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Tandospirone prevents anesthetic-induced respiratory depression through 5-HT$_{1A}$ receptor activation in rats

Meng-ran Song[1,2], Ming-zhi Huang[1,3], Wei-jie Tao[1,2], Zheng Yong[1]✉ & Rui-bin Su[1]✉

Respiratory depression is a side effect of anesthetics. Treatment with specific antagonists or respiratory stimulants can reverse respiratory depression caused by anesthetics; however, they also interfere with the sedative effects of anesthetics. Previous studies have suggested that tandospirone may ameliorate respiratory depression without affecting the sedative effects of anesthetics. Therefore, we evaluated whether tandospirone (0.1–8 mg/kg) ameliorates respiratory depression in a rat model under anesthesia. The protein kinase A redistribution method was used to determine whether tandospirone activates $\alpha_{2a/2c}$ and μ receptors. The effects of tandospirone (10 μM) on $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_2\delta$ GABA receptor current modulation were explored by two-electrode voltage clamping. Prophylactic tandospirone administration reduced respiratory depression caused by anesthetics in rats. Tandospirone (0.1–8 mg/kg) increased SaO_2 in rats treated with fentanyl (80 μg/kg) or midazolam (80 mg/kg) ($P < 0.05$). The ability of tandospirone to prevent respiratory depression was inhibited by the 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptor antagonist WAY100635 (1 mg/kg) ($P < 0.05$). Co-administration of tandospirone with dexmedetomidine or fentanyl did not affect $\alpha_{2a/2c}$ or μ receptors activation. Tandospirone (10 μM) did not affect $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_2\delta$ GABA receptor modulation ($P < 0.05$). Overall, tandospirone ameliorated respiratory depression caused by anesthetics in rats through 5-HT$_{1A}$ receptor activation.

Keywords 5-HT$_{1A}$, Tandospirone, Respiratory depression, Fentanyl, Midazolam, Dexmedetomidine

Anesthetic drugs are widely used for anesthetic induction and intraoperative stabilization, and they play a crucial role in surgical practice. Widely used anesthetic agents include opioids, such as fentanyl[1]; benzodiazepines, such as midazolam[2]; and α_2-adrenergic receptor agonists, such as dexmedetomidine[1]. However, respiratory depression is a common side effect of anesthetic drugs[3], and it can lead to death in people with opioid overdose. Compared with the normal breathing pattern, the respiratory rhythm during depression is fast and shallow with transient pauses. When high doses of midazolam or dexmedetomidine are administered in a single session, respiratory depression can occur. This phenomenon seriously threatens patient health and safety and increases the medical burden.

Physiological antagonists can reverse life-threatening respiratory depression[4]. Examples include naloxone (often used with fentanyl), flumazenil (often used with midazolam), and atipamezole (often used with dexmedetomidine). However, in addition to their ability to reverse respiratory depression, these agents reverse the sedative and analgesic effects of anesthetics[5]. Moreover, naloxone, which is an opioid antagonist, is associated with acute withdrawal symptoms[6]. Abdelal et al. suggested that multiple naloxone administrations are needed to reverse opioid overdose[7], which increases the potential for violent behavior in patients with reversal of sedation after intravenous opioid overdose[8]. Similarly to naloxone, the antagonistic effects of flumazenil are not limited to the reversal of respiratory depression. Flumazenil also blocks the sedative effects of midazolam[9]. When flumazenil blocks the effects of high-dose midazolam and other long-acting benzodiazepines, the danger of re-sedation should be considered, which occurs as the concentration of antagonist decreases[10]. Furthermore, atipamezole has been demonstrated to have undesirable side effects, including severe hypotension and bradycardia, when used at a concentration of 200 μg/kg in cats[11]. Respiratory depression caused by dexmedetomidine has not

[1]Beijing Key Laboratory of Neuropsychopharmacology, State Key Laboratory of Toxicology and Medical Countermeasures, Beijing Institute of Pharmacology and Toxicology, Taiping Road 27, Beijing 100850, China. [2]School of Pharmacy, Henan University, Kaifeng 475004, China. [3]Nanjing University of Chinese Medicine, Nanjing 210000, China. ✉email: yongzhabc@126.com; ruibinsu@126.com

received much research attention. These observations suggest that agents that can prevent respiratory depression without reducing the sedative and analgesic effects of anesthetics are urgently needed.

With this goal in mind, we performed a preliminary literature search, which revealed that tandospirone may have the potential to improve respiratory depression without affecting sedation and analgesia[12]. Tandospirone has shown promising anxiolytic effects in animal models, and it is now marketed for the treatment of anxiety disorders[13]. Tandospirone exerts its anxiolytic effects by activating postsynaptic 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptors coupled with G-proteins ($G_{i/o}$)[14], which inhibit the activity of adenylate cyclase, thereby reducing cyclic adenosine monophosphate (cAMP) and inhibiting protein kinase A (PKA)-mediated protein phosphorylation[15]. We intend to use this experimental system to (i) investigate whether tandospirone citrate ameliorates respiratory depression induced by fentanyl, midazolam, and dexmedetomidine in rats; (ii) evaluate the quantitative and spatiotemporal nature of the amelioration of respiratory depression by tandospirone citrate; and (iii) investigate the mechanism through which tandospirone citrate ameliorates respiratory depression. Through this study, we hope to evaluate whether tandospirone citrate has a broad-spectrum effect on improving respiratory depression. The findings of this study will inform whether tandospirone could be a useful agent to counteract respiratory depression caused by widely used anesthetics without interfering with the sedative and analgesic effects of these anesthetics.

Materials and methods

Animals

Male Sprague–Dawley rats (weighing 180–220 g) were provided by Beijing Huafukang Biotechnology Co. Ltd. (Beijing, China). The rats were housed in a controlled environment with a 12-hour/12-hour light–dark cycle at a constant temperature of 24 °C ± 2 °C in transparent boxes. The lights were switched on at 07:00, and the animals had unrestricted access to food and water and could move around freely.

Drugs

Tandospirone citrate and dexmedetomidine citrate were prepared by TargetMol Biotechnology Co. (Boston, MA, US). Midazolam hydrochloride was purchased from Yichang Renfu Pharmaceutical Co. (Hubei, China). Fentanyl hydrochloride (purity > 99%) and atipamezole (purity > 99%) were synthesized at our institution. WAY100635 was purchased from Biyuntian Biotechnology Co. (Shanghai, China), naloxone from McLean Biochemical Technology Co. (Shanghai, China), nikethamide from Boselle Biotechnology Co. (Beijing, China), and flumazenil from the China Institute for Food and Drug Control (Beijing, China). Fetal bovine serum was purchased from Life Technologies Co. (Shanghai, China). Phosphate-buffered saline and 0.25% trypsin solution were obtained from Sai Aomei Cell Technology Co. (Beijing, China). Forskolin was obtained from Sigma-Aldrich Co. (St. Louis, MO, US). Bis-benzimide H-33,342 trihydrochloride nuclear staining solution was obtained from Abe Medical Equipment Trading Co. (Shanghai, China). Hydrochloric acid (HCl), formaldehyde, sodium chloride (NaCl), potassium chloride (KCl), HEPES buffer, magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), $MgCl_2$ hexahydrate, and sodium pyruvate were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

Tandospirone, dexmedetomidine, fentanyl, nikethamide, and naloxone were dissolved individually in saline (0.9% NaCl), and flumazenil was dissolved in 2.5% dimethyl sulfoxide and Tween-80 (2.5%) prior to the experiment. Midazolam was first dissolved in deionized water and adjusted to pH 3 with HCl. All drugs were injected intravenously at a volume of 0.1 mL/100 g.

