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Electroencephalographic effects induced by choline pivaloyl esters in scopolamine-treated or nucleus basalis magnocellularis lesioned rats

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Abstract

The electroencephalographic (EEG) effects of two choline pivaloyl esters, [2-(2,2-dimethylpropionyloxy)ethyl]trimethylammonium iodide (1) and [2-(2,2-dimethylpropionyloxy)ethyl]trimethylammonium 2,2-dimethylpropionate (2), were evaluated in scopolamine-treated or nucleus basalis magnocellularis (NBM) lesioned rats. In scopolamine-treated animals, Compounds 1 and 2 prevented or reduced EEG effects, such as increased amplitude of total spectra and high-voltage spindle (HVS) activity as well. Furthermore, choline esters showed a noticeable effectiveness in reversing the EEG changes produced in rats by AMPA-induced lesion of NBM. Indeed, Compounds 1 and 2 were able to induce EEG desynchronisation, a significant decrease in the total EEG power (0.25–16 Hz) and in the lower frequency delta and theta bands (0.25–3 and 3–6 Hz, respectively). The EEG effects produced by Compounds 1 and 2 were well comparable with that evoked by Tacrine, used as a reference compound. The results of the present work allow us to put forward the hypothesis that the EEG effects observed are most likely mediated through the stimulation of the cholinergic neurotransmission ensuing from enhanced cerebral levels of acetylcholine (ACh) consequent upon acetylcholinesterase (AChE) inhibition by choline pivaloyl esters.

Keywords: Cholinergic neurotransmission; Electroencephalography; Lesioned nucleus basalis magnocellularis; AChE Inhibitors

1. Introduction

Alzheimer disease (AD) is a neurodegenerative disorder affecting major brain areas and is characterised by progressive decline in cognitive functions and behavioural disturbances, as a consequence of severe impairment in central cholinergic transmission in addition to dysfunctions in other neurotransmitter systems, including serotoninergic, noradrenergic, dopaminergic and excitatory aminoacidergic systems (Katzman, 1986; Terry and Katzman, 1994).

Clinical symptoms present in AD are associated with a progressive degeneration in forebrain and, particularly, in the nucleus basalis magnocellularis (NBM). The degeneration of cell bodies in NBM leads to the loss of neuronal projection to cortex (Whitehouse et al., 1981, 1982), impairing the mechanism of cortical activation involved in attention, learning and memory (Lo Conte et al., 1982). Furthermore, progressive neuronal loss results in a reduction of the brain levels of acetylcholine (ACh) and ACh biosynthetic enzyme choline acetyltransferase (ChAT) (Perry et al., 1978), which correlate with the severity of cognitive dysfunctions (Katzman, 1986).

Cortical activation is correlated with electroencephalographic (EEG) desynchronisation, i.e., with the presence of low-amplitude EEG waves associated with a decrease in lower frequency EEG spectrum components (delta and theta bands, range 0.25–3 and 3–6 Hz, respectively), and the fall of the total EEG power (Stewart et al., 1984; Walen et al., 1994).

Otherwise, AD patients evidenced a slower cerebral electrical activity, as shown by EEG synchronisation characterised by the presence of large-amplitude waves, associated with a decrease in high-frequency components (alpha1,

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alpha2 and beta bands, range 6–9, 9–12 and 12–16 Hz, respectively) and an increase in total EEG power (Brenner et al., 1988; Helkala et al., 1991; Soininen et al., 1991; Kuskowski et al., 1993; Miyauchi et al., 1994; Pozzi et al., 1995).

It is noteworthy that a selective lesion of rat NBM, induced by excitotoxic agents (Muir et al., 1993), produces a reduction of the number of neurons and changes the normal EEG spectral power in the frontal cortex (Lo Conte et al., 1982). Indeed, in lesioned animals, EEG analysis shows an increase in slow waves and high-voltage spindle (HVS) activity, which is due to a deficiency in the cholinergic control arising from NBM (Riekkinen et al., 1991). Anticholinergic drugs, such as scopolamine, also induce EEG effects similar with that produced by NBM damage (Sunderland et al., 1985). Therefore, qualitative and quantitative analyses of EEG activity may be a useful diagnostic tool to evaluate the functional decline occurring in AD and the effectiveness of new potential therapeutic agents (Schreiter-Gasser et al., 1993; Elmstahl et al., 1994; Stam et al., 1996; Lopez et al., 1997).

