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Differential cholinergic modulation of synaptic encoding and gain control mechanisms in rat hippocampus

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Abstract

Recent studies have highlighted a variety of cognitive effects caused by cholinolytic drug injections into different cortical structures. These findings were largely interpreted as evidence for location-specific cholinergic modulation of synaptic encoding mechanisms. Here, using evoked field responses in anaesthetized rat dorsal hippocampus we show that in addition to reinforcement of synaptic connections (long-term potentiation, LTP), endogenous acetylcholine also regulates firing gain of CA1 pyramidal neurons (EPSP-spike potentiation). Gain augmentation upon increase in cholinergic drive involves evoked synchronous firing at both apical and basal afferent projections, unlike enhancement of activity-induced LTP constrained to the basal afferent system. These data indicate that acetylcholine can act as an effective input and gain controller in the hippocampus. Modulation of synaptic plasticity would determine the relative dominance of afferent inputs while the facilitation of synchronous firing is likely to promote a more generalized spread of excitation and long range communication within the limbic cortex.

Keywords: Acetylcholine; Basal dendrites; Gain control; Population discharge; Disinhibition

1. Introduction

Acetylcholine (ACh) is perhaps the most versatile modulator of brain activity and function. The integrity of the forebrain cholinergic system is critical for an array of high integrative processes, which include development and plasticity of cortical representations, mechanisms of attention and memory formation (McGaughy et al., 1996; Sarter et al., 2005). Choline acetyltransferase (ChAT) positive afferents, primarily originating from neurons of the basal forebrain (BF) area, provide a major source of ACh for various forebrain structures (Mesulam et al., 1983; Zaborszky et al., 1999). Recent studies have highlighted the location-specific action of endogenous ACh in numerous cortical areas (Bunce et al., 2004; Gil et al., 1997; McGaughy et al., 2005; Turchi et al., 2005), which have been interpreted as evidence linking the behavioral effects of ACh with its focal modulation of synaptic encoding mechanisms that underlie the formation of new memories (Hasselmo, 2006). Such location-specific action of ACh contrasts with an earlier postulated diffuse model of brain state and activity regulation by endogenous cholinergic drive, exhibiting typical features of reticular modulator systems with distributed effects on a broad range of neuronal processes (Fibiger, 1982; Sarter and Bruno, 1997).

The hippocampus, like other allocortical structures, receives dispersed cholinergic innervations primarily from rostral portions of the BF (Amaral and Kurz, 1985; Frotscher and Leranth, 1985). Recent topographic analysis on a light microscopic level however disclosed the laminar organization of putative cholinergic axons and varicosities within the CA1 region of the rat dorsal hippocampus, with their highest density in the principal cell layer and stratum oriens (Aznavour et al., 2002). Such heterogeneous distribution of ACh positive elements is consistent with the functional diversity and location/input-specific effects of ACh on neuronal processing within the hippocampal CA1 area as revealed in electrophysiological reports *in vitro* (Hasselmo and Schnell, 1994; Shimono et al., 2000) and *in vivo* (Buzsaki et al., 1981; Leung, 1980; Rovira et al., 1983).

The present *in vivo* study compares the effect of the muscarinic cholinergic receptor antagonist scopolamine and

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the endogenous cholinergic drive enhancer physostigmine on activity-dependent long-term potentiation (high-frequency stimulation (HFS)-induced long-term potentiation (LTP)) and synaptically driven synchronous firing (evoked population spike), respectively, in CA1 pyramidal neurons in urethane-anaesthetized rats. As cholinergic drive is preserved under urethane anaesthesia in the rat (Stewart and Fox, 1989), we examined whether and how it affects HFS-induced LTP and evoked population spikes at two major basal and apical afferent projections in the CA1 area of the dorsal hippocampus.

2. Materials and methods

Experiments were performed in male Wistar rats ($n = 45$, 250–350 g) under the license approved by the Department of Health, Republic of Ireland. The method of electrode implantation was similar to that described previously (Ovsepian et al., 2004; Ovsepian, 2006). Briefly, under deep urethane anaesthesia (1.5 g/kg, i.p., supplemented as necessary), the animal was fixed in the stereotaxic frame with bregma and lambda leveled. Steel screws were mounted over the cerebellum and the frontal lobe for ground and stimulation anode, respectively. Monopolar teflon-coated electrodes were lowered into the dorsal hippocampal area through small holes in the scull for eliciting field excitatory postsynaptic responses (fEPSPs) and population spike via activation of afferent pathways within the stratum oriens and stratum radiatum (P3.8-L4.4), which were monitored in the ipsilateral stratum radiatum and stratum pyramidale, respectively (P2.2-L2.6). The depth of electrodes was optimized using electrophysiological criteria (phase reversal of basal-dendritic fEPSP compared to that elicited by activation of apical dendrites) (Leung, 1979) (Fig. 1A1 and A2). Test stimuli consisting of cathodal rectangular pulses of 0.2 ms were delivered to the stimulation site at a rate of 0.033 Hz. The fEPSPs were recorded at approximately half the maximum initial slope; the population spike responses at approximately 50% of their maximum amplitude. Based on pilot studies, in which the number of pulses and their intensity in HFS protocols for LTP induction were systematically adjusted, the HFS conditioning protocol was set at the level, which reliably induced sub-maximal LTP (20–35% at 45–60 min post-HFS recording) at basal (10 trains of 10 stimuli at 100 Hz; inter-train interval, 2 s, delivered at the test-pulse intensity) and apical (10 trains of 10 stimuli at 100 Hz; inter-train interval 2 s, delivered at the 150% of test-pulse intensity) afferent systems in the CA1 area. We employed a relatively mild protocol for LTP induction since synaptic plasticity induced by weak conditioning stimulation is especially sensitive to cholinergic modulation (Dringenberg et al., 2007; Huerta and Lisman, 1996). Body temperature was monitored by means of a rectal probe and maintained at 37.0 ± 2 °C via the use of an electrical heating pad. At the end of each experiment, the location of electrodes was routinely marked by the application of a strong DC current of 10–15 mA (10–20 s). Afterwards, the animal was euthanasied by administration of a high dose of urethane (3.5 g/kg, i.p.). The brain was removed from the skull cavity and fixed overnight in a 4% paraformaldehyde solution. Subsequently, coronal slices were cut (100 μm) for verification of the electrode location. The Axograph 4 (Molecular Devices) package was used for data acquisition and triggering stimulation current pulses. Analog signals were filtered at 1–5 kHz, digitized at 10 kHz and saved on a Macintosh computer for subsequent off-line analysis. The LTP magnitude is expressed as the mean percentage of pre-HFS baseline fEPSP initial slope ± S.E.M. Population spike amplitude was quantified as half the sum of the falling and rising phase amplitudes of the population spike. The Student's *t*-test was used for statistical analysis and *p*-values <0.05 were considered significant (Microsoft Excel). Scopolamine (20 nmol/5 μl), physostigmine hemisulfate (30 nmol/5 μl) and saline (0.9% NaCl 5 μl) (Sigma, St. Louis, MO, USA) were injected intracerebroventricularly (i.c.v.) slowly (over 2-min period) via a custom-made cannula lowered into the lateral ventricle (P0.8-L1.4) fixed by dental cement on the skull.

