plays essential roles in adipogenesis and glucose homeostasis and is a molecular target of insulin-sensitizing drugs (Lihman et al., 1995; Spiegelman, 1998; Willson et al., 2001). Furthermore, it was recently reported that PPARγ activation prevented septic myocardial dysfunction by reducing pro-inflammatory cytokines, apoptosis, and necroptosis in the myocardium (Peng et al., 2017). DEX may be a useful candidate to not only inhibit postoperative inflammatory responses but also prevent various other complications following surgery.

The present study has some limitations. The first limitation is that the specific mechanism for the increase of 15d-PGJ_2 and PPARγ mRNA expression via α_2 adrenoceptors by DEX is

not clear in the present study. The possibility is that DEX influences an anti-inflammatory pathway of the COX cascade resulting in accelerating 15d-PGJ_2 production and PPARγ mRNA expression. Further studies will be needed for clarifying the further detail mechanism. The second limitation is that the concentration (10 μM) of DEX in the present study is high, at 100-times the clinically relevant concentration. However, we demonstrated the dose-dependent effects of DEX on LPS-induced inflammatory productions and gene expression in mouse macrophage-like cells. These results suggest that DEX could suppress the inflammatory signals at even lower concentrations.

In conclusion, the present study showed that DEX dose-dependently inhibited LPS-induced inflammatory responses, including TNFα, IL-6, and PGE_2 productions and COX-2 mRNA expression, in the RAW264.7 cells. Furthermore, DEX directly or indirectly increased 15d-PGJ_2 production and PPARγ mRNA expression under COX activation, and the effect of DEX was reversed by yohimbine, a selective antagonist of α_2 adrenoceptors and T0070907, a selective PPARγ antagonist. These findings of the present study suggest that DEX inhibits LPS-induced inflammatory responses through PPARγ activation following binding to α_2 adrenoceptors. The possible mechanism for anti-inflammatory action of DEX is shown in Fig. 8. PPARγ plays essential roles in adipogenesis and glucose homeostasis.

Therefore, DEX may have the ability to modulate not only inflammatory responses but also diabetic and atherogenic actions.

Declaration of competing interest

The authors declare no conflict of interest related to the present study.

Funding

This work was supported by JSPS (Japan Society for the Promotion of Science)

KAKENHI Grant Number 17K17248.

Acknowledgments

Not applicable.

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Legends

Fig. 1. Dose-dependent effects of dexmedetomidine (DEX) on TNFα (A) and IL-6 (B) productions and cyclooxygenase (COX) -2 mRNA expression (C) after 6 h incubation in RAW264.7 cells.

TNFα and IL-6 levels in the supernatants of the cells and COX-2 mRNA expression in the cells following 6 h incubation with only LPS at 10 ng/ml, LPS + DEX at 1, 10, and 50 μM were measured. Data are presented as the mean ± S.D. (n = 5 for each group). **P < 0.01, ***P < 0.001, ****P < 0.0001 compared with only LPS (0). Differences among the values were analyzed using one-way ANOVA followed by Dunnett's test.

Fig. 2. Effects of yohimbine (YOH) to reverse the action of dexmedetomidine (DEX) on LPS-stimulated TNFα (A) and IL-6 (B) productions and cyclooxygenase (COX) -2 mRNA expression (C) in RAW264.7 cells.

TNFα and IL-6 levels in the supernatants of the cells and COX-2 mRNA expression in the cells following 6 h incubation with only LPS at 10 ng/ml, LPS + DEX at 10 μM, LPS + DEX at 10 μM + YOH at 100 μM, and LPS + YOH at 100 μM were measured. Data are presented as the mean ± S.D. (n = 5 for each group). *P < 0.05, ****P < 0.0001 compared with each

group. Differences among the values were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Fig. 3. Effects of dexmedetomidine (DEX) and yohimbine (YOH) to reverse the action of DEX on prostaglandin E_2 (PGE_2) (A) and 15-deoxy-delta-12,14-prostaglandin J_2 (15d-PGJ_2) (B) productions in RAW264.7 cells.

PGE_2 and 15d-PGJ_2 levels in the supernatants of the cells following 6 h incubation with only D-MEM, only DEX at 10 μM, only LPS at 10 ng/ml, LPS + DEX at 10 μM, and LPS + DEX at 10 μM + YOH at 100 μM were measured. Data are presented as the mean ± S.D. (n = 5 for each group). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 compared with each group. Differences among the values were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Fig. 4. Effects of dexmedetomidine (DEX) and yohimbine (YOH) to reverse the action of DEX on peroxisome proliferator-activated receptor gamma (PPARγ) mRNA expression in RAW264.7 cells.

PPARγ mRNA expression in the cells following 6 h incubation with only DEX at 10 μM, only LPS at 10 ng/ml, LPS + DEX at 10 μM, LPS + DEX at 10 μM + YOH at 100 μM, and LPS + YOH at 100 μM were measured. Data are presented as the mean ± S.D. (n = 5 for each group). ****P < 0.0001 compared with each group. Differences among the values were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Fig. 5. Effect of a selective COX-2 inhibitor on the action of dexmedetomidine (DEX) to peroxisome proliferator-activated receptor gamma (PPARγ) mRNA expression in RAW264.7 cells.

PPARγ mRNA expression in the cells following 6 h incubation with only DEX at 10 μM, only LPS at 10 ng/ml, LPS + DEX at 10 μM, LPS + DEX at 10 μM + NS-398 at 1 μM were measured. Data are presented as the mean ± S.D. (n = 5 for each group). *P < 0.05, **P < 0.01 compared with each group. Differences among the values were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Fig. 6. Effects of a peroxisome proliferator-activated receptor gamma (PPARγ) antagonist, T0070907, to reverse the action of dexmedetomidine (DEX) on LPS-stimulated TNFα (A) and IL-6 (B) productions in RAW264.7 cells.

TNFα and IL-6 levels in the supernatants of the cells following 6 h incubation with only LPS at 10 ng/ml, LPS + DEX at 10 μM, LPS + DEX at 10 μM + T0070907 at 100 μM, and LPS + T0070907 at 100 μM were measured. Data are presented as the mean ± S.D. (n = 5 for each group). ****P < 0.0001 compared with each group. Differences among the values were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Fig. 7. Effects of thiazolidinedione (TZD) and the combination of dexmedetomidine (DEX) and TZD on LPS-induced TNFα production in RAW264.7 cells.

TNFα levels in the supernatants of the cells following 6 h incubation with only LPS at 10 ng/ml, LPS + DEX at 10 μM, LPS + TZD at 10 μM, LPS + DEX at 10 μM + TZD at 10 μM were measured. Data are presented as the mean ± S.D. (n = 8 for each group). **P < 0.01, ****P < 0.0001 compared with each group. Differences among the values were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test.

Fig. 8. The possible mechanism for anti-inflammatory action of dexmedetomidine.

COX: cyclooxygenase, DEX: dexmedetomidine, IKK: inhibitor of nuclear factor-κB kinase, NF-κB: nuclear factor-κB, PG: prostaglandin, PPARγ: peroxisome proliferator-activated receptor gamma, 15d-PGJ_2: 15-deoxy-delta-12,14-prostaglandin J_2