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Combined Neuroprotective Effects of Propofol and Dexmedetomidine on Endoplasmic Reticulum Stress-mediated Apoptosis in SH-SY5Y Cells

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Abstract : Propofol is a short-acting intravenous anesthetic agent. Dexmedetomidine, a highly selective α_2-adrenergic receptor agonist, has a well-known sedative effect. Both agents exhibit cytoprotective effects in the nervous system under ischemic conditions. Recently, the combination of propofol plus dexmedetomidine was used for the sedation of mechanically ventilated patients in an intensive care unit, but there are few experimental reports of the protective effects of the propofol plus dexmedetomidine combination in cells. Meanwhile, intraoperative brain ischemia–reperfusion induces endoplasmic reticulum (ER) stress-mediated apoptosis. The aim of the present study was to clarify molecular details underlying the neuroprotection afforded by the combination of propofol plus dexmedetomidine against thapsigargin (TG)-induced ER stress in human neuroblastoma SH-SY5Y cells, and whether the combination provided more efficient neuroprotection. TG was used to generate ER stress in SH-SY5Y cells. Cells were pretreated with propofol or dexmedetomidine, individually or in combination, for 1 h before cotreatment with TG for 20 h. There was a significant increase in $[Ca^{2+}]_i$, caspase activation, and the expression of ER stress biomarkers in TG-induced apoptotic cells. The increase in $[Ca^{2+}]_i$ and the induction of ER stress by TG were suppressed by pretreatment with propofol, dexmedetomidine, and their combination. The dexmedetomidine-induced reduction in caspase activity and ER stress biomarkers was inhibited by pretreatment with an α_2-adrenergic receptor antagonist, but was enhanced by pretreatment with a cAMP inhibitor. Treatment with the propofol plus dexmedetomidine combination exhibited the strongest protection against TG-induced apoptosis. These results demonstrate that the combination of propofol plus dexmedetomidine at clinically relevant concentrations suppresses ER stress-induced apoptosis in neuroblastoma SH-SY5Y cells. The findings suggest that the combination of propofol plus dexmedetomidine within a clinically relevant concentration range may be used safely in patients.

Key words : neuroprotection, propofol, dexmedetomidine, ER stress-mediated apoptosis, thapsigargin

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Introduction

The 2013 Clinical Practice Guideline For Management of Pain, Agitation and Delirium in Adult Patients in Intensive Care Unit[1] recommends the use of non-benzodiazepine-based sedation and adequate analgesia to prevent agitation and delirium. Propofol and dexmedetomidine are known to produce less agitation and delirium. As agitation and delirium are associated with mortality and many adverse events, they must be prevented[2]. Strategies to reduce the unwanted effects of anesthetic agents are important.

Propofol, a $GABA_A$ receptor agonist, is a short-acting intravenous anesthetic agent that is used for anesthesia and sedation but has no analgesic action. It is the preferred sedative in intensive care units (ICU) because it offers advantages over benzodiazepines in terms of lack of accumulation, rapid onset, and rapid recovery after withdrawal. Reported adverse effects of propofol include hypotension, bradycardia, respiratory depression, and hypertriglyceridemia[3]. Furthermore, because prolonged use of high-dose propofol causes propofol infusion syndrome (PRIS), the prolonged use of high-dose propofol should be avoided[3].

As propofol does not have any analgesic action, combination with an adjuvant to provide analgesia is required. Furthermore, propofol has been reported to exhibit dose-dependent cardiodepressive effects[4]. Thus, propofol is commonly used in combination with other drugs that provide adequate analgesia[5].

Dexmedetomidine is a highly selective α_2-adrenergic receptor agonist with both sedative and analgesic properties that is devoid of respiratory depressant effects[6]. Dexmedetomidine is an alternative to GABA receptor agonists for use as a sedative in the ICU. In addition to producing sedation, dexmedetomidine reduces concurrent analgesic requirements while maintaining a patient's arousability and not compromising respiratory drive[7]. Propofol and dexmedetomidine produce less agitation and delirium compared with benzodiazepines, so the combination of propofol plus dexmedetomidine is used in the ICU to maintain spontaneous respiration while providing adequate sedation. Several studies have reported that dexmedetomidine infusion significantly reduces propofol requirements during the induction and maintenance of general anesthesia[8]. In addition, there are many reports that propofol has neuroprotective effects against trauma and under ischemic conditions[9, 10], and we have reported that propofol has neuroprotective effects against endoplasmic reticulum (ER) stress-mediated apoptosis[11]. In addition to its well-proven sedative effects, dexmedetomidine has been shown to exert cell-protective effects in nervous tissue under ischemic conditions[12]. Recently, the combination of propofol plus dexmedetomidine has been used to sedate mechanically ventilated patients in the ICU[13]. However, the relative benefits, and potential harm, of using these two drugs remain controversial. Furthermore, few experiments have investigated the neuroprotective effects of the combination of propofol plus dexmedetomidine in cells.

