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Mecamylamine Reduces Some EEG Effects of Nicotine Chewing Gum in Humans

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PICKWORTH, W. B., R. I. HERNING AND J. E. HENNINGFIELD. *Mecamylamine reduces some EEG effects of nicotine chewing gum in humans.* PHARMACOL BIOCHEM BEHAV **30**(1) 149–153, 1988.—Spontaneous EEG was recorded in nine cigarette smokers who had been abstinent from tobacco for 12 hr. Subjects were treated with a capsule containing either centrally acting nicotine blocker, mecamylamine (10 mg), or placebo. At each of three 60-min intervals after the capsule was ingested, the subjects chewed two pieces of gum containing a total of 0, 4 or 8 mg of nicotine. Nicotine and mecamylamine dose combinations were randomized across subjects. Two three-minute periods of spontaneous EEG were recorded before the capsule and before and after gum chewing from bipolar electrode montages at the following positions: C_z-T_5, C_z-T_6, C_z-F_7 and C_z-F_8. During one period the subjects relaxed with eyes closed, in the other period they performed a math task with eyes open. When the drugs were given individually, mecamylamine decreased beta power and nicotine gum (4 and 8 mg) increased alpha frequency. Mecamylamine pretreatment prevented the increase in alpha frequency caused by the 4 mg gum dose but not the 8 mg dose. Alpha power was increased by the 8 mg gum dose and that increase was prevented by mecamylamine. Self-reported ratings of the "strength" of the gum were significantly diminished by mecamylamine pretreatment. The data are consistent with the results of earlier studies which indicate that the effects of tobacco administration and withdrawal are mediated by central actions of nicotine.

Cigarette smoking Drug dependence Drug interaction Electroencephalogram Human subjects Mecamylamine Nicotine Nicotine gum

MECAMYLAMINE is a ganglionic blocking drug that antagonizes peripheral and centrally mediated effects of nicotine and has proven to be useful in assessing the role of central nicotinic receptors in response to tobacco [30]. The use of mecamylamine in studies of the effects of nicotine is analogous to the use of opiate antagonists such as naloxone in studies of the opiate receptors [5]. For example, it has been shown that mecamylamine initially increases the self administration of smoked nicotine in humans [20]. The effects of chronic mecamylamine on cigarette smoking in humans has not been systematically studied. Chronic mecamylamine treatment in animals decreased the self-administration of intravenous nicotine [6]. The discriminative stimulus properties of nicotine are reduced by mecamylamine pretreatment in squirrel monkeys [29], rats [26] and humans [7]. It appears to be the effects of mecamylamine on nicotine receptors in the central nervous system which are critical to its blockade of behavioral responses induced by nicotine since nicotine antagonists that don't readily cross the blood-brain barrier do not antagonize effects of nicotine [31].

Electroencephalographic (EEG) measures proved a noninvasive index of the activity of centrally acting drugs in humans [13]. The electrocortical manifestations of nicotine administration have been extensively studied in humans and animals [3]. Nicotine gum in deprived heavy smokers caused increases in alpha (7.25–14 Hz) and beta (14.25–25 Hz) frequencies and decreased theta (4–7 Hz) power [23]. The EEG effects of the nicotine gum were similar to those of smoked tobacco [10, 12, 32]. The purpose of the present study was to determine if mecamylamine could antagonize the effects of nicotine gum on the spontaneous EEG in tobacco-deprived cigarette smokers.

METHOD

Subjects

Nine male smokers with a mean age of 33.8 years (22 to 49) and weight of 71.9 kg (58.4 to 84.3) resided on the clinical ward for the duration of the study. They had histories of regular smoking for an average of 18.3 years (8 to 30) and scores on the Fagerstrom Tolerance Questionnaire [4] which averaged 7.7 (3 to 11). The subjects were recruited from the local community in response to newspaper advertisements promising payment for participation in a research program. The subjects were informed that the purpose of the study was to determine the effects of gum delivered nicotine on the EEG; they were specifically told that the research did not have treatment benefits for the cessation of smoking. The subjects were given a thorough medical and psychiatric examination prior to their participation in the study. They provided informed consent in accordance with the Department of Health and Human Services guideline for the protection of human subjects.

