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Dexmedetomidine Reduces Long-term Potentiation in Mouse Hippocampus

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Background: **Dexmedetomidine (Precedex; Abbott Laboratories, Abbott Park, IL) is a selective α2-adrenergic agonist that also has affinity for imidazoline receptors. In clinical studies, dexmedetomidine has sedative effects and impairs memory, but the action of dexmedetomidine on synaptic plasticity in the brain has yet to be established. In the present study, the authors investigated the effects of dexmedetomidine on two forms of synaptic plasticity—long-term potentiation (LTP) and paired-pulse facilitation—in the CA1 region of mouse hippocampal slices.**

Methods: **The authors recorded Schaffer collateral-evoked field excitatory postsynaptic potentials from mouse hippocampal slices in CA1 stratum radiatum. The slope of the rising phase of the field excitatory postsynaptic potential was used to estimate the strength of synaptic transmission.**

Results: **Application of dexmedetomidine for 20 min before "theta burst" stimulation dose-dependently attenuated LTP, and half-inhibitory concentration of dexmedetomidine was 28.6 ± 5.7 nM. The inhibitory effect of dexmedetomidine on LTP was not abolished by an α2-adrenoceptor antagonist (yohimbine), an imidazoline type 1 receptor and α2-adrenoceptor antagonist (efaroxan), an α1-adrenoceptor antagonist (prazosin), or a γ-aminobutyric acid type A receptor antagonist (picrotoxin). However, an imidazoline type 2 receptor and α2-adrenoceptor antagonist (idazoxan) completely blocked the dexmedetomidine-induced attenuation. Furthermore, 2-benzofuranyl-2-imidaloline, a selective imidazoline type 2 receptor ligand, reduced LTP. 2-(4,5-dihydroimidaz-2-yl)-quinoline, another imidazoline type 2 receptor ligand, abolished the 2-benzofuranyl-2-imidaloline-induced attenuation, but the inhibitory effect of dexmedetomidine on LTP was not abolished by 2-(4,5-dihydroimidaz-2-yl)-quinoline. Dexmedetomidine did not affect paired-pulse facilitation.**

Conclusion: **Dexmedetomidine impairs LTP in area CA1 of the mouse hippocampus *via* imidazoline type 2 receptors and α2-adrenoceptors.**

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DEXMEDETOMIDINE is a highly selective α2-adrenergic agonist (binding affinity for α2:α1 = 1620:1, Ki = 1.08 nM, as measured by displacement of [^{3}H]clonidine)[1] with both sedative and analgesic effects. Dexmedetomidine-induced sedation is accompanied by little respiratory depression and is easily reversed with verbal or physical stimuli. Therefore, dexmedetomidine is used clinically in intensive care units to sedate intubated patients. In rats, α2-adrenoceptor activation impairs spatial navigation in a water maze,[2] whereas blocking these receptors facilitates learning and memory.[3,4]

Activity-dependent synaptic plasticity is considered a cellular mechanism for learning and memory.[5] Whereas α2-adrenoceptor activation can reduce noradrenergic synaptic transmission and block synaptic plasticity at glutamatergic synapses of the basolateral amygdala,[6] the role of α2-adrenoceptors in hippocampal synaptic plasticity has not been well studied.[7] The three subtypes of α2-adrenoceptors in the brain are $\alpha2_{A/D}$, $\alpha2_B$, and $\alpha2_C$.[8] $\alpha2_{A/D}$ and $\alpha2_C$ are densely expressed in the hippocampus[9] where they inhibit noradrenaline release.[10] Because it was suggested that endogenous noradrenaline release is required for maintenance of hippocampal CA1 long-term potentiation (LTP),[11] presynaptic inhibition of noradrenaline release by dexmedetomidine may impair hippocampal LTP. However, this hypothesis has yet to be tested directly.

Dexmedetomidine has an imidazoline structure and affinity for imidazoline receptors.[12,13] Three subtypes of imidazoline receptors are expressed in the brain, namely imidazoline type 1, type 2, and nontype 1/type 2 (subtype 3). [^{3}H]idazoxan binding, which reveals imidazoline type 2 receptors, is densely distributed in the brain—both in neurons and glial cells[14,15] and in the hippocampus including the CA1 field[16]; however, it is unclear whether these receptors modulate synaptic transmission in the hippocampus.

In the present study, we investigated the effects of dexmedetomidine on synaptic transmission and plasticity in the CA1 field of mouse hippocampal slices. Dexmedetomidine attenuated LTP induced by θ-burst stimulation (TBS) through activation of α2-adrenoceptors and imidazoline type 2 receptors.

Materials and Methods

Hippocampal Slice Preparation and Electrophysiological Recording

All experiments were performed in strict accordance with the regulations of the National Center of Neurology and Psychiatry for animal experiments. Hippocampal

