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# Polypeptide Influences on Attention, Memory and Anxiety in Man[1,2]

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MILLER, L. H., A. J. KASTIN, C. A. SANDMAN, M. FINK AND W. J. VAN VEEN. *Polypeptide influences on attention, memory and anxiety in man.* PHARMAC. BIOCHEM. BEHAV. 2(5) 663–668, 1974. – The effects of two polypeptides, $ACTH_{1-24}$ and $ACTH_{4-10}$ on a variety of bioelectric and behavioral measures of attention, memory and anxiety in human subjects were examined within the context of a disjunctive reaction time paradigm. $ACTH_{1-24}$ had no effect on any of the measures involved; $ACTH_{4-10}$, however, served to improve visual memory, decrease anxiety, reinstitute a previously habituated alpha blocking response in the occipital EEG, and generally influence the occipital EEG toward a pattern consistant with increased attention. The results were taken to suggest a direct polypeptide influence on CNS attentional mechanisms.

ACTH Polypeptide Autonomic EEG Attention Memory Anxiety Human

IT is becoming increasingly apparent that short-chain polypeptides such as melanocyte stimulating hormone (MSH) and analogous adrenocorticotrophic hormone (ACTH) fractions ($ACTH_{1-10}$, $ACTH_{4-10}$) influence both behavior and the electrophysiological activity of the brain. (It should be noted that the heptapeptide known as $ACTH_{4-10}$ (Met-His-Phe-Arg-Try-Gly) is a constituent of both ACTH and MSH. DeWied has identified this segment

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with the direct CNS effects of these two hormones.) In the rat, such polypeptides render conditioned avoidance [2, 3, 5, 13] and appetitive [12] behaviors resistant to extinction, facilitate reversal learning [10,14], and interfere with conditional learning [16]. These peptides also enhance short-term visual, but not verbal memory, in human subjects [9]. Arousal [13], emotionality [12], memory [2, 3, 5, 9, 13], and attention [10,14], have been invoked as psychological explanations of these various behavioral effects.

The attentional demands of behavioral tasks have been reported to interact with MSH to significantly increase the magnitude of somatic evoked potentials in a mixed group of subjects, consisting mainly of patients with hypopituitarism of varying etiology [9 . Other human EEG studies report a disintegration of alpha activity that could be related to attentional processes [8]. Animal EEG studies report an increase in intermediate frequency, high voltage activity [6,11], which some authors speculate may be related to activation of neural processes underlying memory functions [14].

These behavioral and neurophysiological findings indicate that ACTH and its fractional analogues have direct, extra-adrenal effects on central nervous system (CNS) functioning. In fact, glucocortico-steroids have been shown to have effects opposite to those of ACTH and its analogues [4]. In order to provide additional definition of the putative direct CNS influences of ACTH and its analogues, two experiments were conducted: one involved an ACTH fraction ($ACTH_{1-24}$) with maximal adrenocorticotrophic activity; the other involved a fraction practically devoid of such properties ($ACTH_{4-10}$). (The $ACTH_{4-10}$ (01-63) was kindly supplied by Dr. Henk van Riezen of Organon International BV, The Netherlands.) Both peptides were compared with saline controls in terms of their effects on short-term visual memory, anxiety state, and disjunctive reaction time as well as their concurrent effects on the contingent negative variation [18], occipital and frontal EEG, digital and cephalic pulse volumes, heartrate, electrodermal activity, and respiration.