3. Results

Fig. 1A1–B2 illustrates synaptically evoked fEPSPs and population spikes of CA1 pyramidal neurons in the dorsal hippocampus. Activation of the apical afferent pathway caused a current sink in the stratum radiatum detected as a negative waveform, in contrast to basal-dendritic fEPSP evoked by stimulation of afferents within the stratum oriens, which resulted in a phase-reversed positive field potential (Fig. 1A1 and A2). Such phase-reversed fEPSPs in the CA1 area of the dorsal hippocampus have been described previously (Klooster-man et al., 2001). The population spike evoked in the CA1 pyramidal cell layer by stimulation of basal and apical pathways has a characteristic tri-phasic waveform, with negative deflection that increases with the intensity of stimulus (Fig. 1B).

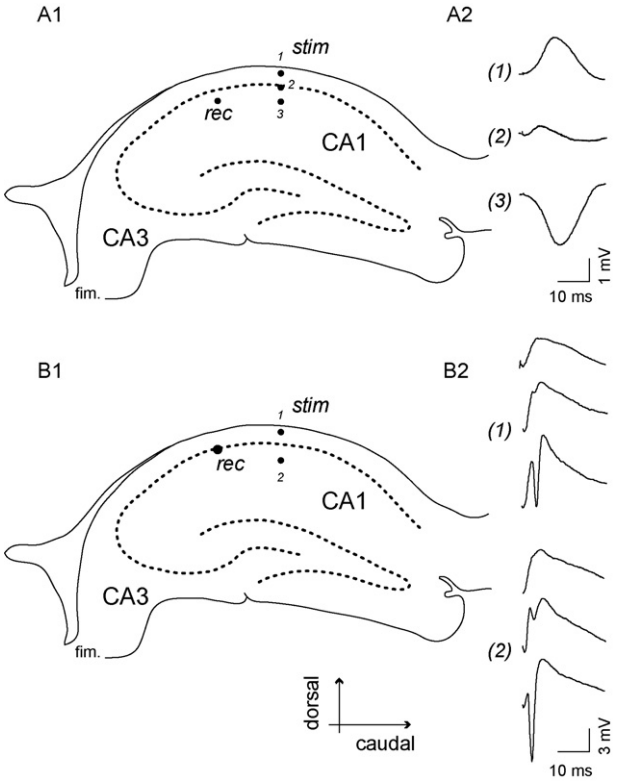

Fig. 1. Experimental design and recording of evoked field potentials (fEPSPs and population spikes) in rat dorsal hippocampus. (A1) Schematic diagram of a sagittal hippocampal section; approximate location of stimulation (stim.) and recording (rec.) sites are marked by black dots. (A2) A phase reversal of fEPSPs in stratum radiatum corresponds to the fEPSPs evoked by st. oriens (1), st. pyramidale (2) and st. radiatum (3) stimulation, respectively. CA1, CA3 refer to the CA1 and CA3 areas, respectively; fim. refers to fimbria. (B1) Schematic diagram of a sagittal hippocampal section of evoked population spike experiments; an approximate location of stimulation (stim.) and recording (rec.) sites marked by black dots. (B2) Population spikes evoked in stratum pyramidale by stimulation of st. oriens (1) and st. radiatum (2). Traces illustrate changes in evoked response upon increase in stimulation current-pulse intensity (1 and 2 from top to bottom).

Earlier work in rats showed differential sensitivity of excitatory synaptic transmission at CA1 basal and apical inputs to cholinergic modulation *in vivo* (Buzsaki et al., 1981; Leung, 1980; Rovira et al., 1982). More recently, it has been reported that ACh regulates HFS-induced synaptic plasticity in CA1 basal synapses via activation of muscarinic receptors (mAChRs) (Leung et al., 2003). To examine whether the endogenous cholinergic drive via a mAChR mechanism also