slices were prepared from 20- to 30-d-old male C57BL/6J mice as reported.[17,18] Briefly, animals were decapitated under halothane anesthesia, and the hippocampus was rapidly removed. Transverse slices (400 μm thick) were cut using a Vibratome 3000 (Vibratome, St. Louis, MO) in cold (2.5°C) sucrose Ringer's solution containing (in mM): 234 sucrose, 26 $NaHCO_3$, 2.5 KCl, 0.5 $CaCl_2$, 1.25 NaH_2PO_4, 10 $MgSO_4$, and 11 glucose (pH 7.3-7.4), oxygenated with 95% O_2/5% CO_2. The slices were incubated in artificial cerebrospinal fluid (ACSF) containing (in mM): 26 $NaHCO_3$, 124 NaCl, 3.0 KCl, 2.0 $CaCl_2$, 1.2 KH_2PO_4, 1.3 $MgHPO_4 \cdot 7H_2O$, and 10 glucose (pH 7.3–7.4) at room temperature for at least 90 min before use. During this incubation, halothane used for anesthesia in our experiments would be washed out in incubation media, ACSF. Therefore, although volatile anesthetics have suppressive effects on LTP in the hippocampus,[19] the influence of halothane was expected to be minimal in our study. A single slice was transferred to the recording chamber and perfused with ACSF at a rate of 2-3 ml/min (30 ± 2°C). ACSF was continuously bubbled with 95% O_2/5% CO_2. Field excitatory postsynaptic potentials (fEPSPs) were recorded from stratum radiatum of the hippocampal area CA1 using a glass micropipette (1–2 MΩ) filled with ACSF. Electrical signals were amplified using a MultiClamp 700B amplifier (Axon Instruments, Union City, CA), filtered at 10 kHz, digitized at 10 kHz, and acquired with Clampex software (version 9.2, Axon Instruments). A bipolar stainless steel stimulating electrode was placed in the stratum radiatum to stimulate the Schaffer collateral pathway. In all experiments, stimulus intensity was adjusted to produce a fEPSP that was 40–50% of the maximal amplitude. The duration of the stimulus was 0.10–0.15 ms. fEPSPs for which the 40–50% amplitude was >1 mV were used for data analysis. The strength of synaptic transmission was determined by measuring the slope of the fEPSP during the rising phase (20–60%). The average fEPSP slope during the 10 min before LTP induction was taken as baseline, and all values were normalized to this baseline (normalized fEPSP slope). The average of normalized fEPSPs slope from 55 to 60 min after induction was taken as a measure of the maintenance of LTP. The stimulus frequency during the baseline was 0.033 Hz. LTP was induced by TBS (15 bursts of four pulses at 100 Hz, delivered at an interburst interval of 200 ms). Paired-pulse facilitation (PPF) was induced by delivering two pulses with a 20-, 50-, 70-, 100-, or 200-ms interpulse interval.

Drug Solutions

All drugs were prepared as stock solutions and dissolved in ACSF on the day of the experiment. Antagonists were bath-applied >10 min before and during dexmedetomidine application with the exception of picrotoxin, which was only perfused for the 10 min before and during LTP induction. Dexmedetomidine was obtained from Abbott Japan (Osaka, Japan). Yohimbine, idazoxan, efaroxan, and prazosin were purchased from Sigma (St. Louis, MO). 2-benzofuranyl-2-imidaloline (2-BFI) and 2-(4,5-dihydroimidaz-2-yl)-quinoline (BU-224) were purchased from Tocris-Cookson (Bristol, UK). Picrotoxin was purchased from Research Biochemicals Incorporated (Natick, MA).

Statistical Analysis

Baseline transmission and PPF between ACSF (the average of normalized fEPSPs slope from 15 to 20 min after perfusion of ACSF) and 50 nM dexmedetomidine (the average of normalized fEPSPs slope from 25 to 30 min after switching to dexmedetomidine) were compared using a nonparametric Mann–Whitney U test. The effects of different concentrations of dexmedetomidine (0, 25, 35, 50, and 100 nM) on LTP were compared using a nonparametric one-way ANOVA followed by Dunn's multiple comparison test. Statistical comparisons of the data between groups (vehicle *vs.* dexmedetomidine) in the presence of yohimbine + WAY100635, efaroxan, idazoxan, BU-224, prazosin, and picrotoxin were performed using a nonparametric Mann–Whitney U test. Comparison among the effects of antagonists on LTP was performed using a nonparametric one-way ANOVA followed by Dunn's multiple comparison test. Comparison of the difference in the dexmedetomidine effect on LTP in the presence of antagonists was performed using a nonparametric one-way ANOVA followed by Dunn's multiple comparison test. Comparisons of the data obtained in the presence of ACSF, 2-BFI, BU-224, and BU-224 + 2-BFI were performed using a nonparametric one-way ANOVA followed by Dunn's multiple comparison test. The concentration of dexmedetomidine that produces 50% of maximal inhibition (IC_{50}) was calculated from a fitting curve of our data to Hill equations (nonlinear regression analysis using Graphpad-Prism software version 4.00; Graphpad Software, Inc., San Diego, CA). The number of animals tested is indicated by n. Probability values (P) <0.05 were considered statistically significant. The mean ± SEM is shown in all figures.

Results

Effect of Dexmedetomidine on Basal Synaptic Transmission and PPF

Figure 1 shows the effect of dexmedetomidine on basal synaptic transmission and PPF (n = 5). After 20 min of baseline recording in ACSF, dexmedetomidine (50 nM) was applied. There was no change in the slope of the first (ACSF *vs.* dexmedetomidine; 1.00 ± 0.01 *vs.* 0.99 ± 0.02, $P > 0.05$) or second (ACSF *vs.* dexmedetomidine; 1.94 ± 0.07 *vs.* 1.96 ± 0.08) fEPSP in the presence of dexmedetomidine, indicating that basal synaptic transmission and PPF were unaffected. Dexmedetomidine also did not significantly affect the PPF ratio at other interpulse intervals (control *vs.* dexmedetomidine; 20, 50, 70,