## EXPERIMENT 1

### *Method*

Twenty healthy, young (22–24 years), male medical student paid volunteers were randomly assigned to either experimental (N = 10) or control groups (N = 10) in double blind fashion. Subjects arrived at the laboratory and were administered the rod and frame test [19] and the trait section of the State-Trait Anxiety Inventory (STAI) [17] to provide data as to proneness to suggestion and to anxiety. Transducers for the measurement of cephalic pulse amplitude (CPA), digital pulse amplitude (DPA), galvanic skin potentials (GSP), heartrate (HR), frontal and occipital EEG, contingent negative variation (CNV) and respiration were attached and subjects were taken to an 80 dB sound-attenuated room where they were seated in a comfortable reclining armchair. Subjects were then presented with a fixed fore-period, disjunctive reaction time task (DRT) consisting of 25 response and 25 nonresponse trials. The various bioelectric measures detailed above were recorded throughout the period, with particular attention being paid to responses occurring within the 4 sec interstimulus interval (ISI) of the DRT. Following this initial DRT procedure, subjects were administered the State section of the STAI and a modified (1 sec exposure, 30 sec delay) Benton Visual Retention Test (BVRT) [1] prior to receiving an i.v. injection of either 0.5 mg of $ACTH_{1-24}$ (100 units activity/mg) dissolved in 0.9% NaCl or placebo (diluent). Bioelectric measures were again recorded throughout the postinjection DRT. At the conclusion of the second DRT procedure (approximately 1 hr postinjection), subjects were administered the State sectionof the STAI and a second, equivalent, form of the modified BVRT.

Data were statistically analyzed within the framework of a 2 (drug) × 2 (pre–postinjection) repeated measures of analysis of variance.

### *Results*

No differences were found in rod and frame and trait anxiety scores between experimental and control groups. $ACTH_{1-24}$ was not found to have a significant effect on any of the psychological or physiological variables studied.

## EXPERIMENT 2

### *Method*

Another 20 subjects were drawn from the same population as in Experiment 1 and all procedures were identical except that the experimental group received an i.v. injection of 10 mg $ACTH_{4-10}$ dissolved in 2 ml 0.9% NaCl; controls received an injection of 2 ml diluent.

Data were analyzed in the same fashion as in Experiment 1.

### *Results*

Significance levels of the results from Experiments 1 and 2 are summarized in Table 1. Means and standard errors of variables affected by $ACTH_{4-10}$ are presented in Table 2. $ACTH_{4-10}$ improved performance on the BVRT to a marginally significant degree ($p<0.10$) as per analysis of variance procedures. Further analysis employing $t$ tests for correlated observations revealed that while both control and experimental groups showed improved performance on their second BVRT testing, only the experimental group improved significantly ($p<0.02$).

Both the experimental and control groups reported a reduction in state anxiety on their second testing with the experimental group reporting the greater reduction. Analysis of variance indicated only a marginally significant effect of $ACTH_{4-10}$ on anxiety reduction ($p<0.10$) but correlated $t$ tests yielded probability values of $p<0.05$ for the experimental group and $p<0.20$ for the controls.

The occipital EEG ($0_2-0_1$, Int. 10–20 system) was passed through four bandpass filters (<3 Hz, 3–7 Hz, 7–12 Hz and 12+ Hz) and analysed in terms of the power output (RMS) of the filters. Controls showed a pre- to post-injection decline in the power output of the 12+ Hz and the 7–12 Hz band, but showed an increase in the output of the 3–7 Hz band. The $ACTH_{4-10}$ group, on the other hand, exhibited an increase in the power output of the 12+ Hz and 7–12 Hz bands coupled with a slight decrease in the output of the 3–7 Hz band. The effect of $ACTH_{4-10}$ on the power output of both the 12+ Hz and 7–12 Hz frequency bands was significant at the 0.001 level.

Visual inspection of occipital EEG patterns changes following injection of $ACTH_{4-10}$ or diluent showed (Fig. 1) that the Alpha (7–12 Hz) blocking response

TABLE 1

DIRECTION OF ACTH RELATED PRE–POST CHANGES IN DEPENDENT VARIABLES COMPARED TO CONTROLS: EXPERIMENTS 1 AND 2

| | $ACTH_{1-24}$ | $ACTH_{4-10}$ |
|---|---|---|
| Galvanic Skin Potential | * | * |
| Galvanic Skin Resistance | * | * |
| Respiration | * | * |
| Heartrate | * | * |
| Cephalic Pulse Amplitude | * | * |
| Digital Pulse Amplitude | * | * |
| Contingent Negative Variation | * | * |
| EEG ($0_2-0_1$) | | |
| 1. Percent/time | | |
| a. 0–3 Hz | * | * |
| b. 3–7 Hz | * | † |
| c. 7–12 Hz | * | ‡ |
| d. 12 + Hz | * | ‡ |
| 2. Power output (RMS) | | |
| a. 0–3 Hz | * | * |
| b. 3–7 Hz | * | † |
| c. 7–12 Hz | * | ‡ |
| d. 12 + Hz | * | ‡ |
| Benton Visual Retention | * | ‡ |
| State Anxiety | * | † |
| Disjunctive Reaction Time | * | * |

