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Enhancement of the synchronized firing of CA1 pyramidal cells by medial septum preconditioning: Time-dependent involvement of muscarinic cholinoceptors and $GABA_B$ receptors

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Abstract

Activation of the medial septum (MS) enhances the synchronized firing of pyramidal cells in the hippocampus. At least two distinct mechanisms might be recruited in this process: GABAergic inhibition of hippocampal inhibitory interneurons and cholinergic enhancement of pyramidal cell excitability. In the present study, a field potential recording in urethane anaesthetised rats was used to show that MS preconditioning with brief high-frequency burst stimulation markedly enhanced Schaffer collateral/commissural (SC/C) synaptically evoked population spikes in the CA1 area without significant alteration in the initial slope of SC/C field excitatory postsynaptic potentials (fEPSPs). An early phase (<∼125 ms) of the facilitation of neuronal discharge was inhibited by intracerebroventricular (i.c.v.) injection of the $GABA_B$ receptor antagonist CGP 52432 (200 nmol/5 μl). In contrast, the muscarinic cholinoceptor antagonist scopolamine (20 nmol/5 μl) inhibited a later phase (∼150–300 ms) of the population spike facilitation. These findings support an important role for both cholinergic and GABAergic mediated septal drive in the tuning of signal conversion within the hippocampus as well as in securing the cortical feedback from the hippocampus.

Keywords: Medial septum; $GABA_B$; Acetylcholine; Hippocampus; In vivo; Disinhibition; Excitability

Activation of the medial septum (MS) facilitates the population discharges of hippocampal principal cells. Both cholinergic and GABAergic mechanisms [4,18] have been invoked to explain this effect, although in vivo evidence that clearly distinguishes between them is lacking. Krnjevic et al. [16,18] demonstrated that the cholinergic component of the septo-hippocampal pathway has a disinhibitory effect on fimbrial/commissural response, which might be explained by the presynaptic cholinergic inhibition of GABA release in the CA1 area. This forecast was supported by combined immunocytochemical and electron microscopy studies, which indicate that presynaptic muscarinic receptors are present on many GABA-containing terminals in area CA1 (e.g. [25]). By reducing GABA release, muscarinic heteroreceptors in this location might mediate the sepal disinhibition of the pyramidal neurons. More recently, Toth et al. [30] using combined septo-hippocampal slices from adult rats pharmacologically identified the inhibitory postsynaptic currents (IPSCs) in GABAergic interneurons in the area CA3 conditioned by stimulation of septo-hippocampal afferents. This finding suggested a new mechanism of septal ascending modulation of neuronal activity in the hippocampus, through the postsynaptic inhibition of GABAergic interneurons, as predicted by anatomical studies (e.g. [10]).

Previous reports of MS priming of hippocampal firing in vivo examined changes in fimbrial/commissural-evoked responses [17,26]. The present study assessed the effect of septal preconditioning on the Schaffer collateral/commissural (SC/C) induced population discharge of CA1 pyramidal cells. It was found that MS preconditioning markedly facilitated the population discharge of CA1 pyramidal neurons evoked by the stimulation of the SC/C pathway in the stratum radiatum without significant changes in the fEPSP initial slope. Similar to the facilitation of fimbrial/commissural responses [17,26], the enhancement of the SC/C population spike amplitude (PSA) was significantly inhibited by scopolamine. In addition, the selective $GABA_B$ receptor antagonist CGP 52432 also reduced the MS facilitation of SC/C PSA. Analysis of the time profile

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of MS-conditioned enhancement of synchronized discharge showed that whilst scopolamine greatly depressed the later phase, the effect of CGP 52432 was more evident in the early, rapid-onset phase of septo-hippocampal facilitation.

Experiments were performed on adult male Wistar rats weighing 250–350 g (Bio Resources Unit, Trinity College, Dublin) under license from the Department of Health and Children, Ireland. The method of implantation of the stimulation electrodes in the medial septum and in the stratum radiatum of the CA1 region under urethane (1.5 g/kg, i.p.) anaesthesia was similar to that described previously [24]. A recording electrode was lowered into the stratum pyramidale (P2.8–L3.4) to register the fEPSP/population spikes in response to test pulse SC/C stimulation. Body temperature was maintained at 37 °C. After the recording session, a direct current (15 mA, 30–45 s duration) was injected through the electrodes and their location was subsequently verified in 300 μm sections of the brain. A schematic diagram in Fig. 1A illustrates the approximate sites of the stimulating and recording electrodes within the septo-hippocampal area. The intensity of the SC/C test pulse stimulus was adjusted to evoke either a fEPSP (at half the threshold for a population spike generation), or a population spike about one-third of maximum in amplitude. The fEPSP initial slope and populations spike amplitude were measured before and after septal brief conditioning by a high-frequency train (seven pulses at 0.2 ms duration and intensity of 6–8 mA, which corresponded to twice the threshold to evoke a detectable septal slow field potential in stratum pyramidale) at a frequency of 150 Hz (inter-impulse interval ∼7.5 ms). PSA was quantified as 1/2 of the sum of the amplitudes of the falling and rising phases of the spike. The interval between the first pulse of the MS preconditioning barrage and test pulse stimulus delivered to the SC/C pathway ("post-MS preconditioning interval") was varied between 50 and 500 ms. In experiments involving the local administration of saline (5 μl), CGP 52432 (200 nmol/5 μl) [6] and scopolamine (20 nmol/5 μl) [5] a custom-made cannula was lowered into the lateral ventricle (P0.8–L1.4) and fixed by dental cement on the skull. The infusion of drugs was performed slowly over a 2-min period. The changes in the PSA and fEPSPs initial slope were expressed as the percentage of stable baseline recording for at least 10 min (±S.E.M.). Signals were filtered above 3 kHz and digitized at sampling rate of 20 kHz. Student's paired and unpaired *t*-tests were used for statistical analysis and *P*-values <0.05 were considered as statistically significant.