Historically, cholinesterase inhibitors (ChEI) are the first and most developed drugs studied for AD treatment; to date, they are the main agents indicated for AD therapy. The mechanism of their action is based upon the restoration of the cholinergic neurotransmission by preventing ACh hydrolysis and increasing its cerebral levels (recent reviews on ChEI: Giacobini, 2000; Grutzendler and Morris, 2001; Doody et al., 2001).

The aim of the present work was to evaluate the effects of two choline pivaloyl esters, namely, [2-(2,2-dimethylpropionyloxy)ethyl]trimethylammonium iodide (1) and [2-(2,2-dimethylpropionyloxy)ethyl]trimethylammonium 2,2-dimethylpropionate (2) (Carelli et al., 2000), on EEG changes induced in rats by NBM lesion or scopolamine treatment. Indeed, owing to the ability to enhance the central cholinergic tone recently reported for Compounds 1 and 2 (Rispoli et al., 2004), we had good reasons to hope that these compounds could also be able to restore the EEG pattern in impaired animals.

2. Materials

2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), hydrobromide, 9-amino-1,2,3,4-tetrahydroacridine (Tacrine, THA), hydrochloride hydrate and scopolamine were purchased from Sigma-Aldrich (Milano, Italy). All other chemical and biological reagents and solvents used were of the highest commercially available grade.

3. Animals

Male Wistar rats (weighing 200–250 g; Harlan Italy, Udine, Italy) were used in the experiments. All animals were housed and treated according to the guidelines for care and use of experimental animals of the European Community Council, Directive (86/609/EEC).

4. Surgical procedures

To perform the recording of EEG cortical activity, the animals anaesthetised with intraperitoneal chloral hydrate (400 mg/kg) were fixed in a stereotaxic frame and implanted with four surface electrodes onto the frontoparietal region of the neocortex (two electrodes for each hemisphere), which were fastened to the skull with dental cement. A reference electrode, placed onto the nasal bone, was used as ground. After surgery, all animals were housed 48 h to allow complete recovery. On the day of the experiment, the animals were placed in the recording cage and left for a minimum of 15 min prior to the recording session.

The bilateral and selective damage of NBM of the anaesthetised male Wistar rats was stereotaxically performed (coordinates: AP = − 0.9 from bregma; L = 2.6; V = 7.4 from skull, according to the atlas of Paxinos and Watson, 1980) by intracerebral infusion of excitotoxic AMPA, which was injected (1.3 μg/0.5 μl) with a Hamilton syringe through a Teflon tube connected by an injector (26 gauge, rate of microinfusion 0.1 μl/min). No AMPA was injected in the sham-operated rats. After lesion, the animals were housed for two weeks; thereafter, they were stereotaxically implanted with cortical electrodes. Forty-eight hours later, electrocortical activity was continuously monitored, recorded and quantitatively analysed in both hemispheres.

Brain histological preparations were performed to verify the site of injection and to quantify the neuronal loss in the NBM. Briefly, the rats were anaesthetised and transcardially perfused with 0.1 M phosphate buffer (pH 7.4) and then treated with 40% paraformaldehyde dissolved in phosphate buffer. Brains were then removed, postfixed and finally mounted in a freezing microtome. Thick sections (30 μm) containing the nucleus basalis were cut and processed by means of ChAT immunohistochemistry. The quantitative analysis of NBM ChAT activity was carried out using a computerised image analysis system (Axiophot Zeiss microscope equipped with a Vidas Kontron system). The cortical ChAT immunoreactivity was reduced to 45% ($P < .01$) in the frontal cortex and to less than 35% ($P < .05$) in the parietal cortex when compared with the control animals in both hemispheres. Furthermore, analysis showed a significant reduction (45%; $P < .01$) of NBM cholinergic neurons in both hemispheres of lesioned rats.

5. EEG analysis

In the EEG experiments on intact rats, the number of tested animals was seven for each drug and each dose. Control groups of animal received in the same way the

same amount of the vehicle alone. Physiological saline was used as vehicle for the intraperitoneal administration of drugs.

