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Pharmacology, Biochemistry and Behavior 69 (2001) 165–172

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Effects of metrifonate on the hippocampal theta rhythm of freely moving intact and MS-lesioned mice

S. Ikonen, H. Tanila*

Department of Neuroscience and Neurology and the University Hospital, University of Kuopio, P.O. Box 1627, 70211 Kuopio, Finland

Received 6 November 2000; received in revised form 10 January 2001; accepted 6 February 2001

Abstract

Changes in hippocampal electroencephalogram (EEG) have been suggested to be closely associated with spatial learning ability. Spatial learning can be improved in medial septal (MS)-lesioned mice by metrifonate, a cholinesterase inhibitor. We designed this study to investigate the effects of metrifonate on the hippocampal theta oscillation of intact and MS-lesioned mice. Intact and MS-lesioned C57BL mice were treated with acute injections of metrifonate (doses: 15, 50 and 100 mg/kg ip). These included a dose that considerably improved spatial memory of MS-lesioned mice in our earlier study. In addition, subtype selective muscarinic agents, BIBN-99, AF267B and AF150(S) were used. Recordings of hippocampal theta during movement and awake immobility revealed a dramatic reduction of theta in the lesioned animals. Metrifonate induced prominent changes in the EEG of intact mice, but not of MS-lesioned mice. The effect of metrifonate was not mimicked by two selective M_1-agonists and was augmented by a combined injection of a selective M_2-antagonist. These data suggest that improved spatial learning by the cholinesterase inhibitor metrifonate is unrelated to its effects on the hippocampal EEG. These two effects may be mediated through different muscarinic receptor subtypes.

Keywords: Metrifonate; EEG; Theta; Septum; Lesion; Muscarinic

1. Introduction

Hippocampal theta rhythm is a regular electroencephalographic 4–12 Hz oscillation in the hippocampus and related structures. The medial septum (MS) has been considered the pacemaker of hippocampal theta since the discovery of rhythmically bursting cells in the MS of rabbits (Petsche et al., 1962). The original observation has been confirmed by several studies showing that mechanical and excitotoxic lesions of the septohippocampal axis abolish theta in the hippocampus (Andersen et al., 1979; Leung et al., 1994; Winson, 1978). The role of cholinergic muscarinic receptors in the regulation of theta is also well established. Atropine, a nonselective muscarinic antagonist, attenuates theta (Kramis et al., 1975). Conversely, cholinergic muscarinic agonists can induce theta when injected into the septum (Monmaur and Breton, 1991) and even when applied on a hippocampal slice preparation (Konopacki et al., 1987).

The theta rhythm has been implicated in spatial learning as septal lesions that abolish the theta rhythm also impair maze learning (Winson, 1978). Cholinergically induced theta-frequency oscillations have been shown to promote synaptic plasticity in hippocampal slices (Huerta and Lisman, 1993). Furthermore, changes in theta rhythm as a result of memory processing have been recorded in rats (Givens, 1996; Givens and Olton, 1995) and, recently, also in humans (Tesche and Karhu, 2000). Moreover, memory enhancement in aged animals by cholinergic agonists has been linked to changes in theta rhythm (Markowska et al., 1995). We have reported earlier that cholinesterase inhibitor metrifonate reverses spatial learning deficit in MS-lesioned mice but not in mice with a lesion in the dorsal hippocampus (Ikonen et al., 1999), which suggests that the site of action of metrifonate is the dorsal hippocampus. Noticing that electrolytic MS lesions that dramatically impaired water maze learning in our earlier study never encompassed all cholinergic cell groups in the MS and the diagonal band, we assume that metrifonate acts on this mouse model by boosting the remaining septohippocampal cholinergic projections. A recently reported study on anaesthetized rats suggested that modulation of hippocampal theta may be a

* Corresponding author. Tel.: +358-17-162014; fax: +358-17-162048.
E-mail address: heikki.tanila@uku.fi (H. Tanila).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00495-6

necessary action of cognition enhancers (Kinney et al., 1999). Therefore, a rational hypothesis to be tested is that metrifonate would improve spatial learning of MS-lesioned mice by restoring the hippocampal theta rhythm. The objectives of this study were (a) to study the effects of metrifonate on hippocampal theta of freely moving intact mice, (b) to study whether metrifonate at doses that reversed spatial learning impairment after MS lesion reinstates theta in these animals and (c) to assess the role of different cholinergic receptors in mediating the actions of metrifonate. Our approach was to record hippocampal electroencephalogram (EEG) from both intact and MS-lesioned mice under the effect of metrifonate and other selective and nonselective cholinergic agents.

