EEG Effects of Physostigmine and Choline Chloride in Humans

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Abstract. Seventeen normal volunteers received either 0.5 mg, 1.5 mg, or 2.5 mg physostigmine i.v. in a placebo-drug-placebo single-blind design. EEG was recorded simultaneously and analyzed by computerized spectral analysis. Eleven healthy elderly volunteers (mean age = 69.1 years) with mild memory impairment were treated with placebo, followed by oral choline chloride (either 8 g/day for 3 weeks, or 16 g/day for 1 week), and then, again, placebo. Recordings of spontaneous EEG and EEG event-related potentials (contingent negative variation) were obtained during both placebo and choline treatments. The larger doses of physostigmine produced an increase in low frequency activity and a slowing of the peak alpha frequency. Oral choline chloride had no effect on the EEG as measured by spectral analysis, but appears to have differential effects on contingent negative variation (CNV) amplitude and reaction time, depending upon the initial CNV amplitude.

Key words: Physostigmine — Choline chloride — EEG — Spectral analysis — Contingent negative variation

Studies with agents that selectively increase or decrease central cholinergic activity indicate that cholinergic mechanisms are involved in many cognitive and behavioral activities, including memory, affective state, and the control of movement. In normal subjects a low dose of the cholinesterase inhibitor, physostigmine, improved storage of information into long-term memory (Davis et al., 1978b) while higher doses of physostigmine (Davis et al., 1976b) and the anti-cholinergic scopolamine impaired memory performance (Drachman and Leavitt, 1974). There have also been reports that choline chloride, a metabolic precursor of acetylcholine, may produce some behavioral improvement in patients suffering from Alzheimer's disease (Boyd et al., 1977). In patients with manic-depressive psychosis, it has been shown that physostigmine can reverse manic symptoms (Davis et al., 1978a; Janowsky et al., 1973). In patients with tardive dyskinesia or Huntington's chorea, the frequency of abnormal movements can be diminished by cholinomimetics (Davis et al., 1976a).

In spite of the interesting findings obtained using cholinomimetics in studies of brain functioning and psychopathology, few systematic studies of the EEG effects of cholinomimetics in humans have been conducted. Those available (Grob et al., 1947; Rowntree et al., 1950; Lesny and Votja, 1960; Pfeiffer et al., 1963; Goldstein and Beck, 1965; Reiger and Okonek, 1975; Sitaram et al., 1976) have produced inconsistent results and are difficult to compare due to differences in the drugs used, dosage range, subject populations, and EEG assessment techniques.

Most of the human EEG studies have used visual inspection to delineate drug-induced changes. Computerized EEG spectral analysis and event-related potential construction offer additional means of assessing the neurophysiologic effects of psychoactive compounds. EEG spectral analysis provides information about the frequency composition of the spontaneous electrical activity of the brain and, therefore, is an indicator of electrophysiologic changes associated with drug administration (Fink, 1963). Computerized averaging techniques and repeated sensory stimulation can delineate averaged evoked responses from the background EEG activity. These averaged evoked responses reflect neurophysiologic aspects of sensory and cognitive processing of stimuli (Pfefferbaum et al., 1978), are altered by psychoactive drugs (Roth et al., 1977), and can be correlated with behavioral responses (Kutas et al., 1977).

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This report presents EEG findings from two studies involving the administration of cholinergic agents. The first study investigated the EEG changes, as measured by spectral analysis, produced by short-acting cholinergic stimulation (i.v. physostigmine) in young normal subjects. In the second study, elderly patients with mild memory impairment were treated with the longer-acting cholinergic agent, choline chloride, and effects on the spontaneous EEG were assessed using spectral analysis. In addition, a convergent evoked potential-cognitive task was employed to further investigate the electrophysiologic correlates of behavior.

**Experiment I: Physostigmine in Normal Subjects**

**Materials and Methods**

Seventeen male volunteers, 21–30 years old, participated in the study. The protocol employed a placebo-drug-placebo single blind design completed in a single session. Four subjects received only 0.5 mg physostigmine (one 0.5-mg injection), four received 1.5 mg (three 0.5-mg injections), and nine received 2.5 mg (five 0.5-mg injections). Table 1 outlines the experimental protocol for all three dose groups.