Seven groups of seven animals each were used for the EEG measurements on NBM-lesioned rats: a group of NBM-lesioned untreated rats, four groups of NBM-lesioned animals receiving compounds 1, 2, THA and vehicle, respectively, and two control groups, including intact and sham-operated animals, respectively.

The cortical-derived EEG activity over the total spectral range (0.25 to 16 Hz) was continuously monitored and recorded, for at least 120 min, in awake and freely moving animals, by a computerised apparatus (Vega 24, ESA-OTE Biomedica, Firenze, Italy). EEG analysis was performed on the whole spectrum and on preselected frequency bands (delta 0.25–3; theta 3–6; alpha$_1$ 6–9; alpha$_2$ 9–12; and beta 12–16 Hz) as well. Five artifacts-free epochs of 4 s each were selected following baseline recording and post-treatment period, digitised and quantitatively analysed by a Berg–Fourier EEG recorder (ESA-OTE Biomedica) using Fast Fourier Transform (FFT). Statistical analysis of the data was performed on the EEG signal amplitude and every range of frequencies, comparing each group with a control by analysis of variance (ANOVA), followed, if significant, by the post hoc Tukey-Kramer test for multiple comparison. In each group, the baseline EEG activity versus EEG recording, after drug administration, was evaluated.

6. Results

6.1. Effects of Compounds 1 and 2 on EEG pattern in intact rats

A dose–response study was initially carried out to find the dose of the two choline esters inducing the most significant EEG effects. For both Compounds 1 and 2, 60 μmol/kg proved to be the best dose, which, while not producing any sign of cholinergic hyperactivity, was nevertheless able to significantly modify the baseline EEG pattern. The highest dose tested (600 μmol/kg) produced, 15 min after injection, severe tremor, diarrhoea, dyspnea, convulsion and EEG spikes. Death from respiratory failure occurred in more than 50% of animals, even 24 h later. The lowest dose tested (6 μmol/kg) did not produce any EEG change.

The injection of Compound 1 (60 μmol/kg ip) produced, 15 min after administration in awake and freely moving rats, a behavioural response of alert, EEG desynchronisation and significant ($P<.05$) decrease in the total power of the EEG spectrum (Fig. 1) and in the lowest frequency bands (0.25–3 and 3–6 Hz) as well (Figs. 2 and 3), lasting 15 min. Compound 2 (60 μmol/kg ip) also induced, within 15 min from its administration in rats, alert response, EEG desynchronisation and significant ($P<.01$) fall in the total EEG power (Fig. 1) and in the lowest frequency band (0.25–3 and 3–6 Hz; Figs. 2 and 3), lasting 30 min. ANOVA

