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Cholinergic/GABAergic interaction in the production of EEG theta oscillations in rat hippocampal formation *in vitro*

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Abstract. The generation of EEG theta rhythm (RSA) in the hippocampal formation is a prime example of rhythmic activity involving central mechanisms of oscillation and synchrony. Cholinergic nature of the *in vitro* and *in vivo* induced RSA has been undoubtedly established. Recently, we have demonstrated *in vitro* that the hippocampal formation theta rhythm resulted from interaction between the cholinergic and GABAergic systems. In the present study we have provided additional *in vitro* evidence that the hippocampal GABA-A receptors are actively involved in the mechanism of theta production. Specifically, we demonstrated that bicuculline - GABA-A antagonist significantly augmented carbachol induced theta response increasing amplitude and power of rhythmical slow waves. In separate experiments the carbachol+bicuculline induced RSA were studied in the presence of muscarinic M1 and M2 antagonists - pirenzepine and gallamine (respectively) and GABA-A agonist - muscimol. Both pirenzepine and muscimol antagonized induced theta oscillations and gallamine was found to be completely ineffective in blocking this EEG response. The results provided evidence for M1 cholinergic/GABA-Aergic interaction in mechanisms responsible for theta production.

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INTRODUCTION

Theta rhythm (RSA) dominates in the EEG activity recorded from the hippocampal formation of mammals (Bland 1986, Bland and Colom 1993). It consists of the rhythmical sinusoidal slow waves of large amplitude (1.0-1.5 mV in rodents). Research utilizing hippocampal slice preparations maintained *in vitro* has shown that bath perfusion of the slices with carbachol, acetylcholine or eserine resulted in the production of theta oscillations (Konopacki et al. 1987a,b, 1988, 1992). This cholinergically induced RSA pharmacologically and physiologically closely resembles theta activity naturally occurring in the *in vivo* conditions (Bland 1986, Smythe et al. 1992, Bland and Colom 1993). A detailed study provided evidence for muscarinic receptors to be actively involved in the mechanism responsible for generation of cholinergic *in vitro* theta rhythm (Konopacki et al. 1988, 1992).

The medial septum and vertical limb of diagonal band of Broca (MS/vDBB) appear to be extremely important for the generation of theta rhythm. As it was first proposed by Petsche et al. (1962) and supported by more recent data (Stewart and Fox 1990) the neurons of the MS/vDBB act as a pacemaker for the hippocampal RSA. A confirmation for this view derives from the facts that electrolitic (Green and Arduini 1954, Rawlins et al. 1979, Sainsbury and Bland 1981) or reversible septal lesions induced by local anesthetic - procaine (Colom et al. 1991, Smythe et al. 1992, Lawson and Bland 1993) abolish theta in the hippocampal formation. Furthermore, electrical or chemical (carbachol, CCH) stimulation of the medial septum can produce hippocampal slow wave oscillations in the hippocampal formation ((Stumpf 1965, Monmaur and Breton 1991, 1993, Lawson and Bland 1993). Neuroanatomical studies have demonstrated the existence of a projection from the medial septal area to the hippocampus *via* fibres travelling in the fimbria and fornix (Raisman 1966, Meibach and Sigel 1977). While the cholinergic nature of the septo-hippocampal pathway (aproximately 50% of fibres) has been well documented (Lewis and Shute 1967, Baisden et al. 1984, Amaral and Kurz 1985, Wainer et al. 1985) the GABAergic component has only recently attracted attention. In rats the non-cholinergic component of the septo-hippocampal projection (about 30% of fibres) was shown to be GABAergic (Köhler et al. 1984, Amaral and Kurz 1985). This projection innervates mostly GABAergic interneurones in the hippocampal formation (Freund and Antal 1988, Gulyas et al. 1991). These GABAergic interneurones have been postulated to mediate feed--forward and feed-back inhibition of the hippocampal formation principal neurons (Andersen et al. 1980, Buzsaki 1984). This inhibition is an essential point of the hypothesis concerning the generation of the hippocampal theta rhythm. According to this hypothesis, theta oscillations result from dynamic balance between two neuronal systems - the cholinergic and GABAergic (Colom et al. 1991, Smythe et al. 1992). The cholinergic projection provides the afferent excitatory drive to the hippocampal theta cells and GABAergic projection acts to reduce the overall level of inhibition by inhibiting the activity of hippocampal GABAergic interneurones. Both mechanisms are essential for the generation of the hippocampal theta field potentials: earlier experiments demonstrated that only the combination of CCH and GABA-A antagonist bicuculline (BICU) produced trains of theta oscillations in the septally deafferented hippocampus. Intrahippocampal microinfusions of carbachol or bicuculline alone failed to evoke any hippocampal slow wave oscillations when MS/vDBB was reversible suppressed by procaine (Colom et al. 1991, Smythe et al. 1992).