Intraoperative brain ischemia−reperfusion causes ER stress, decreasing glucose, energy, and ER Ca^{2+} availability, eventually leading to the induction of apoptosis. Insults such as ischemia or hypoxia not only induce changes in the respiratory, circulatory, and nervous systems in the entire

body, but they also damage individual cells. Ischemia after surgery causes ER stress and leads to rapid activation of the unfolded protein response (UPR)[14].

The aim of the present study was to clarify the molecular mechanisms underlying the neuroprotection afforded by the combination of propofol plus dexmedetomidine against thapsigargin (TG)-induced ER stress in human neuroblastoma SH-SY5Y cells, and to determine whether the combination provides more efficient neuroprotection.

Materials and methods

Drugs and reagents

Propofol (2,6-diisopropylphenol), dexmedetomidine (5-[(1S)-1-(2,3-dimethylphenyl)ethyl]-1H-imidazole, hydrochloride), TG, Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 were purchased from Wako Pure Chemical Industries (Osaka, Japan). Fetal bovine serum (FBS), atipamezole and 2′,5′-dideoxyadenosine (ddAdo) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibiotic-antimycotic (penicillin G, streptomycin, amphotericin B) were obtained from Life Technologies (Carlsbad, CA, USA). All other chemicals used in the present study were of the purest grade available commercially.

Cell culture and drug treatment

SH-SY5Y cells (human neuroblastoma ; EC-94030304) were obtained from the European Collection of Authenticated Cell Cultures (ECACC ; London, UK). Cells were cultured in DMEM / Ham's F-12 containing 10% FBS and antibiotic-antimycotic solution and maintained under a humidified atmosphere of 5% CO_2 and 95% air at 37℃. To evaluate the effects of TG, cells were incubated in DMEM / Ham's F-12 with or without 1 μM TG (used as a control) for 20 h. SH-SY5Y cells are known to be sensitive to TG treatment[15] and prolonged exposure of the cells to TG will induce apoptosis. In addition, we have reported previously that ER stress is induced in SH-SY5Y cells following treatment with 1 μM TG[11]. In the present study, cells were pretreated with propofol (1, 5, 10 μM) or dexmedetomidine (1, 10, 100, 1000 nM), individually or in combination (prop + dex), for 1 h before cotreatment with 1 μM TG for a further 20 h. In some experiments, cells were incubated with the α_2-adrenergic receptor antagonist atipamezole (0.3 μM) or the adenylyl cyclase inhibitor ddAdo (100 μM) for 10 min prior to treatment with propofol, dexmedetomidine, or prop + dex. All treatments were performed under sterile conditions.

Caspase-3 and -4 activities

Caspase-3 and -4 activity was determined fluorometrically using respective synthetic peptide substrates. SH-SY5Y cells that had been pretreated with propofol (1, 5, 10 μM), dexmedetomidine (1, 10, 100, 1000 nM), or prop + dex for 1 h before incubation with TG for another 20 h were rinsed with cold phosphate-buffered saline (PBS), resuspended in chilled cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA), and incubated on ice for 10 min. Then, after incubation, reaction buffer containing 10 μM dithiothreitol (Medical & Biological

Laboratories, Aichi, Japan) and the respective specific peptide substrate were added to each sample, and incubated at 37℃ for 2 h. The substrates used for caspase-3 (Kamiya Biochemical, Seattle, WA, USA) and caspase-4 (BioVision Technology, Chester Springs, PA, USA) were Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD-AFC) and Leu-Glu-Ala-Asp-AFC (LEVD-AFC), respectively. The AFC released by the enzyme reaction was measured spectrophotometrically at excitation and emission wavelengths of 405 and 505 nm, respectively, on a Spectra Max 340i3 (Molecular Devices, Sunnyvale, CA, USA). Protein content was determined using the Bio-Rad Protein Assay with bovine serum albumin (BSA) as the standard.

Determination of C/EBP homologous protein

After 20 h exposure to TG following 1 h pretreatment with propofol (1 μM), dexmedetomidine (1, 10, 100 nM), or prop + dex, cells were extracted with a cold cell lysis buffer and sonicated using an ultrasonic cell disrupter. For quantitative determination of C/EBP homologous protein (CHOP) in SH-SY5Y cell lysates, an ELISA kit for DNA damage inducible transcript 3 (CHOP; Uscn Life Science, Wuhan, China) was used.

