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BRIEF COMMUNICATION

Influence of Cholinesterase Inhibitors on Cortical Slow-Wave Activity in Aging Nonhuman Primate

L. JAIME FITTEN,*†[1] K. M. PERRYMAN,* J. O'NEILL‡ AND E. HALGREN‡

*Psychiatry and †Medicine Services, Department of Veterans Affairs Medical Center, 116A-9, 16111 Plummer St., Sepulveda, CA 91343
‡Psychiatry Service, Wadsworth Veterans Administration Medical Center, and Departments of Psychiatry, Biobehavioral Science, and Medicine School of Medicine, University of California, Los Angeles, CA 90024

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FITTEN, L. J., K. M. PERRYMAN, J. O'NEILL AND E. HALGREN. *Influence of cholinesterase inhibitors on cortical slow-wave activity in aging nonhuman primate.* PHARMACOL BIOCHEM BEHAV 49(1) 235–239, 1994. – Substantial evidence has now accumulated suggesting that the cognitive decrements characteristic of Alzheimer's disease and, to a lesser degree, of normal aging, may result, at least in part, from degenerative changes in the cholinergic system innervating archi- and neocortices. This evidence for cholinergic degeneration in AD has provided the key rationale for many recent clinical trials utilizing cholinergic agents for the purpose of palliating cognitive loss. The basal forebrain cholinergic system plays an important function in electrocortical activation associated with behavioral arousal and cognitive functions. We recorded electrocortical changes from nonhuman primates following administration of potentially clinically useful cholinergic agonists as well as an antagonist. The cholinesterase inhibitors tacrine (THA) and, to a lesser extent, physostigmine (PHYSO) and amodiaquine (AMDQ), caused an upward shift in the frequency of the resting electrocortical activity, although scopolamine significantly slowed the activity below baseline levels. We believe these findings support the concept that the cholinergic system may play an important role in cognitive processes associated with cortical activation.

Cholinesterase Inhibitors EEG ECoG Cortical slow waves Cortical activation Aging monkeys

A LARGE body of biochemical, morphologic, and behavioral evidence has led to the belief that memory and other higher cortical functions are, in part, modulated by the rich cholinergic innervation of the hippocampus and neocortex which originates in basal forebrain nuclei (9). Certain cognitive disorders, such as Alzheimer's disease (AD) and, to a lesser extent, normal aging changes, could then result from various degrees of deafferentation of this cortical cholinergic innervation (4,5). Other neurotransmitter systems are also undoubtedly involved in the modulatory process, and the interaction of the various systems with each other will likely be important (14,34), but a current view is that the cholinergic system may have a preeminent role (9). This is further supported by recent work that has shown that the basal forebrain cholinergic system is the only neurotransmitter system affected in direct proportion to the cognitive decline in AD (16). Taken together, then, these lines of evidence for cholinergic involvement in neurodegenerative conditions such as AD have provided the key rationale for the many recent clinical trials utilizing cholinergic agents for the purpose of palliating cognitive loss.

It has been clearly demonstrated in the rat that large irregular EEG cortical slow waves result from damage to the nucleus basalis (30,32). In contrast, Webster and Jones (1988) have shown that the destruction of the brain stem cholinergic

[1] To whom requests for reprints should be addressed.

groups (e.g., CH5, CH6) does not cause EEG slowing. Recently, it was proposed that basal forebrain cholinergic innervation to the neocortical pyramidal cells can prevent the build up of slow waves through its effect on the calcium-mediated opening of the potassium channels and blockade of the after-hyperpolarization potential (AHP) (10,21,23,27). Interestingly, cholinergic drugs have also been able to shift the EEG background activity back to more normal patterns both in animals and AD patients (1,10,19,25,28,33,36,38).

In normal humans and animals, centrally acting anticholinergic drugs, such as scopolamine, induce acute attentional and memory deficits similar to those seen in AD patients (20). These deficits, as well as those resulting from nucleus basalis lesions, may be reversed by centrally acting cholinesterase inhibitors such as physostigmine (3,6,7,35). Other investigators (6–8,15) have shown memory improvement in older monkeys following cholinesterase inhibitor therapy, and modest cognitive improvements have been described in many clinical trials (12,13,27,31).

In this study we examine the accelerating influence of two known memory enhancing cholinesterase inhibitors [physostigmine (PHYSO) and tacrine (THA)] on electrocorticogram (ECoG) background rhythmic activity in monkey. We compare these effects to those of the previously untested cholinesterase inhibitor Amodiaquine (AMDQ) and to the classic anticholinergic scopolamine (SCOPOL).