Rat model of respiratory depression

The MouseOX Murine Plus Oximeter System was used to measure real-time arterial oxygen saturation (SaO_2) in rats[16]. Before the experiment, the neck was shaved to expose the skin, and the rats were placed in a clear Plexiglas tank with bedding. The rats wore a clear neck collar to simulate the study condition and were allowed to move freely. After at least 30 min of acclimatization, the basal SaO_2 was recorded using an infrared probe. Tandospirone was intravenously injected at 0.1, 0.5, 1, 2, 4, or 8 mg/kg 10 min before starting the experiment. Intravenous fentanyl (80 μg/kg) was administered 5 min after tandospirone injection, and continuous recording of SaO_2 began 5 min after tandospirone injection. The vital signs were transmitted to the computer in real-time, including SaO_2, respiratory rate, and heart rate. The parameters were measured for 15 s every 5 min for a total of 45–60 min, and the average of each 15-second recording was obtained.

PKA redistribution

Chinese hamster ovary (CHO) cells were cultured in 200 μL F12 medium with 10% fetal bovine serum under standard conditions (37 °C, 5% carbon dioxide) to regulate cell density up to approximately 80%. After preparation of the cell suspension, a small portion of the mixture (one-third to one-quarter) was mixed with a small volume of medium in a pipette sink. The CHO cells were inoculated into black opaque 96-well plates and cultured overnight. The next day, drug administration began once the cell attachment rate had reached 80%.

In the experimental group, 40 μM tandospirone was added to each well. In the positive control group, 40 μM dexmedetomidine or fentanyl was added to each well. The co-administration group received 40 μM tandospirone and 40 μM dexmedetomidine or fentanyl. Additionally, 40 μM forskolin was added to each well and left to react for 10 min. The control group was untreated, and 200 μL F12 medium was added to complete the reaction. Once the reaction was complete, 12% formaldehyde solution was applied to each well for 20 min for fixation. The samples were stained with nucleic acid dye and kept in the dark for 3 h. Fluorescence detection was performed using high-content screening equipment, and the particle size and area were calculated[17].

Two-electrode voltage clamp

Sexually mature female African clawed frogs (*Xenopus laevis*) were purchased from the Kunming Institute of Zoology, Chinese Academy of Sciences. The frogs were reared according to standard experimental procedures. The water was changed three times per week, and a temperature of 18–23 °C was maintained. The frogs were housed in a 12-hour/12-hour light–dark cycle and were fed three times per week. To obtain Xenopus oocytes, the frogs were anesthetized by covering the entire body with crushed ice for at least 30 min. Then, the skin and muscle layers of the right leg were incised (incision length 1 cm) on the ventral side to fully expose the ovary. The oocytes were removed and placed in OR-2 solution (82 mM NaCl, 2.5 mM KCl, 5 mM HEPES buffer, and 1 mM $MgCl_2$ at pH 7.6). The oocyte clumps were treated with 1 mg·mL^{-1} collagenase (Sigma-Aldrich Co.) in OR-2 for 1 h. Stage V mature oocytes were observed and carefully selected under a microscope with a 10-fold volume of OR-2 solution. The selected oocytes were cultured in ND-96 (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES buffer at pH 7.6) overnight at 16 °C. The next day, microelectrode glass capillary tubes (#5206, VitalSense, Wuhan, China) were pulled using a two-stage glass micropipette puller (Narishige, Tokyo, Japan) for microinjection. The complementary RNA mixture was collected in sterile enzyme-free centrifuge tubes at a ratio of $\alpha_1:\beta_2:\gamma_2 = 1:1:1$ or $\alpha_4:\beta_2:\delta = 1:1:1$, and 40 ng of the mixture was injected into each oocyte using the microinjection pump. The cells were then cultured in OR-2 solution for 2–4 days, and the cell culture solution was changed daily. Current recordings were made after 2 days of synaptic $\alpha_1\beta_2\gamma_2$ gamma amino-butyric acid (GABA) receptor expression. Current recordings were made after 4 days of extrasynaptic expression of $\alpha_4\beta_2\delta$ GABA receptors[18].

Statistical analysis

The MouseOx Plus software was used to disaggregate, filter, average, and export Microsoft Excel spreadsheets from the .txt files generated by MouseOx. The statistical analyses and figure preparation were performed using GraphPad Prism 9 software (GraphPad, San Diego, CA, US). All data are presented as the average of 15-second recordings obtained at 5-minute intervals. The groups were compared by two-way analysis of variance (dose × time) followed by Bonferroni's post hoc test. The data are expressed as the mean ± standard error of the mean. $P < 0.05$ was considered statistically significant.

Results

Tandospirone reduced fentanyl-induced respiratory depression in rats

Fentanyl-induced respiratory depression was significantly reduced in the tandospirone group compared with the vehicle control group ($^{\Phi}P < 0.0001$, $^{\Omega}P < 0.001$, $^{\$}P < 0.01$, $^{*}P < 0.05$ vs. vehicle control group). Tandospirone at 0.1, 0.5, 1, 2, 4, or 8 mg/kg significantly reduced respiratory depression in a dose-dependent manner over 60 min (Fig. 1a). Within 5 min of fentanyl injection, SaO_2 decreased significantly in the vehicle control group from 95.78% ± 0.52–37.62% ± 1.44% (the difference in the decline as a proportion of the baseline value was 39.28%) ($^{\Phi}P < 0.0001$). However, SaO_2 in the 2 mg/kg tandospirone group decreased less, from 95.78% ± 0.26–63.60% ± 2.38% (the difference in the decline as a proportion of the baseline value was 66.40%) ($^{\Phi}P < 0.0001$ vs. vehicle control group).