Eukaryotic translation-initiation factor 2α phosphorylation

Phosphorylation of eukaryotic translation-initiation factor 2α (eIF2α) was evaluated using an eIF2α ELISA kit (Cell Signaling Technology) according to the manufacturer's instructions. SH-SY5Y cells (1×10^6 cells/ml) were pretreated with propofol (1 μM), dexmedetomidine (1, 10, 100 nM), or prop + dex for 1 h, followed by 20 h exposure to TG. Cells were fixed and blocked as directed by the manufacturer, and incubated with anti-phosphorylated (p-) eIF2α (Thr^{183}/Tyr^{185}) or anti-eIF2α, which are included in the kit. Cells were then examined spectrophotometrically at 405 nm with a Spectra Max 340pc (Molecular Devices).

Determination of cAMP levels

Concentrations of cAMP were determined using a Cyclic AMP ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. SH-SY5Y cells were pretreated with propofol (1 μM), dexmedetomidine (1, 10, 100 nM), or prop + dex for 1 h, followed by 20 h exposure to TG. In addition, in some experiments, SH-SY5Y cells were pre-incubated with atipamezole for 10 min before pretreatment with propofol or dexmedetomidine, after which cAMP levels were determined using the ELISA kit according to the manufacturer's instructions.

Determination of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ levels were evaluated using a FLIPR Calcium 5 Assay Kit (Molecular Devices). In these studies, SH-SY5Y cells were loaded with FLIPR reagent diluted in Hank's balanced salt solution + 20 mM HEPES buffer (pH 7.4) for 1 h at 37℃. After SH-SY5Y cells had been incubated with the FLIPR reagent, changes in $[Ca^{2+}]_i$ were measured by monitoring the fluorescence signals of FLIPR with excitation and emission wavelengths of 485 and 525 nm,
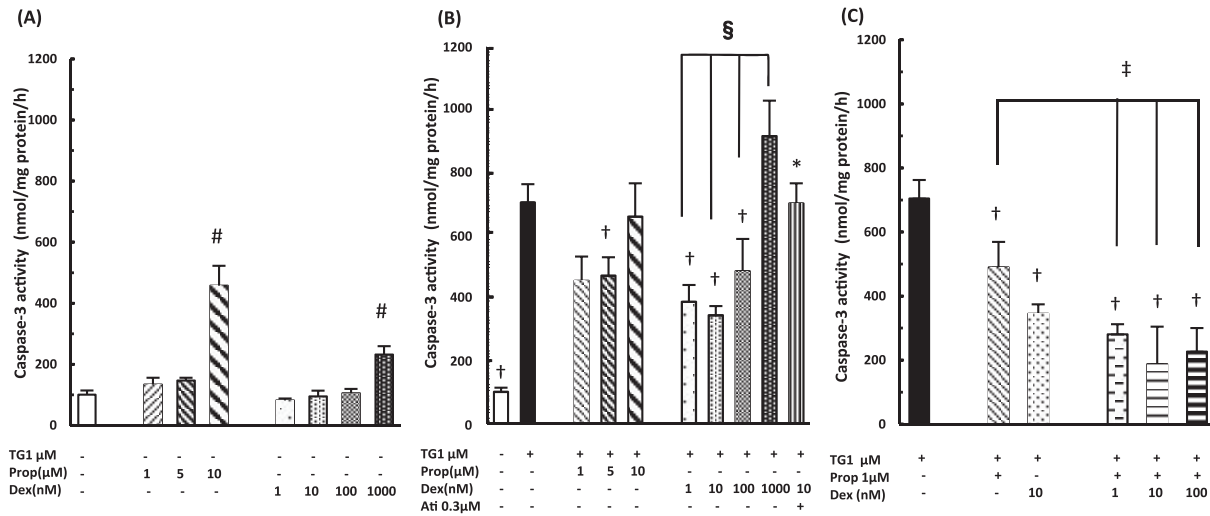
Fig. 1. Measurement of caspase-3 activity

Caspase-3 activity was measured using the peptide substrate Asp-Glu-Val-Asp-7-amino-4-trifluoromethylcoumarin (DEVD-AFC). (A) Effects of propofol (Prop; 1, 5, 10 μM) and dexmedetomidine (Dex; 1, 10, 100, 1000 nM) on caspase-3 activity in SH-SY5Y cells. $^{\#}P < 0.05$ compared with control cells. (B) Effects of Prop (1, 5, 10 μM) and Dex (1, 10, 100, 1000 nM) on caspase-3 activity in thapsigargin (TG)-treated apoptotic SH-SY5Y cells, as well as effects of the α_2-adrenergic receptor antagonist atipamezole (Ati) in response to Dex (10 nM) and TG (1 μM). (C) Effects of pretreatment of SH-SY5Y cells with the combination of Prop (1 μM) + Dex (1, 10, 100 nM) on TG-induced increases in caspase-3 activity. Data are the mean + SEM of 11−59 samples. $^{\#}P < 0.05$ compared with control cells; $^{\dagger}P < 0.05$ compared with 1 μM TG-treated cells; $^{\ddagger}P < 0.05$ compared with 1 μM TG + 1 μM Prop-treated cells; $^{\S}P < 0.05$ compared with 1 μM TG + 1000 nM Dex-treated cells; $^{*}P < 0.05$ compared with 1 μM TG + 10 nM Dex-treated cells.

respectively, using Meta Xpress Image Acquisition (Molecular Devices).