*No change.
†Significant ($p<0.05$) decrease compared to saline controls.
‡Significant ($p<0.05$) increase compared to saline controls.

commonly seen in the ISI of behavioral tasks such as the one employed tended to habituate over the course of the preinjection trials in both groups. Controls tended to recover this habituated response briefly following saline injection but the recovery was short-lived. The $ACTH_{4-10}$ group, however, displayed a postinjection recovery of the alpha blocking response over the course of the postinjection trials that was quite robust. $ACTH_{4-10}$ subjects also exhibited significantly ($p<0.01$) more 7–12 Hz activity (% time occurrence) in the postinjection intertrial intervals (ITIs) than did controls.

$ACTH_{4-10}$ was not found to have a significant effect on any of the other bioelectric measures examined. The CNV for both response and nonresponse conditions did tend to be somewhat more negative ($p<0.15$) following $ACTH_{4-10}$ injections, however.

Anecdotal data were also of interest. Post experiment interviews revealed that 8 of 10 experimental subjects believed they had received something other than saline, while 3 of 10 control subjects incorrectly stated they had received something other than saline. The remarks of 2 subjects from the experimental group are of particular interest: one stated "Wow! I sure didn't get saline." In response to questions regarding his psychological state he reported that he felt very alert, but very relaxed, and felt that he could study very effectively in his present condition: "I feel like I could go home and study now and it would really stick". Another subject reported a similar mental state which seemed to be somewhat less intense than that of the first.

No significant differences were found between groups in either Rod and Frame ($ACTH_{4-10}$ $\bar{X}$ = 2.56, S.E. = 0.42, Saline $\bar{X}$ = 2.77, S.E. = 0.46) or trait anxiety ($ACTH_{4-10}$ $\bar{X}$ = 35.7, S.E. = 1.56; Saline $\bar{X}$ = 34.9, S.E. = 2.3) scores.

## DISCUSSION

$ACTH_{1-24}$ had no effect on any of the behavioral or bioelectric measures taken. $ACTH_{4-10}$, on the other hand, improved visual memory, decreased anxiety and produced significant changes in occipital EEG patterns within the experimental group. The differences in the effects of these two short-chain polypeptides, however, could well be a function of the duration of the postinjection period over which the measures were averaged. Although both fractions apparently have direct CNS effects, the $ACTH_{1-24}$ fraction also has maximal adrenocortical stimulating properties. Reports on the CNS effects of adrenal steroids indicate that they are opposite in nature to those of the polypeptides tested [4,20]. Thus, the averaging of $ACTH_{1-24}$ and steroid effects could well have concealed their individual influences on CNS activity.

Neither polypeptide influenced the measures of autonomic activity observed. This would tend to weaken the viability of constructs such as generalized emotionality and arousal as psychological explanations of the behavioral effects of these short-chain polypeptides. Explanations invoking general orienting reflex [15] activity are likewise unsupported by our data.

The replication of an earlier finding of improved visual memory following injection of $ACTH_{4-10}$ strengthens memory, and perhaps attention, as possible explanatory constructs. The initial study [9] involved mainly patients with hypopituatarism of various etiologies and the polypeptide involved was synthetic $\alpha$ MSH. The finding of significant imporvement in visual memory of the $ACTH_{4-10}$ group in the present study indicates the generality of short-chain polypeptide effects on short-term visual memory across a wide range of subjects.

The postinjection recovery and persistance of previously habituated EEG arousal response patterns generally elicited by novel stimulation is very much in agreement with the findings of Endröczi *et al.* [7] who reported a recovery of a stimulus-specific pattern of EEG arousal following i.v. injection of $ACTH_{1-24}$ and $ACTH_{1-10}$ which they interpreted as representing a disinhibition of CNS activating mechanisms. $ACTH_{1-24}$, however, did not elicit such stimulus-specific EEG response patterns in their study.