Study Design

From the evening before until the conclusion of the test sessions the subjects were not allowed to use tobacco or to

consume caffeine containing substances. This pretest deprivation period lasted approximately 12 hr and was monitored by research staff; exhaled carbon monoxide levels averaged 11 ppm (range 5 to 21) the morning of the study. After application of the EEG leads, a baseline recording session was obtained and the subjects were given a capsule containing either placebo or mecamylamine, 10 mg. At hourly intervals for 3 hours after swallowing the capsule the subjects were given two pieces of gum to chew. Chewing was monitored by the staff and the pace of chewing was governed by a tone presented every 2 sec for 10 min; subjects were instructed to bite the gum once at each sounding of the tone. EEG recordings were taken immediately before and after each of the three bouts of gum chewing. Various combinations of gum containing either 0 or 4 mg of nicotine were used to produce doses of 0, 4 and 8 mg nicotine. The order of nicotine doses and the presentation of the capsule were randomized across subjects. Each subject was tested on four occasions separated by at least 48 hr. On two test days measures of spontaneous EEG were obtained; on the other two test days event-related potentials were measured. The results of those latter experiments have been presented elsewhere [9]. All experiments were conducted under double blind conditions of drug administration.

Measurements

Ten minutes before administration of the capsule and at 60, 120, 180, 240 min thereafter, blood pressure and heart rate were measured in the seated subject. At these times a symptom checklist for possible subjective effects was completed by the subject. In addition, after each dose of gum, the subjects were asked to rate their desire to smoke, their desire for another dose of gum, the similarity of effects of the gum to cigarettes, and the strength of the gum, on a previously described questionnaire [19].

Recordings of EEG were obtained from the following bipolar lead: C_z–T_5, C_z–T_6, C_z–F_7, and C_z–F_8. Two three-minute recordings were obtained: in one recording the subject was instructed to keep his eyes closed and to relax. In the other recording the subject was instructed to keep his eyes open and to perform sequential subtraction ("mental arithmetic"). The EEG was collected and analyzed with a Nicolet Pathfinder II. The computer-based analysis software continuously acquired 4-second epochs from each of the four bipolar leads. The EEG was digitized at 256 Hz and samples with artifact were automatically rejected. The digitized EEG was converted into the frequency domain using a fast Fourier transform. For each one-minute sample the computer printed the power (amplitude, μV^2) and peak frequency (resolution=0.25 Hz) in the following frequency bands: delta, 0.25–3.75 Hz; theta 4–7 Hz; alpha 7.25–14 Hz and beta, 14.25–25 Hz. The three one-minute samples were averaged and the data were entered into a computer for statistical analysis.

Statistical Analysis

Separate repeated measure analysis of variance were performed for EEG measure (power and frequency) on the mecamylamine effects and the interactions of the mecamylamine and nicotine gum using the BMDP statistical package. For the mecamylamine effect, the factors were: capsule (placebo and active); time (pre and 50 min post); status of eyes (open and closed); and electrodes (F_7, F_8, T_5, T_6 all to C_z). For the mecamylamine-nicotine interaction, the factors were: capsule (placebo and active); time (before and after gum); status of eyes (open and closed); electrode (F_7, F_8, T_5, T_6 all to C_z) and the nicotine dose (0, 4 and 8 mg). Post hoc paired t-tests were used for specified comparisons of factors. The data from the subjective questionnaire and the cardiovascular findings were analyzed by t-tests.

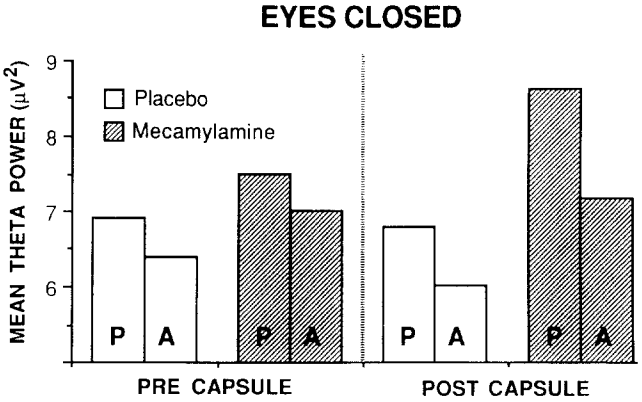

FIG. 1. Mecamylamine-induced changes in theta (4–7 Hz) power in posterior (P) T_5–C_z, T_6–C_z and anterior (A) F_7–C_z, F_8–C_z derivations. Bars are mean power (n=9) before and 60 min after capsules of mecamylamine (shaded) or placebo (open).