The cholinergic/GABAergic interactions were also addressed in our recent *in vitro* study (Konopacki and Gołębiewski 1993). We demonstrated that bicuculline facilitated the effect of subtreshold concentration of carbachol in inducing *in vitro* RSA. This carbachol/bicuculline induced theta was supressed by muscarinic blocker - atropine sulphate and a GABA-A agonist - muscimol.

In the present study we have extended our preliminary observations. We have provided evidence for the hippocampal M1 cholinergic and GABA-A-ergic receptors to be actively involved in the

mechanism responsible for the generation of the *in vitro* hippocampal theta oscillations.

METHODS

Forty two hippocampal formation slices prepared from 38 male Wistar rats (100-150 g) were used in the present study. The slices were prepared and maintained in a gas-liquid interface as previously described (Konopacki and Gołębiewski 1993). Oxygenated and prewarmed artificial cerebrospinal fluid (CSF: composition in the mM see ref. Konopacki et al. 1987 a) with or without drugs was continuously perfused through the bath chamber at the rate of 1.5 ml min^{-1}. Concentrations of 50 μM carbachol (previously determined as threshold, Konopacki et al. 1992) and carbachol with bicuculline (CCH+BICU, 50μM+50μM) were used to induce theta activity. Muscarinic antagonists: M1 - pirenzepine (PIR, 10 μM), M2 - gallamine (GAL, 50μM) and GABA-A agonist - muscimol (MUSCI, 50μM) were used to determine subtype of receptors actively involved in the mechanism responsible for production of cholinergic/GABAergic theta *in vitro*. All the agents were obtained from Sigma Chemical Corp. The drug solutions were made fresh prior to each experiment using artificial CSF. The glass recording electrode (5-10 MΩ, 2.0 M NaCl) was typically positioned in the stratum moleculare of the dentate gyrus of the hippocampal formation. The recorded signals were filtered (0.001-10.0 kHz, band pass) and amplified (x 1,000) using Grass Instrument P-15 preamplifier operating in the differential mode. EEG activity were simultaneously displayed with the use of a digital oscilloscope (Kikusui 7061, Japan) and stored on FM tape recorder (Vetter Co. Model 420) for subsequent off-line computer analysis. The trains of hippocampal theta activity were led into Spike 2 software (Cambridge Electronic Design Ltd, England) for spectral EEG analysis (FFT).