Short high-frequency MS preconditioning facilitated the synchronized discharge of pyramidal cells evoked by SC/C stimulation in a manner similar to the well-documented septal enhancement of the fimbrial/commissural responses in the CA1 area [17]. The enhancement of PSA was most evident at the near threshold range of the SC/C test pulse stimulation although a clear increase of PSA was also observed at the higher intensity. The facilitation of SC/C PSA was regularly accompanied by a slow up to 100 ms positive waveform generation in the CA1 stratum pyramidale in response to the septal high-frequency train, which was often followed by the rebound in opposite polarity. This slow septal response was relatively consistent from site to site and in general did not exceed 0.5 mV in amplitude. Fig. 1B shows representative traces of PS/fEPSPs recording in the CA1 stratum pyramidale without and with septal preconditioning barrage. At a stimulation intensity that evoked an approximately 30% maximum SC/C PSA, the population spike was markedly enhanced by MS barrage preconditioning. The extent of PSA enhancement depended on the time-interval between the septal preconditioning train and the SC/C shock, with the highest facilitation at intervals of approximately 75–120 ms (∼169 ± 6% of maximum enhancement at 105 ms post-MS preconditioning interval, $n = 6$, $P < 0.05$ paired *t*-test, compared to the unconditioned SC/C population spike), which gradually diminished to a quarter of its peak facilitation by 300 ± 12 ms and with only residual facilitation persisting at 400 ms ($P > 0.05$ paired *t*-test, compared to the unconditioned SC/C population spike) (Fig. 1C). Despite the fact that the facilitation of the synchronized discharge of pyramidal cells in the CA1 area was optimal in location where septal burst conditioning evoked the finest slow waveform, there was no apparent correlation between the septal waveform amplitude and its potency to facilitate the population discharge of CA1 pyramidal cells. Indeed, robust facilitation of the SC/C PSA was observed occasionally after single-pulse MS preconditioning, the intensity far below that of the threshold for generation of a noticeable septal slow field potential in the CA1 area. Remarkably, there was no detectable effect of MS burst preconditioning on the initial slope of the SC/C fEPSPs measured in the somatic layer of the CA1 area (e.g. 98 ± 4% and 101 ± 2% at 75 and 200 ms post-MS preconditioning, $n = 6$, $P > 0.05$ paired *t*-test, compared to the unconditioned SC/C fEPSPs; Fig. 1D).

Next, the involvement of muscarinic cholinoceptor and $GABA_B$ receptor in the septal facilitation of pyramidal cell population discharge in area CA1 was tested. Both types of receptors are expressed abundantly in the CA1 area [2,14] and are involved in modulation of neuronal excitability in this location [7,23]. Previously, MS preconditioning was found to facilitate the fimbrial/commissural population spike in the CA1 area in vivo in a scopolamine-sensitive manner, although even at high doses, scopolamine did not fully block the septal facilitation [17]. More recently, single-pulse conditioning stimulation of septo-hippocampal fibres in combined slices in vitro has been reported to occasionally induce a slow $GABA_B$-mediated IPSC within the inhibitory interneurons in the hippocampal CA3 area [30]. Along with anatomical studies exposing targeted innervations of the hippocampal inhibitory interneurons by GABAergic afferents arising from MS [10,13] inhibition of hippocampal GABAergic cells conditioned by the stimulation of ascending septal afferents [30] provides a rational for the scopolamine-resistant facilitation of synchronized firing of CA1 pyramidal cells [17]. To test the possible contribution of $GABA_B$ and muscarinic cholinoceptors in septal facilitation of synchronized firing of CA1 pyramidal neurons driven by SC/C input stimulation, the effect of MS preconditioning on SC/C population discharge was measured under control conditions and after the local (i.c.v.) administration of the $GABA_B$ receptor antagonist CGP 52432 (200 nmol/5 μl), and the non-selective muscarinic cholinoceptor antagonist scopolamine (20 nmol/5 μl). Fig. 2 summarizes the time profile measurements of SC/C PS facili-