Control of hippocampal theta rhythm by serotonin: Role of 5-HT2c receptors

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ABSTRACT

The hippocampus plays an important role in learning and memory and has been implicated in a number of diseases, including epilepsy, anxiety and schizophrenia. A prominent feature of the hippocampal network is the capability to generate rhythmic oscillations. Serotonergic modulation is known to play an important role in the regulation of theta rhythm. 5-HT2c receptors represent a specific target of psychopharmacology and, in particular, the behavioral effects of the 5-HT2c receptor agonist mCPP have been thoroughly tested. The present study used this compound and the selective 5-HT2c receptor antagonist SB-242084 to elucidate the role of 5-HT2c receptors in the generation of hippocampal oscillations. Hippocampal EEG was recorded and the power in the theta frequency range was monitored in different behaviors in freely-moving rats and after brainstem stimulation in anesthetized animals. We found that in freely-moving rats, mCPP suppressed hippocampal theta rhythm and the effect was stronger during REM sleep than during waking theta states. Under urethane anesthesia, mCPP decreased the power for both spontaneous and elicited theta rhythm in a dose-dependent manner and the 5-HT2c antagonist reversed this effect. The results of this study demonstrate that 5-HT2c receptors are important element of the serotonergic modulation of hippocampal theta oscillations and thus pharmacological interactions with these receptors can modulate physiological and pathological processes associated with limbic theta activity.

SB-242084 = 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl carbamoyl]indoline mCPP = 1-(3-chlorophenyl)piperazine dihydrochloride

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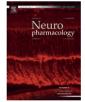© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Hippocampal theta rhythm is one of the most studied neuronal network oscillations due to its presumed role in various high-level brain functions. A close connection has been revealed between hippocampal theta activity and affective or explorative behavior, in sensory filtering and various domains of cognitive processes based on extensive studies on rodents (Pare and Gaudreau, 1996; Buzsaki, 2002; Wyble et al., 2004; Kunec et al., 2005). Characteristic hippocampal theta rhythm (an approximately sinusoidal field potential oscillation in a 5–10 Hz frequency range) can be recorded not only in the hippocampus, but also in closely connected regions of the limbic circuitry, including the ventro-medial prefrontal cortex, brain regions associated with cognitive and affective functions (Siapas et al., 2005; Sigurdsson et al., 2010). Hippocampal theta activity in humans have been also demonstrated and, although it shows some

differences in oscillations patterns to rodent theta activity, its role in human brain has been confirmed to the same type of functions preclinical experimental studies suggested (Kahana et al., 1999; Raghavachari et al., 2001; Cantero et al., 2003).

Neuronal network mechanisms involved in hippocampal theta generation, as well as its regulation via various afferent systems have attracted great interest over the last years. The critical networks of GABAergic and cholinergic neurons within the hippocampus and medial septum-diagonal band of Broca (MSDB) are well established, modulatory roles of monoaminergic and peptidergic afferents have been also intensively studied (Vertes and Kocsis, 1997). Among the afferent projections serotonergic neurons provide a dense innervation of both the hippocampus and the MSDB; 5-HT containing axons form perisomatic and peridendritic baskets and asymmetric synaptic contacts on parvalbumin GABAergic neurons (Leranth and Vertes, 1999). Inhibition of activity of medial raphe 5-HT neurons evokes hippocampal theta oscillation in both anaesthetized rats (Vertes et al., 1994; Kinney et al., 1995) and in freely-moving cats (Marrosu et al., 1996), indication that 5-HT neurons play an inhibitory role in

