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$G_{\alpha i}$ and $G_{\beta\gamma}$ subunits have opposing effects on dexmedetomidine-induced sedation

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ABSTRACT

Dexmedetomidine (DMED) is a potent and highly selective α_2-adrenergic receptor agonist and is widely used for short-term sedation. However, the mechanism of DMED-induced sedation has not been deciphered. In the present study, we investigated the mechanism of $G_{\alpha i}$ and $G_{\beta\gamma}$ subunits on DMED-induced sedation. An ED_{50} of DMED-induced loss of righting reflex (200.0nmol/kg) was increased to 375.0 or 433.3nmol/kg after pre-treatment with cAMP analog dbcAMP (50nmol/5 μl/mouse, i.c.v.) or the phosphodiesterase 4 inhibitor rolipram (100nmol/5 μl/mouse, i.c.v.). Conversely, the ED_{50} of DMED-induced LORR decreased to 113.6 or 136.5 nmol/kg after pre-treated with $G_{\beta\gamma}$ subunit inhibitor M119 (100 mg/kg, i.p.) or gallein (100 mg/kg, i.p.) respectively. Administration of dbcAMP, rolipram, gallein or M119 alone had no effect on LORR. Gallein (10 μM) significantly inhibited forskolin-stimulated cAMP accumulation in α_{2A}-AR -CHO cells. Compared with $G_{\beta\gamma}$ subunit inhibitors or DMED alone, $[Ca^{2+}]i$ and pERK1/2 was significantly increased after co-administration with $G_{\beta\gamma}$ subunit inhibitors and DMED. DbcAMP (5 μM) or rolipram (5 μM) alone had no effect on ERK1/2 phosphorylation, but decreased DMED-induced ERK1/2 phosphorylation after co-administration with DMED. $G_{\beta\gamma}$ subunit inhibitor treatment increased DMED-induced phosphorylation of CREB, whereas dbcAMP or rolipram had no effect on pCREB induced by DMED. From our results we conclude that, $G_{\beta\gamma}$ subunit may inhibit DMED-induced sedation through the cAMP and pERK1/2 pathway.

1. Introduction

α_2-adrenergic receptors (α_2-AR) are a heterogeneous group of receptor , whose ligands are the catecholamine neurotransmitters noradrenaline and epinephrine that mediate presynaptic inhibitory actions in the central nervous system (CNS). The α_2-ARs consist of three pharmacologically distinct subtypes: α_{2A}, α_{2B} and α_{2C}-AR. The α_{2D}-AR subtype is the rat homologue of the human α_{2A}-AR (Schwartz and Clark, 1998). α_{2A}-AR and α_{2C}-AR subtypes are predominately expressed in the CNS (Handy et al., 1993; Nicholas et al., 1993; Berkowitz et al., 1994), while α_{2B}-AR subtype is present in the peripheral tissues (Handy et al., 1993; Berkowitz et al., 1994) as well as expressed in low amounts in certain discrete areas of the CNS (Nicholas et al., 1993).

Dexmedetomidine (DMED) is a potent and high selective agonist of α_2-adrenergic receptors. It is widely used in veterinary anesthetic and for short-term sedation of patients in the clinical because of its broad spectrum of functions which include neuroprotective, antinociceptive, analgesia and sedation (Ramsay and Luterman, 2004; Jalowiecki et al., 2005; Cotecchia et al., 1990; Kamibayashi and Maze, 2000; Walker et al., 2005). Well-designed experiments performed in knockout animals have shown that DMED-induced analgesia, sedation, hypotension and body temperature reduction were mediated by activating α_{2A}-AR subtype (Lakhlani et al., 1997). For instance, DMED, clonidine, moxonidine-induced hypotensive effect and bradycardia depend on α_{2A}-AR for its action (MacMillan et al., 1996; Altman et al., 1999). In addition, DMED is unable to induce antinociceptive action on α_{2A}-AR-knockout mice (Lakhlani et al., 1997; Hunter et al., 1997). As reported in 2007, to analyze the role of the central and peripheral α_2-adrenoceptor subtypes in mice, the authors of the paper demonstrated that D79N mutant mice with dysfunctional α_{2A}-AR subtype could not response to DMED but α_{2C}-AR knockout mice still showed sedative action induced by DMED (Gyires et al., 2007a, b).