2. Materials and methods

2.1. Animals, surgery and implantation technique

These experiments were conducted on 25 young (4–5 months) female C57BL/6J//Kuo mice. The mice were housed individually after the surgery. The environmental conditions were controlled and constant (21 ± 1°C, humidity at 50 ± 10%, light period 0700–1900 h). Food and water were freely available. The study plan was approved by the Department of Social and Health Affairs of the Provincial Government of Eastern Finland.

MS (A: 0.8 mm, M: 0.0 mm, D: −4.0 mm; relative to the bregma) lesions were made by passage of an anodal DC current (1 mA, 15 s) via tungsten electrodes (diameter 62.5 μm, 1.0 mm tip uninsulated). Sham-lesioned mice were treated identically, but no current was applied. The lesioning technique was exactly the same as in our earlier studies (Ikonen et al., 1999). Hippocampal electrodes (100 μm in diameter, stainless steel) were implanted to the left hemisphere at A: −2.2 mm (from bregma), M: 1.5 mm (from the midline) and D: −1.5 mm (from the dura). The longer electrode was aimed at the hippocampal fissure and the shorter at the CA1 stratum oriens (electrode tip separation 500 μm). One stainless steel screw on the frontal bone served as a ground electrode. The implant was fixed to the skull with dental cement and three anchor screws. The mice were deeply anaesthetized with a 1:1 mixture of Dormicum (Roche, Espoo, Finland) and Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) (subcutaneously) and for analgesia received 0.1 mg/kg of buprenorfine (Temgesic; Reckitt and Colman, Hull, England) (subcutaneously) after the surgery. The mice were allowed to recover for 2 weeks before the start of the first experiments.

2.2. Drugs

Metrifonate (a donation from Bayer, Wuppertal, Germany) was dissolved in 5% sodium citrate (pH 5.5) and injected intraperitoneally (ip) at 0, 15, 50 and 100 mg/kg 30 min before the recordings. The acetylcholinesterase inhibition in mouse hippocampus induced by a single dose of metrifonate 50 mg/kg ip is 40%, and with 100 mg/kg ip it is 71% (our unpublished results). Atropine (Leiras, Turku, Finland), a nonselective muscarinic antagonist, was injected intraperitoneally at 25 mg/kg (20 ml/kg). BIBN-99 (a donation from Dr. Karl Thomas, Biberach/Riss, Germany), a selective M_2-antagonist, was dissolved in 0.9% NaCl and injected subcutaneously (sc) at 0.5 mg/kg. AF267B (a donation from Dr. A. Fisher, Ness-Ziona, Israel), a selective M_1-agonist, was dissolved in H_2O and administered per os at 1, 5 and 25 mg/kg. AF150(S) (a donation from Dr. A. Fisher), another selective M_1-agonist (Brandeis et al., 1995), was dissolved in PBS (pH 7.4) and administered per os at 5 mg/kg. All drug doses, solvents and routes of administration were selected on the basis of earlier pharmacological studies in vivo (Brandeis et al., 1995; Ikonen et al., 1999; Kramis et al., 1975; Quirion et al., 1995; A. Fisher, personal communication).

2.3. EEG recordings

EEG recordings during movement took place in a cylinder (diameter 70 cm, height 50 cm). The free movement of the mice was encouraged by novel objects. EEG was recorded during walking between the objects. EEG during awake immobility was recorded, while the mouse was placed on top of an upside-down bucket. Before the actual recordings, the mice were familiarized with the recording environment and the test situation by performing two recording sessions with vehicle injections.

A dual-channel JFET on the headstage acted as a source follower. The signal (1-s sweeps) was amplified 1000–4000 times, filtered (1–100 Hz) and digitized at 1 kHz. The data sampling and analysis was performed off-line by a commercial software (DataWave Technologies, Longmont, CO, USA). Four 1-s sweeps were combined and seven epochs of 4 s were sampled for FFT analysis.