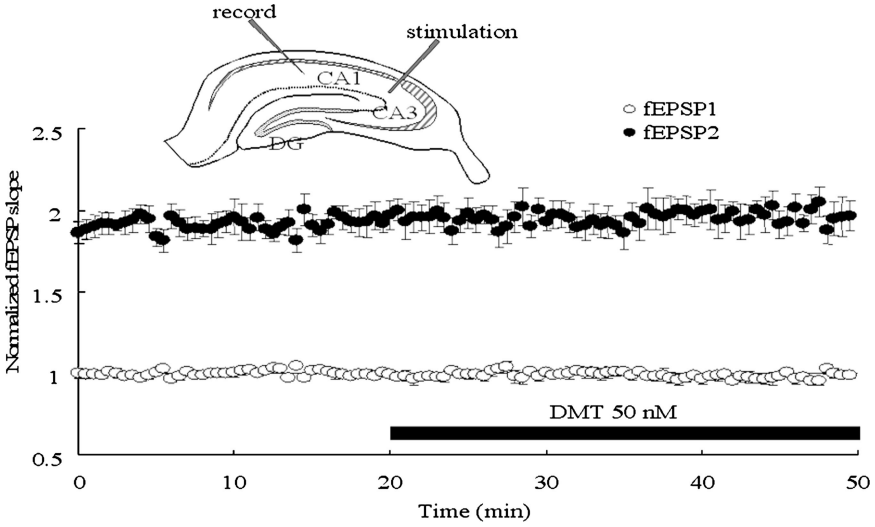

Fig. 1. Synaptic transmission and paired-pulse facilitation are not affected by dexmedetomidine (DMT). *Open* and *filled circles* indicate the first and second responses of pulses (50-ms interpulse interval), respectively. After 20 min of recording in artificial cerebrospinal fluid (ACSF), the solution was changed to DMT (50 nM) dissolved in ACSF. DMT had no effect on either the first or second response ($P > 0.05$, n = 5). The *insert* shows a schematic of the placement of recording and stimulating electrodes. The recording electrode was placed in the stratum radiatum of the CA1, and the stimulating electrode was placed in the stratum radiatum to stimulate the Schaffer collateral pathway. DG = dentate gyrus; fEPSP = field excitatory postsynaptic potential.

100, and 200 ms: 1.72 ± 0.05 *vs.* 1.80 ± 0.09; 1.84 ± 0.05 *vs.* 1.79 ± 0.05; 1.76 ± 0.08 *vs.* 1.81 ± 0.02; 1.64 ± 0.10 *vs.* 1.57 ± 0.05; 1.41 ± 0.05 *vs.* 1.38 ± 0.02; n = 4 for each).

Effect of Dexmedetomidine on LTP

It has been reported that hippocampal LTP by TBS may be linked to the exploratory behavior of rodents in spatially novel environments.[20–22] Thus, we used this pattern of high-frequency stimulation to induce LTP in our experiments. As has been reported by others,[22,23] TBS induced robust and persistent LTP under control conditions (fig. 2, *open circles*). When 50 nM dexmedetomidine was perfused during TBS, maintenance of LTP was suppressed. LTP induction was measured at the peak, which occurred within 10 min post-TBS. The maximal normalized fEPSP slope was 2.17 ± 0.10 and 1.86 ± 0.10 for control and dexmedetomidine, respectively (n = 8 for each, $P > 0.05$). Maintenance of LTP was assessed 60 min post-TBS. The normalized fEPSP slope was 1.82 ± 0.07 under control conditions and 1.38 ± 0.03 in the presence of dexmedetomidine (n = 8 for each; $P < 0.001$). Similar experiments were performed using 25, 35, and 100 nM dexmedetomidine (fig. 3, n = 8 for each). The effect of 100 nM dexmedetomidine was similar to that of 50 nM dexmedetomidine, suggesting saturation of the action. The IC_{50} of dexmedetomidine for LTP 60 min after TBS was 28.6 ± 5.7 nM as calculated from the dose–response curve in figure 3. Because the variance of slope values was much greater at the peak than at 60 min post-TBS, 60 min post-TBS was used for the pharmacological analysis described below. We tested the effect of dexmedetomidine when it was applied after induction of LTP. In this case, dexmedetomidine (50 nM) did not affect LTP (control *vs.* dexmedetomidine perfused after LTP induction; 1.78 ± 0.09 *vs.* 1.85 ± 0.14, $P > 0.05$, n = 6 for each, data not shown), suggesting that dexmedetomidine acts during induction of LTP.

Effect of Dexmedetomidine on LTP in the Presence of the α2-Adrenoceptor Antagonist Yohimbine

It is well established that dexmedetomidine is a potent agonist of α2-adrenoceptors. Thus, to address the mech-

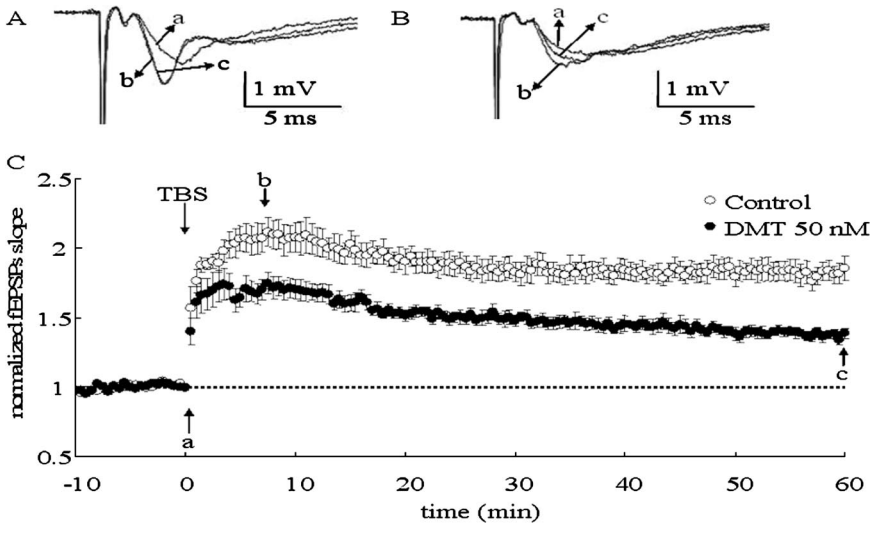

Fig. 2. Dexmedetomidine (DMT) suppresses long-term potentiation. (*A*) Sample traces recorded in the absence of DMT just before θ-burst stimulation (TBS) (a), immediately after TBS (b), or 60 min after TBS (c). (*B*) Sample traces recorded in the presence of DMT (50 nM) at the same three time points. (*C*) Maintenance of long-term potentiation was significantly reduced by DMT ($P < 0.001$, n = 8, *filled circles*) compared with control (n = 8, *open circles*). fEPSP = field excitatory postsynaptic potential.