Statistical analysis

All measurements were repeated three times. Results are expressed as the mean + SEM. The effects of various treatments were compared with untreated control cells using one-way analysis of variance (ANOVA) and post hoc Dunnett's test. $P < 0.05$ was considered significant.

Results

Effects of propofol and dexmedetomidine on caspase-3 activity

TG-induced ER stress-mediated apoptosis was detected by determining caspase-3 activity (Fig. 1). In SH-SY5Y cells, there was no significant effect on caspase-3 activity following incubation of cells with either 1 and 5 μM propofol or 1, 10, and 100 nM dexmedetomidine. However, when cells were incubated with 10 μM propofol or 1000 nM dexmedetomidine, caspase-3 activity increased significantly compared with activity in untreated SH-SY5Y cells (Fig. 1A). After 20 h incubation with TG, caspase-3 activity increased significantly in SH-SY5Y cells compared with untreated control cells. Pretreatment of SH-SY5Y cells with propofol (1, 5 μM) or dexmedetomidine (1, 10, 100 nM) before 20 h exposure to TG significantly suppressed the TG-induced increase in caspase-3 activity. These observations indicate that propofol or dexmedetomidine can inhibit TG-induced apoptosis, but that a high concentration of
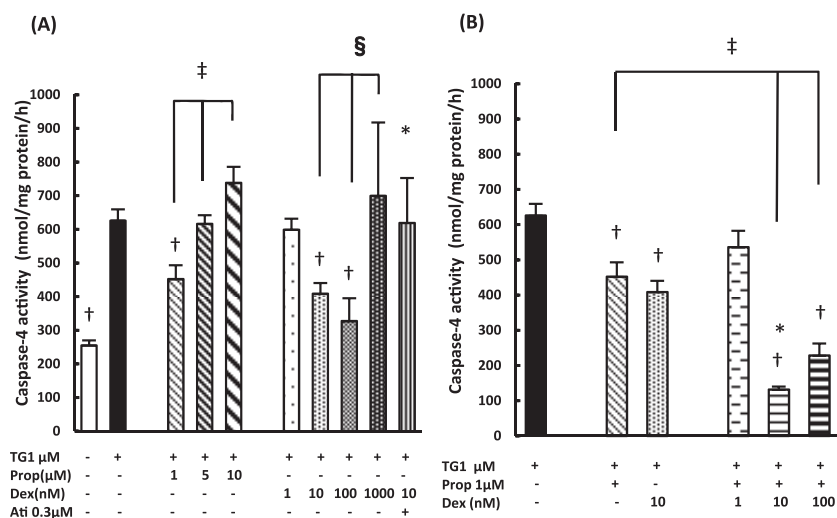
Fig. 2.　Measurement of caspase-4 activity

Caspase-4 activity was measured using the peptide substrate Asp-Glu-Val-Asp-7-amino-4-trifluoromethyl coumarin (DEVD-AFC). (A) Effects of propofol (Prop ; 1, 5, 10 μM) and dexmedetomidine (Dex ; 1, 10, 100, 1000 nM) on caspase-4 activity in thapsigargin (TG)-treated apoptotic SH-SY5Y cells, as well as effects of the α_2-adrenergic receptor antagonist atipamezole (Ati) in response to Dex (10 nM) and TG (1 μM). (B) Effects of pretreatment with the combination of Prop (1 μM) + Dex (1, 10, 100 nM) on TG-induced increases in caspase-4 activity. Data are the mean + SEM of 9–63 samples. $^{†}P <$ 0.05 compared with 1 μM TG-treated cells ; $^{‡}P < 0.05$ compared with 1 μM TG + 1 μM Prop-treated cells ; $^{§}P < 0.05$ compared with 1 μM TG + 1000 nM Dex-treated cells ; $^{d}P < 0.05$ compared with 1 μM TG + 10 nM Dex-treated cells.

dexmedetomidine (1000 nM) can itself induce ER stress-induced apoptosis.

The effects of dexmedetomidine can be inhibited by the highly specific α_2-adrenergic receptor antagonist atipamezole[16]. In the present study, pretreatment of SH-SY5Y cells with atipamezole prior to exposure of cells to 10 nM dexmedetomidine and TG significantly increased caspase-3 activity compared with TG + dexmedetomidine (10 nM) treatment (Fig. 1B). These findings support the notion that the protective effect of dexmedetomidine against ER stress-induced apoptosis is mediated through α_2-adrenergic receptors.

