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ACTH 4–9 Effects on the Human Visual Event-Related Potential

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SANDMAN, C. A., C. BERKA, B. B. WALKER AND J. VEITH. *ACTH 4–9 effects on the human visual event-related potential.* PEPTIDES 6(5) 803–807, 1985.—Three oral doses (5, 10 and 20 mg) of an analog of ACTH 4–9 were compared with a vehicle control and d-amphetamine (10 mg). In a double-blind procedure, five men and five women were tested at weekly intervals with each treatment. In each session, four visual event-related potentials (ERPs) were recorded at hourly intervals. Visual ERPs were averaged from the electroencephalogram recorded from the left and right hemisphere. Dosage, time after administration, hemisphere of the brain and sex of the subject influenced the ERP. The ACTH 4–9 analog decreased amplitude of P100 but increased integrated activity of the ERP. This effect peaked at 60 minutes then "recovered." The effects of the peptide were more pronounced with doses of 5 and 10 mg, in the right hemisphere of men and in the left hemisphere of women. The findings indicated that the ACTH 4–9 analog influenced components of ERP commonly related to the perceptual/attentional state of the organism in a sexually dimorphic manner.

Peptides Visual event-related potentials Sex differences Attention MSH/ACTH and brain laterality

EARLY research with rats indicated that injections with MSH or ACTH fragments produced high amplitude, 4–9 Hz EEG activity [9]. The initial study of somatosensory event-related potentials (ERPs) suggested that MSH increased the amplitude of early components (P100) in both endocrine and neurological patients [6]. In a later study involving a behavioral task, the continuous performance task (CPT), injections of ACTH 4–10 in normal volunteers resulted in increased latency and decreased amplitude of the P200 complex after visual stimulation [7]. Even though these studies differed in several ways, changes in early components, rather than later components, were augmented by the naturally occurring MSH/ACTH peptide. The studies of Born *et al.* [3] and Rockstroch *et al.* [8] also suggested disinhibition of exogenous components by ACTH-like molecules. In addition Rockstroh *et al.* [8] indicated that only simple tasks may enhance later components (e.g., P300) in the presence of ACTH. The current experiment was designed to assess the time course of three doses of a long-acting ACTH 4–9 analog on the visual ERP. These effects were compared with placebo and a positive control, d-amphetamine. The d-amphetamine was employed as a "control" to test the belief that the ACTH fragments may possess undifferentiated activational properties. Further, since sexually dimorphic responses have been suggested for MSH/ACTH peptides [2,12], both men and women served as subjects.

METHOD

Subjects

Five normal men and five normal women between the ages of 22 and 37 gave informed consent to serve as subjects. All of the participants were knowledgeable about the action of peptides on brain and behavior. The subjects were healthy and none had a history of medical complications.

Procedure

In a double-blind, crossover procedure, the subjects received 0, 5, 10 and 20 mg of the ACTH 4–9 analog or the

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TABLE 1

LATIN-SQUARE DESIGN TO CONTROL FOR EFFECTS OF ORDER OF TREATMENT IN STUDY OF ACTH 4–9 ANALOG ON EVENT-RELATED POTENTIALS OF THE BRAIN

	Treatment Weeks				
Subjects	1	2	3	4	5
1,6	Placebo	5 mg	10 mg	20 mg	d-amp
2,7	5 mg	10 mg	20 mg	d-amp	Placebo
3,8	10 mg	20 mg	d-amp	Placebo	5 mg
4,9	20 mg	d-amp	Placebo	5 mg	10 mg
5,10	d-amp	Placebo	5 mg	10 mg	20 mg

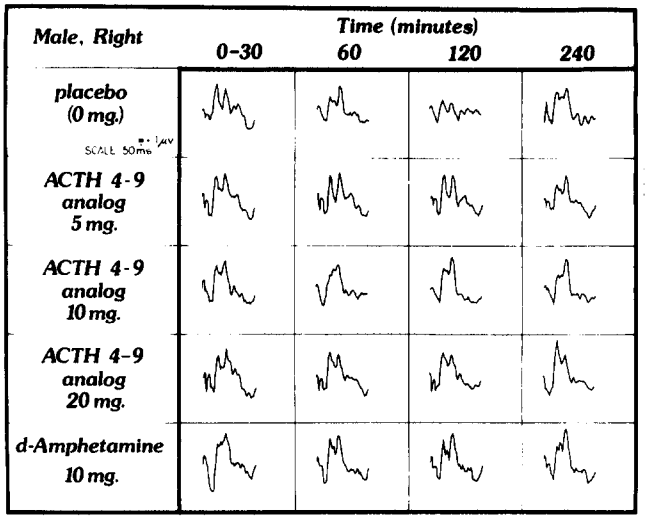

FIG. 1. Evoked responses recorded from the right hemisphere of men for each dose and temporal delay after treatment.