METHOD

Subjects and General Surgical Techniques

Two adult, colony born and bred, healthy, female *Macaca radiata* (bonnet) monkeys, ages 18 and 22 years, and weighing 4–6 kg, participated in this study. Both subjects originated from the UC Davis Regional Primate Center and have resided at Sepulveda at least 7 years under strictly controlled environmental conditions. Both animals are behaviorally sophisticated subjects employed in a concurrent cognitive study in which electrophysiological slow-wave potentials are also collected from the cortical surface.

Initially, the subjects underwent sterile operative procedures under sodium thiopental (20 mg/kg/IV) and ketamine hydrochloride (5 mg/kg/IV) anesthesia for stereotaxic implantation of epidural electrodes. The potentials collected for this study were recorded from epidural surfaces using eight (four on each side) stainless steel screws threaded into the calvarium and covered with dental acrylic. In the absence of a specific atlas for *M. radiata*, the atlas of Szabo and Cowan (37) for the cynemologous monkey (*M. fascicularis*) was used for stereotaxic localization of epidural electrode placements (coordinates frontal at AP +30, lat. 10; central at AP +5, lat. 10; parietal at AP 0.0, lat. 15; occipital at AP +5, lat. 10). Previously, through in vivo MRI imaging and postmortem histological studies conducted to ascertain subcortical recording sites in *M. radiata*, we have determined that the morphology and morphometric characteristics of numerous brain structures in *M. fascicularis* closely approximate those of *M. radiata*. In particular, major sulci and gyri of the lateral and frontal cortex of *M. radiata* are within 2–3 mm of those in *M. fascicularis*. Thus, for the purpose of locating general frontal, parietal, temporal, and occipital cortices for epidural electrode placements in *M. radiata*, the Szabo and Cowan atlas was deemed appropriate.

After electrode implantation, a miniature nine pin Amphenol connector was also embedded into the acrylic pedestal that provided electrical continuity for recording. Both monkeys were given the antibiotic sodium cefazolin (100 mg/kg/IM) and the corticosteroid dexamethasone (1 mg/kg/IM) 24 h prior to surgery and daily for 1 week following the operative procedure as prophylaxis for infection and postoperative edema.

Recording Procedures

EEG slow wave activity was recorded differentially from frontal (F)-central (C), central-parietal (P), and parietal-occipital (O) epidural electrodes on each hemisphere. GRASS model 7PS11J physiological instrumentation was employed to amplify slow-wave cortical activity between 0.3 Hz and 100 Hz. Electrocorticograms (ECoG) were digitally recorded using a Vetter model 3000 multiplexer-demodulator and a Panasonic model PV1461 VHS video recorder. Successive 4 s epochs of ECoG activity were digitized by a 80286 PC in conjunction with a Data Translation model DT2801, 12 bit converter sampling at a rate of 1024 points per second. A C language compiled fast Fourier transformation (FFT) was used to quantify the relative (percent of total power) energy associated with 4 Hz bin width ECoG bands and to compute dominant (maximum relative power) cortical rhythms associated with each pharmacological intervention. Two to three artifact-free, 4 s epochs were averaged from each postinjection recording period associated with a specific pharmacological intervention for each animal.

ECoG activity was recorded from each monkey while seated in a primate restraint chair in a ventilated, soundproof, low-level illumination enclosure. The subjects had previously been adapted to this recording environment for well over 12 months of daily training and testing in another study. Prior to each pharmacological intervention, the monkeys were transferred to the chair and an Amphenol connector was attached to their pedestal. The animals were permitted a 15-min period of additional adjustment before commencing a baseline 5-min ECoG recording period.

Drug Administration

Each subject received the following cholinesterase inhibitors parenterally (IM) and sequentially, on different days after baseline recordings: AMDQ (2 mg/kg), PHYSO (0.1 mg/kg), THA (1.5 mg/kg). The anticholinergic SCOPOL (0.08 mg/kg) was similarly administered. Vehicle consisted of Ringer's solution (2 ml IM). The initial dose ranges from which the above doses were taken were chosen for each drug based on past experience with monkeys using these compounds (15) and on numerous literature reports for behaving animals. Actual doses used in the preliminary dose-finding period were SCOPOL (0.05, 0.08, 0.1 mg/kg), THA (0.9, 1.5, 2.4 mg/kg), PHYSO (0.05, 0.07, 0.1 mg/kg), and AMDQ (1.2, 2.0, 3.6 mg/kg). In each case the dose that produced the most significant EEG and EKG changes with the fewest autonomic and behavioral side effects (e.g., vomiting, marked inactivity) was chosen as the dose to be administered.