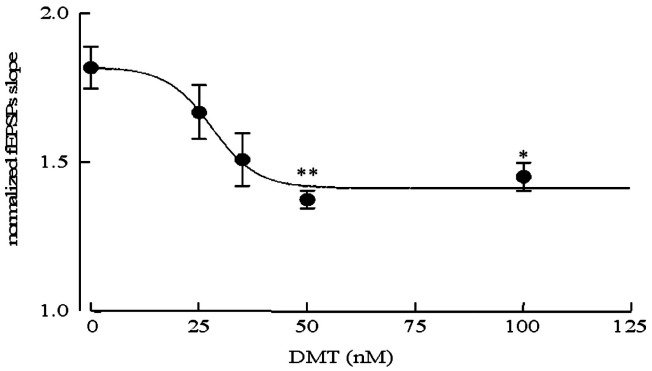

Fig. 3. The effect of dexmedetomidine (DMT) on long-term potentiation is dose-dependent. Maintenance of long-term potentiation, measured 60 min after θ-burst stimulation (TBS, 60 min), was reduced by 50 nM (n = 8, **$P < 0.01$, *post hoc* test after two-factor analysis of variance) and 100 nM (n = 8, *$P < 0.05$, *post hoc* test after two-factor analysis of variance) DMT, but 25 and 35 nM DMT had no significant effect (n = 8 for each). Control, 25, 35, 50, and 100 indicate 0, 25, 35, 50, and 100 nM DMT, respectively. Sigmoid line indicates concentration-response curve for long-term potentiation inhibition by DMT. The half-inhibitory concentration of DMT and Hill coefficient were 28.0 ± 4.3 nM and -0.0825. fEPSP = field excitatory postsynaptic potential.

anism of dexmedetomidine action, we blocked α2-adrenoceptors using yohimbine (2 μM) and induced LTP in the presence or absence of 50 nM dexmedetomidine (fig. 4). Yohimbine has a high affinity for α2-adrenoceptors (equilibrium K_D of 10 nM),[24] and 2 μM yohimbine completely reversed the effects of noradrenaline on LTP in mouse amygdala *in vitro* [6]. To inhibit yohimbine activation of 5HT1a receptors,[25] a 5HT1a receptor antagonist, WAY100635 (1 μM) was coperfused with yohimbine. The combination of yohimbine and WAY100635 did not completely block the dexmedetomidine effect (fig. 4). We also tested a 10-fold higher concentration of yohimbine (20 μM), but LTP induction was severely inhibited at this concentration (mean potentiation was 1.12 at 60 min post-TBS, n = 2), thus preventing reliable assessment of dexmedetomidine action.

Effects of Efaroxan and Idazoxan on Dexmedetomidine-mediated Reduction of LTP

Because the combination of yohimbine and WAY100635 did not abolish the effect of dexmedetomidine on LTP, we suspected that another receptor system also could be involved. Dexmedetomidine has been reported to have affinity for imidazoline receptors as well as α2-adrenoceptors.[12,13] We thus tested the α2-adrenoceptor and imidazoline type 1 receptor antagonist efaroxan (10 μM). This concentration of efaroxan was chosen because it completely blocked the suppressive effect of noradrenaline on perforant-path fEPSPs in mouse hippocampal dentate gyrus *in vitro*[26]. LTP was induced in the presence of efaroxan with or without 50 nM dexmedetomidine (fig. 5). Efaroxan did not completely block the dexmedetomidine effect.

Next, LTP was induced in the presence of 10 μM idazoxan, an α2-adrenoceptor and imidazoline type 2 receptor antagonist (fig. 6). In the presence of idazoxan, 50 nM dexmedetomidine did not inhibit LTP, suggesting that α2-adrenoceptors and imidazoline type 2 receptors mediate the effect of dexmedetomidine on LTP.

Effect of 2-BFI and BU-224 on LTP

To clarify the role of the imidazoline type 2 receptor in LTP, the effect of 2-BFI and BU-224, selective imidazoline type 2 receptor ligands, on LTP were investigated (fig. 7). 2-BFI at 10 μM, which is approximately the IC_{50} of monoamine oxidase (MAO) inhibition in adipocytes,[27] reduced LTP significantly (n = 8, $P < 0.05$), but BU-224 did not have a significant effect ($P > 0.05$, n = 8). The action of 10 μM 2-BFI was reversed by 10 μM BU-224 (n = 8).

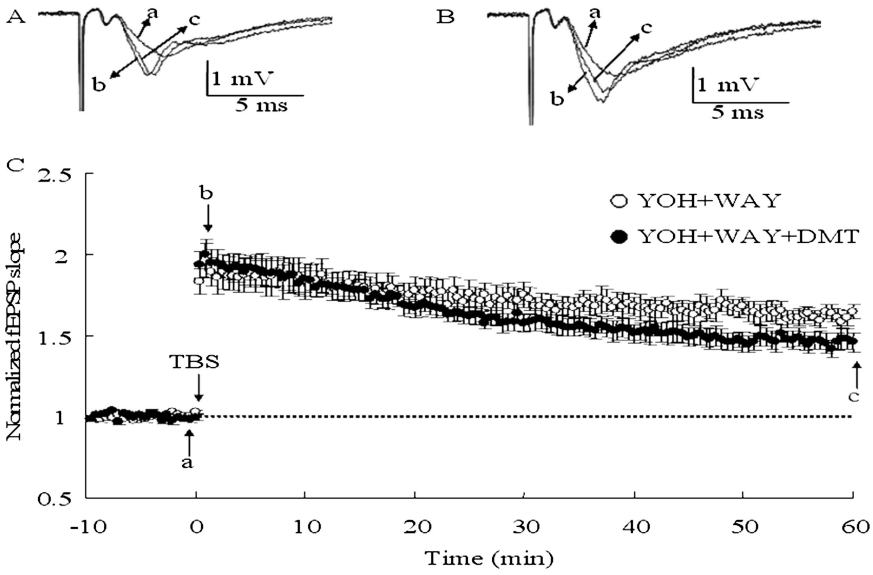

Fig. 4. Dexmedetomidine (DMT) attenuates long-term potentiation in the presence of yohimbine (YOH) and WAY100635 (WAY). (*A*) Sample traces recorded in the presence of yohimbine (2 μM) and WAY100635 (1 μM) just before θ-burst stimulation (TBS) (a), immediately after TBS (b), or 60 min after TBS (c). (*B*) Sample traces recorded at the same time points in the presence of yohimbine (2 μM), WAY100635 (1 μM), and DMT (50 nM). (*C*) DMT suppressed long-term potentiation in the presence of yohimbine and WAY100635 (n = 8, $P < 0.05$, *filled circles*) compared with vehicle (n = 8, *open circles*). fEPSP = field excitatory postsynaptic potential.