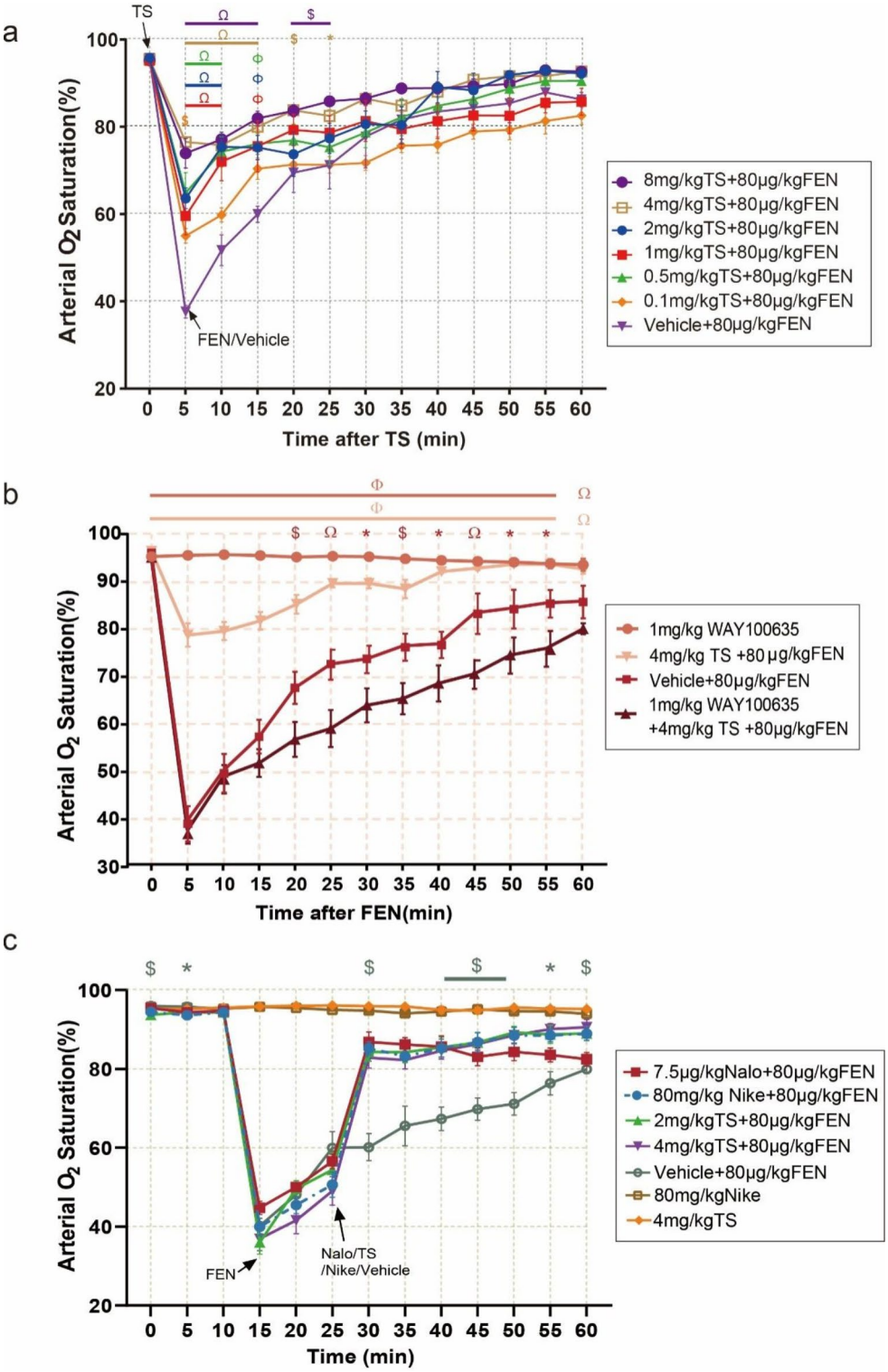
The 5-HT_{1A} receptor antagonist WAY100635 (Fig. 1b) completely blocked the ameliorative effects of tandospirone on respiratory depression. Figure 1b shows that WAY100635 alone did not change the baseline SaO_2 in rats, and the drug did not improve respiratory depression when used alone. In the group treated with intravenous tandospirone (Fig. 1c), the marked decrease in SaO_2 (from 94.94% ± 0.51–43.53% ± 3.30% [the difference in the decline as a proportion of the baseline value was 45.85%]) 5 min after fentanyl injection (80 μg/kg intravenous) indicated that the respiratory depression model had been successfully established, and there was no significant difference between Vehicle + 80 μg/kg FEN groups. However, the SaO_2 increased from 50.19% ± 3.07–79.66% ± 1.67% (the difference in the decline as a proportion of the baseline value was 33.05%) when fentanyl was injected 10 min after 2 mg/kg tandospirone ($^{\Phi}P < 0.0001$). Respiratory depression caused by fentanyl was reversed by naloxone, resulting in the rats regaining consciousness.

Tandospirone improved dexmedetomidine-induced respiratory depression in rats

Three minutes after dexmedetomidine administration (Fig. 2a), SaO_2 decreased from 95.75% ± 0.46–62.89% ± 2.83% (decrease of 65.68% from baseline). SaO_2 recovered to 77.19% ± 3.91% at 33 min (80.62% of baseline). Dexmedetomidine decreased SaO_2 from 95.78% ± 0.30–84.51% ± 3.10% at 3 min ($^{\Phi}P < 0.0001$) (88.23% of baseline). SaO_2 recovered to 81.89% ± 3.46% at 33 min (85.50% of baseline). There was no significant change in SaO_2 after the administration of 8 mg/kg tandospirone. Compared with the vehicle control group, pre-injection of WAY100635 completely blocked the ability of tandospirone to improve respiratory depression (Fig. 2b). A 6-minute baseline measurement was made prior to the experiment. A considerable reduction in SaO_2 was recorded 6 min after atipamezole injection in all groups (Fig. 2c), implying that the model of respiratory depression was proficiently established. These experimental results demonstrate that atipamezole blocked respiratory depression caused by dexmedetomidine.

Tandospirone reduced midazolam-induced respiratory depression in rats

No significant differences in SaO_2 were observed between the groups of rats prophylactically injected with tandospirone (Fig. 3a). In the vehicle control group, 3 min after midazolam administration, SaO_2 decreased from 94.91% ± 0.86–69.82% ± 3.01% (73.56% of baseline). In the group that received 2 mg/kg tandospirone, the SaO_2 only decreased from 96.18% ± 0.44–95.67% ± 0.28% after 3 min (99.47% of baseline). Tandospirone at 4 or 8 mg/kg was effective at reducing respiratory depression induced by midazolam ($^{\Phi}P < 0.0001$). A 6-minute baseline measurement was made prior to the experiment, and a considerable reduction in SaO_2 was recorded 6 min after flumazenil injection in all groups (Fig. 3b), implying that the model of respiratory depression was proficiently



established. After 9 min, dexmedetomidine was administered. The graph illustrates that tandospirone (2 mg/kg) was as effective as the specific antagonist flumazenil (1.8 mg/kg) at reducing respiratory depression caused by midazolam, and it surpassed the effect of nikethamide (80 mg/kg). SaO_2 decreased more in rats administered WAY100635 before tandospirone and midazolam injection than in the vehicle control group, suggesting that WAY100635 completely blocked the ability of tandospirone to reduce respiratory depression.

