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THE RELATIONSHIP OF ENDOGENOUS ACTH LEVELS TO VISUAL – ATTENTIONAL FUNCTIONING IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

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SUMMARY

The within subject experimental approach of either doubling cortisone medication dose or withdrawing steroid treatment for 36 hr preceding behavioral testing was found to induce corresponding significantly elevated or suppressed plasma ACTH levels, as measured by radioimmunoassay, in six of eight adults diagnosed as having congenital adrenal hyperplasia (CAH). During the session characterized by elevated ACTH levels, the CAH patients exhibited significantly reduced median reaction times on the Sternberg Item Recognition Task. Their response pattern was suggestive of facilitated visual attentional functioning and/or overt motor response capacity rather than alteration of simple cognitive processing. Moreover, Sternberg performance was significantly correlated with endogenous ACTH levels but not with levels of plasma cortisol or cortisone replacement medication. This enhancement of performance paralleled a previous finding of improved performance on the Sternberg paradigm by normal adults following exogenous administration of ACTH 4-10 (Ward *et al.*, 1979). Further analysis of the Sternberg performance suggests that other variables such as mineralocorticoid treatment, type of CAH impairment, and sex may act to moderate the degree of ACTH-related facilitation on this task. Performance on other visual and verbal attention and memory tasks, found earlier to be sensitive indices of exogenous administration of ACTH 4-10 and related fragments, was not significantly altered by manipulation of endogenous ACTH levels in these CAH patients.

INTRODUCTION

PROBLEMS involved in the examination of the influence of ACTH on behavior have included the attempt to differentiate the impact of this adenohipophyseal neuropeptide from that of concomitant variation of adrenal corticosteroids on behavioral functioning. However, this difficulty has been largely surmounted by the recognition that the fourth to tenth amino acid sequence of ACTH (ACTH 4-10) may share at least some of the behavioral influences of the larger hormone, yet it does not stimulate steroidogenesis (Sandman *et al.*, 1977; Veith *et al.*, 1978a).

Administration of ACTH 4-10 to healthy men has been shown to enhance selective visual attention, as measured by a concept formation task (Sandman *et al.*, 1975, 1977) and tests of visual memory (Miller *et al.*, 1974, 1976; Sandman *et al.*, 1975). In contrast, a study employing women (Veith *et al.*, 1978a) revealed that administration of ACTH 4-10 increased verbal memory processing without the enhancement of visual attention

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previously found in men. These results suggest that the peptide may act to facilitate sexually dimorphic modalities.

Recent work has been directed towards further clarification of the behavioral influences of the peptide. The Sternberg Item Recognition Task (SIRT) (Sternberg, 1967, 1969) permits the delineation of changes in visual attention and stimulus encoding as compared to memory processing. Men and women, following administration of ACTH 4-10, exhibited a pattern of reduced reaction times suggestive of facilitated attention rather than memory (Ward *et al.*, 1979). On this basis, it may be postulated that ACTH and related peptide fragments adaptively serve to modulate human behavioral functioning. To examine this proposal further, the present study employed an adult clinical population of patients with congenital adrenal hyperplasia (CAH), whose hypothalamic-pituitary-adrenocortical (HPAC) functioning permitted experimental manipulation of ACTH levels.

CAH, the result of a recessive autosomal genetic defect, is relatively uncommon; estimates of its occurrence have ranged from 1:5,000 to 1:15,000 (New *et al.*, 1981). While at least five types of CAH have been identified (Migeon, 1977), two types account for 95% of all cases (Gregory & Gardner, 1976). The most common type, the simple virilizing form, is due to a partial 21-hydroxylase enzyme deficiency in the zona fasciculata of the adrenal cortex and is characterized by high levels of pregnanetriol, a precursor of cortisol, and low levels of cortisol (Cope, 1972). The salt-losing or salt-wasting form, which may induce Addisonian-like crises if untreated, is thought to be a result of a deficiency of the same enzyme in the zona glomerulosa as well as in the zona fasciculata (New *et al.*, 1981). This syndrome is characterized by decreased circulating aldosterone levels, resulting in increased plasma renin activity (Rosler *et al.*, 1977), along with the disruption of cortisol synthesis.