RESULTS

Effects of Mecamylamine

The effects of mecamylamine were determined by comparing EEG measures obtained before capsule administration and 50 min after the capsule (i.e., prior to the first gum dose). Compared to placebo pretreatment, mecamylamine produced several significant effects on the EEG. Specifically, theta power tended to increase (drug, $F=3.16$, $p=0.1$) and the increase was most pronounced in the posterior electrodes (Fig. 1). Power in the beta frequency was significantly decreased by mecamylamine (drug × time, $F=4.99$, $p=0.05$). There was no significant drug effects on alpha or delta power. Alpha frequency was reduced by time ($F=5.09$, $p=0.05$) however, the drug × time interaction was not significant ($F=2.28$, $p=0.17$). As shown in Fig. 2, the decrease in alpha frequency was sustained over the duration of the daily session. Beta frequency decreased over time ($F=8.64$, $p=0.02$) but there was no significant drug interaction. Theta frequency was not changed by the administration of mecamylamine, however, a significant interaction (drug × electrode, $F=4.89$, $p=0.01$) occurred on the measure of delta frequency. Neither mecamylamine nor placebo pretreatment significantly changed heart rate or blood pressure, although systolic blood pressure appeared to have decreased somewhat following the mecamylamine administration (Fig. 3). Mecamylamine pretreatment had minimal subjective effects. Although six of the subjects reported feeling a drug effect after mecamylamine five of the subjects gave the same response after the placebo capsule. Effects that occurred more frequently after the mecamylamine included: sleepy, dry mouth, blurred vision and dry eyes. These symptoms were most frequently reported 100 to 160 minutes after the capsule.

Effects of Nicotine Gum

The effects of nicotine gum were assessed by analyzing

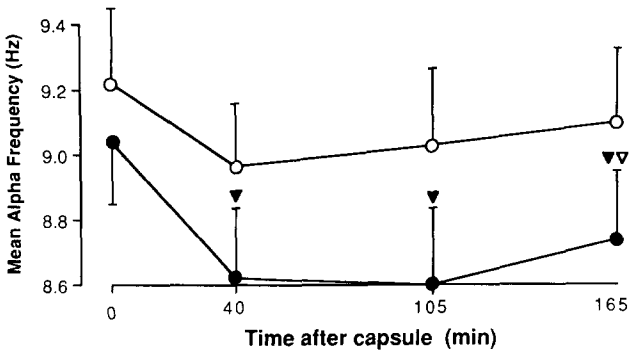

FIG. 2. Mean alpha frequency (n=9) before and after capsules of placebo (open) or mecamylamine (filled). Points are mean±SEM. Statistical significance determined by paired t-test. ▽: $p<0.05$ vs. placebo; ▼: $p<0.05$ vs. baseline; ●: mecamylamine, 10 mg; ○: placebo.

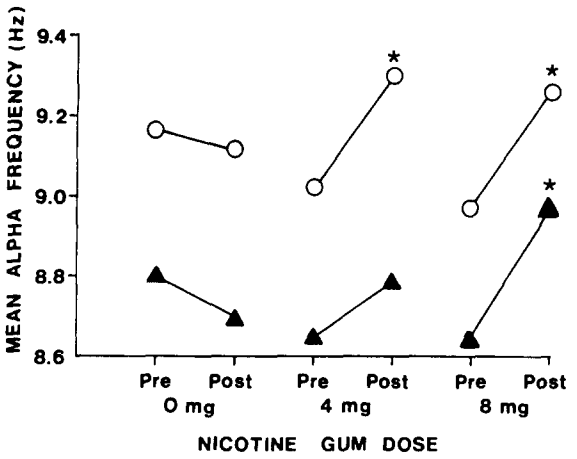

FIG. 4. Mean (N=9) alpha frequency before and after nicotine gum in subjects treated with placebo (○) or mecamylamine (▲); * indicates value is significantly different from pre-gum value $p<0.05$ (paired t-test).