CHO-α2a/2c-PKAcat-enhanced green fluorescent protein (EGFP) cell redistribution experiments

In inactive cells, fluorescent particles exist in a highly aggregated state. After activation by forskolin, intracellular adenylate cyclase is activated, increasing intracellular cAMP and activating PKA, and the fluorescent fusion proteins change from an aggregated to a dispersed state. The results show that the number of fluorescent

◀**Fig. 1**. Pharmacodynamic ability of tandospirone to ameliorate fentanyl-induced respiratory depression and effect of the 5-HT_{1A} receptor antagonist WAY100635. (**a**) The rats were administered intravenous tandospirone (0.1, 0.5, 1, 2, 4, or 8 mg/kg) or vehicle and monitored continuously for 60 min at 5-minute intervals after fentanyl injection (80 μg/kg). The data are presented as the mean ± standard error of the mean ($n = 5$ rats per group) ($^{\Phi}P < 0.0001$, $^{\Omega}P < 0.001$, $^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the vehicle control group). (**b**) Pre-administration of WAY100635 (1 mg/kg) to block tandospirone (4 mg/kg) followed by fentanyl injection showed that WAY100635 completely blocked the ability of tandospirone to reduce respiratory depression. The data are presented as the mean ± standard error of the mean ($n = 5$ rats per group) ($^{\Phi}P < 0.0001$, $^{\Omega}P < 0.001$, $^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the group injected with WAY100635, tandospirone, and fentanyl). (**c**) The rats were acclimatized for 15 min and then injected with fentanyl (80 μg/kg) to establish the respiratory depression model. Combinations of naloxone (7.5 μg/kg), nikethamide (80 mg/kg), tandospirone (2 or 4 mg/kg), and vehicle were administered 15 min later. Fentanyl-induced respiratory depression was significantly reduced in all but the vehicle control group. The data are presented as the mean ± standard error of the mean ($n = 5$ rats per group) ($^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the nikethamide-injected group). 5-HT_{1A}, 5-hydroxytryptamine; FEN, fentanyl; Nalo, naloxone; Nike, nikethamide; TS, tandospirone.

particles was markedly lower in CHO-α_{2a}-PKAcat-EGFP cells than in cells from the vehicle control group when forskolin treatment was used alone (Fig. 4a). In the tandospirone treatment group (1×10^{-5} to 1×10^{-10} M), the number of fluorescent particles significantly decreased at a concentration of 10^{-9} mol·L^{-1}, indicating that forskolin fully activated the cells. PKA shifted from aggregated to dispersed, indicating that tandospirone did not interact with $\alpha_{2a/2c}$ receptors. Dexmedetomidine (1×10^{-5} to 1×10^{-9} mol·L^{-1} M) counteracted the activation by forskolin, indicating that dexmedetomidine bound to $\alpha_{2a/2c}$ receptors and inhibited PKA redistribution (Fig. 4b). In the tandospirone group (1×10^{-5} to 1×10^{-11} mol·L^{-1}), there was no significant difference in the number of fluorescent particles after the addition of dexmedetomidine (1×10^{-7} mol·L^{-1}) compared with the forskolin alone group. Moreover, tandospirone did not influence the $\alpha_{2a/2c}$ receptor binding effect of dexmedetomidine, indicating that tandospirone did not act directly on $\alpha_{2a/2c}$ receptors.

CHO-μ-PKAcat-EGFP cell redistribution experiments

Fentanyl activated μ receptors on CHO-PKA-cat-EGFP cells in a concentration-dependent manner. Significant redistribution of cellular fluorescent particles was observed in the fentanyl group compared with the forskolin group (1×10^{-6} to 1×10^{-10} mol·L^{-1}). In the tandospirone group (1×10^{-5} to 1×10^{-9} mol·L^{-1}), there was no significant difference in the number of fluorescent particles compared with the forskolin group. In both the tandospirone group (1×10^{-5} to 1×10^{-11} mol·L^{-1}) and the fentanyl group (1×10^{-7} mol·L^{-1}), there was no significant difference in the number of fluorescent particles when compared with the forskolin group. Moreover, tandospirone prophylaxis did not impact the μ-receptor binding effect of fentanyl, suggesting that tandospirone did not act directly on μ receptors.

Effect of tandospirone on α1β2γ2 and α4β2δ GABAA receptor currents

In Xenopus oocytes, which express synaptic and extrasynaptic receptors (Fig. 5a and b, respectively), the inward currents induced by GABA (1 μM) through the $\alpha_1\beta_2\gamma_2$ GABA receptor subtype were -89.44 ± 23.73 nA. The antagonist bicuculline almost completely blocked GABA-induced currents. The inward currents through the $\alpha_4\beta_2\delta$ GABA receptor subtype were -208.49 ± 40.17 nA, as measured by dual-motor voltage clamp with the cells clamped at −70 V. Midazolam (100 μM) increased the 1 μM GABA-evoked current amplitude, and both GABA receptor subtypes showed the same enhancement effect at this dose (Fig. 5d and e). Tandospirone (10 μM) decreased the 1 μM GABA-mediated inward chloride current, and for $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_2\delta$ GABA receptor modulation, the inhibition rate was $-5.59\% \pm 6.28\%$ and $-38.07\% \pm 7.80\%$, respectively. Tandospirone reduced the modulation of GABA receptor currents by endogenous GABA, with a significant decrease in extrasynaptic receptor currents. The combination of tandospirone (10 μM) and midazolam (100 μM) reduced the 1 μM GABA-induced current amplitude, and for $\alpha_1\beta_2\gamma_2$ receptor and $\alpha_4\beta_2\delta$ receptor modulation, the inhibition rate was 85.54% ± 18.74% and 62.68% ± 24.16%, respectively. However, for $\alpha_1\beta_2\gamma_2$ receptors, the difference in current amplitude induced by 1 μM GABA compared with 10 μM tandospirone was not statistically significant. The results suggest that tandospirone may regulate GABA receptor current, but not enough to affect the efficacy of midazolam.

