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ORIGINAL ARTICLE

Dexmedetomidine suppresses long-term potentiation in the hippocampal CA1 field of anesthetized rats

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Abstract

Purpose The aim of this study was to evaluate the effect of dexmedetomidine (DEX) on hippocampal synaptic activity in vivo.

Methods The adult rats used for this study received a intraperitoneal bolus injection of 3, 10, 30, or 100 μg/kg of DEX or an equivalent volume of saline. Electrophysiological recording of the hippocampal CA1 region was initiated 20 min after drug administration. The results are expressed as the percentages of the population spike amplitude measured just before high-frequency stimulation (HFS). The electrophysiological data were analyzed with an area under the curve (AUC) of 10–60 min after HFS. Moreover, to investigate the sedative dose of DEX in rats, we recorded the duration of loss of spontaneous movement after the administration of each dose of DEX.

Results Intraperitoneal administration of DEX at doses of 30 and 100 μg/kg induced a range of sedative effects. The AUC measurements were significantly lower in the 30 and 100 μg/kg groups than in those injected with vehicle (vehicle: 8.81 ± 0.49, $n = 7$; DEX 30 μg/kg: 6.02 ± 0.99, $n = 6$; DEX 100 μg/kg: 5.10 ± 0.43, $n = 5$; $P < 0.05$).

Conclusion The results of our in vivo study reveal that sedative doses of DEX impaired the induction of hippocampal long-term potentiation (LTP). These findings may signify a causal link between DEX-induced sedative action and hippocampal LTP suppression, providing a better understanding of the mechanisms underlying the DEX-induced sedative and/or amnestic effect.

Keywords Dexmedetomidine · Synaptic plasticity · Long-term potentiation · Hippocampus

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Introduction

Dexmedetomidine (DEX) is a potent, highly selective alpha-2-adrenergic (α_{2A}) agonist [1] that has both sedative and analgesic effects. DEX has several clinical advantages over other commonly used anesthetics with gamma-aminobutyric acid (GABA)-ergic and/or glutamatergic properties, such as the induction of sedation with minimal respiratory suppression. In addition, DEX-induced sedation is easily reversed with verbal or physical stimuli. Accordingly, results from recent studies indicate that the use of DEX as a primary sedative in critical care and as the sole anesthetic for certain surgical cases is expanding [2, 3].

Similar to other anesthetic agents, DEX has a potent amnestic effect. For example, Ebert et al. [4] found that patients treated with sedative doses of DEX suffered from suppression of recall and recognition memory, and Prior et al. [5] reported that DEX impairs memory even at a low dose. It is unclear how and where DEX acts in the brain to impair memory; however, a recent study using functional magnetic resonance imaging showed that DEX inhibits hippocampal activity in a region-specific fashion during learning [6]. Given that the hippocampus is crucially involved in learning and memory functions, these results led us to postulate that DEX acts on hippocampal functions.

The electrophysiological profile of DEX in the hippocampus has not been well characterized. Several studies

have investigated the influence of DEX on hippocampal long-term potentiation (LTP), a mechanism involved in information storage during learning and memory [7]. For example, Takamatsu et al. [8] showed that DEX impairs LTP induction in area CA1 of mouse hippocampal slices. However, no report has extrapolated the DEX-induced LTP suppression observed in animal studies to clinical situations. We postulated that an in vivo study would provide a more clinically relevant experimental rat model of human clinical settings compared to slice preparation.

Therefore, we performed an in vivo electrophysiological study in which we investigated the impact of DEX on hippocampal functions, with a focus on the DEX-induced sedative effect. Elucidating the effect of a sedative dose of DEX on the hippocampal LTP in an anesthetized rat may help to characterize the mechanism underlying DEX-induced sedation and/or amnesia in clinical settings.