Under TG-induced ER stress, there was a tendency for greater suppression of caspase-3 activity with prop + dex (10 nM) compared with propofol or dexmedetomidine treatment alone, but the differences did not reach statistical significance (Fig. 1C).

Effects of propofol and dexmedetomidine on TG-induced ER stress

Caspase-4 activity

Fig. 2 shows changes in caspase-4 activity in SH-SY5Y cells. TG significantly increased caspase-4 activity, and this increase was significantly suppressed by both propofol (1 μM), similar to previous findings[10], and dexmedetomidine (1, 10, 100 nM). When SH-SY5Y cells were pretreated with atipamezole prior to exposure to dexmedetomidine (10 nM) and TG, caspase-4
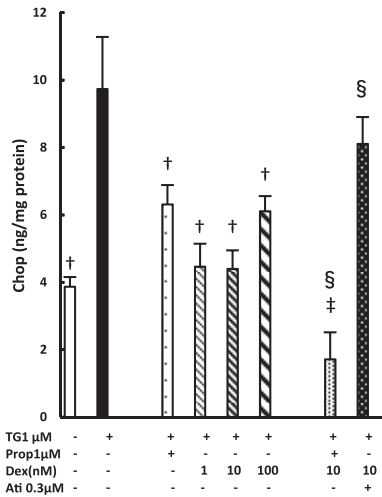
Fig. 3. Detection of C/EBP homologous protein (CHOP)

CHOP levels were evaluated using an ELISA kit. The effects of propofol (Prop ; 1 μM) and dexmedetomidine (Dex ; 1, 10, 100 nM) on CHOP levels in thapsigargin (TG)-treated apoptotic SH-SY5Y cells are shown, in addition to the effects of the α_2-adrenergic receptor antagonist atipamezole (Ati) on responses to Dex (10 nM) and TG (1 μM). Data are the mean + SEM of 6–30 samples. $^\dagger P < 0.05$ compared with 1 μM TG-treated cells ; $^\ddagger P < 0.05$ compared with 1 μM TG + 1 μM Prop-treated cells ; $^\S P < 0.05$ compared with 1 μM TG + 10 nM Dex-treated cells.
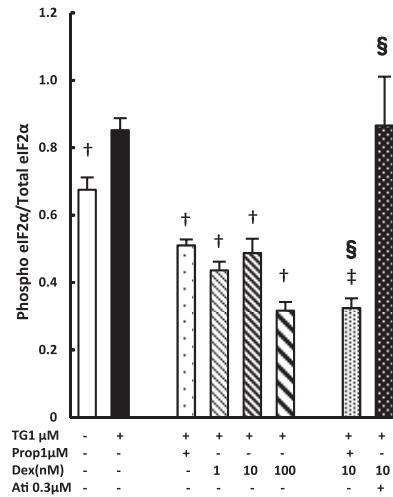

Fig. 4. Detection of eukaryotic translation-initiation factor 2α (eIF2α) phosphorylation

Phosphorylation of eIF2α was determined using an ELISA kit. The effects of propofol (Prop; 1 μM) and dexmedetomidine (Dex ; 1, 10, 100 nM) on eIF2α phosphorylation in thapsigargin (TG)-treated apoptotic SH-SY5Y cells are shown, in addition to the effects of the α_2-adrenergic receptor antagonist atipamezole (Ati) in response to Dex (10 nM) and TG (1 μM). Data are the mean + SEM of 6–28 samples. $^\dagger P < 0.05$ compared with 1 μM TG-treated cells ; $^\ddagger P < 0.05$ compared with 1 μM TG + 1 μM Prop-treated cells ; $^\S P < 0.05$ compared with 1 μM TG + 10 nM Dex-treated cells.

activity was significantly increased (Fig. 2A). Under TG-induced ER stress, treatment with prop + dex (10 nM) had a greater inhibitory effect on TG-induced increases in caspase-4 activity than treatment with either propofol or dexmedetomidine alone (Fig. 2B).

CHOP levels

Fig. 3 shows changes in CHOP levels in SH-SY5Y cells. TG significantly increased CHOP levels in SH-SY5Y cells, and this was significantly suppressed by propofol (1 μM) and dexmedetomidine (1, 10, 100 nM) pretreatment. Under TG-induced ER stress, prop + dex treatment resulted in a greater reduction in TG-induced increases in CHOP levels than treatment with either propofol or dexmedetomidine (10 nM) alone. Incubation of cells with atipamezole before exposure to dexmedetomidine (10 nM) and TG significantly increased CHOP levels.