With regard to the novel application of AMDQ as a potentially centrally active cholinesterase inhibitor, the initial dose range used in the dose-finding phase was chosen based on experience gained from a concurrent early phase II clinical trial with mild AD patients conducted by our group. AMDQ has been used overseas as an antimalarial, and was recently identified as an effective cholinesterase inhibitor with desirable pharmacological properties, e.g., serum half life ca. 8 h (18). In addition, its abilities to block cellular K^+ channels in

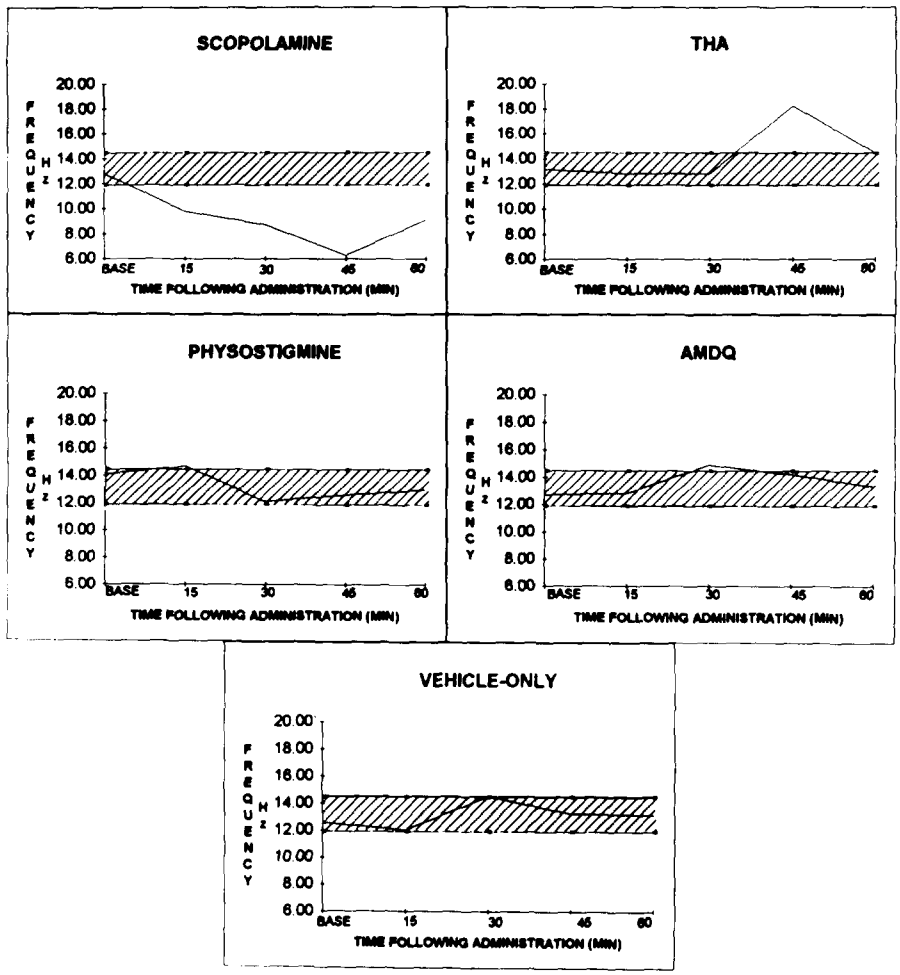

FIG. 1. Dominant electrocortical frequency following IM injections of tacrine (THA 1.5 mg/kg), physostigmine (PHYSO 0.1 mg/kg), amodiaquine (AMDQ 2 mg/kg), scopolamine (SCOPOL 0.08 mg/kg), and vehicle-only (2 ml Ringer's solution). The shaded area represents three standard errors (90% confidence interval, CI) above and below the mean baseline frequencies of both monkeys: No. 19383, age 22, and No. 19358, age 18. At the time point of maximum EEG effect for each drug, the change from baseline for THA and SCOPOL is well outside the 99% CI, and at the 95% and 90% CI for AMDQ and PHYSO, respectively.

a manner similar to 4-aminopyridine thereby enhancing neurotransmitter and neuromodulator release (2), and to partially reverse hypoxia-induced behavioral deficits in mice (17), give AMDQ two additional characteristics that may be important in enhancing communication in a debilitated CNS. E. Roberts and colleagues (personal communication) have tested numerous substances for their capacity to inhibit acetylcholinesterase and block voltage-gated K^+ channels. They selected for further study only those substances that inhibited K^+ transport in submillimolar concentrations (10^{-3} M or less) and inhibited acetylcholinesterase in micromolar (10^{-6} M) or lower concentrations. Only five substances met the above criteria. In order of potency they were: a) AMDQ; b) 9-aminoacridine; c) quinacrine; d) chloroquine and d) THA.

