The Effects of Different Concentrations of the α₂-Adrenoceptor Agonist Medetomidine on Basal Excitatory Synaptic Transmission and Synaptic Plasticity in Hippocampal Slices of Adult Mice

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BACKGROUND: α₂-Adrenoceptor agonists are used frequently in human and veterinary anesthesia as sedative/analgesic drugs. However, they can impair cognition. Little is known about the concentration-dependent effects of α₂-adrenoceptor agonists on synaptic plasticity, the neurophysiological basis of learning and memory. Therefore, we investigated the effects of different concentrations of medetomidine, an α₂-adrenoceptor agonist, on basal excitatory synaptic transmission and on 2 forms of synaptic plasticity: paired-pulse facilitation (PPF) and long-term potentiation (LTP).

METHODS: Evoked field excitatory postsynaptic potentials were recorded in Schaffer fibers-CA1 pyramidal cell synapses of mouse hippocampal slices, and the initial field excitatory postsynaptic potentials slope was measured. For basal synaptic transmission and PPF, increasing concentrations of medetomidine (1–200 μM) were applied to each slice. For LTP experiments, individual slices were used for each tested concentration of medetomidine (0.1–0.4 μM), where LTP induction and LTP maintenance were measured.

RESULTS: The lower tested concentrations of medetomidine decreased LTP in a concentration-dependent manner, whereas greater concentrations were required to decrease fiber volley amplitude and basal excitatory synaptic transmission. PPF was only affected by the greatest concentration (200 μM).

CONCLUSIONS: Medetomidine decreased LTP in the mouse hippocampus, in accordance with the ability of medetomidine to induce memory deficits. (Anesth Analg 2015;120:1130–7)

Alpha₂-adrenoceptor agonists are used frequently in human and veterinary clinical anesthesia.1,2 These drugs produce sedation and analgesia, reduce anesthetic requirements, and improve perioperative hemodynamic stability.3,4 Medetomidine is a potent and selective α₂-adrenoceptor agonist with an α₂/α₁ selectivity ratio of 1620/1, as measured by the displacement of [³H]clonidine.5 Its active isomer is dexmedetomidine (the dextro-enantiomer),6 which has the pharmacologic activity of dexmedetomidine, and dexmedetomidine in humans and medetomidine in animals provide a good anesthetic stability and reduce postanesthetic delirium and agitation.6,7 However, (dex)medetomidine can affect learning and memory in humans and rodents,10–12 which as in agreement with the finding is in agreement with the impact of the pharmacologic manipulation of the noradrenergic system on memory performance.15–17

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plasticity in the adult hippocampus is yet to be established to provide a neurophysiologic correlate of memory impairment caused by (dex)medetomidine administration.

The purpose of this study was to evaluate the effect of different concentrations of the α2-adrenoceptor agonist medetomidine on basal excitatory synaptic transmission and on short-term (PPF) and long-term synaptic plasticity (LTP) in the CA1 region of the adult mouse hippocampus, an age group more routinely subject to anesthesia and less prone to develop neurotoxicity on exposure to anesthetics than the more commonly studied younger brain.

METHODS
All procedures were provided ethical approval from the Portuguese competent authority for animal protection, Direcção Geral de Veterinária (Lisbon, Portugal).

Animals and Hippocampal Slice Preparation
The experiments were performed on hippocampal slices from 5- to 6-month-old female BALB/c mice. The mice were housed with controlled temperature (21–23°C) and relative humidity at 55%. The animals were euthanized by cervical dislocation followed by decapitation; the brain was removed rapidly and the hippocampi dissected free in ice-cold artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl 124, KCl 3, NaH2PO4 1.25, glucose 10, NaHCO3 26, MgSO4 1, CaCl2 2, gassed with 95% O2 and 5% CO2 (pH = 7.4). Slices (400-μm thick) were cut perpendicular to the long axis of the hippocampus with a McIlwain tissue chopper (Mickle Laboratory Engineering Co Ltd, Guildford, UK) and maintained for at least 60 minutes in gassed aCSF solution at room temperature (23–25°C).