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hippocampal theta generation. Furthermore, recently, it has been shown that 5-HT2c receptor activation inhibits, whereas 5-HT2c receptor blockade facilitates hippocampal theta oscillations of MSDB neurons and hippocampal field potentials in anaesthetized rats (Hajos et al., 2003a). Although these findings indicate a potential regulatory role of 5-HT2c receptors in hippocampal network oscillations, they do not reveal what type of hippocampal theta is impacted by 5-HT2c receptors in relation to function or behavior. Recently we have shown that inhibition of norepinephrine (NE) re-uptake by reboxetine modulates hippocampal theta oscillation in a stage- and behavioral dependent manner. Enhanced NE transmission augments theta oscillations in anaesthetized rats, inhibits rapid eye movement (REM) sleep-associated theta oscillation, and in awake rats could either increase or decrease theta power depending on current, on-going behavior (Hajos et al., 2003b; Kocsis et al., 2007). Therefore, in the present study, we evaluated the effects of the 5-HT2c receptor agonist mCPP hippocampal theta oscillation in 2 experiments, i.e. in freely-moving rats where 5–10 Hz theta rhythm is related to sleep-wake behavior and in anesthetized rats where theta in the 4–6 Hz range appears spontaneously and can be elicited in a wider range (4–8 Hz) by brainstem stimulation (Kramis et al., 1975; Vertes and Kocsis, 1997; Buzsaki, 2002; Li et al., 2007).

2. Methods

2.1. Animals

This study included two series of experiments, one conducted under urethane anesthesia and the other in freely behaving rats. Male Sprague-Dawley rats (body weight 350–450 g) were kept under standard, temperature and humidity controlled laboratory conditions with food and water ad libitum in a 12 h light/12 h dark cycle. All experiments were performed in accordance with National Institute of Health guidelines and were approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center.

2.2. Surgery

Rats for acute experiments were anesthetized using urethane (750 mg/ml, 1.5 g/kg body weight, i/p) and those for survival surgery were given a mixture of ketamine and xylasine (35–45 and 5 mg/kg, respectively, i/p). In each rat, pairs of stainless steel wires were implanted for recording hippocampal EEG on both sides at AP −3.7 mm, Lat ±2.2 mm, DV −3.5 mm, relative to bregma, and fixed to the skull with dental acrylic. The tips of the electrodes were separated by ∼1 mm and positioned so the deeper electrode reached below the hippocampal fissure and the shorter would be located in the CA1 oriens/pyramidal layer. Stainless steel screws were fixed in the skull over the cerebellum and olfactory bulb to serve as ground and reference electrodes. Rats for freely-moving recordings also received an electrode to record EEG over the frontal cortex and a pair of EMG electrodes for polysomnography whereas in anesthetized rats an additional pair of wires was implanted in the nucleus reticularis pontis oralis (AP −7.8 mm, Lat 1.8 mm, DV −8.0 mm), for electrical stimulation. Saline injection has no effect on the animals' status and/or EEG.

2.3. Drugs

In experiments under urethane anesthesia, two or three intraperitoneal drug injections were made in each rat. The injections started with the lowest dose of the 5-HT2c agonist mCPP in all rats (0.15 mg/kg, n = 11) and continued with the medium dose (0.3 mg/kg, n = 10). A third injection was made in 7 rats, of either the highest dose of mCPP (0.6 mg/kg, n = 3) or of a combination of 0.3 mg/kg mCPP and the selective 5-HT2c antagonist SB-242084 (1 mg/kg, n = 4). In chronic experiments, mCPP was injected intraperitoneally (i.p.) in doses of 1 mg/kg (n = 12) and in 2 mg/kg (in three rats). The injections were repeated 2 or 3 times in each rat in different days of recording, alternating with saline injection as vehicle control, with a separation of at least 2 days between consecutive injections. The initial doses were chosen on the basis of previous studies, e.g. (Hajos et al., 2003a; Kantor et al., 2005).

2.4. Electrophysiological recordings

In the experiments using anesthetized rats, hippocampal EEG was recorded on both sides throughout the experiment. The signals were amplified and filtered between 0.15 and 100 Hz (Model 3500, A-M Systems). RPO stimulation started after a 30–40 min control recording of spontaneous EEG. For electrical stimulation of the RPO, 200 us square waves were used at 100 Hz, for 10 s (AMPI Master 8 stimulator with IsoFlex constant current unit). The stimulus intensity varied between animals and were set in each individual experiment using the well-known linear characteristics between stimulus intensity and theta frequency (see e.g. Li et al., 2007). Thus, RPO was stimulated at different intensities to identify the threshold to elicit theta rhythm in the hippocampus and the intensity necessary to elicit the largest response, i.e. above which the frequency no longer increases. Test stimulations then used two stimulus intensities, one eliciting the maximum response and the other set at 50% of the effective range between the threshold and maximum (usually less than 0.7 mA). Both types of stimuli were applied at least 5 times in the control period and then in each drug condition. Between injections, we waited until normal EEG returned, including full recovery of spontaneous and elicited theta oscillations. This recovery period usually lasted 1–2 h.