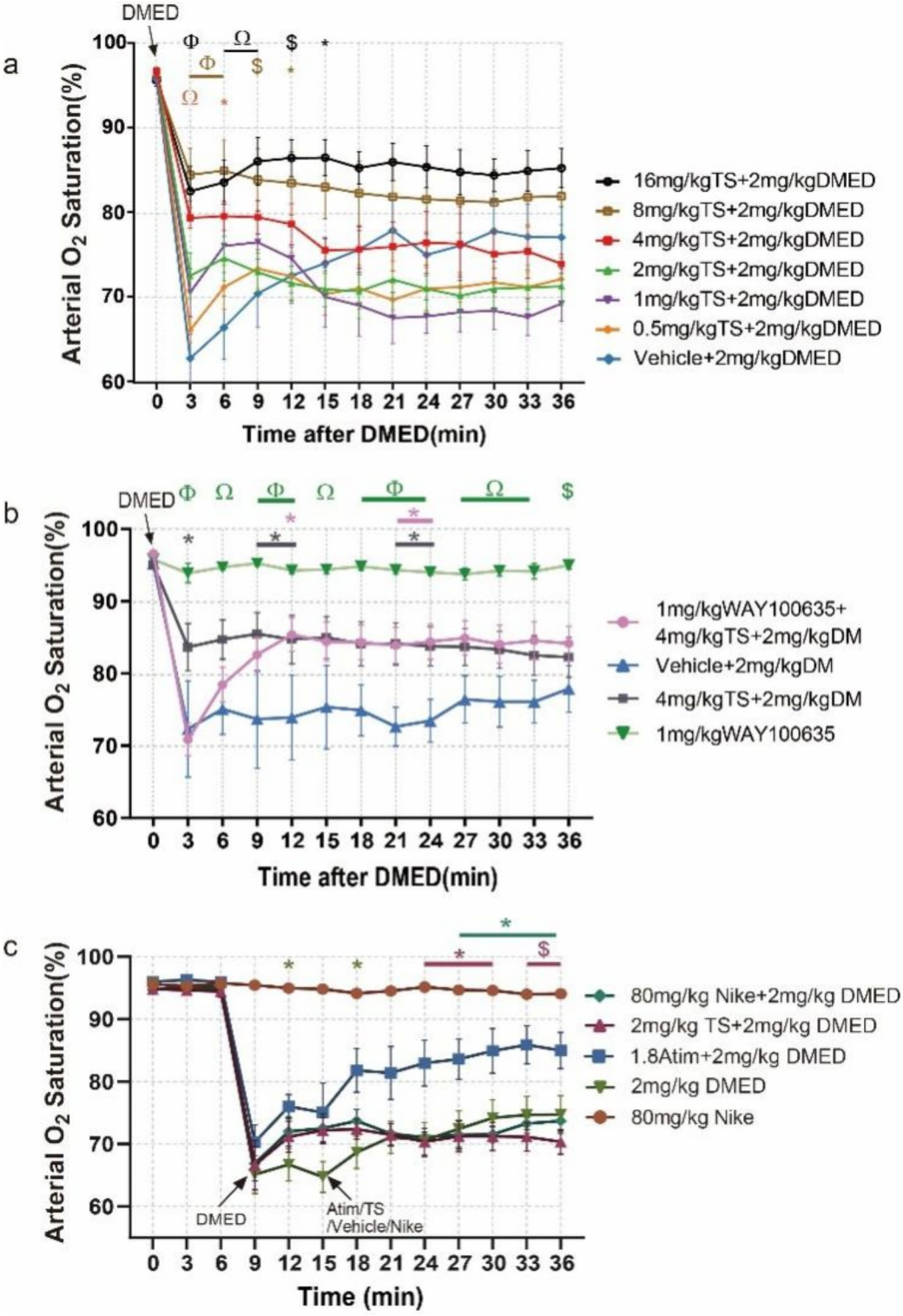
Discussion

Pharmacodynamics study of tandospirone to improve respiratory depression

In this study, we established the quantitative and temporal ability of tandospirone to improve respiratory depression caused by anesthetics in rats. Prophylactic administration of tandospirone significantly reduced respiratory depression caused by fentanyl, midazolam, and dexmedetomidine; increased SaO_2; and accelerated recovery in rats. Tandospirone showed broad-spectrum amelioration of respiratory depression caused by three widely used anesthetics.

Tandospirone exerts counteracting effector receptor mechanisms

Tandospirone improved respiratory depression caused by anesthetic drugs that act on multiple and diverse receptor targets. The mechanism of tandospirone can be explored from two perspectives: direct effects and non-direct effects. Direct effects imply that tandospirone may act directly on the receptors that are targeted by anesthetics that cause respiratory depression, functioning as a specific agonist or antagonist. Non-direct



effects suggest that tandospirone may not directly act on the receptor targets of these anesthetic drugs, instead influencing the downstream signaling pathways of these receptor targets or directly regulating the respiratory center to prevent respiratory depression. To clarify the mechanism of tandospirone as a receptor antagonist, the PKA redistribution test was used to observe whether tandospirone has direct or antagonistic effects on the α_{2a}- and α_{2c}-adrenergic receptors (dexmedetomidine target) and the μ-opioid receptor (fentanyl target). The regulatory effect of tandospirone on the $GABA_A$ receptor (midazolam target) was also investigated using the dual-electrode voltage clamp system. Furthermore, the improvement in multi-drug-induced respiratory depression afforded by tandospirone was examined after pretreatment with the 5-HT_{1A} receptor antagonist WAY100635 to determine whether the effect of tandospirone was related to 5-HT_{1A} receptor activation.

Opioids primarily act on three types of receptor (μ, δ, κ) to exert their biological effects. Currently, it is thought that opioid-induced respiratory depression is mainly mediated by μ receptors expressed on respiratory

◀**Fig. 2**. Pharmacodynamic ability of tandospirone to ameliorate dexmedetomidine-induced respiratory depression and effect of the 5-HT_{1A} antagonist WAY100635. (**a**) The rats in each group were administered intravenous tandospirone (0.5, 1, 2, 4, 8, or 16 mg/kg) or vehicle and continuously monitored for 36 min at 3-minute intervals after dexmedetomidine administration (2 mg/kg). The data are presented as the mean ± standard error of the mean ($n = 5$–7 rats per group) ($^{\Phi}P < 0.0001$, $^{\Omega}P < 0.001$, $^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the vehicle-injected group). (**b** Pre-administration of WAY100635 (1 mg/kg) to block tandospirone (4 mg/kg) followed by fentanyl injection showed that WAY100635 partially blocked the effects of dexmedetomidine (2 mg/kg), reducing respiratory depression. The data are presented as the mean ± standard error of the mean ($n = 5$ rats per group) ($^{\Phi}P < 0.0001$, $^{\Omega}P < 0.001$, $^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the WAY100635, tandospirone, and dexmedetomidine-injected group). (**c**) In the dexmedetomidine (2 mg/kg) group, only the specific antagonist atipamezole (1.8 mg/kg) reduced dexmedetomidine-induced respiratory depression, whereas nikethamide (80 mg/kg), tandospirone (2 mg/kg), and vehicle did not. The data are presented as the mean ± standard error of the mean ($n = 5$ rats per group) ($^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the nikethamide-injected group). 5-HT_{1A}, 5-hydroxytryptamine; Atim, atipamezole; DMED, dexmedetomidine; Nike, nikethamide; TS, tandospirone.

neurons in the central nervous system. The respiratory centers of the brain stem, such as the pre-Bötzinger complex and the parabrachial nucleus, are involved in the generation of respiratory patterns and express opioid receptors. After exposure to exogenous fentanyl, μ receptors are activated and respiratory depression occurs[19]. Additionally, opioids can inhibit the rhythmic activity of key neuronal networks, such as the pons and spinal cord, sometimes even leading to their disappearance, hyperpolarization, and deinhibition[20].