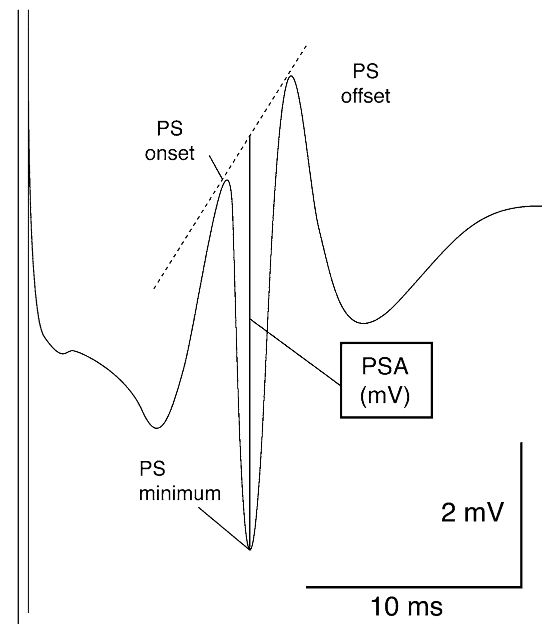


Fig. 1 Measurement of CA1 field response components. A response recorded from the CA1 region after the stimulation of Schaffer collaterals, showing a stimulus artifact (leftmost deflection) followed by a small fiber response and large excitatory postsynaptic potentials (positive, upward wave) and a population spike (*PS*; downward, negative wave). The maximal vertical distance from the tangent to the spike trace was recorded as the PSA. *PSA* Population spike amplitude

Materials and methods

General methodology

Adult male Wistar rats (10–12 weeks old), obtained from Shizuoka Laboratory Animal Center in Hamamatsu, Japan, were housed in a room maintained at 22–25 °C, with a 12-h dark/light cycle and free access to food and water. The rats were handled in accordance with the Guidelines for the Care and Use of Laboratory Animals at Hokkaido University, Graduate School of Medicine. Before the electrophysiological study was started, each rat was weighed and assigned to a treatment group. DEX was obtained from Hospira Japan Co., Ltd (Osaka, Japan) and diluted in 0.9 % saline before being administered intraperitoneally (i.p.) at a volume of 5 mL/kg. The treatment groups were as follows: Group 1, DEX (100 μg/kg, i.p.); Group 2, DEX (30 μg/kg, i.p.); Group 3, DEX (10 μg/kg, i.p.); Group 4, DEX (3 μg/kg. i.p.) and vehicle control (0.9 % saline at 5 mL/kg, i.p.).

Electrophysiological recordings

Electrophysiological recordings were obtained as described previously [9]. The rats were anesthetized with 1.2 % isoflurane in a mixture of 21 % O_2 and 79 % nitrogen through a tracheal catheter. Rats were immobilized with 1.2 % isoflurane. We previously reported that isoflurane did not affect population spike amplitude (PSA) at doses of up to 1.0 minimum alveolar concentration (approx. 1.4 % for adult rats) [10]. In our preliminary study, hippocampal LTP was well established at 1.2 % isoflurane (data not shown), leading us to suppose that 1.2 % of isoflurane was sufficient to induce anesthesia in our electrophysiological study. Rats were placed in a stereotaxic apparatus with the bregma and lambda in the same horizontal plane, and body temperature was maintained at 37 ± 0.5 °C throughout the recording period. The concentration of isoflurane and expired CO_2 tension were monitored continuously through a tracheal catheter using an anesthetic gas monitor (model 5250RGM; Datex-Ohmeda, GE Healthcare, Madison, WI), and expired CO_2 tension was maintained between 35 and 45 mmHg. A monopolar recording electrode was inserted into the pyramidal cell body layer of hippocampal region CA1 (5.0 mm posterior and 3.0 mm lateral to the bregma; approx. 2.3 mm ventral to the dura) in order to record the extracellular PSA. A bipolar stimulating electrode was inserted into the ipsilateral Schaffer collaterals (3.0 mm posterior and 1.5 mm lateral to the bregma; 2.8 mm ventral to the dura) to deliver the cathodal stimulus currents (frequency 0.1 Hz, pulse duration 250 μs). A single electrical stimulation evoked action potentials in the Schaffer collaterals, resulting in activation of pyramidal cells in the CA1 region, and extracellular PSA was recorded (MacLab and PowerLab, ADInstruments, Sydney, Australia). The maximal vertical distance from the tangent to the spike trace was recorded as the PSA (Fig. 1). To adjust the test stimulation, we recorded changes in PSA caused by varied stimulus intensities; the intensity of the test stimulation was fixed to produce a half-maximal response for each rat. After establishing a stable baseline for 20 min, each dose of DEX or saline was administered i.p., and 20 min after