The latency of the effect described by Endröczi *et al.* [7] was much longer than observed in our present study. In their study an EEG effect was not evident in the case of $ACTH_{1-24}$ until the day following injection. The effects of $ACTH_{1-10}$ were observed to occur earlier however. $ACTH_{1-24}$ was not found to have a CNS effect. In our study the CNS effect was apparent in 15–30 min for most $ACTH_{4-10}$ subjects, and no effect was seen in the $ACTH_{1-24}$ subjects. Perhaps retesting on the day following injection would have shown an effect for $ACTH_{1-24}$ as

TABLE 2

MEANS AND STANDARD ERRORS FOR EEG, VISUAL MEMORY AND ANXIETY MEASURES FOR EXPERIMENTAL AND CONTROL GROUPS OF EXPERIMENT 2

| | $ACTH_{4-10}$ | | | | Saline | | | |
|---|---|---|---|---|---|---|---|---|
| | Pre | | Post | | Pre | | Post | |
| Variable | $\bar{X}$ | (SE) | $\bar{X}$ | (SE) | $\bar{X}$ | (SE) | $\bar{X}$ | (SE) |
| Occipital EEG (overall) | | | | | | | | |
| % times | | | | | | | | |
| 0–3 Hz | | | | | | | | |
| 3–7 Hz | 76.6 | (1.19) | 75.7 | (1.11) | 74.0 | (1.79) | 76.3 | (1.99) |
| 7–12 Hz | 77.6 | (1.49) | 79.88 | (2.3) | 75.3 | (2.17) | 76.7 | (2.47) |
| 12 + Hz | 80.5 | (1.29) | 84.38 | (2.16) | 74.33 | (3.77) | 70.0 | (3.45) |
| Power Output | | | | | | | | |
| (RMS)* | | | | | | | | |
| 0–3 Hz | | | | | | | | |
| 3–7 Hz | 55.8 | (2.6) | 53.5 | (3.3) | 49.0 | (3.0) | 54.0 | (4.0) |
| 7–12 Hz | 58.1 | (3.3) | 62.8 | (3.8) | 58.3 | (3.5) | 57.3 | (5.3) |
| 12 + Hz | 65.7 | (3.5) | 69.7 | (6.1) | 53.3 | (6.3) | 51.8 | (6.12) |
| (Intertrial Interval) | | | | | | | | |
| % time | | | | | | | | |
| 0–3 Hz | | | | | | | | |
| 3–7 Hz | 78.0 | (2.0) | 76.2 | (1.2) | 75.2 | (1.8) | 80.2 | (2.00) |
| 7–12 Hz | 79.4 | (1.9) | 82.1 | (1.5) | 76.1 | (2.0) | 77.2 | (2.6) |
| 12 + Hz | 75.3 | (1.4) | 76.9 | (2.0) | 77.2 | (3.4) | 75.3 | (3.5) |
| BVRT (errors) | 5.1 | (0.71) | 4.3 | (0.39) | 4.1 | (0.28) | 4.0 | (0.47) |
| State Anxiety | 40.4 | (3.19) | 37.2 | (1.97) | 38.47 | (2.31) | 37.6 | (1.63) |

*RMS power output reported in computer units.

well as a continuation of the $ACTH_{4-10}$ effect, but we were unaware of Endröczi's findings at the time we were collecting our data.

The heptapeptide, $ACTH_{4-10}$, appears to interact with the attentional demands of the environment to effect a disinhibition of CNS activating mechanisms in some unspecified way. Behaviorally, this postulated interaction would be reflected in sustained attention during repetitive tasks, with consequent enhancement of visual memory, and the development of overlearned conditional responses highly resistant to extinction. Our data do, however, indicate that the activation observed in CNS specific and does not include the autonomic nervous system. Our anecdotal data support these conclusions and suggest that the subjects receiving $ACTH_{4-10}$ were relaxed, alert, and resistant to attentional fatigue and boredom. It should be noted that our EEG data were generated by the primary visual areas of the brain which gives our results a unity that is quite seductive and suggestive.