Order of drug administration was randomly selected for each monkey. The interval between each drug administration was 1 week. For each drug trial a baseline recording was obtained 10 min prior to the giving of the specific drug. After injection, ECoG recordings were made at 15, 30, 45, and 60 min. Each recording consisted of 3 to 4 min of continuous ECoG while the animal remained seated in the chair in the dimly illuminated enclosure. Through a one-way observation port, the subjects were monitored for level of alertness as well as for abnormal behaviors not ordinarily exhibited by them in those conditions. Any abnormal behaviors exhibited in the enclosure were recorded and evaluated for signs of early behavioral and/or general toxicity.

RESULTS

Behavioral Observations

During the administration of the selected "best" doses of cholinesterase inhibitors (THA, PHYSO, AMDQ), no behavioral abnormality was noted, with the exception of a very mild decrease in the random and spontaneous movements normally observed when monkeys are chaired in the enclosure, drug-free, prior to testing. During the recordings, monkeys re-

mained alert, with eyes open, and no nausea, vomiting, diarrhea, or salivation were noted. However, THA and PHYSO caused approximately a 20% decrease in the resting heart rate of the subject that was monitored for cardiac changes. The decreased heart rate gradually returned to normal over a period of 15 min for physostigmine and 30 min for THA.

ECoG Findings

Findings are summarized in Fig. 1. As expected, SCOPOL slowed the ECoG frequency beginning shortly after administration of the drug and lasting for about 45 min. Thereafter, a return to baseline was noted. THA had the opposite effect on the ECoG. However, the onset of the increased frequency of the dominant rhythm did not start until 30 min after injection. The upward frequency shift reached a maximum level at about 45 min postinjection. PHYSO and AMDQ demonstrated marginal increases in frequency occurring at 15 min and 30 min postinjection, respectively. Differences in latency of onset of CNS action and duration are probably related to differences in rate of absorption from muscle, serum transport, passage across the blood–brain barrier, and other pharmacokinetic differences. Drug-induced changes in background frequency were compared against a baseline-derived 90% confidence limit based on multiple baseline measures in two monkeys. The limit is shown as the shaded areas in Fig. 1. Highly significant differences are noted particularly at the point of maximum EEG effect for THA and SCOPOL. AMDQ and PHYSO appear to induce a marginally significant EEG effect at the doses administered (see Fig. 1).

DISCUSSION

Our results indicate that centrally active cholinesterase inhibitors, particularly THA, increase the frequency of the dominant background rhythm in monkey after parenteral administration. In contrast, anticholinergics such as SCOPOL reduce the frequency. Cholinergics have been reported to improve cognitive performance modestly in both animals and humans, and anticholinergics are known to impair cognitive functions such as attention and memory. Thus, there appears to be some relationship between increases in electrocortical frequency and cognition, although the details of that relationship are largely unknown.

A theory to explain the influence of acetylcholine (ACh) on cortical slow waves through nucleus basalis action on neocortical pyramidal cells has been proposed (11). In this theory, calcium mediated potassium channels are opened in pyramidal cell somata following action potentials (22,27) and GABAergic inhibition from neighboring interneurons (24). According to this perspective, the opening of these channels generates an afterhyperpolarization potential (AHP) in the somatic membrane creating a dipole source of a volume-propagated electric disturbance, which is negative on the surface and positive in depth due to the orientation of the pyramidal cell. The long time constant of the potassium channels allows the AHPs from numerous synchronously activated individual pyramidal cells, to summate to large local field positivities which take the form of a slow wave when they reach the surface.

ACh would then act to inhibit the calcium-mediated opening of the potassium channels (21,27). Thus, cholinergic innervation to neocortical pyramidal cells from the basal nucleus can blockade the AHP and thereby effectively prevent the buildup of slow waves (10). When ACh influence is reduced, pyramidal cells return to the burst mode, fire less frequently due to the greater AHP, which leads to slow wave generation (22,23). In normal aging, and more dramatically in AD, the general level of hippocampal and neocortical ACh input is reduced. This has been offered as a possible explanation for the greater prevalence of EEG slow waves in normal-aged and more dramatically, in AD subjects (4). It is possible that a higher level of neuronal activation is required for high fidelity information transfer across the association cortices that subserve cognitive functions. EEG slowing may signal a diminution of that fidelity.

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