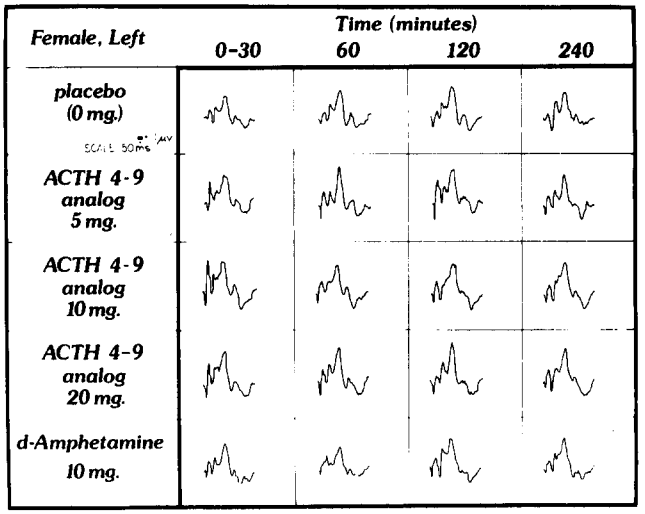

FIG. 2. Evoked responses recorded from the left hemisphere of women for dose and temporal delay after treatment.

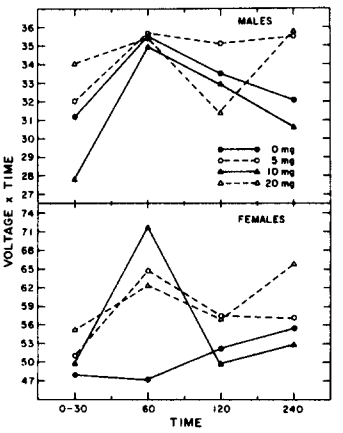

FIG. 3. Effects of different doses in men and women of the ACTH 4–9 analog on measures of area-under-the-curve (power).

positive control, 10 mg d-amphetamine (kindly supplied in coded forms by Organon, Oss, The Netherlands). The different doses and compounds were given at the same time each session, exactly one week apart. Thus, each subject participated for five successive weeks. The order of treatment was balanced by a Latin-square design (see Table 1). Each session was five hours long and was conducted between 8 a.m. and 1 p.m. Subjects were instructed to refrain from drugs and stimulants (such as coffee) for 24 hours prior to testing and to eat breakfast 45 minutes before the test session.

The subjects were tested in a sound attenuated and electrically shielded room. The recording, programming and stimulus presentation equipment were housed in an adjacent room. Subjects were reclined in a comfortable chair and instructed to keep their eyes open and fixate on a dot on a screen in front of them. They were asked to limit muscle movement and to breathe normally. A masking "white" noise was delivered through headphones to eliminate extraneous auditory distractions. One hundred flashes of light (20 msec), separated by at least 4 seconds, were presented to subjects as they viewed the dot.

The experimental session was divided into four time periods. The periods corresponded to the time after receiving the capsule and the procedures for each period were identical. The testing periods after the treatment were: 0–30 minutes; 60 minutes; 120 minutes and 240 minutes.

EEG. Bilateral ERP's were recorded from the right and left occiput for each subject with monopolar O_1 and O_2 placements referenced to linked mastoids according to the international 10–20 system. The electrode sites were swabbed with acetone and rubbed with EEG cream before electrodes were attached. The electrodes (Ag/AgCl) were filled with EEG cream and fixed to the skull with collodium. The electrode pairs were matched for impedence with any pair exceeding 10,000 Ω being replaced. Recording of the EEG was done with a Grass, Model 7, polygraph equipped with appropriate preamplifiers and driver amplifiers. The signal was amplified by a Grass AC preamplifier with the low frequency filters set at 1 Hz (which corresponds to a time constant of 0.1 sec) and the high frequency filter set at 35 Hz [5]. Two channels of the EEG from the right and left hemisphere were recorded on a Sony FM tape recorder for off-line computer analyses.

