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Microinjection of procaine and electrolytic lesion in the ventral tegmental area suppresses hippocampal theta rhythm in urethane-anesthetized rats

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Abstract

The midbrain ventral tegmental area (VTA), a key structure of the mesocorticolimbic system is anatomically connected with the hippocampal formation. In addition mesocortical dopamine was found to influence hippocampus-related memory and hippocampal synaptic plasticity, both being linked to the theta rhythm. Therefore, the aim of the present study was to evaluate the possible role of the VTA in the regulation of the hippocampal theta activity. The study was performed on urethane-anesthetized male Wistar rats in which theta rhythm was evoked by tail pinch. It was found that unilateral, temporal inactivation of the VTA by means of direct procaine injection resulted in bilateral suppression of the hippocampal theta which manifested as a loss of synchronization of hippocampal EEG and respective reduction of the power and also the frequency of the 3–6 Hz theta band. Depression of the power of the 3–6 Hz component of the EEG signal was also seen in spontaneous hippocampal EEG after procaine. The permanent destruction of the VTA by means of unilateral electrocoagulation evoked a long-lasting, mainly ipsilateral depression of the power of the theta with some influence on its frequency. Simultaneously, there was a substantial increase of the power in higher frequency bands indicating decrease of a synchrony of the hippocampal EEG activity.

On the basis of these results indicating impairment of synchronization of the hippocampal activity the VTA may be considered as another part of the brainstem theta synchroning system.

Keywords: Ventral tegmental area; Hippocampal theta rhythm; Procaine; Electrolytic lesion

1. Introduction

Hippocampal field activity (EEG) is controlled by a multisynaptic system involving several sites in the lower brainstem, diencephalon and telencephalon (for review see [9,10,62]). Theta rhythm, that is highly synchronized electrical activity of the hippocampus at a 3–12 Hz frequency range in freely moving rodents, is critically dependent on the integrity of the ascending pathway originating in the brainstem reticular formation (nucleus reticularis pontis oralis (RPO) [27,34,39,59,60,63] and pedunculopontine tegmental nucleus (PPN) [23,38,63]), synapsing in the midline diencephalon (posterior hypothalamus (PH) [9,24,26,41,64,65], medial and lateral mammillary nuclei [2,29,33], supramammillary nucleus (SuM) [25,26,44,66], dorso-medial hypothalamus [13,64]) passing through the lateral hypothalamus [21] to the medial septal area and from the later region reaching the hippocampal formation. As research progresses still other brain structures are incorporated into the theta controlling system: the ventral tegmental nucleus of Gudden [6,28], the fasciculus retroflexus [56] and the raphe nuclei [22,57]. Recently, we found [20] that the midbrain ventral tegmental area (VTA) is involved in the regulation of both hippocampal and cortical EEG activity in conscious rats performing behaviors (such as exploratory sniffing) which are easily elicitable by VTA stimulation and usually accompanied by hippocampal theta rhythm. The present study was aimed at evaluating more precisely the possible role of VTA in the regulation of the hippocampal electrical activity, particularly in hippocampal field activity at theta frequency. We used a standard experimental model of sensory-elicited theta rhythm recorded from the dorsal hippocampus in urethane-anesthetized rats. Under urethane survives only atropine-resistant (cholinergically medi-

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ated) so called theta 2, the one which in the rat is recorded during the paradoxical phase of sleep [49,58,61] and in waking during attentive immobility [30,48,58]. This commonly used model enables analysis of the ability of hippocampus to synchroneous activity in strictly controlled conditions which are difficult to meet in freely behaving animals in which the theta is frequently mixed with other types of EEG waves. Numerous studies [8,22,26,33,36,38,40,57] proved that this is the valid model to demonstrate the involvement of definite brain areas in the theta regulation.

The VTA which is composed of the dopaminergic projection neurons of the A10 group and the non-dopaminergic (mainly GABA-ergic) pericaria [12,40], has numerous anatomical connections with the hippocampal formation [14,16] and dopamine is involved in the modulation of hippocampal synaptic plasticity as assessed by long term potentiation (LTP) and long term depression (LTD) (for review see [18,32]). Mesocortical dopamine in the dorsal and ventral subiculum have been found to be involved in memory [15]. As both LTP and memory are linked to the hippocampal theta system [1,17,54] it seemed reasonable to study the possible role of the VTA in the regulation of the theta.