RESULTS

Earlier experiments revealed that in the hippocampal slice preparations 50 μM of carbachol was capable of evoking trains of rhytmical slow wave oscillations (Konopacki et al. 1987a,b, 1988). In the present study we have supported this observation: 50 μM of carbachol always induced repeateable trains of theta activity. Just in the same slices (after 10-20 min washout with artificial CSF when CCH induced theta activity was no longer apparent) slow wave oscillations were evoked by perfusion of carbachol and bicuculline. Carbachol and carbachol+bicuculline induced trains of theta were usually recorded after 10-15 min of perfusion and were completely eliminated after 15-20 min washout with artificial CSF (Fig. 1, upper panel). The majority of this oscillations were near sinusoidal in shape. In contrast to CCH induced theta activity, CCH+BICU evoked trains of theta were stationary (i.e. did not vary in frequency and amplitude over the train duration). However, in some experiments they started with a higher frequency and lower amplitude and then declined to a lower frequency and higher amplitude. The histograms of power frequency analysis of the theta trains are shown in Fig. 1. (bottom panel). The mean amplitude and power (±SE) of the CCH induced theta trains were 474.3±30.0 μV and 2718.7±218.0 μV^2 (respectively, Table I). The same parameters for theta oscillations evoked by perfusion of CCH+BICU significantly ($P<0.05$) increased to 891.5±74.0 μV and 3929.2±326.1 μV^2, respectively. The frequency of theta induced by CCH+BICU vs. frequency of theta after CCH perfusion insignificantly decreased (7.8±0.5 Hz vs. 7.6±0.6 Hz). Some details concerning the comparison of the changes in frequency, amplitude and power of in CCH and CCH+BICU theta elicited were shown in Table I.

To determine whether CCH+BICU induced theta oscillations were mediated both by M1 and GABA-A receptors in separate experiments the evoked slow waves were studied in the presence of M1 muscarinic blocker - pirenzepine, M2 muscarinic blocker - gallamine and GABA-A agonist - muscimol. The results of this procedure are shown in Fig. 2 and Table I. Both pirenzepine and muscimol antagonized cholinergic/GABA-Aergic induced theta slow waves. Gallamine, in contrast, was