In both types of CAH, the cortisol deficiency resulting from the lack of 21-hydroxylase triggers a marked elevation of ACTH to the extent that cortisol levels are raised to normal, along with abnormally high levels of its precursors including progesterone, 17-hydroxyprogesterone and 21-deoxycortisol. A portion of the precursors are androgenic; approx. 5-10% are transformed into testosterone (Migeon, 1977).

The purpose of the present study was to investigate the influence of circulating ACTH levels on cognitive functioning in individuals with CAH. Adequate cortisone medication can suppress the elevated ACTH levels along with biosynthesis of cortisol precursors. However, in the absence of daily treatment, suppression of ACTH is quickly removed and levels become rapidly and significantly elevated. Thus, this clinical population provides a rare opportunity to permit manipulation of endogenous ACTH levels without concomitant cortisol changes, due to the limited capacity to synthesize this glucocorticoid.

By manipulating their steroid replacement medication, we tested the patients during periods in which their plasma levels of ACTH were either markedly suppressed or elevated. Performance measures, previously found to be sensitive to administration of fragments contained within ACTH, were incorporated into the study in order to test the hypothesis that elevated endogenous ACTH levels result in a similar facilitation of behavioral functioning. In addition, radioimmunoassays (RIAs) were employed to ascertain levels of both ACTH and cortisol preceding and following behavioral testing.

Finally, in order to assess possible practice effects and to permit further comparisons, an external control group consisting of healthy siblings of the CAH patients and unrelated individuals was included.

METHODS

Subjects

Initial contact with persons diagnosed as having primary CAH was accomplished with the assistance of several endocrinologists within the community of Columbus, Ohio. The physicians were requested to screen their medical files for former and present CAH patients, ruling out all individuals under the age of 16, those having documented histories of mental retardation or those with long-term psychological disturbances. Letters then were sent to those considered to meet the selection specifications, and each was asked to contact the primary investigator if the patient had any further questions or was interested in participating.

In total, eight patients with confirmed diagnoses of CAH were recruited (Table I). Three of the volunteers were male and the remaining five were female. The mean age of the subjects was 21.25 yr with a range of 17–29 years. Five of the participants were initially diagnosed as having CAH before the age of 1 yr, at which time appropriate treatment was initiated. The remaining subjects were diagnosed and treated before the age of seven. Four of the five female subjects had undergone corrective surgery of their external genitalia in early childhood. In addition, four (three females and one male) of the eight participants were being treated for the salt-losing form of CAH. All individuals were under the care of a physician and were receiving daily medication.

TABLE I. TYPE AND DOSE OF STEROID REPLACEMENT MEDICATION AND EXPERIMENTAL MANIPULATION IN CAH PATIENTS

Patient	Sex	Diagnosis	Type of medication prescribed	Dose levels prescribed	Dose levels preceding	
					Session 1	Session 2
1	F	Simple CAH	Cortisone acetate	10 mg a.m. 5 mg noon 25 mg p.m.	20 mg a.m. 10 mg noon 50 mg p.m.	None
2	M	Simple CAH	Prednisone	5 mg a.m. 2.5 mg p.m.	10 mg a.m. 5 mg p.m.	Placed on cortisone acetate during week preceding Session 2 (25 mg a.m./12.5 mg p.m.). No medication for 32 hr preceding Session 2.
3	F	Salt-loser	Prednisone	2.5 mg noon 5 mg p.m.	5 mg noon 10 mg p.m.	Placed on cortisone acetate during week preceding Session 2 (20 mg a.m./20 mg p.m.). No cortisone medication for 32 hr preceding Session 2. Only fludrocortisone acetate (0.1 mg/a.m. prescribed for 32 hr preceding Session 2.
			Fludrocortisone acetate	0.1 mg a.m.	0.1 mg a.m.	
4*	M	Simple CAH	Cortisone acetate	25 mg a.m.	50 mg a.m. (Exhibited ACTH elevation.)*	Physician had changed medication to 10 mg prednisone/day which was discontinued 32 hr preceding Session 2. (Failed to exhibit ACTH elevation.)* (Continued)