Two separate experiments were performed. In Experiment 1, the intact ($n = 8$) and MS-lesioned ($n = 9$) mice were treated with metrifonate 15, 50 and 100 mg/kg or saline in a counterbalanced order. Each recording was followed by a wash-out period of 1–5 days. A control saline recording was performed before and after the drug study to ensure that the baseline EEG was reestablished after the treatments. After vehicle injections, the wash-out time was at least 1 day; after metrifonate 15 or 50 mg/kg it was at least 2 days and after metrifonate 100 mg/kg it was at least 5 days. The mice received the injections 30–40 min before the recordings. Recordings with a single administration of atropine (Leiras, 25 mg/kg ip, 30 min before the recording) were performed after the metrifonate recordings were completed. With the exception of atropine recordings, the experimenter was blind to the treatment of the mice.

In Experiment 2, another group of mice ($n = 8$) was used to examine more closely the mechanism of action of metrifonate by using various muscarinic agonists and antagonists. The drugs used were (1) metrifonate 100 mg/kg, (2) a combination of atropine (25 mg/kg, 60 min before the recording) and metrifonate (100 mg/kg, 30 min before the recording), (3) a combination of BIBN-99 (0.5 mg/kg, 60 min before the recording) and metrifonate (100 mg/kg, 30 min before the recording), (4) AF267B (1, 5 and 25 mg/kg, 30 min before the recording) and (5) AF150(S) (5 mg/kg, 30 min before the recording). Before each drug recording, the reestablishment of the baseline was determined by performing a vehicle recording.

2.4. Statistical analysis

A Fast Fourier Transformation was performed over 0.1–50 Hz with a resolution of 0.5 Hz. The power of frequency bands of interest was divided by the total power over 0.1–50 Hz. The parameters analyzed for movement-related theta were relative power in the 4–12 Hz theta band, maximum power within 5–11 Hz band and frequency at maximum power. Because theta during awake immobility occurs only in brief bouts and no distinct theta peak is present in the frequency spectrum, drug-induced frequency shifts during awake immobility were determined only indirectly by dividing the theta band into lower 4–7.5 Hz and upper 8.5–12 Hz bands and analyzing the relative powers separately. In addition, the upper/lower theta band ratio, as well as the relative power in the total 4–12 Hz theta band, was analyzed.

In Experiment 1, the baseline differences between the groups were analyzed by comparing three vehicle recordings using the analysis of variance (ANOVA) for repeated measurements as a test of between-subjects effects. The effects of metrifonate was assessed by analyzing one saline recording and the recordings with different drug doses with the analysis of variance (ANOVA) for repeated measurements as a test of within-subjects effects. In Experiment 2, the effects of metrifonate, atropine, BIBN-99, AF267B and AF150(S) were analyzed by comparing a drug recording with the preceding vehicle recording with a paired-samples t test. To compare the drug effects despite variations in the baseline across days, an index value was calculated that shows the relative change in the EEG when compared to the baseline ((value after drug administration – baseline value)/baseline value). The index values were compared with a paired-samples t test.