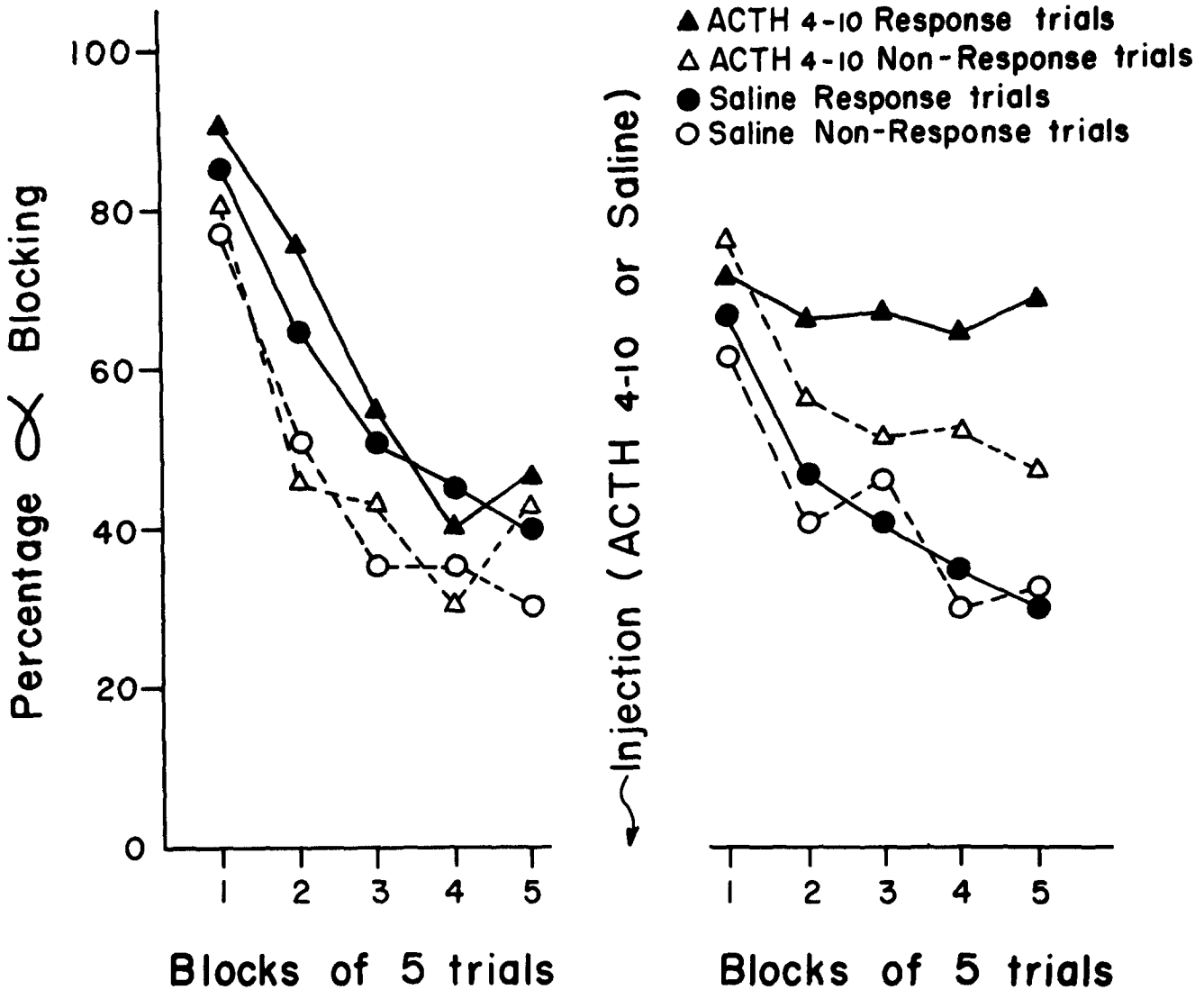


FIG. 1. Percentage of trials in which an EEG alpha blocking response was present during response and nonresponse trials in controls and experimental subjects. Note that postinjection alpha blocking occurs in a much higher percentage of trials in the experimental group than in the control groups.

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