TABLE I continued

Patient	Sex	Diagnosis	Type of medication prescribed	Dose levels prescribed	Dose levels preceding	
					Session 1	Session 2
5	F	Salt-loser	Cortisone acetate	25 mg a.m. 25 mg p.m.	none	50 mg a.m. 50 mg p.m.
			Fludrocortisone acetate	0.1 mg/day	0.1 mg/day	0.1 mg/day
6	F	Simple CAH	Prednisone	10 mg/day	Placed on 25 mg a.m. and p.m. of cortisone acetate during week preceding Session 1. Medication discontinued 32 hr preceding Session 2.	20 mg/day prednisone
7**	M	Salt-loser	Cortisone acetate, i.m. (aqueous suspension)	50 mg/3 times per week	No injections for 4 days preceding testing (Failed to exhibit ACTH elevation)	50 mg/day for 3 days preceding testing
			Fludrocortisone acetate	0.1 mg a.m. 0.1 mg p.m.	0.1 mg a.m. 0.1 mg p.m.	0.1 mg a.m. 0.1 mg p.m.
8**	F	Salt-loser	Cortisone acetate	20 mg a.m. 15 mg noon 20 mg p.m.	none (Failed to exhibit ACTH elevation)	40 mg a.m. 30 mg noon 40 mg p.m.
			Fludrocortisone acetate	0.1 mg/day	0.1 mg/day	0.1 mg/day

*Data reversed.

**Data discarded.

The cortisol replacement medication employed varied and is summarized in Table I. Of the four CAH patients diagnosed as salt-losers, all were being treated with an additional salt-retaining steroid, fludrocortisone acetate (typically 0.1 mg daily). Since this medication, having potent mineralocorticoid properties, is critical to the maintenance of renin – angiotensin system functioning, salt-losing patients continued to use it at their prescribed dose level throughout testing.

The CAH patients were randomly divided into one of two groups. The first group was requested to take twice their normal dose level of steroid replacement medication beginning 36 hr before the first session (Session 2xC). For the 36 hr preceding the second session (Session 0-C), these individuals were instructed to ingest no steroid medication. The second group of CAH patients was given opposing medication manipulation instructions, thereby permitting a counterbalanced design. The primary investigator was aware of the two patient groups but not of each individual's order of medication manipulation. Instead, a physician's assistant knew this information and was available to the patients for further instructions if necessary.

Because of the different replacement regimes, the exact experimental medication manipulation was determined on an individual basis with the advice of an endocrinologist (see Table I). Patients treated with longer acting forms of replacement medication required preliminary temporary treatment with cortisone acetate to increase the likelihood of ACTH non-suppression when all medication was discontinued. At the end of each of the two test sessions, each patient was asked about their compliance with the medication instructions; all responded affirmatively.

The external control group, who received no medication manipulation, consisted of four siblings of CAH individuals and four unrelated individuals, all of whom were matched to the CAH patients on race, age and sex. The siblings were recruited at the same time as the CAH patients; the unrelated subjects were drawn from a group of students enrolled in a university introductory psychology course. All control subjects were in good health; the siblings of CAH patients had undergone earlier medical examination to insure they did not have the syndrome.

All subjects were not informed of the nature of the experimental hypotheses until termination of testing. Each was paid \$20.00 for participating, with the exception of the unrelated control subjects who received four experimental credit units in their psychology course. All those scoring in the upper 50% on the Sternberg Item Recognition Task received an additional \$5.00 in an attempt to increase motivation. Any major travel expenses were reimbursed along with any costs incurred by medication changes required by the study.

The two sessions were scheduled at least 4 days apart and occurred during the late afternoon (1600 hr – 1830 hr). All participants were asked to abstain from any drug which might alter mental alertness for the 24 hrs preceding each session. Eating, drinking and cigarette smoking was not permitted during the testing period. Preceding the first session, informed written consent was obtained.

Endocrine assessment

Multiple determinations of circulating ACTH and cortisol levels were done. At both the initiation and end of each test session, 30 ml of blood was drawn from the antecubital vein of each subject by a qualified assistant. To prevent any undue stress, only one attempt at successful venipuncture was permitted during each collection.