The VTA projections are directed to both ventral (temporal) and dorsal (septal) hippocampal formation although to a different extent. The majority of the VTA inputs terminate in the ventral hippocampus, however, a certain number of VTA neurons project to the subicular and CA1 fields in the dorsal hippocampus [14,16]. Although both ventral and dorsal hippocampi produce the theta rhythm [46], this field activity pattern is most frequently recorded, probably due to the convenience of electrodes implantation, from the dorsal hippocampus. Certain functional differences were found between the dorsal and ventral hippocampus [e.g. 5,37] which follow differences in anatomical connections, with the ventral hippocampus being linked to the cortical and subcortical limbic structures, hypothalamus and the limbic-related endocrine and autonomic functions, and the dorsal hippocampus being linked to the sensorimotor cortex, the structures of the so called Papez circuit and to the spatial and contextual memory. However, in many instances [e.g. 7] the dorso-ventral differences are quantitative rather than absolute, relative to a substantial number of intrahippocampal projections connecting its dorsal and ventral part [3,51]. This undoubtedly concerns the theta rhythm which have been found to be driven by the common (septal) pacemaker throughout its dorso-ventral axis [46]. As was found by Gasbarri et al. [15] dopamine depletion impairs specifically spatial memory, the function anatomically connected with the dorsal hippocampus from which the theta rhythm is usually recorded. Besides, electrical stimulation of the VTA was found to elecit intensity-dependent field potential in the dentate gyrus of the dorsal hippocampal formation and to increase the amplitude of population spikes evoked by the perforant path stimulation [11]. Therefore, we decided to evaluate the effect of the VTA inactivation on the dorsal, rather than ventral hippocampal field activity.

In the present study, the sensory-elicited theta was analyzed in the hippocampal EEG before and after unilateral intra-VTA administration of procaine or destruction of the VTA tissue by means of electrocoagulation. For the first time we provide evidence that such manipulations suppress the hippocampal theta rhythm.

A preliminary report of the present work was published in abstract form [42].

2. Materials and methods

2.1. Animals

Male Wistar rats weighing approximately 270–450 g were used. The animals were randomly divided into the PRO group ($n = 5$), receiving an intra-VTA procaine injection, and the ELE group ($n = 8$), subjected to unilateral electrolytic lesion of the VTA. Two additional rats were lesioned outside the VTA area to control for anatomical specificity of the observed effects. In one sample animal, the spread of the injection fluid was assessed by injecting 0.5 μl of aqueous solution of the alcian blue dye through the VTA cannula.

2.2. Surgery

The animals were anesthetized with urethane (Urethane, Sigma 1.5 g/kg i.p.) and positioned in the frame of a stereotaxic apparatus. A unilateral recording electrode was implanted in the stratum moleculare of the dorsal blade of the dentate gyrus of the dorsal hippocampus in accordance with standard stereotaxic procedure. The hippocampal monopolar electrode consisted of stainless steel wire of 0.2 mm diameter insulated along its entire length with the exception of the flat-cut tip. The following Paxinos and Watson [45] stereotaxic coordinates (skull levelled) were used for implantation: 3.3–3.6 mm posterior to the bregma, 1.6–2.4 mm lateral to the midline and 3.2–3.3 mm ventral to the skull surface. Stainless steel skull screws positioned in the frontal pole of the skull over the olfactory bulbs and the anterior frontal area, where electrical activity is minimal (stereotaxic coordinates: 5.2–5.7 mm anterior to the bregma, 1.0 mm lateral to the midline and 1.0 mm ventral to the skull surface) served as ground and reference electrodes. All electrodes were fixed to the skull surface with dental acrylic. A small hole was drilled over the VTA to allow subsequent insertion of an injection cannula (the left-side cannula in two rats, the right-side cannula in three rats) or a lesion electrode (the left-side electrode in four rats, the right-side electrode in four rats). The stereotaxic coordinates for VTA implantation were: 4.8–5.1 mm posterior to the bregma, 0.9–1.0 mm lateral to the midline and 7.8–8.1 mm ventral to the skull surface. The two control rats were implanted with lesion electrodes above the VTA (stereotaxic coordinates: 5.1 mm posterior to the bregma, 1.0 mm lateral to the midline and 6.8 mm ventral to the skull surface). As the injection cannula, a needle (0.4 mm diameter, with the flat-cut tip) of a 10 μl Hamilton syringe was used. The syringe was placed in the stereotaxic holder with a microinfusion pump (Kopf Instruments). The lesion electrodes consisted of a stainless steel wire, 0.3 mm diameter, insulated along its entire length with the exception of the flat-cut tip.

2.3. Experimental procedure

Urethane-anesthetized rats maintained at 37 °C were fixed in the stereotaxic apparatus. EEG was recorded from the hippocampal electrodes during the whole experimental period using a Medicor electroencephalograph as a preamplifier (bandpass 0–70 Hz) and the EEG DigiTrack computer system (ELMIKO, Poland) to register EEG signal (sampling rate 240 Hz) on the computer hard disk. The animals were maintained on a plane of anesthesia at which spontaneous theta rhythm was not present in the hippocampal EEG but could be elicited by sensory stimulation. At this level of anesthesia no motor or respiratory signs of pain or even perception of sensory stimuli produced either by a tail pinch or by other aspects of experimental procedure (skull surgery, stereotaxic apparatus pressure) have ever been noticed. The level of anesthesia was controlled by monitoring the frequency of breathing.

Sensory stimulation which was a tail pinch lasting 60 s was applied three to four times at 10 min intervals in the preinjection period (PRO group) and seven times at 10 min intervals in the prelesion recordings (ELE group). A tail pinch was produced by a plastic clamp (always the same) which was positioned on