Table 1 Sedative effects of dexmedetomidine in the rat

Dose of DEX (μg/kg)	Spontaneous movement reduction (the number of rats/all rats (%))[a]						
	Time after administration (min)						
	5	15	30	45	60	75	90
3	0/6 (0.0)	3/6 (50.0)	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)	0/6 (0.0)
10	0/5 (0.0)	3/5 (60.0)	2/5 (40.0)	3/5 (60.0)	0/5 (0.0)	0/5 (0.0)	0/5 (0.0)
30	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	5/6 (83.3)	5/6 (83.3)	4/6 (66.7)
100	5/6 (83.3)	6/6 (100.0)	5/6 (83.3)	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)	6/6 (100.0)

Groups of rats were injected intraperitoneally (i.p.) with dexmedetomidine (DEX) and observed for 5, 15, 30, 45, 60, 75, and 90 min after the administration

[a] Values are expressed as the number of rats displaying a loss of spontaneous movement/all rats, with the percentage of animals given in parenthesis

drug administration, LTP was induced by applying high-frequency stimulation (HFS; 10 trains at 1 Hz each, composed of 8 pulses at 400 Hz) at the same intensity as the test stimulus. The mean of five PSA measurements before and after the HFS were then plotted every 5 min for 60 min after the HFS.

Acute sedative effects in rats

We investigated the sedative dose of DEX on Wistar strain rats. Because DEX-induced sedation is easily reversed by physical stimuli [11], we determined the depth of sedation in the rats by using the loss of spontaneous movement as an endpoint, according to results from our previous study [12]. The loss of spontaneous movement was recorded for 90 min after administration of each dose of DEX. These results are expressed as the percentage of animals that presented a given behavioral sign.

Statistical analysis

All experimental values are presented as the mean ± standard error of the mean. Electrophysiological measurements are expressed as a percentage of the PSA value measured just before HFS, then analyzed with an area under the curve (AUC) of 10–60 min after HFS, using one-way analysis of variance with Bonferroni's multiple comparisons test. Probability values were considered to be significant at $P < 5$ %. Statistical analyses were performed using GraphPad Prism software version 5.0 (GraphPad Software, Inc., San Diego, CA).