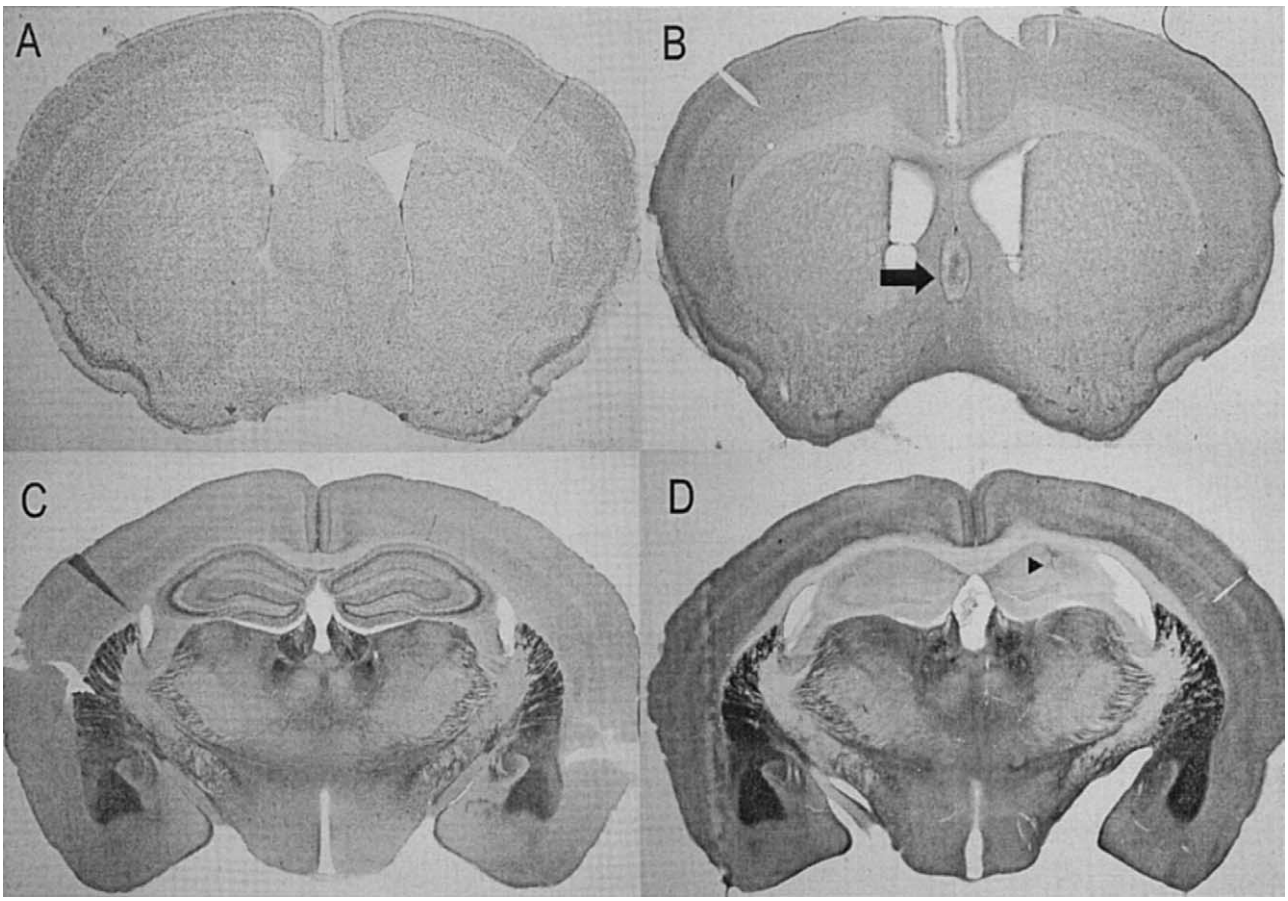

Fig. 1. A Nissl staining of the septal area of a control mouse (A) and MS-lesioned mouse (B). AChE stainings of a control mouse (C) and MS-lesioned mouse (D). In (D), the electrode mark has been indicated by a filled triangle. The EEG spectra of the same mice can be seen in Fig. 2.

2.5. Histological methods

After the recordings, the mice were deeply anaesthetized. To mark the tip location with iron deposits, an anodal current of 30 μA (3 s) was passed through the electrodes. The mice were decapitated, the brains were removed and immersed for 1–2 days in 4% formaldehyde solution. Vibratome sections of 50 μm were cut and the sections encompassing the MS area were stained with cresyl fast violet to confirm the location of the lesion. The iron deposits around the electrode tips were visualized with the Prussian Blue reaction. Some of the remaining hippocampal sections were processed with acetylcholinesterase staining in order to confirm the decrease of acetylcholine-containing fibers in the hippocampus.

3. Results

3.1. Histology

All lesioned mice accepted to this study had lesions that encompassed the MS. The lesion resulted in striking loss of AChE staining in the hippocampus (Fig. 1). The histology confirmed the locations of the longer hippocampal electrodes between deep stratum radiatum and hippocampal fissure. The fact that the electrode pairs straddled the CA1 pyramidal cell layer was also observed by the characteristic phase shift (about 180°) between the long and the short hippocampal electrode (Brankack et al., 1993).

3.2. Control mice and the effects of the lesion

3.2.1. Movement

The EEG of the control mice during movement was characterized by a sharp theta peak of 8.5 ± 0.13 Hz (mean ± S.E.M.) in frequency, which was nearly abolished by the lesion (Figs. 2 and 3). The lesioned animals had a dramatically reduced total theta power (4–12 Hz) (controls: 69.1 ± 3.5, lesioned: 45.2 ± 3.7 [percentage of total power, mean ± S.E.M.]; $F_{1,15} = 21.538$, $P < .001$) and maximum power (controls: 44.8 ± 3.2, lesioned: 20.8 ± 3.5; $F_{1,15} = 25.210$, $P < .001$). Lesioning also shifted the frequency of the maximum power from 8.5 ± 0.13 to 7.0 ± 0.33 Hz ($F_{1,15} = 10.920$, $P < .01$) (Figs. 2 and 3).