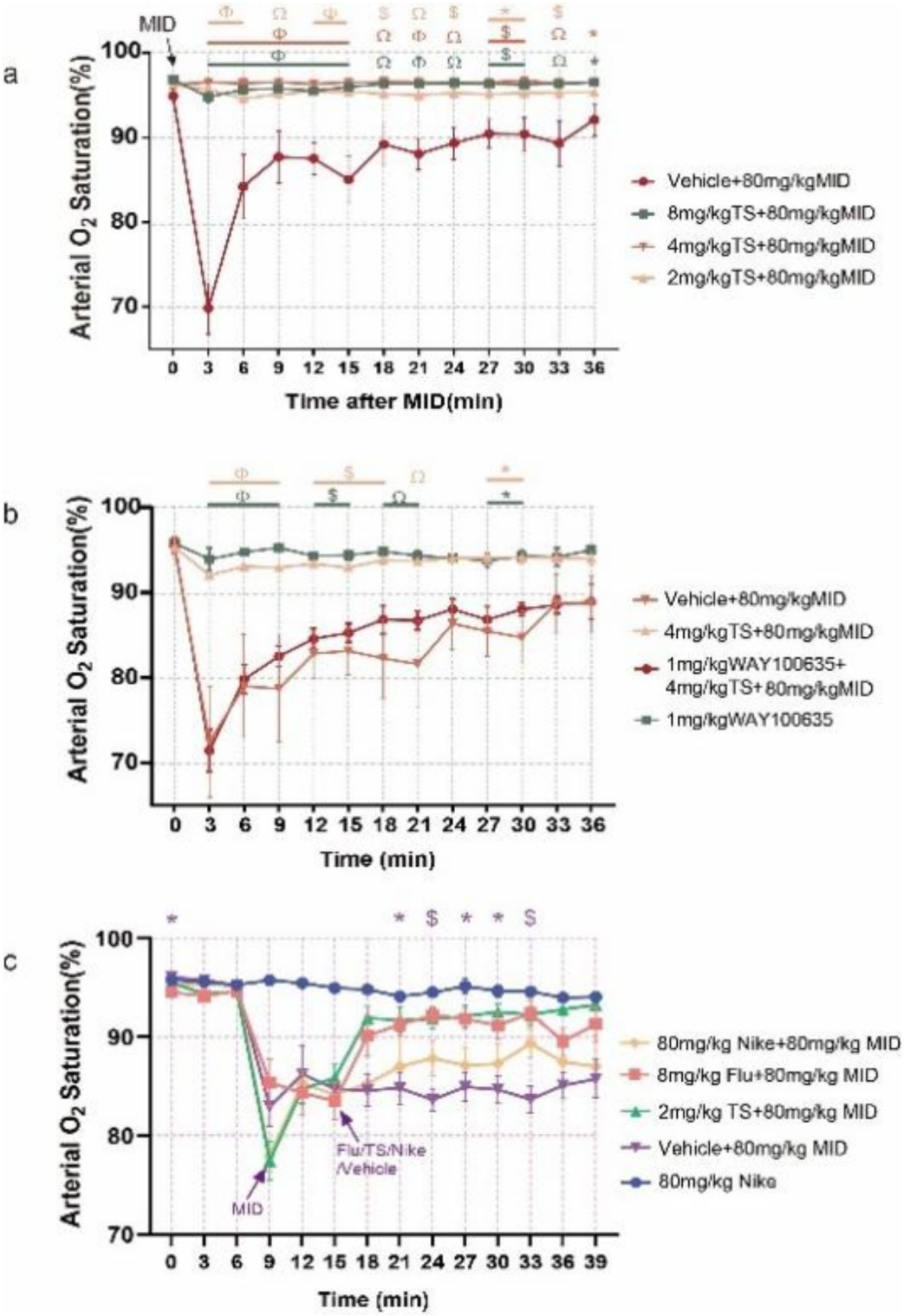
α_2-adrenergic receptors are found throughout the central and peripheral nervous systems, particularly in the locus coeruleus, spinal cord, ventral lateral coracoid medulla, and spinal dorsal horn[21]. Dexmedetomidine mainly inhibits neuronal discharge by activating α_2-adrenergic receptors in the brain and spinal cord, reducing sympathetic nerve tone. It has a sedative and analgesic effect, and it also exerts a hypnotic effect by activating presynaptic and postsynaptic α-adrenergic receptors in the central part of the locus coeruleus, inducing an unconscious state that is similar to that of natural sleep while maintaining spontaneous breathing and upper airway tone[22]. This result suggests that α_{2a}- and α_{2c}-adrenergic receptors may be involved in the action of dexmedetomidine.

In the PKA redistribution experiment, dexmedetomidine activated CHO cells in a concentration-dependent manner, and intracellular fluorescent particles remained aggregated. We established that tandospirone did not act directly on α_{2a}- and α_{2c}-adrenergic receptors, resulting in a decrease in the area of fluorescent particles and causing PKA redistribution. Meanwhile, fentanyl led to significant PKA redistribution. As a result, the intracellular fluorescent particles changed from an aggregated to a diffuse state. These results suggest that dexmedetomidine activates both α_{2a}- and α_{2c}-adrenergic receptors, while fentanyl activates the μ receptor. However, tandospirone failed to block the activating effect of forskolin, indicating minimal binding to $\alpha_{2a/2c}$-adrenergic receptors and μ receptors. When we fixed the dexmedetomidine concentration in combination with tandospirone, tandospirone did not affect the activation of $\alpha_{2a/2c}$-adrenergic receptors by dexmedetomidine. Similarly, our results confirm that the combination of tandospirone and fentanyl has no impact on the activation of μ receptors by fentanyl. Thus, tandospirone does not appear to regulate respiration by directly acting on μ receptors.

Midazolam is a benzodiazepine that positively modulates the GABA receptor, which is expressed as a pentameric protein. A previous study suggested that midazolam binds to the GABA receptor only in the presence of the γ2 subunit[23]. To validate the modulatory effects of tandospirone and midazolam on both intrinsic and extrinsic subunits at the synapse, we transfected the $\alpha_1\beta_2\gamma_2$ subunit and the $\alpha_4\beta_2\delta$ subunit into Xenopus oocytes. To investigate how tandospirone and midazolam affected GABA receptors, we conducted two-electrode voltage clamp experiments. We found that 1 μM GABA had a significant modulatory effect on $GABA_A$ receptors, resulting in inward chloride current and hyperpolarization. The current amplitude was reduced by tandospirone, while midazolam increased the amplitude of GABA-induced currents. The overall current amplitude was additive when tandospirone was used with midazolam, and tandospirone produced the same effect on current modulation for both $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_2\delta$ GABA receptors. The original current-modulating effect of midazolam on $\alpha_1\beta_2\gamma_2$ and $\alpha_4\beta_2\delta$ GABA receptors was not altered by co-administration of tandospirone.

Tandospirone is primarily an agonist of the 5-HT_{1A} receptor, and we have previously explored ways to enhance respiratory depression by inhibiting the 5-HT_{1A} receptor[14]. The results showed that the 5-HT_{1A} receptor antagonist WAY100635 greatly impeded the ability of tandospirone to ameliorate fentanyl- and midazolam-induced respiratory depression and partially blocked dexmedetomidine-induced respiratory depression. Neither tandospirone nor nikethamide had any effect. Other researchers have demonstrated 5-HT receptor-mediated activation of inwardly rectifying potassium channels in rat dorsal raphe neurons[24]. Tandospirone is also a G-protein-coupled receptor agonist[25]. Activation of 5-HT receptors involves G-proteins in the transduction pathway, and G-proteins may interact directly with potassium channels[26]. However, it remains to be established whether this is the molecular mechanism through which tandospirone ameliorates respiratory depression.