data obtained on days in which no mecamylamine was given (i.e., placebo capsule). Nicotine gum increased the alpha frequency in all channels and a significant interaction between nicotine and time (pre-gum–post-gum) occurred ($F=9.62, p=0.002$) (Fig. 4). Alpha power was reduced by the nicotine gum as indicated by the main effect of time ($F=5.73$, $p=0.04$). The beta power tended to be decreased by the nicotine gum as indicated by the nicotine by time interaction ($F=2.32$, $p=0.13$). Although the gum had no significant effects on delta power or frequency, the theta power tended to be decreased by the gum as indicated by the nicotine by time interaction ($F=2.32, p=0.13$) and the complex interaction of eyes open by nicotine by time ($F=4.34$, $p=0.01$).

Mecamylamine-Nicotine Interactions

The interaction between the effects of mecamylamine and nicotine gum administration were assessed by analyzing data from subjects after mecamylamine pretreatment. Mecamylamine prevented some, but not all, of the nicotine-induced changes. For example, mecamylamine pretreatment

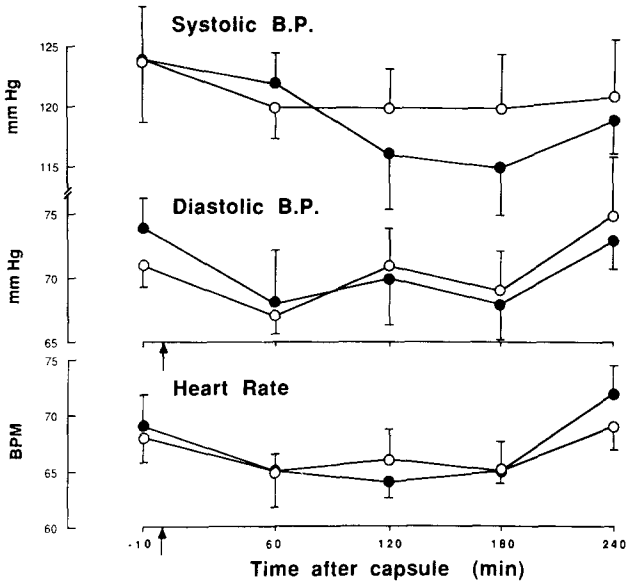

FIG. 3. Effects of mecamylamine and placebo capsules given at ↑ on systolic and diastolic blood pressure and heart rate (BPM=beats per minute). Points shown are mean±SEM; n=9. ●: Mecamylamine, 10 mg; ○: placebo.

attenuated the decrease in theta power usually observed after nicotine gum. In this condition the mecamylamine × nicotine interaction was statistically nonsignificant ($F=0.15$, $p=0.86$). Following mecamylamine pretreatment, alpha frequency did not change after the placebo and 4 mg of nicotine gum, however, mecamylamine did not prevent the increase in alpha frequency after the 8 mg nicotine condition (Fig. 4). Mecamylamine pretreatment did not block any other physiologic effects of the nicotine gum. For example, mecamylamine did not prevent the decrease in alpha or beta frequency caused by the gum.

Self-reported ratings of the gum dose strength was significantly diminished by mecamylamine pretreatment ($t_d=2.530$, $p=0.03$). Mecamylamine did not change ratings of the similarity of gum to cigarettes, desire to smoke, or desire to take another dose of gum.

DISCUSSION

As has been previously reported, nicotine administration to nicotine-deprived cigarette smokers produced EEG effects associated with cortical arousal [3]. That is, alpha and beta frequency increased and theta power decreased. Similar effects have been reported after gum-delivered [23] or intravenous nicotine [15] or inhaled tobacco smoke [10,32]. The effects of mecamylamine on these measures of human EEG activity have not been previously reported. Most interestingly, at a dose that did not significantly alter blood pressure or heart rate, mecamylamine enhanced EEG responses that typically accompany early signs of tobacco withdrawal [10,32]. Specifically, mecamylamine increased theta power and decreased alpha frequency. It remains to be determined if mecamylamine would induce similar EEG changes in non-tobacco-deprived cigarette smokers or in non-smokers.