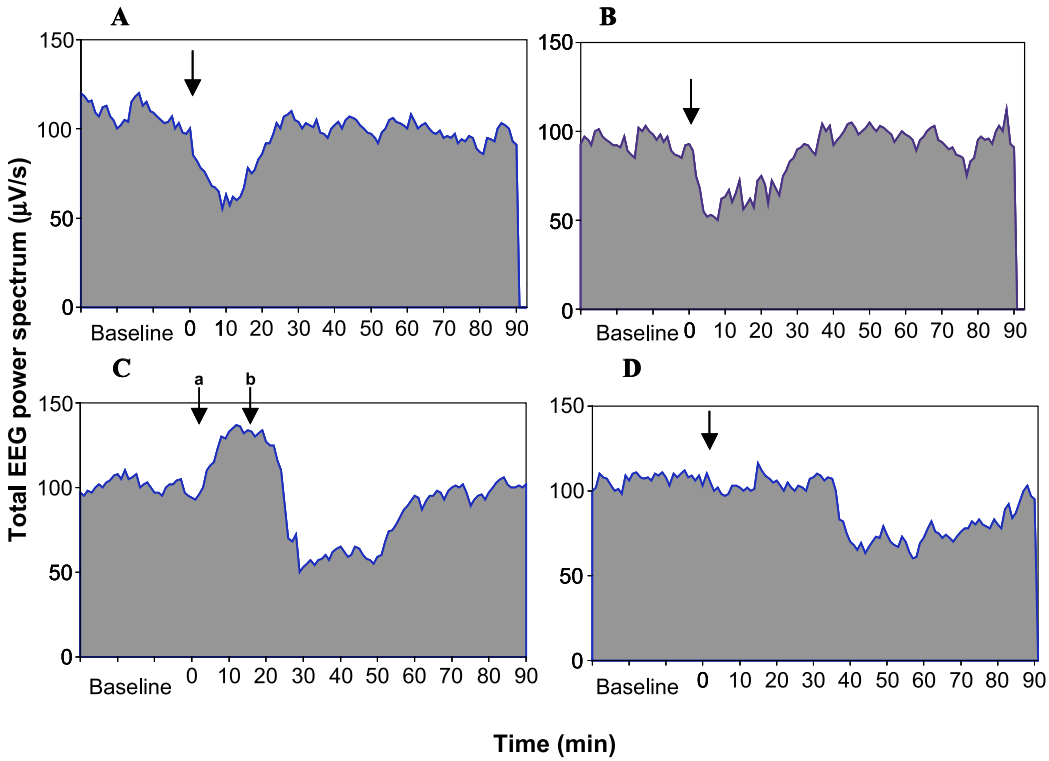

Fig. 1. Time course of cortical-derived EEG activity over total spectral range (0.25–16 Hz, amplitude expressed as μV/s) in intact rats after intraperitoneal injection of (A) Compound 1 (60 μmol/kg, $P<.05$), (B) Compound 2 (60 μmol/kg, $P<.01$), (C) scopolamine (0.2 mg/kg, a) + Compound 2 (60 μmol/kg, b); and (D) THA (5 mg/kg). Arrows show the injection time.

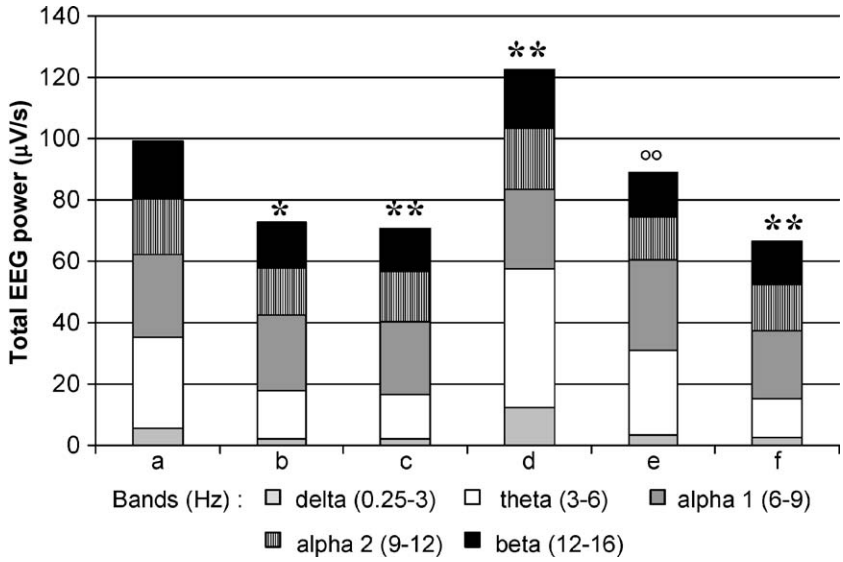

Fig. 2. Quantitative analysis of the total EEG spectrum (μV/s) following intraperitoneal administration in intact rats of (a) vehicle, (b) Compound 1 (60 μmol/kg), (c) Compound 2 (60 μmol/kg), (d) scopolamine (0.2 mg/kg), (e) scopolamine (0.2 mg/kg) + Compound 2 (60 μmol/kg), and (f) THA (5 mg/kg). The contribution of every EEG spectrum component is indicated and expressed as total power fraction. $*P<.05$, $**P<.001$ vs. control, $^{\circ\circ}P<.001$ vs. scopolamine.