Eye blinks. Silver-silver chloride electrodes filled with

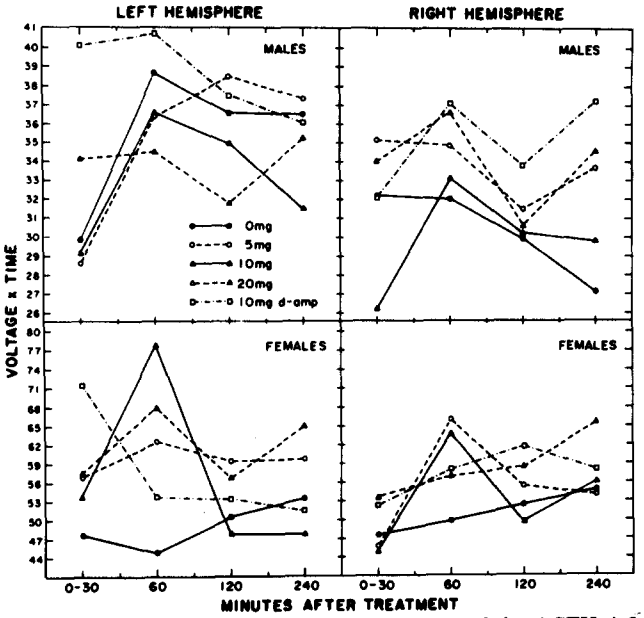

FIG. 4. Power (area-under-the-curve) measures of the ACTH 4–9 analog in the right and left hemisphere of the brain in men and women. (Time on the ordinate refers to the msec duration of the ERP).

EKG Sol were placed below and on the outer canthus of the eye. The signal was amplified with a Grass AC amplifier. All flashes associated with eye movements or blinks were repeated.

Data Reduction

Electrocortical activity associated with the 100 flashes of light for each hemisphere and each period was converted from analog to digital at a sampling rate of 100 Hz for a period of 500 msec following the flash. These data were averaged with a Nova 3/12 computer resulting in 8 ERPs for each session. The ERP was zeroed by subtracting the average of all of the points across time from each sampling period.

Three measurements were taken from each of the ERP's: latency, component amplitude, and area under the curve. Latency refers to the measurement (in msec) from the onset of the flash to the positive peak occurring approximately 100 msec after the flash (P1), approximately 200 msec after the flash (P2), and approximately 300 msec after the flash (P3). The absolute amplitude of each component was measured by calculating the voltage difference between two consecutive peaks of opposite polarity. Area under the curve was measured by integrating time and voltage of the wave form providing an estimate of total power.

RESULTS

Descriptive Data

The effects of dose and time of the ERP's measured from the right hemisphere in men and the left hemisphere in women are illustrated in Figs. 1 and 2. For the men, the most noticeable effect was the emergence of bi-modality after 5 mg of ACTH 4–9 persisting at least 120 minutes. Further, when compared with placebo, all doses of the peptide and d-amphetamine appeared to attenuate ERP habituation. All

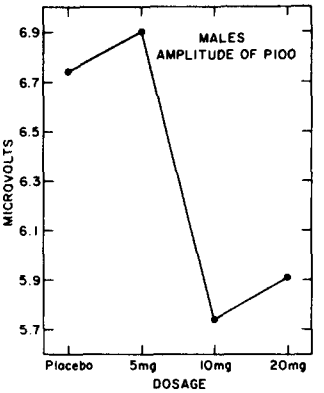

FIG. 5. Amplitude of P100 of the ERP as a function of dosage of the ACTH 4–9 analog in men.

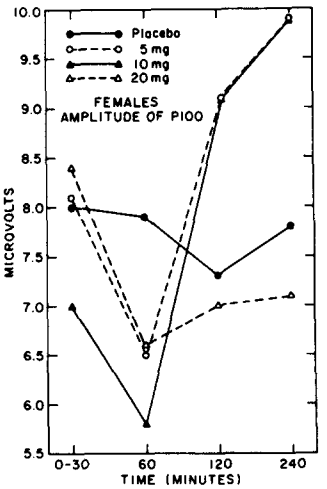

FIG. 6. Amplitude of P100 of the ERP as a function of dosage and time after treatment in women.

doses of the peptide appeared to increase the amplitude of the ERP in women, especially the P200 component.

Statistical Model

The influence of treatment with the MSH/ACTH 4–9 analog was evaluated with a complex factorial design. The influence of sex of subject was included initially as a factor then evaluated separately with a 2 (hemisphere) ×5 (dose/drug) ×4 (time) analysis of variance with repeated measures of all factors. In addition, trend analysis using orthogonal polynomials was computed to permit refined analysis of dosage and of time.