eIF2α phosphorylation

Fig. 4. shows phosphorylation of eIF2α in SH-SY5Y cells. Phosphorylation of eIF2α was significantly increased in TG-treated cells, and this was significantly suppressed by propofol, dexmedetomidine, and prop + dex pretreatment. Under TG-induced ER stress, prop + dex treatment resulted in a greater reduction in TG-induced increases in ER stress compared
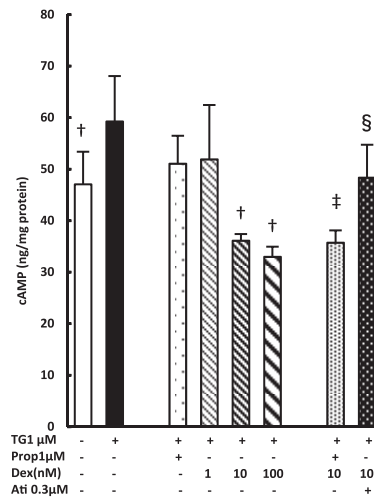
Fig. 5.   Measurement of cAMP levels
cAMP levels were determined using an ELISA kit. The effects
of propofol (Prop; 1 μM) and dexmedetomidine (Dex; 1, 10,
100 nM) on cAMP levels in thapsigargin (TG)-treated apoptotic
SH-SY5Y cells are shown, in addition to the effects of the α_2-
adrenergic receptor antagonist atipamezole (Ati) in response to
Dex (10 nM) and TG (1 μM). Data are the mean + SEM of 6–10
samples. [†]$P < 0.05$ compared with 1 μM TG-treated cells; [‡]$P <$
0.05 compared with 1 μM TG + 1 μM Prop-treated cells; [§]$P < 0.05$
compared with 1 μM TG + 10 nM Dex-treated cells.

with either propofol or dexmedetomidine (10 nM) alone.   When cells were incubated with
atipamezole before being exposed to dexmedetomidine (10 nM) and TG, eIF2α phosphorylation
increased significantly to levels seen following exposure to TG alone (Fig. 4).

Effects of propofol and dexmedetomidine on intracellular cAMP

Dexmedetomidine binds to α_2-adrenergic receptors, which are coupled to Gi-proteins, inhibiting
adenylyl cyclase activity and downregulating cAMP formation [17].   In this series of experiments
we investigated whether cAMP is involved in the protective effects of dexmedetomidine against
TG-induced ER stress-induced apoptosis.   Pretreatment with dexmedetomidine (10, 100 nM)
significantly decreased cAMP levels in SH-SY5Y cells exposed to TG.   When cells were treated
with atipamezole before being exposed to dexmedetomidine (10 nM) and TG, cAMP levels
increased significantly compared with values in the dexmedetomidine plus TG-treated group (Fig.
5).   Because propofol did not alter cAMP levels, the results suggest that cAMP is not involved
in the protective effects of propofol.

Effects of propofol and dexmedetomidine on TG-induced increases in $[Ca^{2+}]_i$

Meta Xpress Images Acquisition was used in the present study to investigate the effects of
propofol, dexmedetomidine, and prop + dex pretreatment on $[Ca^{2+}]_i$ in SH-SY5Y cells exposed
to TG.   In TG-treated cells $[Ca^{2+}]_i$ levels were increased, and increased levels were maintained
over the 180 s of analysis.   In contrast, propofol, dexmedetomidine, and prop + dex pretreatment
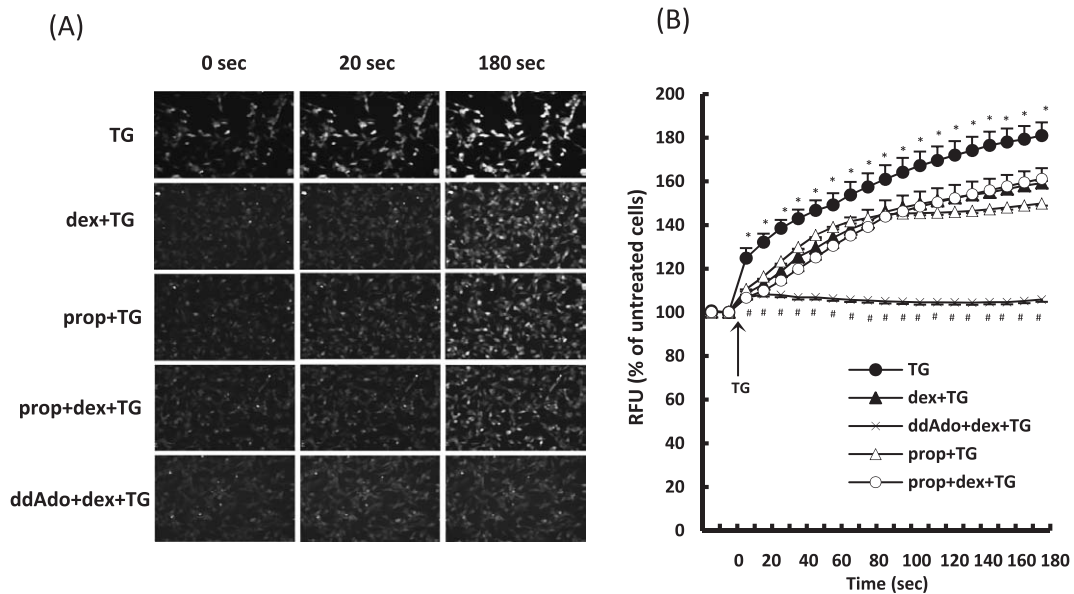
Fig. 6. Measurement of $[Ca^{2+}]_i$ levels