After the mecamylamine pretreatment, some of the EEG effects of the nicotine gum were prevented, others were not. For example, mecamylamine pretreatment prevented the in-

crease in alpha frequency usually seen after the 4 mg gum but it did not prevent the increase in alpha frequency after the 8 mg gum. Mecamylamine attenuated the decrease in theta power after the gum but it did not prevent the decrease in beta power. These data suggest that nicotine changes cortical EEG activity by actions at central regions that vary in their sensitivity to mecamylamine blockade. The dose-related antagonism on the alpha frequency suggests that in some systems a pharmacologic antagonism occurs. Mecamylamine significantly decreased the ratings of gum strength. This finding is analogous to those of other studies in which it has been reported that mecamylamine blocks behavioral effects of nicotine. For example, mecamylamine pretreatment antagonized the discriminative, reinforcing, and punishing properties of nicotine in animals [6]. Similarly, London *et al.* [14] reported that mecamylamine completely antagonized nicotine-induced changes in local cerebral glucose utilization in rats. In cigarette smokers, mecamylamine increased cigarette consumption and impaired performance on the digit symbol substitution test [19, 24, 30]. Henningfield *et al.* [8] reported that mecamylamine prevented changes in skin temperature, pupil diameter and subjective responses caused by intravenous nicotine. The subjective responses of nicotine antagonized by mecamylamine included: desire to smoke, drug liking, amphetamine-like effects and rating of nicotine dose strength. Finally, earlier studies have shown that mecamylamine-treated smokers report less satisfaction from their cigarettes [11,20]. Taken together, these findings indicate that several effects of cigarette smoking are mediated by central actions of nicotine that are antagonized by nicotine blockers.

The 10 mg dose of mecamylamine we used did not cause significant cardiovascular or subjective effects. These results are consistent with those of Nemeth-Coslett *et al.* [20] who also found mecamylamine caused little discomfort at 10 mg and minimal or nonsignificant subjective effects in volunteers given 20 mg. Although we observed no significant cardiovascular or subjective effects, this mecamylamine dose level produced changes in the EEG.

There are alternative explanations for the mecamylamine-induced EEG changes observed in the present study but the most plausible one appears as follows: Mecamylamine may inhibit the effects of residual circulating nicotine at central nicotine receptors. This possibility is consistent with evidence from animal studies which suggest the presence of at least two brain nicotine receptor sites: a C-10, α-bungarotoxin sensitive low affinity receptor [18,21] and a high affinity receptor that binds [^{3}H]-nicotine but is distributed differently than the α-bungarotoxin site [17]. Recent evidence also indicates that mecamylamine blocks nicotine responses at two receptor subtypes which may be those labeled with α-bungarotoxin or [^{3}H]-nicotine [2]. In binding studies mecamylamine did not displace ligands from nicotinic receptors [1, 17, 25] suggesting that the effects of mecamylamine are due to an inhibition of activated ion channels [2]. However, Sloan *et al.* [28] found that mecamylamine binds directly to a low affinity nicotine binding site which they proposed is important for many physiological responses.

Further study will be required to conclusively evaluate the mecamylamine-nicotine interactions that mediate the brain mechanisms responsible for the EEG changes we observed. For example, there have been no studies of the effects of mecamylamine on the EEG of nonsmokers. If it were shown that mecamylamine has similar effects in nonsmokers or in nicotine-tolerant subjects, it could be argued that the antagonism we observed was of a physiologic rather than pharmacologic nature. These studies could clarify the role of nicotine processes in cortical regulation. For example, there is a diffuse distribution of cholinergic neurons throughout the neuraxis [16] which appears to be involved in the regulation of electrophysiologic responses [27] and the cortical EEG [22]. The actions of nicotine on the cortical EEG and the mecamylamine antagonism we observed suggest that nicotine cholinergic mechanisms may be partly responsible for the endogenous regulation of cortical activity.

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