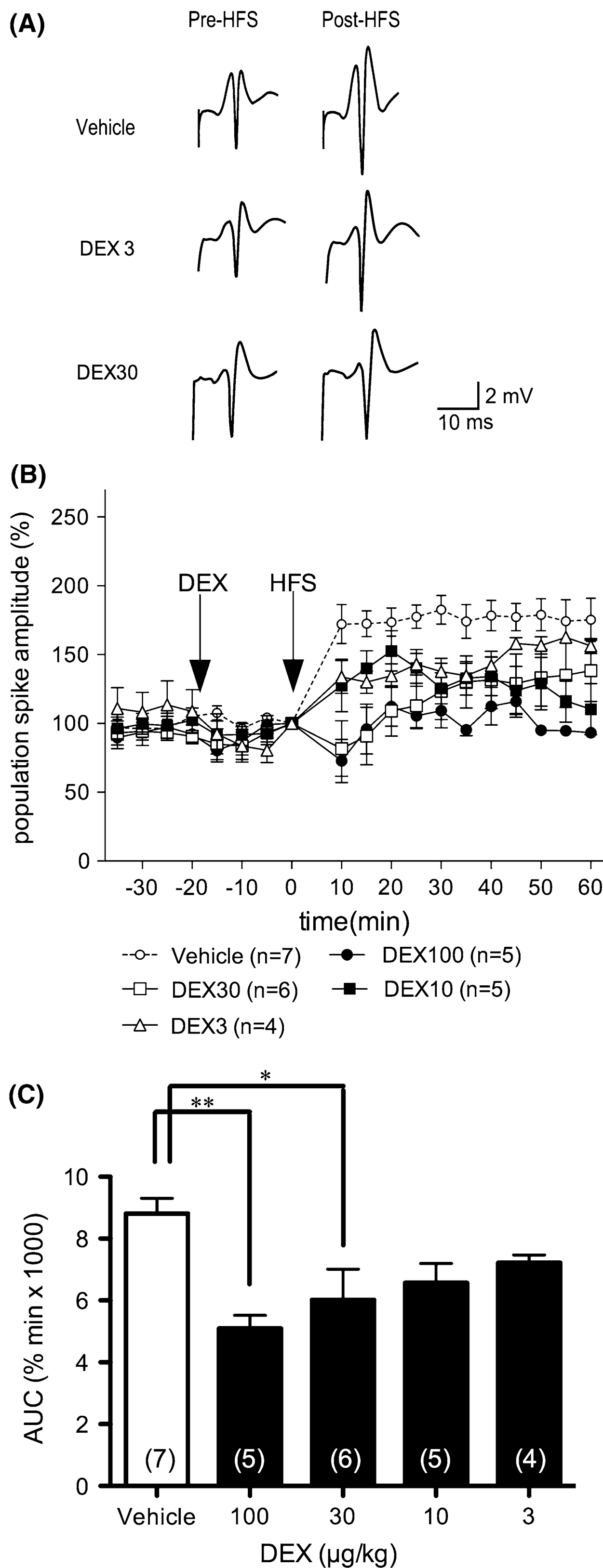
Results

Intraperitoneal administration of higher doses of DEX (30 and 100 μg/kg) to rats induced a range of sedative effects. These effects included rapid sedation that persisted for >90 min after drug administration, as indicated by the loss of spontaneous movement (Table 1). Electrophysiological testing revealed that HFS of the Schaffer collaterals induced long-lasting increases in PSA in the CA1 field, i.e., LTP induction, in the vehicle group. The maximum response recorded was 182.6 ± 10.4 % ($n = 7$; Fig. 2a). The AUC was significantly decreased in the 100 and 30 μg/kg groups as compared to vehicle rats between 10 and 60 min after the HFS (Bonferroni's multiple comparison test, $P < 0.05$; Fig. 2b). This finding suggests that DEX suppresses the induction of LTP [vehicle: 8.81 ± 0.49, $n = 7$; DEX 100 μg/kg: 5.10 ± 0.43, $n = 5$ ($P < 0.01$ vs. vehicle); DEX 30 μg/kg: 6.02 ± 0.99, $n = 6$ ($P < 0.05$ vs. vehicle); Fig. 2b].

Discussion

In our study we demonstrated that a single administration of DEX at a dose of 30 or 100 μg/kg inhibited the induction of LTP in the CA1 region of the adult rat hippocampus. The administration of DEX at concentrations of <30 μg/kg had no significant influence on LTP induction. Moreover, the intraperitoneal administration of DEX at a dose of 30 or 100 μg/kg to rats induced a range of sedative effects. Because the hippocampal LTP was suppressed by these sedative doses of DEX, our findings may signify a causal link between DEX-induced sedative action and hippocampal LTP suppression. To our knowledge, this is the first in vivo demonstration of DEX impairing hippocampal LTP in rats.