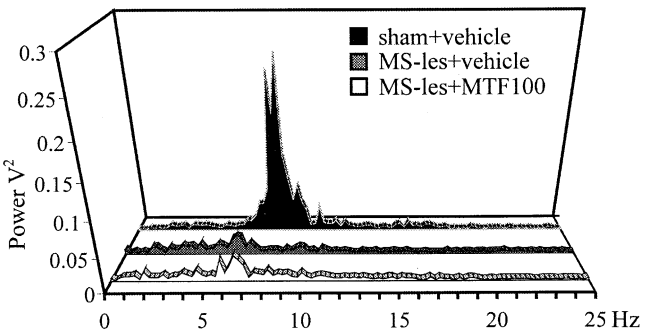

Fig. 2. Examples of hippocampal EEG during movement. EEG power spectra at frequency band 0.1–25 Hz of a control mouse (black) and a MS-lesioned mouse treated with saline (grey) or metrifonate 100 mg/kg (white). Histological stainings of the same mice can be seen in Fig. 1. MTF100 = metrifonate 100 mg/kg.

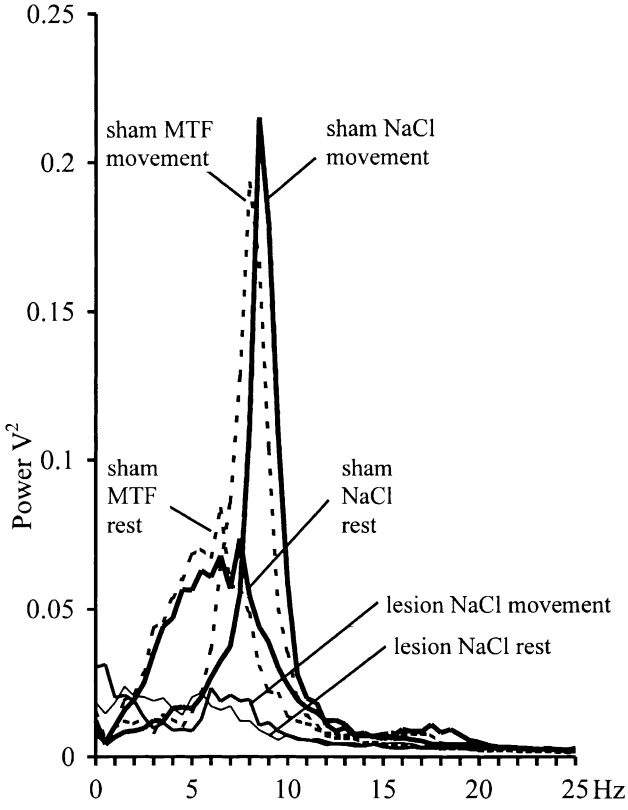

Fig. 3. Average frequency spectra of hippocampal EEG during movement and awake immobility (rest) in sham- and MS-lesioned mice. The solid lines represent spectra of vehicle recordings and the stippled lines represent spectra of recordings with metrifonate 100 mg/kg. MTF = metrifonate 100 mg/kg.

3.2.2. Awake immobility

The analysis of EEG of the control mice during awake immobility showed a peak at 6.4 ± 0.26 Hz, which was much wider and less prominent than the one seen during movement (Fig. 3). The lesion dramatically reduced the total theta power (4–12 Hz) (controls: 63.0 ± 1.1, lesioned: 43.8 ± 2.8; $F_{1,15} = 38.345$, $P < .001$). The analysis of the lower theta band (4–7.5 Hz) revealed that during awake immobility, the theta power of the control animals is mainly concentrated on this band, and the lesioning markedly reduced its power (controls: 37.2 ± 0.6, lesioned: 26.0 ± 1.5; $F_{1,15} = 44.536$, $P < .001$). All animals had only a little power left in the upper theta band (8.5–12 Hz), but still a diminution of the power in the lesioned animals was statistically significant (controls: 15.5 ± 0.6, lesioned: 12.2 ± 1.3; $F_{1,15} = 4.783$, $P < .05$). However, the ratio of the theta bands 8.5–12 and 4–7.5 Hz was unaffected by the

lesion (controls: 0.43 ± 0.02, lesioned: 0.48 ± 0.05; $F_{1,15} = 0.724$, $P > .05$).