analysis showed a very significant Drug × Time interaction [$F(4,112)=4.96$, $P<.001$] and extremely significant Drug × Drug interaction [$F(28,112)=16.4$, $P<.0001$]. The aforementioned effects are quite comparable with that displayed by THA (5 mg/kg ip), 30 min after injection (Figs. 2 and 3). Scopolamine (0.2 mg/kg ip) produced an increase in total EEG power and in cortical EEG slow waves (Figs. 2 and 3) and, furthermore, an enhanced synchronised oscillatory HVS activity. Compounds 1 and 2, administered in scopolamine-treated rats, were able to reduce the cortical slow-wave activity, as well as the increased amplitudes of low-frequency bands (0.25–3, 3–6, 6–9 Hz; Figs. 2 and 3) and total EEG power (Fig. 1). Moreover, choline esters, likewise to THA, suppressed HVS activity induced by scopolamine.

6.2. Effects of Compounds 1 and 2 on EEG pattern in NBM-lesioned rats

NBM-lesioned rats showed a significant ($P<.01$) 45% neuronal loss quantified by ChAT immunochemical analysis

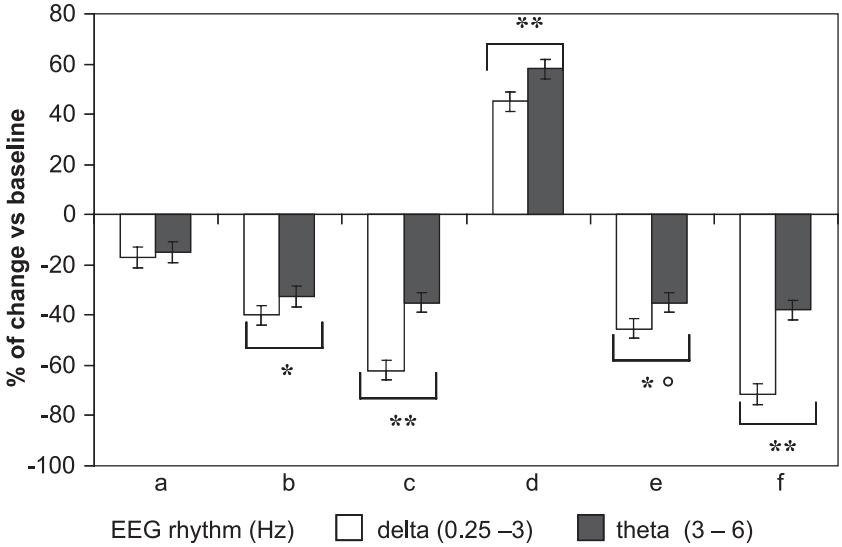

Fig. 3. Effects on EEG bands delta (0.25–3Hz) and theta (3–6Hz) after intraperitoneal administration in intact rats of (a) vehicle, (b) Compound 1 (60 μmol/kg), (c) Compound 2 (60 μmol/kg), (d) scopolamine (0.2 mg/kg), (e) scopolamine (0.2 mg/kg) + Compound 2 (60 μmol/kg), and (f) THA (5 mg/kg). Data are expressed as percent change (mean ± S.E.M.) vs. baseline. $*P<.05$; $**P<.001$, $^{\circ}P<.05$ vs. scopolamine.