Our finding is consistent with the results reported by Takamatsu et al. [8], which showed that DEX impairs LTP induction in mouse hippocampal slices. In contrast, in another in vitro study, Niittykoski et al. [13] observed that the same condition did not reduce the induction and



◀**Fig. 2** **a** The representative recordings of PSA before high-frequency stimulation (*pre-HFS*) and after HFS (*post-HFS*). **b** Time-course of PSA after treatment with dexmedetomidine (*DEX*) (**b**). **c** Area under the curve (*AUC*) for PSA measurements. DEX or saline was administered 20 min before HFS. The AUC were calculated during the first 10–60 min after the HFS. *Vehicle* Saline-treated group, *DEX100*, *30*, *10* and *3* DEX administered at 100, 30, 10, and 3 μg/kg intraperitoneally, respectively. Each value represents the mean ± standard error of the mean. The number of rats tested is given in each column. *$P < 0.05$, **$P < 0.01$

maintenance of LTP in the CA1 area of the rat hippocampus. It is unclear whether the discrepancy between these two in vitro studies reflects differences in species or experimental protocols, such as the recording systems or LTP induction protocol. In any case, the discrepancy between these two reports should be resolved. Our data may facilitate the clarification of the effect of DEX on synaptic plasticity in the hippocampus.

In this study we treated rats with a single DEX injection, whereas DEX is commonly used as a continuous infusion with a stable plasma concentration in clinical practice [14]. However, several studies have reported that a single administration of DEX is sufficient to achieve an effective plasma concentration that is stable over time. In one animal study, the reported effective dose 50 (ED_{50}) of the antinociceptive effect of intraperitoneal DEX was 27.6 ± 5.1 μg/kg, and the antinociceptive effect of a single administration of DEX persisted for up to 90 min [15]. Plasma drug levels also remained high for a prolonged duration in adult rats treated with a single dose of DEX (plasma concentration >1 ng/mL 90 min after one 30 μg/kg dose of DEX) [16]. Notably, a plasma DEX concentration of 1.09 ng/mL is required for sedation in adult rats [16]. Therefore, even a single DEX administration results in a prolonged elevation of plasma DEX levels and a stable sedative effect. Consistent with these findings, our data showed a prolonged sedative effect of a single administration of DEX (over 90 min) at doses of 30 or 100 μg/kg; therefore, we presumed that a DEX dose of >30 μg/kg would be sufficient to sedate the rats throughout all electrophysiological measurements, which required more than 80 min.

We performed an in vivo electrophysiological study with anesthetized rats, which may be a more clinically relevant experimental model of human clinical settings than slice preparations. Slice preparation is an adequate test system for further investigation into the cellular basis of anesthetic action. However, cutting brain slices disconnects the neurons from the sources of their afferences and from the targets of their efferent projections, thereby eliminating the regulation of neuronal activity by serotoninergic projections mainly from the raphe, noradrenaline afferences from the locus coeruleus, acetylcholine innervation from the nucleus basalis, and hormonal modulations [17, 18]—all of which are crucially involved in LTP formation. In addition, several studies have demonstrated that GABA is

involved in triggering the LTP phenomenon. Consequently, disconnecting a structure from its inputs/outputs can lead to divergent results compared to an intact structure. Therefore, in contrast to a slice preparation study, an in vivo electrophysiological study may have clinical implications, providing a realistic picture of the overall functioning of the brain with intact neuronal circuitry. Importantly, our in vivo study revealed that sedative doses of DEX impaired the induction of hippocampal LTP. These findings may signify a causal link between DEX-induced sedative action and hippocampal LTP suppression, providing a better understanding of the mechanisms underlying the DEX-induced sedative and/or amnestic effect.

In conclusion, the findings of our study show that the administration of a sedative dose of DEX altered synaptic plasticity in the rat hippocampus. This in vivo design may provide novel information regarding the basic actions of DEX in the central nervous system.

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