Electrophysiological Recording
A single slice was placed in a submerged recording chamber (1 mL capacity) and superfused at a rate of 3 mL/min with aCSF continuously bubbled with 95% O2 and 5% CO2, which was maintained at a constant temperature of 32.0 ± 0.1°C, to record synaptic transmission and plasticity as previously described.29 To summarize, electrical stimulation was applied through a bipolar tungsten electrode placed over the Schaffer collateral/commissural fibers. Stimulation was applied through a constant current output unit (Grass Photoelectric Stimulus Isolating Unit 6; Grass Technologies, Warwick, RI), connected to a Grass S44 stimulator. The stimulus duration was 0.1 millisecond, and its intensity (4–6 mA) was adjusted to evoke field excitatory postsynaptic potentials (fEPSPs) with 40% to 50% of their maximal amplitude. Evoked fEPSPs were recorded in the stratum radiatum layer of the hippocampus CA1 area using a glass micropipette filled with 4 M NaCl (2–5 MΩ resistance). Signals were amplified 1000-fold, and filtered below at 5 Hz and above 3 kHz, using an ISO-80 isolated bioamplifier (World Precision Instruments, Inc., Sarasota, FL), and digitally recorded at 10 kHz using a Pico Technologies (Cambridgeshire, UK) analog-to-digital converter ADC-42, connected to a Pentium-based PC system running the 1.3 version of the LTP program.30 All data were stored as averages of 8 consecutive responses. Offline analysis was performed with WinLTP program, version 1.11 (WinLTP Ltd., University of Bristol, Bristol, UK), without additional signal filtering, and responses were quantified as the initial slope of the average fEPSPs. In addition, the amplitude of the fiber volley was also measured when recording basal synaptic transmission.

Under basal conditions, stimuli were delivered at a frequency of 0.067 Hz. To elicit PPF, 2 consecutive pulses were applied with a 50-millisecond interpulse interval, and the interval between paired pulses was 15 seconds. PPF was quantified as the ratio of the fEPSP slopes of the second response over the first, for each pair of stimuli. LTP was induced by a high-frequency stimulation (HFS) train (100 pulses at 100 Hz). This train was applied 30 minutes after a stable baseline at 0.067 Hz stimulation was established. This basal stimulation frequency was resumed immediately after application of the HFS. LTP induction was quantified as the ratio of averaged fEPSP slopes of the first 6 minutes after HFS over the averaged fEPSP slopes during the 10 minutes before HFS, and LTP maintenance was quantified as the ratio of the averaged fEPSP slope from 54 to 60 minutes after HFS over the averaged fEPSP slope 10 minutes before HFS.

Drugs Used and Their Administration
Medetomidine ((±)-4-[1-(2,3-dimethylphenyl) ethyl]-1H-imidazole monohydrochloride) solution (Domitor®) was obtained from Pfizer (Porto Salvo, Oeiras, Portugal) and was diluted in aCSF to obtain the desired concentration. For PPF and basal synaptic transmission experiments, each slice was cumulatively exposed to increasing concentrations of medetomidine (1, 2, 4, 8, 12, 24, 48, 100, and 200 μM) for 20 minutes, and the effect of a given medetomidine concentration was determined using the fEPSP recorded in the last 6 minutes of application. For LTP experiments, each slice was exposed to only one concentration of medetomidine (0.1, 0.2, or 0.4 μM). The drug was introduced 30 minutes before induction of LTP and was maintained throughout the experiment. Control slices were perfused with aCSF only.

Statistical Analysis
We analyzed all data (effects of different concentrations of medetomidine on basal synaptic transmission, presynaptic volley amplitude, PPF, LTP induction, and LTP maintenance) by using 2-way analysis of variance (ANOVA), considering animals and medetomidine concentrations as factors. When an overall difference was found, post hoc multiple comparison Dunnett test (2-tailed) was used, with the drug-free condition as the control group. Exact significance (P values) for post hoc comparisons is presented except where indicated. Because of the low number of replicates for each experiment (n = 4), the number of comparisons, and to avoid causing a type I error, a P value <0.01 was considered for statistically significant. The aforementioned statistical analyses were performed using SPSS 21 for Windows (IBM Corporation, Armonk, NY).

The logarithm of the concentration of medetomidine that produces 50% of maximal inhibition (LogIC50) of basal synaptic transmission and of LTP maintenance was calculated by fitting the data by means of a nonlinear least-squares method (the software used was Prism v5.0, GraphPad Software, Inc., San Diego, CA), with a 4-parameter Hill equation, Y = Bottom + (Top − Bottom)/(1 + 10^((LogIC50 − X) * Hill slope)), with nonweighted samples, and with
Effects of Medetomidine on Adult Mouse Brain

the following constraints: bottom = 0% and top = 100% for the basal synaptic transmission; bottom = 100% and top = 148.8% (48.8% increase over baseline being the maximal LTP amplitude recorded in drug-free condition) for the LTP maintenance.