3.3. Drug effects

3.3.1. Movement

The total theta power (4–12 Hz) and maximum theta power were unaffected by metrifonate both in the sham ($F_{3,21} < 0.590$, $P > .05$) and the lesion group ($F_{3,24} < 2.079$, $P > .05$). In the sham group, the frequency of the theta peak was shifted from 8.5 ± 0.13 (vehicle) to 7.3 ± 0.26 Hz (metrifonate 100 mg/kg) ($F_{3,21} = 3.865$, $P < .05$) (Fig. 3). In the lesioned animals, metrifonate did not shift the frequency of the maximum power ($F_{3,24} = 1.408$, $P > .05$). Atropine decreased the total and maximum theta power in the sham group ($T_7 < -1.940$, $P < .001$) and in the lesion group ($T_8 < -3.231$, $P < .05$), but it did not shift the frequency of the maximum power ($P > .05$).

3.3.2. Awake immobility

In Experiment 1, metrifonate increased the total theta power (4–12 Hz) of the sham group ($F_{3,21} = 3.103$, $P < .05$), but not of the lesion group ($F_{3,24} = 1.749$, $P > .05$). Analysis of the theta band 4–7.5 Hz revealed a significant drug by group interaction ($F_{3,45} = 3.388$, $P < .05$). Metrifonate dose-dependently increased the power in this lower theta band of the sham-lesioned animals ($F_{3,21} = 8.914$, $P < .001$), but it had no effect on the lesioned animals ($F_{3,24} = 0.684$, $P > .05$) (Fig. 4A). Drug by group interaction was also found for the theta band 8.5–12 Hz ($F_{3,45} = 5.671$, $P < .01$). Metrifonate decreased the power of upper band theta in the sham group ($F_{3,21} = 12.334$, $P < .001$), but again, it had no effect on the lesion group ($F_{3,24} = 1.211$, $P > .05$). The ratio of the theta bands 8.5–12 and 4–7.5 Hz was decreased by metrifonate in the sham group ($F_{3,21} = 5.617$, $P < .01$), while the lesion group remained unaffected ($F_{3,24} = 0.072$, $P > .05$). Also atropine had a differential effect on the groups as a significant Drug × Group interaction was found ($F_{1,14} = 16.690$, $P < .001$). The total theta power (4–12 Hz) was decreased by atropine in the sham group ($T_7 = -18.965$, $P < .001$), while the lesion group with little remaining theta power was unaffected ($T_7 = -1.594$, $P > .05$). Atropine markedly decreased the amount of theta in the 4–7.5 Hz band of the sham-lesioned mice ($T_7 = -8.283$, $P < .001$), but it had no effect on the lesioned group ($T_8 = 0.084$, $P > .05$). Atropine had no effects in the theta band 8.5–12 Hz ($P > .05$) in either group. As a result, the ratio of the higher and lower theta bands was increased by atropine in the sham group ($T_7 = 6.919$, $P < .001$), but not in the lesion group ($T_8 = -0.412$, $P > .05$).

In Experiment 2 (Fig. 4B), metrifonate was confirmed to increase the power of the theta band 4–7.5 Hz of intact animals ($T_7 = 3.572$, $P < .01$). Atropine injection 30 min