$[Ca^{2+}]_i$ levels were determined using a FLIPR Calcium 5 Assay Kit. Changes in $[Ca^{2+}]_i$ levels are shown in SH-SY5Y cells treated with thapsigargin (TG; 1 μM), TG (1 μM) + propofol (Prop; 1 μM), TG (1 μM) + dexmedetomidine (Dex; 10 nM), and TG (1 μM) + 2′,5′-dideoxyadenosine (ddAdo; 100 μM) + Dex (10 nM). (A) Representative images showing changes in $[Ca^{2+}]_i$ levels in SH-SY5Y cells. Bright, white areas indicate high $[Ca^{2+}]_i$ levels in SH-SY5Y cells. (B) Changes in $[Ca^{2+}]_i$ levels in SH-SY5Y cells. Values are expressed as a percentage of fluorescence intensity before treatment. Data are the mean ± SEM of 30 plates. $^{*}P < 0.05$ compared with 1 μM Prop, 10 nM Dex, or prop + dex-treated cells; $^{\#}P < 0.05$ compared with 1 μM TG + 10 nM Dex-treated cells. RFU, relative fluorescence units.

reduced the magnitude of the TG-induced increase in $[Ca^{2+}]_i$ (Fig. 6). In a previous study we found that propofol treatment significantly inhibited the effects of TG from the early phase[11], and we speculate that in the present study propofol inhibited the TG-induced release of Ca^{2+} from the ER. Pretreatment of cells with dexmedetomidine (10 nM) significantly reduced the TG-induced increase in $[Ca^{2+}]_i$ (Fig. 6). Pre-incubation of cells with the membrane-permeable adenylyl cyclase inhibitor ddAdo (100 μM) further reduced the TG-induced increase in $[Ca^{2+}]_i$ compared with that in cells treated with dexmedetomidine and TG. Therefore, the reduction in cAMP levels by dexmedetomidine pretreatment of TG-treated cells is likely mediated by repression of $[Ca^{2+}]_i$ by dexmedetomidine.

Discussion

In the present study we showed that the combination of propofol plus dexmedetomidine suppressed ER stress-mediated apoptosis of SH-SY5Y cells. When SH-SY5Y cells were treated with prop + dex, ER stress-mediated apoptosis was suppressed to a greater extent than that seen after treatment with propofol or dexmedetomidine alone. In particular, the combination of 1 μM propofol plus 10 nM dexmedetomidine had the strongest neuroprotective effects.

Typically, patients receive 1−2.5 mg / kg propofol as an intravenous bolus injection for the induction of general anesthesia. When a target control infusion (TCI) pump is used, the target

plasma concentration for maintenance of propofol anesthesia is usually between 2.0 and 5.0 μg/ml. However, propofol binds extensively to plasma protein (mean bound: 97%-98%), with 50% being bound to erythrocytes and 48% binding to serum proteins, almost exclusively to human serum albumin. The free fraction of propofol in the blood is in the range 1.2%-1.7% at total concentrations ranging from 0.5 to 32 μg/ml[18].

If dexmedetomidine is administrated by a loading dose followed by infusion at a rate of 0.2-0.83 μg/kg per h, patients in this setting could obtain an effective dexmedetomidine concentration of 0.22-2.50 ng/ml (1.1-12.5 nM). Furthermore, plasma dexmedetomidine concentrations are moderately correlated with the dose administered[19].

Therefore, the concentrations of propofol (1 μM) and dexmedetomidine (10 nM) used in the present study are similar to serum concentrations seen used clinically. On this basis, it is speculated that the combination of 1 μM propofol plus 10 nM dexmedetomidine may exhibit neuroprotective effects in the clinical setting.

TG decreased Ca^{2+} flow into the ER from the cytoplasm and reduced Ca^{2+} concentrations in the ER. When the Ca^{2+} concentration in the ER is reduced, there is an increase in misfolded proteins, causing ER stress. The adaptive response to ER stress is the UPR. When the UPR process works normally, the overloading with abnormal proteins in the ER is reduced and the cell can adapt to the insult. However, after excessively severe insults, cells cannot adapt and apoptosis is induced via ER stress.