Measures of Power

As depicted in Fig. 3, several complex relationships were detected among the independent variables. The main effect of sex of the subject, $F(1,8)=6.17$, $p<0.05$, indicated that men and women generated different ERPs. Inspection of the values on the ordinate of Fig. 3 reveals that women evidenced much higher voltages integrated over time than men. Figure 3 also illustrates the relationships between dosage and

time. The biphasic influence of time is significant, $F(1,8)=10.60$, $p<0.01$, since peptide effects peaked at 60 min. Both the linear and cubic components of dosage were related to quadratic, $F(1,8)=5.02$, $p<0.05$, and cubic, $F(1,8)=9.93$, $p<0.01$, trends of time. These trends are illustrated in Fig. 3. For women, the biphasic relationships between dose and time are very clear when compared with placebo. All doses peaked at 60 minutes, declined by 120 minutes and accelerated at 240 minutes. This pattern is present in males to a lesser degree.

Figure 4 presents data for men and women in the right and left hemisphere for each dose and time. This 4-way interaction is significant, $F(1,8)=13.55$, $p<0.01$, with a linear effect of dose and a biphasic trend of time. For males the effect of the peptide relative to placebo is different for the right and left hemisphere of the brain. The doses of peptide increased power measurements relative to placebo in the right hemisphere, however, in the left hemisphere the relationships are partially reversed. The statistical effect is due primarily to the placebo. The influence of 10 mg of d-amphetamine does not appear to be lateralized since area-under-the-curve is increased in each hemisphere.

The influence of the peptide on the measure of power is more uncomplicated in women than men. For women, the effect of placebo is relatively unchanged over time for both hemispheres. However, the doses of peptide resulted in increased power especially at the 60 minute period as illustrated in Fig. 4. This effect is especially pronounced in the left hemisphere. The influence of d-amphetamine is unremarkable.

Peak amplitude, men. As illustrated in Fig. 5, 10 and 20 mg of the ACTH 4–9 analog significantly, $F(3,12)=3.80$, $p<0.04$, diminished the amplitude of the P100 component in men. The influence of the peptide on P200 in men was reflected in a significant interaction between the linear, $F(1,14)=30.12$, $p<0.005$, and quadratic, $F(1,4)=11.88$, $p<0.03$, components of dosage and time. These effects indicated that all doses of the peptide, especially 5 and 10 mg, enhanced P200 for up to 120 minutes after administration. A nonsignificant trend, $F(1,4)=3.00$, $p<0.15$, suggested that 10 mg increased the amplitude of P200 relative to the other treatments.

Peak amplitude, women. In women the influence of the peptide analog on the amplitude of P100 is illustrated in Fig. 6. The interaction between cubic components of dosage and linear component of time was significant, $F(1,4)=11.63$, $p<0.03$. The response to treatment with placebo was comparatively flat. The effects of 10 mg are most pronounced, characterized by an initial decrease in amplitude (at 60 min) followed by large increases at 120 and 240 minutes. This response is somewhat paralleled by 5 mg. The effects also resulted in initial decreases in P100, however, the recovery is not observed by 120 or 240 minutes.

Peak delay. There were no reliable effects of any of the independent variables on the latency of P1, P2 or P3.

DISCUSSION

The results of the present study indicated that the orally administered analog of MSH/ACTH 4–9 has a dose and time dependent influence on the ERP. Further, the time and dosage effects interact. Specifically, 5 and 10 mg of the peptide analog produced an initial effect on both power measures and P100 which peaked at 60 minutes. Interestingly, the effect is initial suppression of P100 followed by rapid recovery. The effect of 20 mg followed the early time course of 5 and 10 mg but failed to induce "recovery" at 120 and 240 minutes. This effect was more evident in women than in men.

These data may suggest that neural efficiency would be enhanced 60 minutes and later following administration of 5 or 10 mg of ACTH 4–9. No evidence of enhancement in this time period was apparent of the 20 mg dose. Behavioral data from studies of the ACTH 4–9 analog with mentally retarded subjects support this conclusion [11]. The performance of tasks by retarded subjects was significantly enhanced during a 3 hour period by 5 and 10 mg of ACTH 4–9 but significantly depressed by 20 mg. These data suggest either that the 5–10 mg dose is the optimal range for neural enhancement in human subjects or that the dosage interaction with time has not been completely explored. Thus, the 20 mg dose may show "recovery" (and therefore neural/behavioral enhancement) sometime beyond the 240 minutes studied. Further studies are required to explore these possibilities.