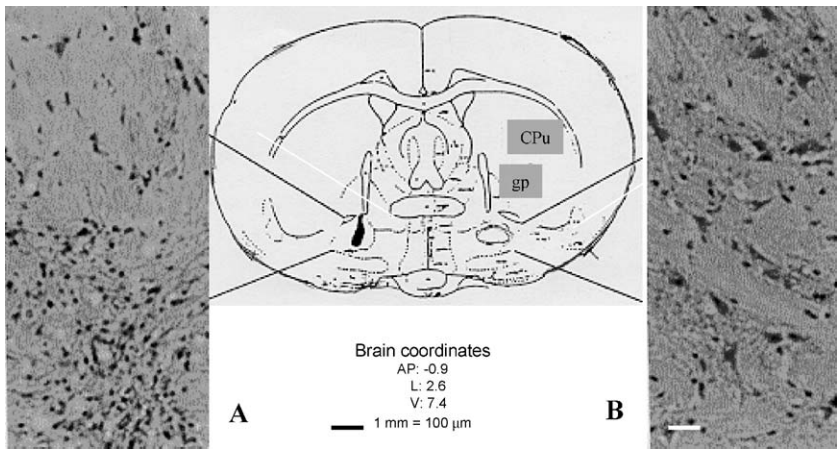

Fig. 4. Low-power photomicrographs (× 40 magnification; 1 mm = 100 μm) of a coronal section through the rat NBM. Cholineacetyl transferase (ChAT) immunostaining was used to show the neuronal loss in NBM after the injection of AMPA (1.3 μg/0.5 μl). Neuronal loss of 45% was observed. The black area in the drawing represents the damaged area (Panel A); the NBM depicted on the left hemisphere (Panel B) serves as the control. Cpu and gp, are caudate–putament and pallidus, respectively.

(Fig. 4). The reduction of cholinergic neurons in NBM-lesioned rats was correlated with an increase in EEG slow waves and HVS activity (Figs. 5 and 6). The last EEG changes were associated with a significant increase ($P < .01$) in total EEG power and in all spectral components, when compared with control and sham-operated animals (Fig. 5).

Treatment of NBM-lesioned rats with Compounds 1 and 2 (60 μmol/kg ip), likewise to THA, reversed, 15 min after the administration, the EEG effects connected with NBM lesions. Indeed, EEG analysis showed a significant fall ($P < .001$) in total EEG power and a considerable decrease ($P < .05$) in the delta power (0.25–3 Hz; Fig. 5). Moreover, choline esters were able to significantly reduce ($P < .05$) HVS activity (Fig. 6). An alert response, correlated with EEG effects, was also observed. ANOVA analysis revealed very significant Drug × Time and Drug × Drug interactions [$F(4,112) = 3.65$, $P < .001$ and $F(49,112) = 12.6$, $P < .0001$, respectively].

7. Discussion

The results presented in this paper show that the choline esters administered to rats are able to penetrate the blood–brain barrier (BBB) and produce specific effects on CNS. Indeed, they were able to modulate the cortical activity inducing EEG desynchronisation and significant changes in the architecture of the EEG tracings, as shown by quantitative EEG analysis. Moreover, a correlate alert response was also evoked. In this regard, it should be noted that alert is a special arousal state due to the stimulation of the cerebral cortex, which induces a condition of attention in

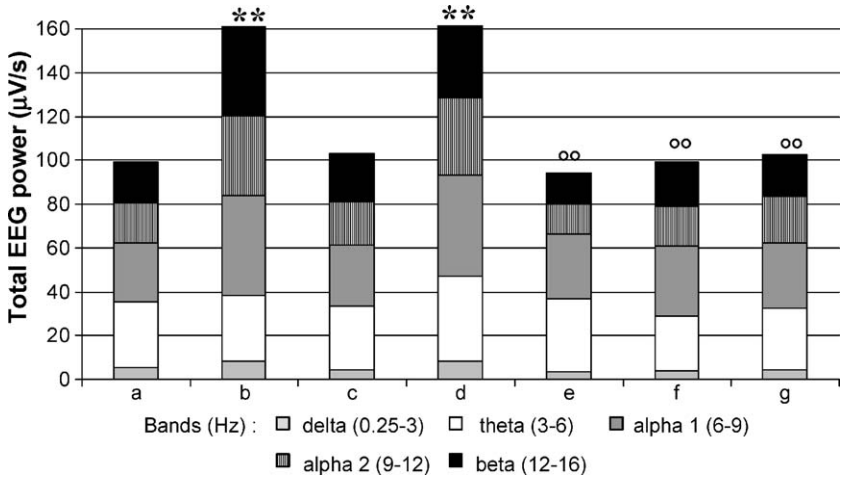

Fig. 5. Quantitative analysis of total EEG spectrum (μV/s) obtained from frontoparietal cortex of rats with bilateral lesion of the NBM. (a) intact rats, (b) NBM-lesioned rats, (c) sham-operated rats, (d) lesioned rats + vehicle, (e) lesioned rats + Compound 1 (60 μmol/kg), (f) lesioned rats + Compound 2 (60 μmol/kg), and (g) lesioned rats + THA (5 mg/kg). The contribution of every EEG spectrum component is indicated and expressed as total power fraction. * * $P < .001$ vs. intact control; ° ° $P < .001$ vs. lesioned animals.