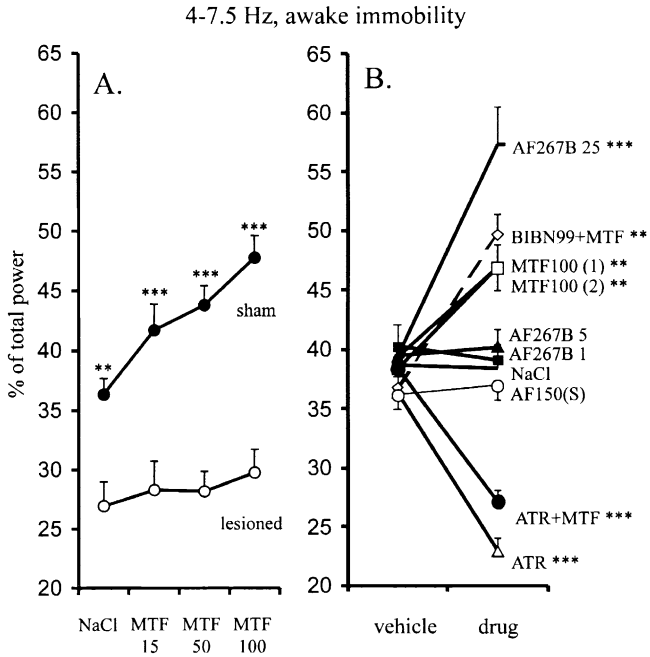

Fig. 4. Theta band 4–7.5 Hz during awake immobility. Part A (Experiment 1): metrifonate increases 4–7.5 Hz activity dose-dependently in intact mice, but not in MS-lesioned mice. Independent samples t test comparing the groups revealed the following significant differences: $^{**}P \leq .01$, $^{***}P \leq .001$. Part B (Experiment 2): the effects of several cholinergic agents on the theta band 4–7.5 Hz of intact mice. For comparison, two recordings (MTF100(1) and ATR) from Experiment 1 were added. Paired t tests comparing the drug recordings to the corresponding saline recordings revealed the following significant differences: $^{**}P \leq .01$, $^{***}P \leq .001$. ATR = atropine; MTF100(1) = metrifonate 100 mg/kg from Experiment 1; MTF100(2) = metrifonate 100 mg/kg from Experiment 2; AF267B 1 = 1 mg/kg, AF267B 5 = 5 mg/kg, AF267B 25 = 25 mg/kg. The values in the Y-axis represent percentage of the total power (0.1–50 Hz). Values are group means ± S.E.M.

before the metrifonate injection blocked the effect completely ($T_7 = -10.066$, $P < .001$). BIBN-99 injection 30 min before the metrifonate injection did not block the effect of metrifonate as the theta power was still increased ($T_7 = 4.930$, $P < .01$); on the contrary, it slightly potentiated the effect ($T_7 = 2.166$, $P = .067$). AF267B at the dose of 1 or 5 mg/kg had no effect ($T_7 > -0.679$, $P > .05$), but a massive dose (25 mg/kg) induced a similar effect in the EEG as metrifonate: the power in theta band 4–7.5 Hz was increased ($T_7 = 5.912$, $P < .001$). This effect was even stronger than the one achieved by metrifonate ($T_7 = 3.458$, $P < .05$). AF150(S) (5 mg/kg) induced no observable changes in the EEG ($T_7 = 0.567$, $P > .05$) (Fig. 4B). None of the drugs at the used doses induced observable adverse effects in the mice during any part of this study.

4. Discussion

We observed that metrifonate, a cholinesterase inhibitor that reversed the spatial memory deficit induced by MS-lesioning in our earlier study (Ikonen et al., 1999), increased the power of the putative cholinergic theta at the range 4–7.5 Hz of the dorsal hippocampus during awake immobility. This effect was absent in mice with a MS lesion. Therefore, it is highly unlikely that restoration of hippocampal theta is the mechanism by which metrifonate improved spatial memory in MS-lesioned mice. In additional experiments, we observed that the effect of metrifonate on hippocampal theta is not mimicked by selective muscarinic M_1-agonists nor is blocked by a selective M_2-antagonist.

The present study indicates that theta rhythm in mouse is not different from that previously found in rat (Sainsbury et al., 1987). During active locomotion, a sharp theta peak was observed around 8.5 Hz, and during awake immobility, a wider and less prominent theta peak was observed around 6.5 Hz. The awake immobility-related theta was eliminated by atropine, which indicates that it is mainly regulated by the cholinergic cells of the septum. The movement-related theta was only partly sensitive to atropine, which suggests that it is regulated also by the noncholinergic component of the septohippocampal axis. The present results also indicate that the effects of MS lesioning on hippocampal EEG are similar in mice as earlier reported in rats (Buzsaki et al., 1983; Kolb and Whishaw, 1977; Sainsbury and Bland, 1981). The electrolytic lesion of the MS, damaging both cholinergic and GABAergic cells, markedly attenuated the theta peak.