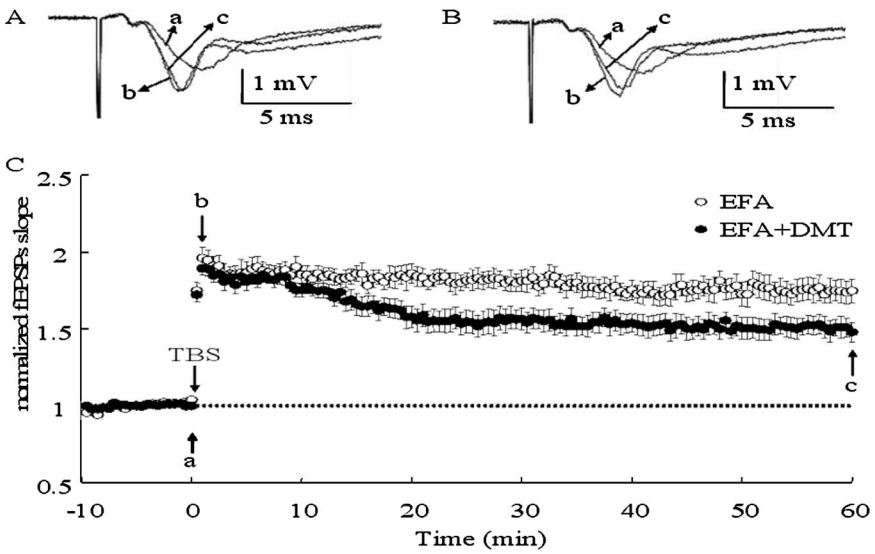

Fig. 5. Dexmedetomidine (DMT) reduces long-term potentiation in the presence of efaroxan (EFA). (*A*) Sample traces recorded in the presence of efaroxan (10 μM) just before θ-burst stimulation (TBS) (a), immediately after TBS (b), or 60 min after TBS (c). (*B*) Sample traces recorded at the same time points in the presence of efaroxan (10 μM) and DMT (50 nM). (*C*) DMT suppressed long-term potentiation in the presence of efaroxan (n = 8, $P < 0.05$, *filled circles*) compared with vehicle (n = 8, *open circles*). fEPSP = field excitatory postsynaptic potential.

Effect of Dexmedetomidine on LTP in the Presence of the Imidazoline Type 2 Receptor Ligand BU-224

The results above show that 2-BFI impaired LTP and that the action was abolished by BU-224. We next investigated the effect of BU-224 on dexmedetomidine-induced LTP inhibition to clarify the role of imidazoline type 2 receptor in dexmedetomidine-induced LTP inhibition (fig. 8). Dexmedetomidine reduced LTP in the presence of 10 μM BU-224 (n = 8, $P < 0.05$) compared with BU-224 alone (n = 8).

Effect of Dexmedetomidine on LTP in the Presence of the α1-Adrenoceptor Antagonist Prazosin

Dexmedetomidine is a highly selective α2-adrenergic agonist that also has weak affinity for α1-adrenoceptors,[1] activation of which can likewise affect induction of hippocampal LTP.[28] Therefore, we used the α1-adrenoceptor antagonist prazosin (1 μM) to investigate the contribution of α1-adrenoceptors to the dexmedetomidine effect. Dexmedetomidine reduced LTP in the presence of prazosin (fig. 9), suggesting that participation of α1-adrenoceptors in the effects of dexmedetomidine is weak.

Effect of Dexmedetomidine on LTP in the Presence of the γ-Aminobutyric Acid (GABA) Type A Receptor Antagonist Picrotoxin

Feedback and/or feed-forward inhibition *via* GABAergic interneurons regulates the induction of LTP.[29] Furthermore, α2-adrenoceptors can regulate GABA release in the hippocampus.[30,31] Thus, we investigated whether the GABAergic system participates in the action of dexmedetomidine. Picrotoxin, a GABA type A receptor antagonist, was perfused for 10 min before LTP induction. Picrotoxin was discontinued after TBS to reduce the generation of epileptiform activity, but dexmedetomidine (50 nM) was perfused throughout the experiment. The presence of picrotoxin during TBS did not block attenuation of LTP by dexmedetomidine (fig. 10). The effects of dexmedetomidine in the presence of antagonists were summarized in table 1.