In chronic experiments, daily recordings started 7–10 days after surgery. The rats were placed in a recording box in the morning and cortical and hippocampal EEG and neck muscle EMG were continuously recorded for 7–8 h. Injections were made after 3–5 h of control recording. Thus, the injection occurred between noon and 2pm, and the observations were limited to daytime recordings when the rats spend most of their time in sleeping or in quiet waking.

2.5. Data analysis

EEG signals were sampled at 256 Hz and power spectra were calculated using fast Fourier transform on 1 s (in stimulation experiments) or 16 s long windows (chronic recordings). In anesthetized rats, theta power was calculated between 4 and 6 Hz i.e. in the range where spontaneous theta oscillations appear in this preparation. Evaluation of the effect of high intensity stimulation also included analysis of a higher, 6–8 Hz, frequency band. Elicited theta was calculated as average of five 10 s segments. Theta was also calculated in 60 s baseline segments before drug injection and the average of 5 such spectra was used to normalize the EEG amplitude between experiments, i.e. EEG power was expressed relative to this baseline average.

In chronic experiments, sleep-wake states were identified in 16 s segments using the level of delta power in the frontal cortex (1–4 Hz) and theta in the hippocampus (5–10 Hz) along with the root-mean square value of the EMG, according to common practice. Rapid eye movement (REM) sleep was identified by atonia and dominance of hippocampal theta rhythm. Identification of slow wave sleep was based on large delta waves in both cortex and hippocampus along with the occurrence of sleep spindles and low EMG activity. Awake state was detected primarily by high level of motor activity (active waking) and/or low amplitude cortical EEG, whereas hippocampal EEG could include theta and non-theta segments, but not delta waves. Since "dominant rhythms" are characterized by increase in power within a certain frequency band and a decrease outside of this band, we used relative power to detect such episodes, i.e. spectral power averaged over theta and delta bands were divided by the total root-mean square value of the signal.

To specifically quantify the effect on the theta generators rather than on the circuits switching between theta and non-theta states, theta segments of the EEG, associated with awake exploratory behavior or REM sleep, were identified when theta power was at least 4 times higher than delta and theta power in the 5–10 Hz frequency range was calculated separately for waking and REM sleep episodes and averaged over 1 h periods. For statistical analyses, Student's t-test and one-way and two-way ANOVA with posthoc Bonferroni pair-wise comparisons or were used.

3. Results

3.1. Theta rhythm under urethane anesthesia

Theta rhythm under urethane anesthesia appears spontaneously and can also be elicited by electrical stimulation of the brainstem reticular formation (Vertes and Kocsis, 1997). Fig. 1A shows the time course of changes in integrated theta power in an example in which mCPP was injected at the height of one of the spontaneous theta episodes. Theta was reduced within minutes of injection whereas the compound had no effect when co-administered with the 5-HT2C antagonist SB-242084. The effect of mCPP was dose-dependent (ANOVA F[4,77] = 10.328, $p < 0.001$); on group average, spontaneous theta power decreased by $6 \pm 5\%$ after 0.15 mg/kg, $31 \pm 5\%$ after 0.3 mg/kg, and by $34 \pm 4\%$ after 0.6 mg/kg (Fig. 1C). Posthoc Bonferroni test showed significant differences between control and 0.3 mg/kg ($t = -5340$, $p < 0.001$) and 0.6 mg/kg ($t = -4.041$, $p = 0.001$) injections, whereas theta in control recordings was not different ($p > 0.05$) from that after injection of mCPP in 0.15 mg/kg dose or in combination with the antagonist.