All results are expressed as mean ± SD, unless stated otherwise.

RESULTS

Effects of Animals on Basal Synaptic Transmission, Fiber Volley Amplitude, PPF, LTP Induction, and LTP Maintenance

No effects of animals as factor were observed regarding all parameters analyzed (2-way ANOVA: P ≥ 0.347) except for PPF ratio (2-way ANOVA: P = 0.00048).

Effects of Different Concentrations of Medetomidine on Basal Synaptic Transmission and on Fiber Volley Amplitude

After 20 minutes of stable baseline recording with aCSF, the application of consecutively increasing concentrations of medetomidine up to 12 μM (1, 2, 4, 8, and 12 μM) did not significantly modify synaptic transmission (control [0 μM] versus each of the aforementioned concentrations individually: all P > 0.999; n = 4), as gauged by the lack of alteration of fEPSP slopes (Fig. 1A). When greater concentrations of medetomidine were applied (24, 48, 100, and 200 μM), synaptic transmission significantly decreased (control [0 μM] versus each of the aforementioned concentrations individually: all P < 0.0001; n = 4). In fact, medetomidine concentrations of 24, 48, 100, and 200 μM inhibited the fEPSP slope by 9.65 ± 1.77%, 18.40 ± 0.94%, 44.31 ± 5.01%, and 92.16 ± 2.77%, respectively (Fig. 1, A–C). This inhibition was completely reverted after washout of medetomidine (control versus washout: P = 0.611; n = 4) (Fig. 1A). The LogIC50, half-maximal inhibitory concentration (IC50), and Hill slope values for the inhibition of the basal synaptic transmission by medetomidine, as calculated from the concentration–inhibition curves shown in Figure 1B, were −4.00 (95% confidence interval [CI], −4.07 to −3.94), 98.9 (95% CI, 85.5–114.4) μM, and 2.46 (95% CI, 1.64–3.30), respectively (n = 4).

Lower concentrations of medetomidine (1, 2, 4, 8, and 12 μM) also failed to modify presynaptic volley amplitude (control [0 μM] versus 1 μM: P > 0.999; control versus 2 μM: P = 0.990; control versus 4 μM: P = 0.995; control versus 8 μM: P = 0.995; control versus 12 μM: P = 0.829; n = 4). By contrast, greater concentrations of medetomidine (24, 48, 100, and 200 μM) significantly decreased the presynaptic volley amplitude (control [0 μM] versus each of the aforementioned concentrations individually: all P < 0.0001, n = 4). In fact, medetomidine concentrations of 24, 48, 100, and 200 μM inhibited the presynaptic volley amplitude by 29.37% ± 7.87%, 38.99% ± 9.10%, 64.32% ± 3.79%, and 84.84% ± 5.39%, respectively (Fig. 1C).

Effects of Different Concentrations of Medetomidine on PPF

Only the greatest concentration of medetomidine tested (200 μM) significantly affected the PPF ratio (control [0 μM] versus 1 μM: P > 0.999; control versus 2 μM: P = 0.991; control versus 4 μM: P = 0.998; control versus 8 μM: P > 0.999; control versus 12 μM: P > 0.999; control versus 24 μM: P > 0.999; control versus 48 μM: P = 0.998; control versus 100 μM: P = 0.941; control versus 200 μM: P < 0.0001; n = 4) (Fig. 2).

Effects of Different Concentrations of Medetomidine on LTP Induction and Maintenance

Under control conditions, HFS increased the fEPSP slope to 230.35% ± 19.25% (n = 4) in the first 6 minutes after its application (LTP induction), relative to baseline (Fig. 3A). When present at a concentration of 0.1 μM, medetomidine did not significantly modify LTP induction (control [0 μM] versus 0.1 μM: P = 0.380; n = 4). At concentrations of 0.2 and 0.4 μM, however, medetomidine significantly decreased LTP induction to 192.19% ± 23.37% and 159.37% ± 13.43%, respectively (control [0 μM] versus 0.2 μM: P = 0.00031; control versus 0.4 μM: P < 0.0001; n = 4) (Fig. 3, B and C). When we explored the effects of medetomidine on LTP maintenance (between 54 and 60 minutes after HFS), no significant differences were detected between LTP amplitude, relative to baseline, in control slices (148.82% ± 2.07%) and in slices treated with 0.1 μM of medetomidine (146.86% ± 1.77%) (control [0 μM] versus 0.1 μM: P = 0.897; n = 4). However, 0.2 μM medetomidine decreased LTP maintenance to 125.09% ± 1.32%, and the greatest tested concentration of medetomidine (0.4 μM) abrogated LTP maintenance (103.03% ± 0.99%) (control [0 μM] versus 0.2 μM: P < 0.0001; control versus 0.4 μM: P < 0.0001; n = 4) (Fig. 3, B and D). The LogIC50, IC50, and Hill slope values for the inhibition of LTP maintenance by medetomidine, as calculated from the concentration–response curves shown in Figure 3D, were −6.69 (95% CI, −6.71 to −6.68), 0.202 (95% CI, 0.194–0.210) μM, and −4.04 (95% CI, −4.83 to −3.25), respectively (n = 4).