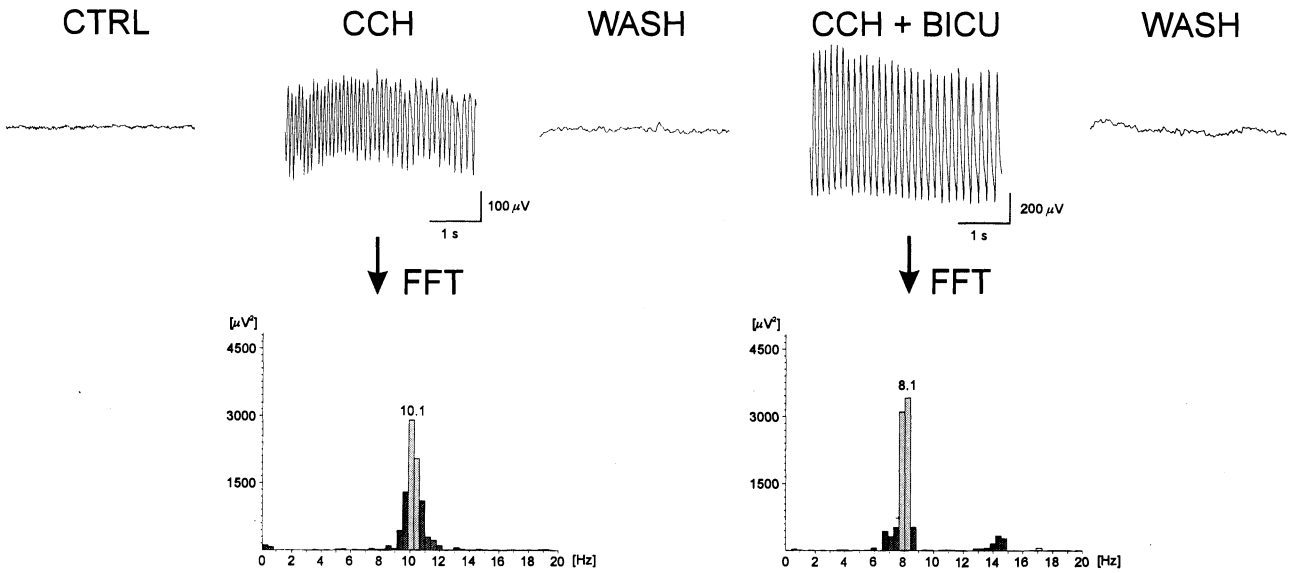

Fig. 1. Cholinergic/GABAergic interaction in generation of the theta oscillations recorded in the hippocampal formation slices. In control conditions (CTRL) hippocampal slices did not manifest any rhythmic activity. Theta oscillations occurred after perfusion of cholinergic agonist - carbachol (CCH, 50 μM) and carbachol with GABA-A antagonist - bicuculline (CCH, 50 μM + BICU, 50 μM). Slow wave activities were usually reversible after 20 min wash with artificial CSF (WASH). The bottom panel shows a power frequency analysis (FFT) of analogue recorded trains of theta oscillations (marked arrow). Please note that: after perfusion with CCH+BICU significant augmentation of EEG response was observed; the second harmonic peak of frequency in CCH+BICU induced EEG response was observed in 60% of the analysed samples.

TABLE I

Amplitude, frequency and power of theta oscillations induced by: carbachol (CCH) or carbachol and bicuculline (CCH+BICU) and the effect of M1, M2 muscarinic blockers: pirenzepine (PIR) and gallamine (GAL) and GABA-A agonist: muscimol (MUSCI)

	Theta rhythm			
Perfusion with	Amplitude (μV)	Frequency (Hz)	Power (μV^2)	n
CCH (50 μM)	474.4±30.0	7.8±0.5	2718.7±218.0	16
CCH+BICU (50+50 μM)	891.5±74.0*	7.6 ± 0.6^{NS}	3929.2±326.1*	13
CCH+BICU+PIR (50+50+10 μM)	–	–	–	13
CCH+BICU+GAL (50+50+50 μM)	867.7±85.6*	8.0 ± 0.5^{NS}	3843.8±404.1*	13
CCH+BICU+MUSCI (50+50+50 μM)	-	-	-	13

Data are presented as a mean ±SE; *Significant difference with carbachol $P<0.05$; NS, not significant; (One-way ANOVA and post hoc Student-Neuman-Keuls multiple range test); *n*, number of experiments. Please note that only pirenzepine and muscimol antagonized carbachol+bicuculline induced theta activity.