When metabolized in vivo, tandospirone produces 1-[2-pyrimidyl]-piperazine (1-PP)[27]. The pharmacodynamic effects of 1-PP remain unclear; however, according to the existing literature, it is hypothesized that 1-PP acts as an α_2-adrenergic receptor antagonist[28]. The results of our preliminary experiment showed that after 5 min of 1-PP administration, there was no significant increase in SaO_2 and no difference in the block of dexmedetomidine compared with the lysophospholipid control group. These results suggest that tandospirone may not exert its effects through 1-PP. We also previously investigated the protective effects of 1-PP against acute death in mice caused by dexmedetomidine. No useful results were obtained; thus, the results have not been published.



This study used a reliable, consistent, and safe model of respiratory depression in rats. However, this study has several limitations that should be considered. First, we did not evaluate the correlations of respiratory rate and tidal volume with SaO_2. Second, the mechanism by which tandospirone reduced respiratory depression without compromising the effects of sedation and analgesia was not evaluated. Finally, the male Sprague–Dawley rats used in this experiment were not differentiated based on their degree of sensitivity to respiratory depression compared with female rats. In previous experiments in male and female rats treated with the opioids heroin and fentanyl, it was found that heroin elicited more extensive and prolonged (lasting for 45–60 min) respiratory depression in female than in male rats[29]. These findings will be evaluated in future experiments, where additional opioids will be tested to investigate the differences in SaO_2 between male and female rats.

◀**Fig. 3**. Pharmacodynamic ability of tandospirone to ameliorate midazolam-induced respiratory depression and effects of the 5-HT_{1A} receptor antagonist WAY100635. (**a**) The rats were administered tandospirone (2, 4, or 8 mg/kg) or vehicle by intravenous injection and continuously monitored for 36 min at 3-minute intervals after midazolam administration (80 mg/kg). The data are presented as the mean ± standard error of the mean ($n = 5$ rats per group) ($^{\Phi}P < 0.0001$, $^{\Omega}P < 0.001$, $^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the vehicle-injected group). (**b**) In the group administered midazolam (80 mg/kg), respiratory depression was alleviated by the specific antagonists flumazenil (1.8 mg/kg) and tandospirone (2 mg/kg). Nikethamide and vehicle had no such ameliorative effect. The data are presented as the mean ± standard error of the mean ($n = 5$ rats per group) ($^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the nikethamide-injected group). (**c**) Pre-administration of WAY100635 (1 mg/kg) to block tandospirone (4 mg/kg) followed by midazolam (80 mg/kg) showed that WAY100635 completely blocked midazolam, leading to an improvement in respiratory depression. The data are presented as the mean ± standard error of the mean ($n = 5$ rats per group) ($^{\Phi}P < 0.0001$, $^{\Omega}P < 0.001$, $^{\$}P < 0.01$, $^{*}P < 0.05$ vs. the WAY100635, tandospirone, and midazolam-injected group). 5-HT_{1A}, 5-hydroxytryptamine; Flu, flumazenil; MID, midazolam; Nike, nikethamide; TS, tandospirone.

Conclusion

Tandospirone ameliorates respiratory depression caused by widely used anesthetics in rats through 5-HT_{1A} receptor activation. Future studies should validate these findings and evaluate whether tandospirone has clinical application value for ameliorating respiratory depression in patients receiving anesthetics.