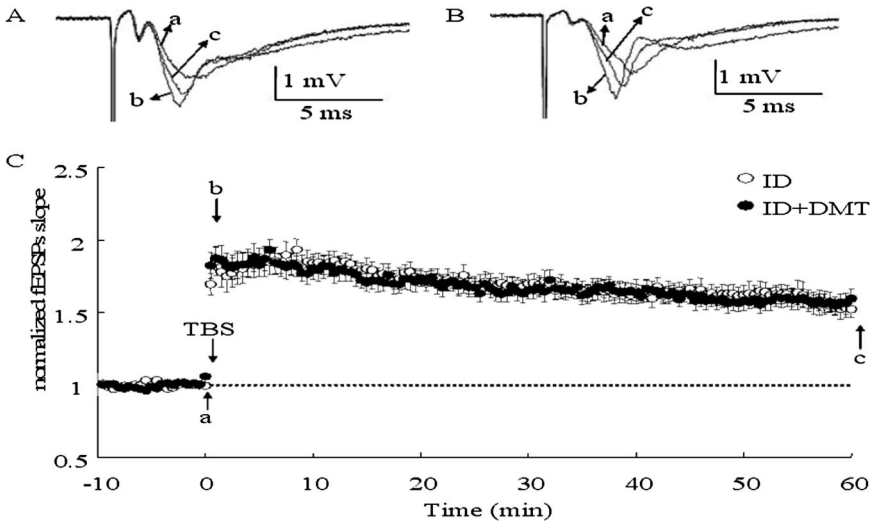

Fig. 6. Dexmedetomidine (DMT) has no effect on long-term potentiation in the presence of idazoxan (ID). (*A*) Sample traces recorded in the presence of idazoxan (10 μM) just before θ-burst stimulation (TBS) (a), immediately after TBS (b), or 60 min after TBS (c). (*B*) Sample traces recorded at the same time points in the presence of idazoxan (10 μM) and DMT (50 nM). (*C*) DMT had no significant effect on long-term potentiation in the presence of idazoxan (n = 8, *filled circles*) compared with vehicle (n = 8, *open circles*). fEPSP = field excitatory postsynaptic potential.

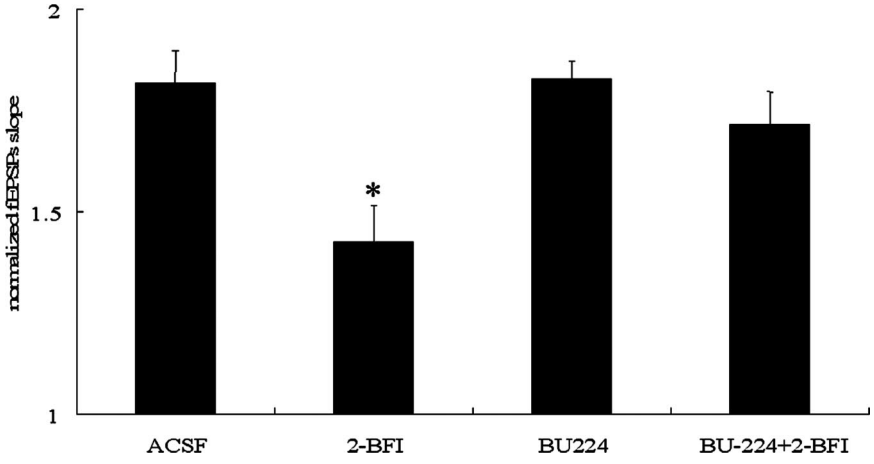

Fig. 7. Effects of the selective imidazoline type 2 receptor ligands, 2-benzofuranyl-2-imidaloline (2-BFI), and 2-(4,5-dihydroimidaz-2-yl)-quinoline (BU-224) on long-term potentiation. 2-BFI (10 μM) suppressed long-term potentiation (n = 8, $P < 0.05$) compared with artificial cerebrospinal fluid (ACSF, n = 8). BU-224 did not significantly effect long-term potentiation (n = 8). 2-BFI (10 μM) did not significantly effect long-term potentiation in the presence of BU-224 (10 μM, n = 8). * $P < 0.05$ compared with artificial cerebrospinal fluid. fEPSP = field excitatory postsynaptic potential.

Discussion

In the present study, we found that dexmedetomidine reduced LTP and, in agreement with previous work,[7] that PPF was unaffected by dexmedetomidine. PPF is thought to occur through presynaptic mechanisms.[32] Therefore, it was suggested that dexmedetomidine does not affect dynamic changes in transmitter release that are required for PPF at Shaffer collateral terminals. However, LTP at Shaffer collateral-CA1 synapses is thought to occur *via* mainly postsynaptic mechanisms.[33,34] Therefore, it seems that dexmedetomidine affects postsynaptic events required for CA1 LTP. Two main postsynaptic events are considered for the mechanism of LTP; one is a targeting of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid-type (AMPA-type) glutamate receptors to the synapses and another is new protein synthesis triggered by high-frequency stimulation. The former causes the early phase of LTP (up to approximately 10 min after high-frequency stimulation), and the latter causes the later phase of LTP.[33–35] Because the effect of dexmedetomidine on LTP was pronounced in the maintenance of LTP, it seems that dexmedetomidine mainly affects the late phase of LTP. β-Adrenoceptor activation facilitates induction of a protein synthesis–dependent late phase of LTP in the CA1.[36] Therefore, reducing noradrenaline release *via* activation of α2-adrenoceptors by dexmedetomidine and subsequent reduction of β-adrenoceptor-induced late-phase LTP is the most plausible mechanism underlying the α2-adrenoceptor-dependent component of dexmedetomidine-mediated reduction of LTP. It is well established that activation of α2-adrenoceptors reduces noradrenaline release in the hippocampus.[10] The importance of endogenous noradrenaline for induction of β-adrenoceptor–induced LTP also has been demonstrated in the hippocampal CA1 field.[11]

α2-Adrenoceptor agonists impair LTP in various brain regions, including mouse amygdala,[6] rat occipital cortex,[37] and spinal dorsal horn.[38] However, the effect of α2-adrenoceptor agonists, such as clonidine and dexmedetomidine, on hippocampal LTP has not been well established. A single report describes the effect of dexmedetomidine on LTP in the hippocampal CA1 field[7]; in that study, dexmedetomidine (50 nM) did not reduce the maintenance of hippocampal LTP in the rat area CA1. In the present study,