The data of this experiment supplement earlier electrophysiological studies of MSH/ACTH fragments [6,7]. Since no cognitive demands were placed on the subjects in the present experiment, the effects of the peptide should emerge between 50–200 msec. Indeed, all of the effects were observed within this time frame, an effect reported earlier for MSH [5] and the ACTH 4–9 analog [3,8]. The fact that later components (P300) of the ERP, which reflect cognitive activity, were not influenced by ACTH 4–10 in one earlier study with cognitive demands [7] and only with a simple paradigm in a second study [8], suggests that the influence of the MSH/ACTH peptides may relate primarily to attentional or perceptual processing [6, 7, 10].

The finding that sex of the subject and hemisphere of the brain are influenced differently by the ACTH fragments complements earlier studies with these peptides. Studies of rats [2,4] indicated that MSH enhanced visual attention only in male animals. Separate studies with normal human subjects indicated that MSH/ACTH 4–10 enhanced visual attention in men [10] but that verbal memory was influenced in women [11]. The sexual dimorphism observed behaviorally may be related to the lateralized influence of the peptide analog on the cerebral hemispheres reported in the present study.

There was no evidence that d-amphetamine, in the time course studied, paralleled the actions of the analog of MSH/ACTH 4–9. Even though behavioral studies [1] have generated similar conclusions, the belief persists that amphetamine and ACTH fragments may have identical actions. The electrophysiological data of this study buttresses the argument that MSH/ACTH fragments are not nonspecific arousing substances but may exert specific behavioral and electrophysiological effects.

ACKNOWLEDGEMENT

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REFERENCES

1. Beckwith, B. E., C. A. Sandman, W. D. Alexander, M. D. Gerald and H. Goldman. D-amphetamine effects on attention and memory in the albino and hooded rats. *Pharmacol Biochem Behav* **2:** 557–561, 1974.
2. Beckwith, B. E., C. A. Sandman, D. Hotherall and A. J. Kastin. The influence of neonatal injections of α-MSH on learning, memory and attention in rats. *Physiol Behav* **18:** 63–71, 1977.
3. Born, J., G. Wolfsdorf, M. Schiebe, B. Rockstroh, H. L. Fehm and K. H. Voigt. Dishabituating effects of an ACTH 4–9 analog in a vigilance task. *Pharmacol Biochem Behav* **21:** 513–519, 1984.
4. Champney, T. F., T. C. Shaley and C. A. Sandman. Effects of neonatal cerebral ventricular injections on learning in male and female albino rats. *Pharmacol Biochem Behav* **5:** 3–9, 1976.
5. Goff, W. R. Human average evoked potentials: Procedure for stimulating and recording. In: *Bioelectric Recording Technique (Part B)*, edited by R. F. Thompson and M. M. Patterson. New York: Academic Press, 1974, pp. 102–156.
6. Kastin, A. J., L. H. Miller, W. D. Gonzalez-Barcena, W. D. Hawley, K. Dyster-Aas, A. V. Schally, M. L. V. DeParra and M. Velasco. Psychophysiologic correlates of MSH activity in man. *Physiol Behav* **7:** 893–986, 1971.
7. Miller, L. H., L. C. Harris, H. van Riezen and A. J. Kastin. Neuroheptapeptide influence on attention and memory in man. *Pharmacol Biochem Behav* **5:** 17–22, 1976.
8. Rockstroh, B., T. Elbert, W. Lutzevberger, N. Birbaumer, H. L. Fehm and K. H. Voigt. Effect of an ACTH 4–9 analog on human cortical evoked potentials in a constant foreperiod reaction time paradigm. *Psychoneuroendocrinology* **6:** 301–310, 1981.
9. Sandman, C. A., P. M. Denman, L. J. Miller, J. R. Knott, A. V. Schally and A. J. Kastin. Electroencephalographic measures of melanocyte-stimulating hormone activity. *J Comp Physiol Psychol* **76:** 103–109, 1971.
10. Sandman, C. A., J. George, J. D. Nolan, H. van Reizen and A. J. Kastin. Enhancement of attention in man with ACTH/MSH 4–10. *Physiol Behav* **15:** 427–431, 1975.
11. Sandman, C. A., B. B. Walker and C. A. Lawton. An analog of MSH/ACTH 4–9 enhances interpersonal and environmental awareness in mentally retarded adults. *Peptides* **1:** 109–114, 1980.
12. Veith, J. L., C. A. Sandman, J. George and V. C. Stevens. Effects of MSH/ACTH 4–10 on memory, attention and endogenous hormone levels in women. *Physiol Behav* **20:** 43–50, 1978.