Activation of G_i-coupled receptors results in its disassociation to free G_α and $G_{\beta\gamma}$ subunits, each of which may further interact with effector molecules (Simon et al., 1991). G_α subunits modulate numerous effectors, including adenylate cyclases and ion channels. $G_{\beta\gamma}$ subunits activate different isoforms of adenylate cyclase and phospholipase C (PLC), phospholipase A2, potassium channels, phosphatidylinositol 3′-

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kinase (Clapham and Neer, 1993; Tang and Gilman, 1991; Taussig et al., 1993). The effect of α_2-ARs on adenylyl cyclase inhibition and on polyphosphoinositide (PI) hydrolysis is mediated by pertussis toxin-sensitive G- proteins (Cotecchia et al., 1990). Activation of G_i or G_q-coupled receptors has been shown to stimulate MAPK activation. MAPK activation may be regulated by multiple pathways via G-protein coupled receptors activated by agonists (Koch et al., 1994). The carboxyl terminus of β adrenergic receptor kinase 1 (βARKct) specifically blocks the signaling mediated by $G_{\beta\gamma}$ subunits. βARKct could block α_{2A}-AR stimulated PI hydrolysis and MAPK activation but not affect α_{1B}-AR- or M1AChR-mediated signaling. These results indicated that $G_{\beta\gamma}$ mediates both PI hydrolysis and MAPK activation. The roles of the α and βγ subunits of $G_{i/o}$ proteins seem to oppose each other: the βγ subunits appear to mediate a voltage-insensitive inhibition via stimulation of phospholipase C/protein kinase, and is unlike the voltage-dependent inhibition that is found in rat superior cervical ganglion neurons (Herlitze et al., 1996; Ikeda, 1996; Delmas et al., 1998). $G_{\beta\gamma}$ subunits derived from $G_{i/o}$ proteins contribute to adrenergic inhibition of Ca^{2+} channels in rat sympathetic neurons (Delmas et al., 1999). Mattera et al. (1987) proposed that $G_{\beta\gamma}$ subunits are general attenuators of basal G protein activity, although little evidence has been demonstrated to support this suggestion. Previous studies have demonstrated that ERK phosphorylation regulates sleep and plasticity in drosophila. When over-expressed as an active form of ERK pan-neuronally in the adult flies demonstrated a significant increase in sleep. In addition, ERK has been shown to regulate cAMP response element binding protein (CREB), a transcription factor that couples synaptic activity to long-term changes in neuronal plasticity (Vanderheyden et al., 2013; Sun et al., 2016; Ravnskjaer et al., 2007). These studies suggest that phosphorylation of ERK and CREB may be important mediators of regulating DMED-induced sedative and hypnotic effect similar to cAMP.

In the present study, the regulation of $G_{\alpha i/o}$ and $G_{\beta\gamma}$ on DMED-induced LORR in mice was investigated. cAMP accumulation was found to play an important role in the regulation of DMED-induced sedation. Furthermore, ERK phosphorylation and intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) was studied under $G_{\beta\gamma}$ subunit inhibitor treatment in α_{2A}-AR -CHO cells. These studies suggest that phosphorylation of ERK and CREB may be important mediators for regulating DMED-induced sedation. The decrease of cAMP accumulation and enhancement of pERK may be the mechanism by which $G_{\beta\gamma}$ subunit inhibition enhances DMED-induced sedation.

2. Materials and methods

2.1. Materials

2.1.1. Animals

Male Kunming mice (20–22 g) were purchased from the Beijing Animals Center (Beijing, China). All animals were housed in alternating 12 h/12 h light/dark cycle room which was maintained in a climate-controlled environment (25 ± 1 °C). Animals were housed in groups of 10 mice per cage with free access to water and food. All experimental procedures were reviewed and approved by Animal Care and Use Committee of Beijing Institute of Pharmacology & Toxicology (Beijing, China). All experimental protocols were approved by and conducted in accordance with the guidelines on the ethical use of animals set by the National Institutes of Health (Bethesda, MD, USA). All efforts were made to minimize the number of animals used and avoid their discomfort.