DISCUSSION

This study showed that the α2-adrenoceptor agonist medetomidine mainly affected LTP in a concentration-dependent manner rather than basal excitatory synaptic transmission and presynaptic volley amplitude, which were only affected by greater concentrations of medetomidine (24, 48, 100, and 200 μM). By contrast, medetomidine only affected PPF at the greatest tested concentration. This finding indicates that medetomidine mostly affects postsynaptic receptors involved in synaptic plasticity and blocks stimulus-induced transmission at greater concentrations, without evident presynaptic effects controlling the evoked release of glutamate.

The concentrations of medetomidine tested in the present study included sedative concentrations in rodents, encompassing concentrations close to those used in clinical human settings. Human plasma concentrations of dexmedetomidine, for sedation, are approximately 1 to 2 ng/mL,31 that is, 0.005 to 0.01 μM. Although the plasma concentration of medetomidine in anesthetized mice has not been determined, a subcutaneous administration of 0.08 mg/kg of medetomidine in rats led to a plasma concentration of 30 ng/mL.32 Considering that the clinical sedative dose in mice is 1 mg/kg,33 the plasma concentrations of medetomidine are expected to be approximately 375 ng/mL, that is, 2 μM. Furthermore, (dex)medetomidine is highly lipid
soluble and preferentially accumulates in the brain, with peak levels 5 times greater than those in the plasma, leading to estimates of brain concentrations of (dex)medetomidine between 0.025 and 0.05 μM in humans and 10 μM in mice. In addition, in this study we used medetomidine that has the same pharmacologic activity of dexmedetomidine but to be equieffective is administrated at double the dose of dexmedetomidine. In this study, a wide range of relevant concentrations of medetomidine (0.1–200 μM) was tested. The lowest concentration of medetomidine tested was nearly equivalent to the dexmedetomidine concentration estimated in human brain tissue. The extrapolation of our results for human and veterinary clinical practice, however, requires extreme caution because the effects of medetomidine in ex vivo conditions may not be the same as in an in vivo setting. Moreover, differences in the routes of administration, doses used, exposure times, and interspecies variations are important variables that also have to be considered.

The most striking effect of medetomidine in excitatory hippocampal synapses was a decrease of the induction and maintenance of LTP in the CA1 region of the adult hippocampus, which was observed with low concentrations of medetomidine. Thus, we observed that concentrations of 0.2 μM and 0.4 μM medetomidine concentration-dependently decreased and completely blocked the maintenance of LTP in the adult hippocampus. Although the effects of α₂-agonists on LTP in the adult hippocampus have not been described previously, it was reported that the α₂-adrenoceptor agonist clonidine dose-dependently reduced LTP elicited in vivo in the occipital cortex of anesthetized rats and ex vivo in amygdala circuits, and dexmedetomidine decreased LTP in hippocampal slices of young mice; in this last study, the half-inhibitory concentration of dexmedetomidine to depress LTP maintenance was approximately 28 nM, whereas in our study, the IC₅₀ was approximately 200 nM. This difference can be explained by the combined facts that only a half of the dose of dexmedetomidine is required to induce similar effects to medetomidine and because the adult brain is less sensitive to neurotoxicity caused by drugs than the young brain. This finding suggests that the concentration of α₂-agonists necessary to induce alterations of LTP and probably memory deficits in adult animals is greater than in younger animals.