The effect of metrifonate on the hippocampal theta in the present study resembled that of physostigmine in rats (Lee et al., 1994): during awake immobility, metrifonate at the dose of 100 mg/kg significantly increased the theta power at the range of 4–7.5 Hz. This effect was shown to be mediated through muscarinic receptors, since it was completely blocked by atropine. A selective M_2-antagonist BIBN-99 at a behaviorally effective dose (Quirion et al., 1995) did not block the effect of metrifonate (rather, the effect was potentiated), which indicates that the effect is not mediated through M_2-receptors. This is consistent with earlier studies showing no effect of M_2-antagonist gallamine on cholinergic theta in the cat (Golebiewski et al., 1993). The potentiation can be explained by the fact that blocking the M_2 autoreceptors leads to an increase in the release of acetylcholine. Our data does not support the role of M_1 receptors as suggested earlier (Barnes and Roberts, 1991; Golebiewski et al., 1993) as M_1 agonists, AF150(S) and AF267B, had no effect on hippocampal theta. The doses used (1–5 mg/kg) were 2–10 fold larger than the doses that produce behavioral effects (Fisher et al., 1998; Ruske and White, 1999; A. Fisher, personal communication). With a considerably higher dose (25 mg/kg), AF267B produced an effect, which is identical to the one obtained with metrifonate. At this very large dose, AF267B looses its M_1 selectivity and may agonize also M_3 (or M_5) receptors (A. Fisher, personal communication), which are structurally closely related to M_1 receptors. Indeed, in a recent study, M_3 receptors have been suggested to mediate the muscarine-induced excitations in the septohippocampal neurons (Liu et al., 1998). Further studies with intrahippocampally injected muscarinic antagonists are needed to elucidate the role of muscarinic receptors in mediating the cholinergic theta in the mouse.

In earlier studies, the loss of hippocampal theta rhythm and spatial memory impairment have been reported to be closely coupled in rats with electrolytic lesions of the MS or fimbria–fornix (M'Harzi and Jarrard, 1992; Winson, 1978). Furthermore, basal forebrain grafts that restore normal AChE staining in the fimbria–fornix-lesioned rats (Bjorklund et al., 1983) and reduce the spatial memory impairment (Dunnett et al., 1982), also partially restore behavior-dependent theta rhythm (Buzsaki et al., 1987; Tuszynski et al., 1990). It has also been shown that various cholinergic agonists and cholinesterase inhibitors produce theta in hippocampal slices (Konopacki et al., 1987), and in vivo when administered systemically (Lee et al., 1994; Valjakka et al., 1991) or microinfused into the hippocampus (Rowntree and Bland, 1986). Collectively, this evidence suggested that the mechanism of the cognition-enhancing effect of metrifonate could be restoration of the hippocampal theta. However, metrifonate failed to have any observable effect on the theta rhythm of MS-lesioned mice. Indeed, this study together with our earlier study (Ikonen et al., 1999) shows a double dissociation between EEG changes and cognitive processes: in intact mice, metrifonate induced EEG changes, but had no cognitive effects, and in MS-lesioned mice, metrifonate reversed the cognitive impairment, but induced no EEG changes. Furthermore, doses of M_1 agonists that improve performance in several cognitive tasks (Fisher et al., 1998; Ruske and White, 1999) did not modulate hippocampal theta in intact animals. Collectively, these findings strongly suggest that modulation of theta is not necessary for cognition enhancing effects of metrifonate.

There are a number of mechanisms by which the metrifonate-induced increase of cholinergic effects can enhance hippocampal plasticity that are not necessarily linked to theta rhythm. Acetylcholine enhances long-term potentiation even without eliciting rhythmic activity (Auerbach and Segal, 1994; Brocher et al., 1992). It mobilizes intracellular calcium by activating the phosphoinositol pathway and augments calcium influx by modulating the voltage gated calcium channels (Felder, 1995). In addition, acetylcholine reduces after-hyperpolarisation (AHP) by affecting calcium-dependent potassium current (Cole and Nicoll, 1983). Reduction of the AHP increases spiking of hippocampal neurons and enhances LTP induction (Behnisch and Reymann, 1998; Norris et al., 1998). It is possible that much lower increase in acetylcholine release is needed for these effects in the dorsal hippocampus after MS lesion than for restoration of the theta rhythm.

Acknowledgments

We thank BSc Tiina Lehto and BSc Rimante Bandzaityte for assistance with the recordings. Iain Wilson is acknowledged for revising the language of the manuscript. This study was supported by the Academy of Finland and the Finnish Cultural Foundation of Northern Savo.

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