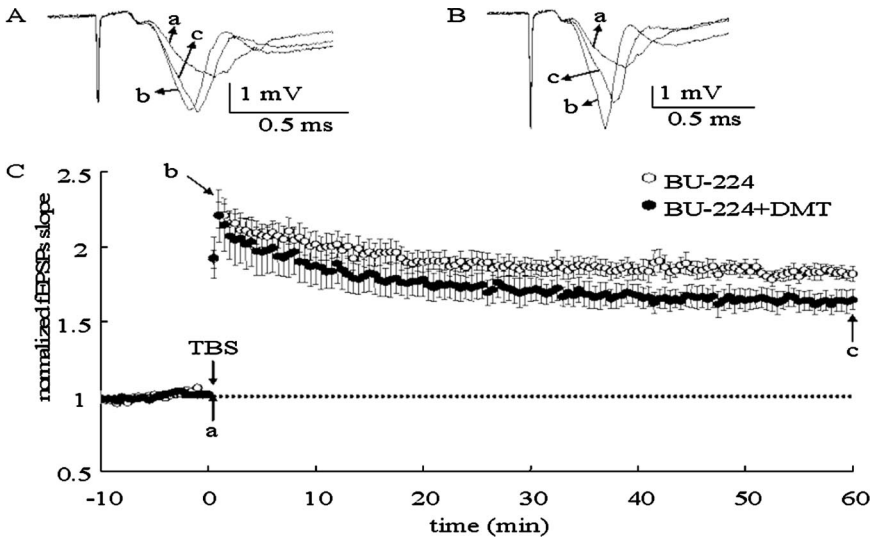

Fig. 8. Dexmedetomidine (DMT) suppresses long-term potentiation in the presence of 2-(4,5-dihydroimidaz-2-yl)-quinoline (BU-224). (*A*) Sample traces recorded in the presence of BU-224 (10 μM) just before θ-burst stimulation (TBS) (a), immediately after TBS (b), or 60 min after TBS (c). (*B*) Sample traces recorded at the same time points in the presence of BU-224 (10 μM) and DMT (50 nM). (*C*) DMT attenuated long-term potentiation in the presence of BU-224 (n = 8, $P < 0.05$, *filled circles*) compared with vehicle (n = 8, *open circles*). fEPSP = field excitatory postsynaptic potential.

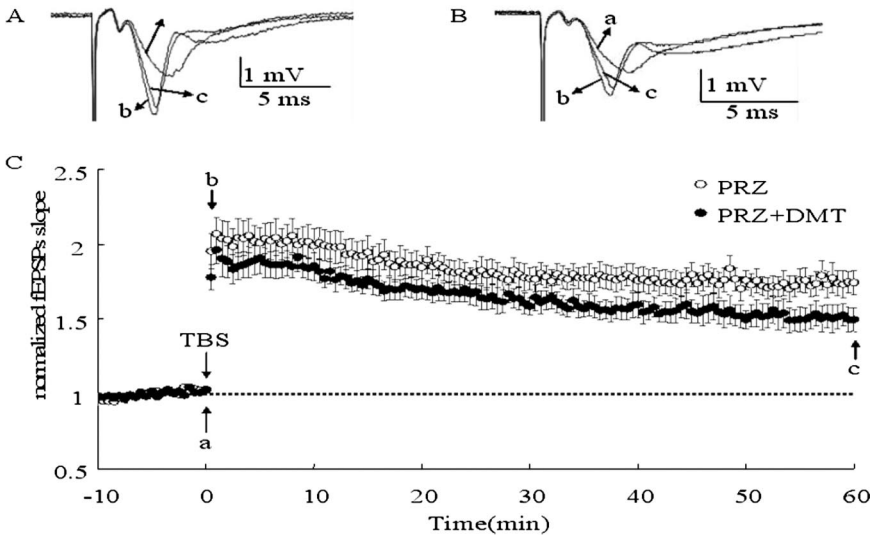

Fig. 9. Dexmedetomidine (DMT) suppresses long-term potentiation in the presence of prazosin (PRZ). (*A*) Sample traces recorded in the presence of prazosin (1 μM) just before θ-burst stimulation (TBS) (a), immediately after TBS (b), or 60 min after TBS (c). (*B*) Sample traces recorded at the same time points in the presence of prazosin (1 μM) and DMT (50 nM). (*C*) DMT attenuated long-term potentiation in the presence of prazosin (n = 8, $P < 0.05$, *filled circles*) compared with vehicle (n = 8, *open circles*). fEPSP = field excitatory postsynaptic potential.

the same concentration of dexmedetomidine attenuated maintenance of hippocampal LTP in the mouse area CA1, but 25 and 35 nM dexmedetomidine had no significant effect. Therefore, slight species differences in dexmedetomidine sensitivity of α2-adrenoceptors or imidazoline type 2 receptors, or differences in receptor expression, most likely account for the observed differences in dexmedetomidine action. Differences in recording systems (an interface type chamber was used in the previous study[7]) as well as in the LTP induction protocol (10 *vs.* 15 bursts of TBS) could also account for this discrepancy.

Our results suggest that not only α2-adrenoceptors, but also imidazoline type 2 receptors, participate in the action of dexmedetomidine on LTP. It has been reported that dexmedetomidine activates imidazoline receptors as well as α2-adrenoceptors,[39] but the physiologic role of the former has not always been addressed. Our results suggest a novel role for activation of imidazoline receptors by dexmedetomidine, namely attenuation of hippocampal LTP in the CA1 field. This action of dexmedetomidine is mediated by imidazoline type 2 receptors. Idazoxan and efaroxan have distinct affinities for imidazoline receptor subtypes: idazoxan is relatively imidazoline type 2-selective, whereas efaroxan is relatively imidazoline type 1-selective.[40] Efaroxan (10 μM) did not completely abolish the action of dexmedetomidine, but idazoxan did. Therefore, imidazoline type 2 receptors are involved in the dexmedetomidine effect. Furthermore, a highly selective imidazoline type 2 receptor ligand, 2-BFI, reduced LTP significantly at the concentration of 10 μM, which is approximately the IC_{50} on MAO activity in adipocytes.[27] These results suggest that the LTP is modulated by imidazoline type 2 receptors. A previous study reported that 2-BFI binds to imidazoline