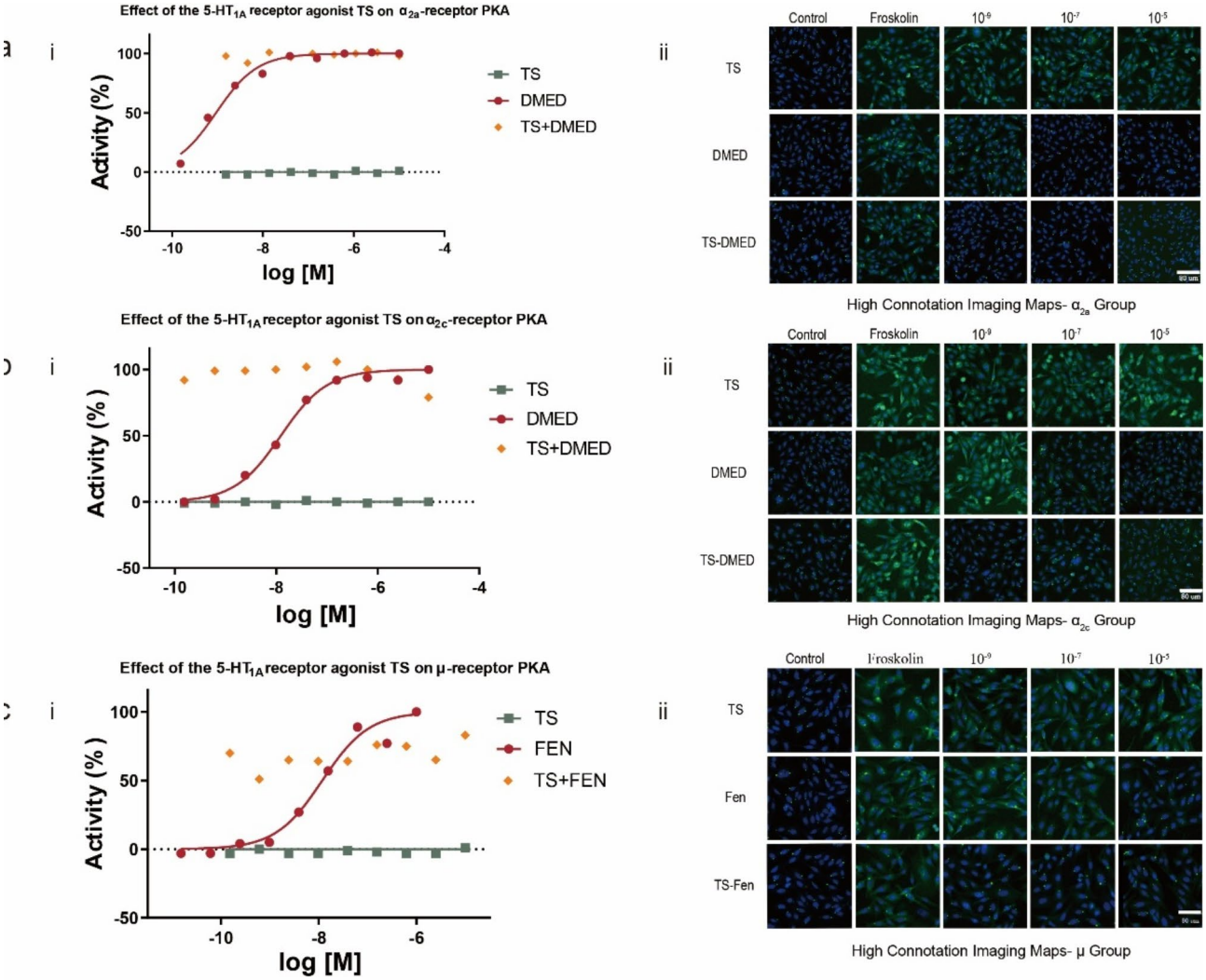


Fig. 4. (**a**) The number of fluorescent particles in the forskolin alone group was markedly decreased in CHO-$\alpha_{2a/2c}$-PKAcat-EGFP and CHO-μ-PKAcat-EGFP cells compared with the control group. (**a**i–**b**ii) Dexmedetomidine activated $\alpha_{2a/2c}$ receptors on CHO-PKAcat-EGFP cells in a concentration-dependent manner causing significant PKA redistribution. (**c** i–**c**ii) Fentanyl activated μ receptors on CHO-PKAcat-EGFP cells in a concentration-dependent manner causing significant PKA redistribution. In the tandospirone group, there was no significant difference in the number of fluorescent particles compared with the forskolin group. The tandospirone group (1×10^{-5} to 1×10^{-9} mol·L^{-1}) and the dexmedetomidine/fentanyl group (1×10^{-7} mol·L^{-1}) demonstrated significant reductions in the number of fluorescent particles compared with the control group. In both the tandospirone group (1×10^{-7} to 1×10^{-11} mol·L^{-1}) and the dexmedetomidine/fentanyl group (1×10^{-7} mol·L^{-1}), there was no significant difference in the number of fluorescent particles when compared with the forskolin group. DMED, dexmedetomidine; FEN, fentanyl; TS, tandospirone,

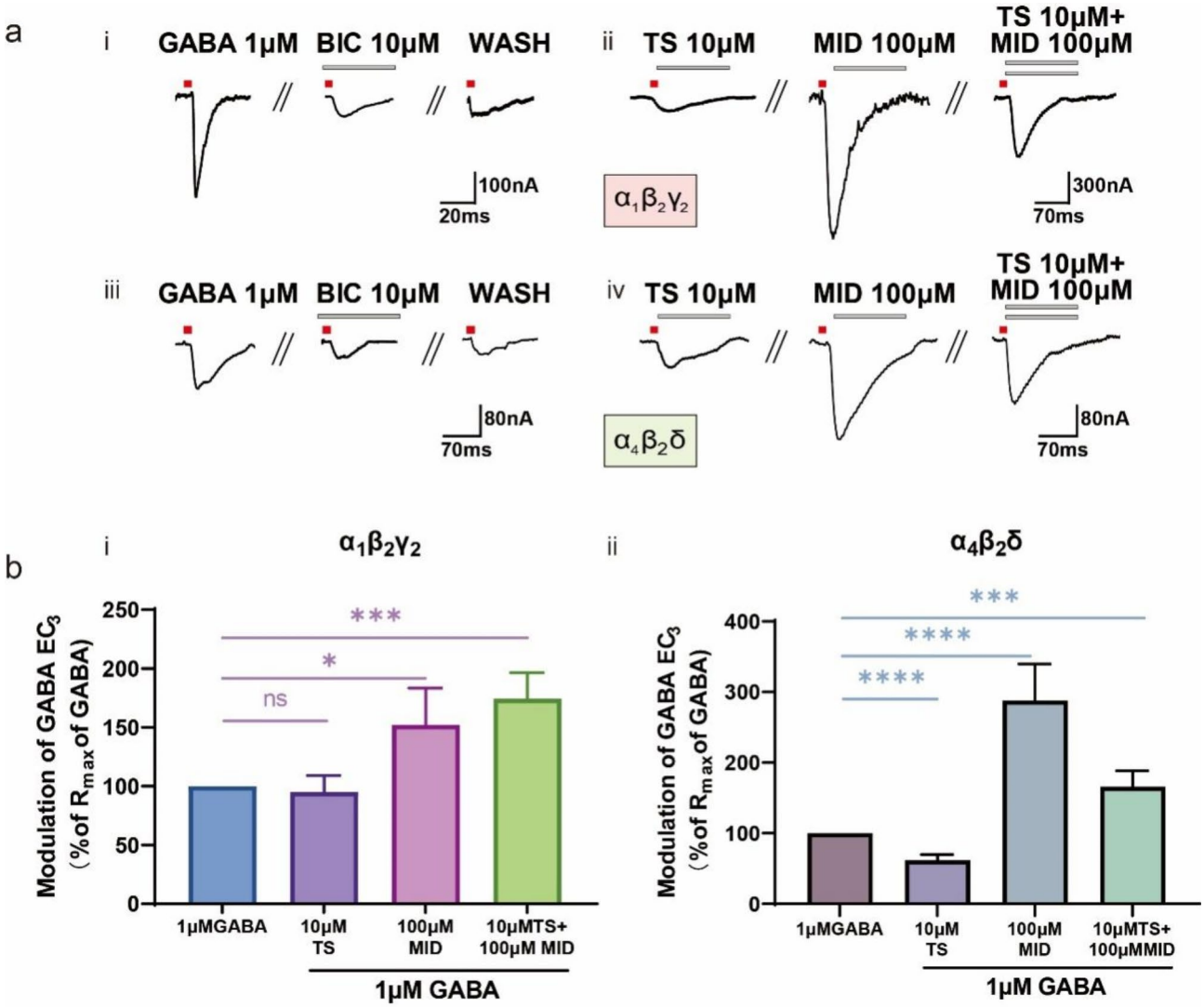


Fig. 5. Effect of tandospirone on midazolam-induced currents. (**a**) Tandospirone (10 μM) reduced 1 μM GABA-mediated inward chloride currents and simultaneously inhibited the enhancement of $\alpha_1\beta_2\gamma_2$ GABA receptor and $\alpha_4\beta_2\delta$ GABA receptor currents by midazolam (i–ii and iii–iv). (**b**) Rate of $\alpha_1\beta_2\gamma_2$ (i) and $\alpha_4\beta_2\delta$ (ii) GABA receptor current modulation by tandospirone. The data are presented as the mean ± standard error of the mean. BIC, bicuculline; GABA, gamma amino-butyric acid; MID, midazolam.

Data availability

Data will be made available on request. Please contact us at yongzhabc@126.com if you wish to retrieve the data.

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Author contributions

MRS: Investigation, Methodology, Validation, Formal analysis, Data Curation, Writing - Original Draft. MZH: Validation, Formal analysis. WJT: Formal analysis, Writing - Original Draft. ZY: Methodology, Writing - Review & Editing, Supervision, Funding acquisition. RBS: Conceptualization, Resources, Supervision, Funding acquisition.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

We confirm that all experiments were performed in accordance with relevant named guidelines and regulations, and that the authors complied with the ARRIVE guidelines.All animal housing and experiments were conducted in strict accordance with the institutional guidelines for care and use of laboratory animals. All experiments were approved by the Institutional Animal Care and Use Committee (approval number: IACUC-DWZX-2023P562).

Additional information

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Correspondence and requests for materials should be addressed to Z.Y. or R.-b.S.