The effects of α₂-adrenoceptor agonists on basal excitatory synaptic transmission in the hippocampus of adults also have not been previously reported. Dexmedetomidine, the active enantiomer of medetomidine, at a concentration of 50 nM did not affect basal synaptic transmission in the hippocampus of young mice (20–30 days), whereas noradrenaline was reported to inhibit excitatory glutamatergic

Figure 1. Effects of different concentrations of medetomidine on basal synaptic transmission in Schaffer fiber-CA1 pyramid synapses from hippocampal slices of adult mice. A, Representative experiment illustrating the time course of the cumulative effects of increasing concentrations of medetomidine on field excitatory postsynaptic potential (fEPSP) slope. B, Average (mean of n = 4) concentration–response curve of the inhibitory effects of medetomidine on fEPSP slopes; in the ordinates, 0% corresponds to the fEPSP slope before medetomidine applications and 100% represents the complete inhibition of fEPSPs (n = 4). Significant (P < 0.00001) inhibition of fEPSP slope was observed after the application of 24, 48, 100, and 200 μM medetomidine. C, Superimposed fEPSPs showing the inhibition of the greater concentrations of medetomidine on fEPSP slope and on presynaptic volley.

May 2015 • Volume 120 • Number 5 www.anesthesia-analgesia.org 1133
Effects of Medetomidine on Adult Mouse Brain

transmission in autaptic synapses through α<sub>2</sub>-receptors<sup>22</sup> but independently of α<sub>2</sub>-receptors in hippocampal slices from juvenile rodents.<sup>23</sup> We observed that medetomidine inhibited basal excitatory synaptic transmission in hippocampal slices of adult mice, but this effect occurred at concentrations 2-fold greater than those affecting synaptic plasticity. In fact, lower concentrations of medetomidine (1–12 μM) did not affect basal excitatory synaptic transmission, whereas only greater concentrations (24–200 μM) decreased basal synaptic transmission in a concentration-dependent manner. Simultaneously, the same concentrations (24–200 μM) also decreased the presynaptic volley amplitude, suggesting that the inhibitory effect of medetomidine on basal synaptic transmission could be explained by an overall inhibition of neuronal activity induced by medetomidine.

The results obtained in the present study also showed that medetomidine concentrations between 1 and 100 μM did not affect CA1 hippocampal PPF, corroborating in adult hippocampal circuits a previous observation that 50 nM dexmedetomidine did not modify PPF in the hippocampal CA1 region of young mice.<sup>28</sup> This finding contrasts with the previously reported ability of dexmedetomidine to decrease the hypoxia-evoked glutamate release from hippocampal slices,<sup>24</sup> which might result from synaptic and nonsynaptic sources (namely involving astrocytes). PPF results from presynaptic mechanisms, and it is used routinely as a presynaptic index for probability of neurotransmitter release.<sup>35,36</sup> Under control conditions in aCSF slices, the response to the second of a pair of stimulation pulses was greater than the first response. Residual presynaptic calcium, after the first stimulation, is responsible for bolstering neurotransmitter release in response to the second stimulation. Therefore, our findings suggest that medetomidine did not affect dynamic changes in transmitter release that are required for PPF at Schaffer collateral terminals, except when the greatest concentration was applied. This result implies that the effects of the lower concentrations of medetomidine on LTP and of moderate concentrations on synaptic transmission are unlikely to result from a presynaptic effect of α<sub>2</sub>-adrenoceptors and possibly involve the action of the most abundantly located postsynaptic α<sub>2</sub>-adrenoceptors<sup>37–39</sup> that have been shown to control ionic conductances, such as hyperpolarization-activated cyclic nucleotide-gated inward current,<sup>40,41</sup> that mainly affect synaptic plasticity but also synaptic transmission in hippocampal circuits.<sup>42,43</sup>

In conclusion, we have shown that α<sub>2</sub>-adrenoceptor agonist medetomidine mainly affects LTP in the CA1 region of the mouse hippocampus rather than presynaptically affecting the release of glutamate to modify short-term plasticity of basal synaptic transmission at excitatory synapses in the hippocampal circuits from adult mice. This provides a neurophysiologic correlate supporting the

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**Figure 2.** Effects of different concentrations of medetomidine on paired-pulse facilitation (PPF) in Schaffer fiber-CA1 pyramid synapses from hippocampal slices of adult mice. A, Time course of the effects of increasing cumulative concentrations of medetomidine on PPF measured as the ratio of field excitatory postsynaptic potential (fEPSP) slopes triggered by the second pulse divided by first pulse (interpulse interval of 50 milliseconds). Only the greatest concentration of medetomidine significantly decreased PPF (P < 0.00001). B, Superimposed fEPSPs
reported deleterious impact of medetomidine on memory performance.

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Effects of Medetomidine on Adult Mouse Brain

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