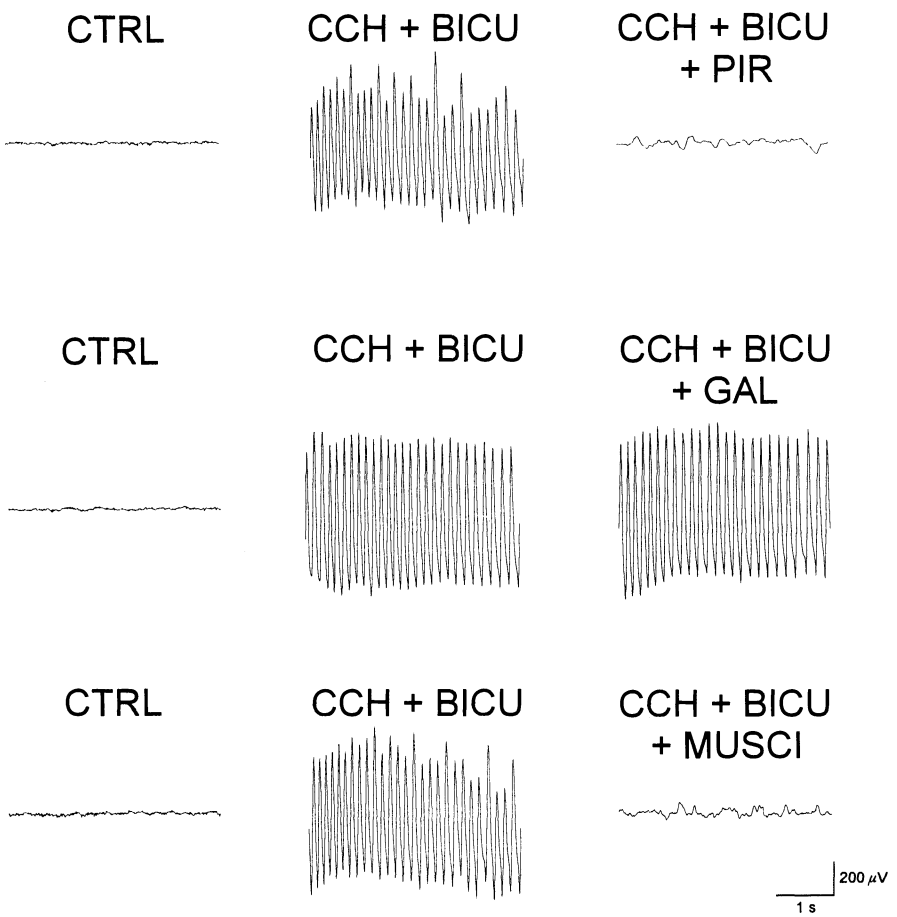

Fig. 2. The effect of muscarinic M1, M2 antagonists - pirenzepine (PIR) and gallamine (GAL) and GABA-A agonist - muscimol (MUSCI) on CCH+BICU induced theta oscillations. Please note that only pirenzepine (10 μM) and muscimol (50 μM) antagonized CCH+BICU (50 μM + 50 μM) induced theta waves. Gallamine (50 μM) was without any effect on induced slow wave oscillations.

without any substantial effect (Fig. 2). Mean amplitude (867.7±85.6 μV), power (3843.8±404.1 μV^2) and frequency (8.0±0.5 Hz) of the CCH+BICU+GAL induced EEG response insignificantly differed from that observed after perfusion of CCH+BICU (NS, Table I).

DISCUSSION

The main findings of the present study were: (1) Hippocamapal slice preparations perfused with 50 μM of carbachol and 50 μM of bicuculline manifested augmentation of induced EEG response. The amplitude and power of the induced theta significantly increased in comparison with the same parameters for CCH induced theta. (2) Perfusion of the rat hippocampal slices with M1 cholinergic blocker - pirenzepine and with GABA-A agonist - muscimol abolished theta rhythm induced by perfusion of CCH+BICU. (3) CCH+BICU induced theta rhythm was still present in the hippocampal slices perfused with M2 cholinergic blocker - gallamine.

These results provide additional evidence for cholinergic/GABAergic interaction in the production of the hippocampal RSA in the *in vitro* conditions. Recently, we have shown (Konopacki and Gołębiewski 1993) that bicuculline (25 μM) GABA-A antagonist facilitated the effect of subthreshold concentration of carbachol (25 μM) in inducing theta waves. Carbachol in subthreshold dose never induced theta oscillations (Konopacki and Gołębiewski 1993).

In the present study bicuculline blocking GABA-A receptors diminished the overall level of inhibition of the hippocampal neuronal network. This diminution of GABAergic inhibition together with threshold excitation of the cholinergic neurones by CCH (50 μM) produced significant augmentation of induced EEG response. The above mentioned *in vitro* study confirmed suggestion proposed by Colom et al. (1991) and Smythe et al. (1992) that appearance of *in vivo* hippocampal RSA requires coactivation of both cholinergic and GABAergic systems. The authors demonstrated that in rats with septally deafferented hippocampal formation only combined CCH+BICU microinfusions were capable of producing theta trains. In the present study CCH+BICU induced theta rhythms had amplitude and power twice as high as CCH evoked theta activity. It may suggest more hippocampal cells to be active during GABA-A induced disinhibition and/or higher synchrony of the cells discharges.

To determine the pharmacological profile of CCH+BICU induced theta we manipulated the hippocampal GABA-A receptors with specific agonist, muscimol and M1, M2 cholinergic receptors with two selective antagonist pirenzepine and gallamine (respectively). The CCH+BICU induced theta oscillations were completely abolished in the presence of muscimol and pirenzepine. Gallamine was ineffective in antagonizing the induced rhythmic oscillations. These findings are consisted with our earlier data obtained on the entorhinal cortex slice preparations (Gołębiewski et al. 1994) and strongly suggest M1/GABA-A mediation of induced theta activity. Further experiments with use of GABA-B blocker, 2-hydroxysaclofen, will allow to specify the involvement of GABA-B receptor in neuronal mechanism responsible for generation of theta in the *in vitro* conditions.

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