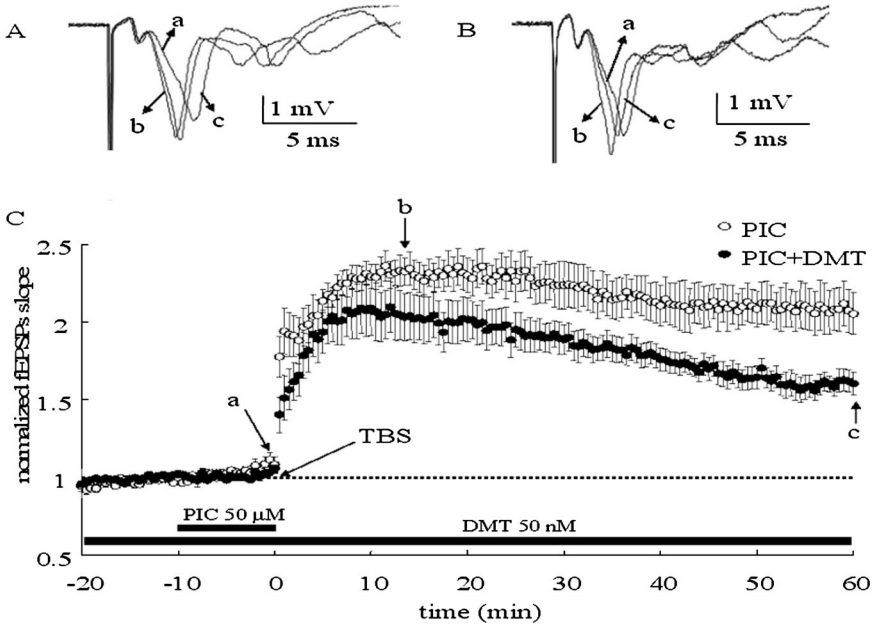

Fig. 10. Dexmedetomidine (DMT) reduces long-term potentiation (LTP) in the presence of picrotoxin (PIC). (*A*) Sample traces recorded in the presence of picrotoxin (50 μM) just before θ-burst stimulation (TBS) (a), immediately after TBS (b), or 60 min after TBS (c). (*B*) Sample traces recorded at the same time points in the presence of picrotoxin (50 μM) and DMT (50 nM). (*C*) DMT suppressed LTP when picrotoxin was present during LTP induction (n = 8, $P < 0.001$, *filled circles*) compared with vehicle (n = 8, *open circles*). fEPSP = field excitatory postsynaptic potential.

Table 1. Normalized fEPSP Slope 60 min after Induction of Long-term Potentiation

	Control	Dexmedetomidine (50 nM)
ACSF	1.82 ± 0.07	1.37 ± 0.03*
Yohimbine + WAY100635	1.62 ± 0.05	1.46 ± 0.06†
Efaroxan	1.75 ± 0.07	1.51 ± 0.06†
Idazoxan	1.56 ± 0.07	1.57 ± 0.07‡
Prazosin	1.75 ± 0.08	1.51 ± 0.08†
Picrotoxin	2.12 ± 0.14	1.60 ± 0.07§‡
BU-224	1.83 ± 0.04	1.63 ± 0.07‡

Values are mean ± SEM, n = 8.

* $P < 0.001$, † $P < 0.05$, and § $P < 0.01$ *vs.* control. ‡ $P < 0.05$ *vs.* artificial cerebrospinal fluid (ACSF) by analysis of variance followed by Dunn's multiple comparison test.

BU-224 = 2-(4,5-dihydroimidaz-2-yl)-quinoline; fEPSP = field excitatory postsynaptic potential.

type 2 receptors as an agonist in the modulation of supraspinal opioid antinociception, whereas BU-224 and idazoxan bind as antagonists.[41] In the present study, 2-BFI inhibited LTP and BU-224 blocked the 2-BFI-mediated inhibition of LTP. Therefore, it is possible that 2-BFI acts as an agonist, whereas BU-224 acts as an antagonist in the modulation of CA1 LTP. However, BU-224 did not completely block dexmedetomidine-mediated LTP reduction. Because idazoxan completely reversed the dexmedetomidine effect, these findings suggest that dexmedetomidine inhibits LTP *via* both imidazoline type 2 receptors and α2-adrenoceptors. α2-Adrenoceptor-deleted mice should be used to confirm the role of imidazoline receptors and α2-adrenoceptors for the suppressive effect of dexmedetomidine on LTP.

The mechanism by which imidazoline type 2 receptors reduce LTP was not addressed in the present study. The selective imidazoline type 2 receptor ligand, 2-BFI, impaired LTP. In the hippocampus, although the mechanism is still unknown, certain previous studies have indicated that 2-BFI reduces noradrenaline levels.[42,43] Therefore, it is possible that imidazoline type 2 receptor activation inhibits hippocampal LTP *via* the reduction of tissue noradrenaline concentrations. Imidazoline type 2 receptors are located on the mitochondrial outer membrane,[44] and these sites are associated with MAO.[45] The hippocampal CA1-field receives dense projections from noradrenergic fibers that originate in the locus coeruleus.[46] The highly selective imidazoline type 2 receptor ligands, BU-224 and 2-BFI, inhibit MAO-A and MAO-B activity in adipocytes.[27] In rat frontal cortex, BU-224 and 2-BFI elevate tissue noradrenaline concentrations.[47] Together, these results suggest the possibility that the effects of BU-224 and 2-BFI on noradrenaline reflect MAO modulation. However, in hippocampus, 2-BFI and BU-224 had different properties on LTP in the present study: 2-BFI reduced LTP, whereas BU-224 had no effect, *per se*, and abolished 2-BFI action. Therefore, it is unlikely that imidazoline type 2 receptors affect LTP *via* MAO activity in the hippocampus.

In conclusion, our findings demonstrate that dexmedetomidine attenuates mouse hippocampal CA1 LTP. Both α2-adrenoceptors and imidazoline type 2 receptors are involved in this dexmedetomidine action. The finding that imidazoline type 2 receptors are involved in the dexmedetomidine-mediated reduction of LTP provides novel information regarding the basic actions of dexmedetomidine in the central nervous system.

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