ER stress can be induced by several different situations, including ischemia-reperfusion and trauma, as well as by many neurodegenerative diseases, so there are many benefits to suppressing ER stress. In a previous study we found that TG treatment was able to generate ER stress[11], thus we decided that the use of TG was the most appropriate way to induce ER stress in the present study.

Propofol is widely used as an intravenous anesthetic and sedative agent. In a previous study, human glioblastoma cells were treated with 300-500 μM propofol[20]. In that study, increases in $[Ca^{2+}]_i$ were observed and the authors reported that propofol induced apoptosis in dose-dependent manner within 24 h[20]. In another study, human promyelocytic leukemia cells were treated with 150-250 μM propofol and, after 5 h, activation of caspase-3, as an indicator of apoptosis, was observed[21]. However, treatment with propofol at a clinically relevant concentration shows neuroprotective effects. Propofol protects nerve, pulmonary epithelial, vascular endothelial, and kidney cells[22-24]. We have reported previously that 5 μM propofol suppresses ER stress-induced apoptosis by inhibiting Ca^{2+} release from the ER[11].

Dexmedetomidine is a potent and highly selective agonist of α_2-adrenergic receptors in the human brain, and its effects include clinical sedation, anesthetic-sparing effects, and analgesia[25]. Recent studies have indicated that dexmedetomidine also exhibits neuroprotective effects against ischemic injury[26] and promotes the survival of nervous tissue that has suffered traumatic injury[27]. However, a study in neutrophils reported that treatment with a high concentration of dexmedetomidine (100 ng/ml; 499.3 nM) accelerated apoptosis, whereas a clinically relevant concentration of dexmedetomidine (1 ng/ml; 4.993 nM) did not affect neutrophil apoptosis[28].

In the present study of ER stress-mediated apoptosis, dexmedetomidine （10, 100 nM） exhibited neuroprotective effects.

Moreover, we have demonstrated in the present study that pretreatment with 10 nM dexmedetomidine significantly decreased the TG-stimulated increase in $[Ca^{2+}]_i$. Pre-incubation of cells with the membrane-permeable adenylyl cyclase inhibitor ddAdo （100 μM） further suppressed $[Ca^{2+}]_i$ levels in cells treated with dexmedetomidine plus TG （Fig. 6）. The neuroprotective actions of dexmedetomidine have largely been attributed to its effect as an agonist at α_2-adrenergic receptors[29, 30].

The α_2-adrenergic receptor is coupled to a Gi-protein complex, which inhibits adenylyl cyclase and Ca^{2+} channels. The result of α_2-adrenergic receptor agonist binding is a reduction in cAMP production and decreased $[Ca^{2+}]_i$[31]. Under ER stress, dexmedetomidine （10, 100 nM） treatment significantly decreased cAMP levels （Fig. 5）. Agonist stimulation of postsynaptic α_2-adrenergic receptors mediates the sedative and analgesic effects of dexmedetomidine[32]. The neuroprotective actions of dexmedetomidine in ER stress-mediated apoptosis have also been attributed to its effect as an agonist at α_2-adrenergic receptors.

In the present study, treatment with the prop + dex combination at a clinically relevant concentration showed the strongest neuroprotective effect against TG-induced ER stress-mediated apoptosis. As mentioned above, the neuroprotective effect of the prop + dex combination against ER stress-mediated apoptosis is caused by a reduction in $[Ca^{2+}]_i$: that is, propofol inhibits Ca^{2+} release from the ER[10], whereas dexmedetomidine binds to α_2-adrenergic receptors, thus decreasing intracellular cAMP levels.

Structurally, dexmedetomidine is an imidazole derivative, and it binds not only to α_2-adrenergic receptors, but also to imidazoline receptors[33]. Therefore, it is necessary to determine whether the neuroprotective effects of dexmedetomidine are mediated via imidazoline receptors in addition to α_2-adrenergic receptors. Further detailed studies are needed to determine how the combination of propofol plus dexmedetomidine provides neuroprotection.

The combination of propofol plus dexmedetomidine is useable because it means that the dose of propofol, which has respiratory suppressive effects and may cause PRIS, can be reduced while maintaining adequate sedation levels. However, the findings of the present study cannot be extrapolated to humans without further in vitro studies that provide further evidence that the combination of propofol plus dexmedetomidine is able to protect nerve tissue under the situations that cause ER stress, such as ischemia−reperfusion, trauma, and neurodegenerative diseases.

In conclusion, the present study has demonstrated that the combination of propofol plus dexmedetomidine has a strong protective effect against ER stress-mediated apoptosis in SH-SY5Y cells. The neuroprotective effect of the propofol plus dexmedetomidine combination is mediated by propofol inhibition of Ca^{2+} release from the ER and dexmedetomidine binding to α_2-adrenergic receptors, which results in decreases in cAMP levels and $[Ca^{2+}]_i$.

Conflict of interest disclosure

The authors declare that they have no conflict of interest.

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