The protocol and recording techniques were the same for all groups of subjects, with the exception of fewer injections at the lower doses. All testing began at 7:30 a.m. Subjects arrived having fasted for at least 8 h and were given a standard breakfast of fruit juice. All subjects were pretreated with 0.5 mg methscopolamine s.c. to block peripheral cholinergic effects. A constant, slow infusion of saline was started in the antecubital vein and all drug injections were given through the i.v. tubing. Prochlorperazine pretreatment (10 mg, p.o.) was given to four of the subjects who received 2.5 mg physostigmine in order to block the nausea and vomiting that occurs with high doses of physostigmine. None of the other subjects received prochlorperazine.

EEG recording began after the methscopolamine had produced a tachycardia of 100 beats/min. The physostigmine (or saline) was administered at 5-min intervals in 0.5-mg (0.5 cc) boluses. The EEG was recorded during the 5-min periods between injections. During the 5-min periods, the subject spent the first 2.5 min with eyes closed and the next 2.5 min reading. This routine was employed during, and for 40 min after, the injections, while EEG recordings continued. The sequence of changing activities was designed to maintain a fairly constant level of arousal and to avoid drowsiness and sleep.

The EEG was recorded from frontal (F3) and occipital (O1) electrode placements with platinum pins referenced to linked mastoid Ag/AgCl disc electrodes. Electrooculogram (EOG) and heart rate were also recorded. The EEG was amplified 10K with amplifiers set at a nominal bandpass of 1–30 Hz (3-dB points of 6-dB/octave roll-off curves). The EEG, EOG, and EKG were constantly monitored on paper and recorded on FM tape.

Only the EEG recorded during the 2.5-min eyes-closed epochs was analyzed, as eye blinking and movement produced significant artifact during the reading periods. The eyes-closed epochs were edited for movement and large eye-blink artifact, leaving 1.5–2.0 min of EEG from each epoch. The epochs were entered into a PDP-11/40 computer and subjected to fast Fourier analysis.

Fourier analysis provides a mathematical description of the EEG, resulting in a quantification of the contribution of each frequency component to the overall EEG, as well as information about the absolute amount of EEG activity (total power). This procedure yielded separate power spectra (for recordings taken every 5 min) with 1/4 Hz resolution for 0–32 Hz components. These data were collapsed into measures of the percentage contribution to the total power of the 0–4 Hz (delta), 4–8 Hz (theta), 8–12 Hz (alpha), and 12–30 Hz (beta) frequency bands. The percent of the total power in each frequency band has been found to provide a more stable measure than the absolute power of each of the frequency bands (Matousek, 1973). In addition, a determination was made of the dominant frequency of the alpha activity (peak alpha frequency) as measured at the occiput, by identifying, in each epoch, the frequency between 8 and 12 Hz (in 1/4 Hz resolution) containing the largest amount of power. The shift of the peak alpha to a lower frequency provided an index of EEG slowing.

**Results**

On questioning, none of the four subjects who received 0.5 mg physostigmine could distinguish the drug from placebo. At the 1.5 and 2.5 mg doses, however, there were significant subjective effects, including dizziness, diaphoresis, difficulty with reading, nausea, and some vomiting. The vomiting produced by 2.5 mg physostigmine was blocked and the nausea significantly attenuated by prochlorperazine pretreatment.

Physostigmine produced neither obvious visible changes in the raw EEG such as described in the direct recordings from animal brains (Kazem, 1977), nor grossly abnormal EEG as seen in some human studies (Reiger and Okonek, 1975). There were, however, changes in the EEG frequency composition as measured by spectral analysis. The drug-induced changes were determined by performing analyses of variance on the percentages of activity in each frequency band. The presence of ‘drug’ main effects and ‘drug x time’ interactions indicated that there were significant EEG changes produced by i.v. physostigmine.

Initial analyses of variance of the effects of physostigmine on activity in the four frequency bands failed to reveal any differences between the two groups receiving 2.5 mg physostigmine (five subjects without and four with prochlorperazine pretreatment). No significant group effects or drug x group interactions were found. Therefore, for this report, the two groups are combined and treated as a single dosage group.

The acute effects of physostigmine were examined by analyzing the data collected in the 5-min epoch immediately following the injection of a given cumulative dose of physostigmine or saline. All 17 subjects received 0.5 mg; 13 received at least 1.5 mg; and 9 received all 2.5 mg of the drug. Thus, by combining dose groups, there were 17 observations at 0.5 mg, 13 observations at 1.5 mg, and 9 observations at 2.5 mg (see Table 1). An analysis of variance was performed for each of these three cumulative dose points, for each of the four frequency bands, and for both leads in order to compare the physostigmine to placebo.

Delta activity increased significantly after 1.5 mg physostigmine as compared to placebo in both frontal