2.1.2. Cells

Chinese hamster ovary (CHO) cells stably expressing the human α_{2A}-AR receptors (α_{2A}-AR-CHO) were cultured in Ham's F12 nutrient medium and 10% fetal bovine serum at 37 °C with humidified atmosphere consisting of 95% air and 5% CO_2

2.1.3. Drugs and materials

The following drugs were purchased from the vendors listed; Dexmedetomidine (Dejiaxin Biological Technology, Jinan, China). dbcAMP and gallein (Absin Bioscience Inc, Shanghai, China). H89 (TargetMol, Shanghai, China). Rolipram (Abcam, USA). BRL44408 and M119, (Sigma, USA). Atipamezole (synthesized at our institute). Dexmedetomidine, atipamezole and BRL44408 were dissolved in sterile physiological saline (0.9% NaCl) to a final concentration used in this study. Gallein, M119, dbcAMP, Rolipram, JP-1302 and ARC239 were dissolved in DMSO (1%~10%). LANCE cAMP 384 Kit (Perkin Elmer, Waltham, USA). Anti-pERK/ERK and Anti-pCREB/CREB (Cell Signaling, USA), Anti-Tubulin (Sigma-Aldrich, USA), Restore Western Blot Stripping Buffer (Pierce, USA), Ham's F12 nutrient medium (Thermo Fisher, USA), fetal bovine serum (HyClone, South Logan, UT, USA). Ca^{2+} imaging was detected using Fluo-4NW Calcium Assay Kits (Thermo fisher, F36206). Changes in Ca^{2+} were recorded using the 20 × objective lens (Hamamastsu C9100-50) of an inverted confocal laser scanning microscopy system (PerkinElmer, UltraView VoX).

2.2. Methods

2.2.1. Loss of righting reflex (LORR)

To study the selective effects of α_{2A}, α_{2B}, α_{2C}-AR antagonist on DMED-induced LORR, mice were pretreated with BRL44408 (7.5–60 nmol/mouse, i.c.v.), JP-1302(24–240 nmol /mouse, i.c.v.), ARC239 (6–100 nmol /mouse, i.c.v.), or atipamezole (3.2–64 nmol/mouse, i.c.v.), 15 min prior to DMED administration (1 μmol/kg, i.v.). To determine the effect of activated $G_{\beta\gamma}$ subunits on DMED-induced LORR, mice were pretreated with $G_{\beta\gamma}$ inhibitor gallein (100 mg/kg, i.p.) or M119 (100 mg/kg, i.p.) 15 min prior to DMED administration (50–900nmol/kg i.v.). Mice were pretreated with dbcAMP (50nmol/mouse, i.c.v.) or rolipram (100nmol/mouse, i.c.v.) were pretreated 15 min prior to DMED administration to study the effect of cAMP accumulation on DMED-induced LORR. The duration of LORR was recorded individually for each mouse. The righting reflex was deemed to be "lost" if the mouse failed to right itself within 60 s of being placed on its back.

2.2.2. ELISA cAMP assays

Stably transfected α_{2A}-AR- CHO cells were harvested using Versene dissociation solution (Gibco, Invitrogen) and washed with Hank's balanced salt solution (HBSS) buffer. The cells were then resuspended at a density of 2×10^6/ml in stimulation buffer (HBSS containing 5 mM HEPES, 0.1% BSA and 0.05 mM 3-isobutyl-1-methylxanthine). Alexa Fluor®647-labeled antibodies (PerkinElmer, Waltham, MA) were added to the final cell suspension prior to the addition of forskolin, in the presence of DMED, gallein or M119. Following incubation at 37 °C for 15 min, detection mix was added and the LANCE signal was subsequently measured after a 1 h incubation step using the VICTOR instrument (PerkinElmer). The LANCE signal obtained at 665 nM was used to directly analyze cAMP standard curves and cellular cAMP levels. The signal at 615 nM was used to identify dispensing or quenching issues. cAMP inhibition was defined with the LANCE signal.

2.2.3. pERK and pCREB assay

α_{2A}-AR- CHO cells were plated onto 60-mm dishes and grown to 80–90% confluence. Cells were serum starved for 4 h and then exposed to solvents, DMED (5 μM), gallein (5 μM) and DMED + gallein (5 μM, respectively) for 5, 15, 30, 60 min, then cooled to 4 °C by washing with ice-cold PBS. Proteins were extracted for 30 min at 4 °C with 0.2 ml of RIPA buffer supplemented with protease inhibitors. The lysates were centrifuged at 15,000 × *g* for 15 min at 4 °C. The whole cell protein was analyzed by western blotting using phospho-p44/42MAPK (ERK1/2) (Thr202/Tyr204) or pCREB antibody. Blots were then stripped and re-probed with p44/42 MAPK (ERK1/2) or CREB antibody. Bands were detected using chemiluminescence (ECL) detection kits. The gray-