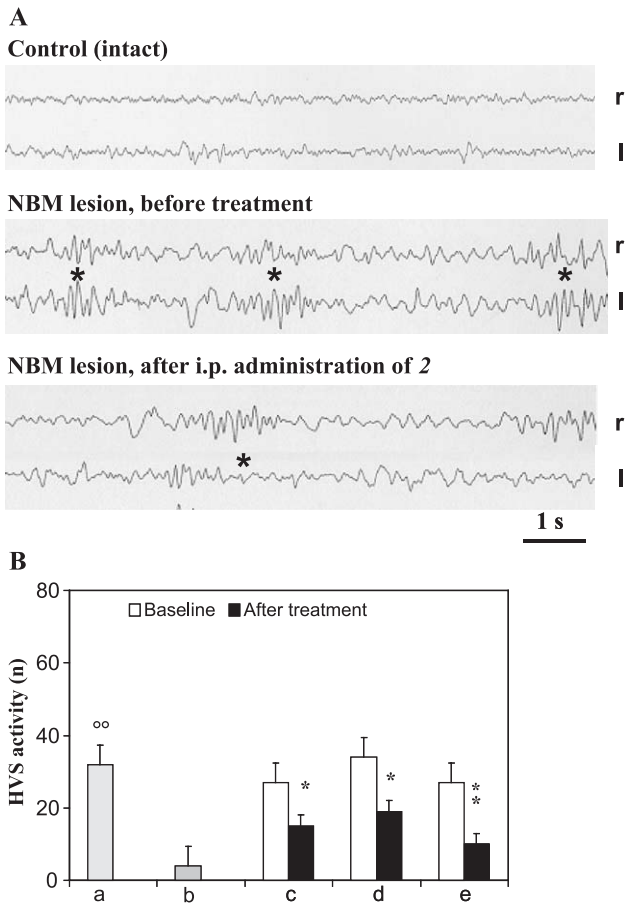

Fig. 6. (A) Time course of EEG tracings obtained from the frontoparietal cortex of rats with bilateral lesions of the NBM; r and l are records (20 s) from the right and left cerebral hemispheres, respectively. Asterisks indicate the HVS oscillations on both tracings. HVS activity appears significantly reduced after intraperitoneal administration of choline ester 2. (B) Histogram showing the reduction of HVS activity in rats with bilateral NBM lesions after treatment with Compounds 1 and 2 and THA. (a) lesioned rats, (b) sham-operated rats, (c) lesioned rats + Compound 1 (60 μmol/kg), (d) lesioned rats + Compound 2 (60 μmol/kg), and (e) lesioned rats + THA (5 mg/kg). Drugs were intraperitoneally injected after 30 min of EEG baseline recording. HVS activity (expressed as n = number of events ± S.E.M./30 min) was recorded for 30 min after treatment. $^{\circ\circ}P < .001$ vs. sham, $^{*}P < .05$ and $^{**}P < .001$ vs. baseline.

man and animals. Therefore, from a physiological point of view, arousal is a process modulated by neuronal network involving different brain areas, such as ascending reticular system and basal forebrain (Casamenti et al., 1986), operating in cortex activation to which cerebral EEG desynchronization effects are also due. Thus, both enhancement in alertness state and modifications of cortical electrical activity can be produced by compounds that are able to increase the cortex activation by the enhancement of the central cholinergic tone.

Owing to the lipophilic character of their 2,2-dimethylpropionyl moieties, choline esters showed short latency in evoking EEG effects, denoting ability to cross fast the BBB. Moreover, the dimethylpropionate anion further enhances the lipophilicity of Compound 2, which, indeed, induced longer lasting EEG effects than Compound 1 did.

Recently, it has been reported (Rispoli et al., 2004) that Compounds 1 and 2 were able to improve cognitive and memory performance in scopolamine-treated or NBM-lesioned rats, as a consequence of their ability to restore cholinergic neurotransmission by acetylcholinesterase inhibition. Therefore, the enhancement of cerebral ACh level would be able to remove scopolamine block on muscarinic receptors and, moreover, stimulate the nicotinic one. Analogously, the described EEG effects induced by choline esters on impaired animals are likely mediated through increase in cholinergic tone.

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