The venous samples were permitted to clot and the sera separated by centrifugation and stored at -20°C until assayed. The cortisol RIAs were completed with the Murphy (1968) technique and the RIAs for ACTH were based on the method of Rees *et al.* (1971).

Behavioral assessment

Following the first venipuncture, behavioral testing was initiated. Whenever available or appropriate, two comparable test forms of each behavioral measure were employed and subjects were randomly assigned to one of the two orders of test presentation. This counterbalanced design was used to reduce order and practice effects. The order of presentation of different tasks was also counterbalanced across subjects and sessions.

Visual memory-attentional assessment

Sternberg (1967, 1969) designed the SIRT as a character classification task consisting of at least two major operations. Operation 1 refers broadly to those events preceding and following Operation 2, including the identification of the stimulus probe, the creation of a cognitive stimulus representation and execution of overt responding. During the second operation, this stimulus image is compared individually and successively to the sequence composed of the cognitive representations of the previously memorized target set. Each comparison results in either a match or no-match binary decision. Only after the entire target set has been compared with the mental representation of the probe is a match or no-match overt response initiated (a component of Operation 1). For this reason, Sternberg labels this as an exhaustive serial-comparison process.

According to Sternberg, changes in performance may be analyzed as resulting from alterations in stimulus encoding and overt response execution (Operation 1), variations in comparison of the stimulus representation with the memory set representation (Operation 2) or both. Ideally, the reaction time function is linear and is an additive function of the two operations, thereby permitting it to be represented as:

$$RT = \alpha \times + \beta$$

The slope (α) is a measure of the median time taken to compare the stimulus representation of the probe with the memorized target set (Operation 2). The zero-intercept (β) (Operation 1) refers to those processes preceding and following the serial comparison, including both the formation of a stimulus representation and the execution of the overt response. Enhanced Operation 1 processes, in theory, should yield a consistent reduction in reaction time across all target set sizes resulting in a linear function parallel to the original but intersecting the ordinate at a point closer to the origin. In contrast, facilitated Operation 2 processes would become more evident with larger target set sizes and would be characterized by a flatter slope. Thus, changes in reaction time (RT) may be analyzed as a function of variation in stimulus encoding and overt responding compared to such cognitive processes as serial comparison and binary decision making.

Preceding the first session, each subject underwent a practice trial with the SIRT. At this time, he/she was randomly assigned to a 'dominant' or 'non-dominant' hand group which remained constant over the two sessions. Complete instructions were then presented. Next, the subject was seated in a darkened, sound attenuated room, facing a cathode ray tube (CRT) which displayed the stimulus item. The two response keys,

corresponding to the right and left hands, were placed directly in front of the subject. Presentation of the stimuli and storage of each subject's responses were controlled by a NOVA 3/12 computer.

The paradigm consisted of four target sets whose size varied from one to four digits. The subject was requested, during a 20 sec period, to memorize the digits comprising the target set. A series of 20 discrete digits were then immediately flashed on the CRT. The subject was asked to respond as quickly as possible by pressing a button indicating whether the displayed digit was contained in the target set that he/she had memorized by pushing the appropriate button. In all cases, the target size of three digits was the first set size to be presented; the other three sets (one, two and four digits) were presented consecutively in a random order. Fifty percent of the test trials were digits contained within the target set (match response). If the subject responded correctly within one second, the next stimulus probe was presented within 300 msec. If an error was made, the word 'error' was presented and when no response was made within 1 sec, the words 'too slow' appeared on the CRT screen. The duration of presentation of these statements was 500 msec.

As mentioned, the practice session consisted of twenty trials across each of the four target sets. The subject's performance was then examined to insure that each was able to successfully complete the task. During both test sessions, subjects were requested to complete the SIRT with each block of the four target sets containing 100 test trials. These data were used later to compute the following dependent measures: median overall reaction time, median reaction time of correct responses, number of correct and wrong responses, and number of errors of omission (no response). Comparison of performance on these five measures was completed using match response trials (the probe was a member of the target set) vs no-match response trials (the probe was not a member of the target set).

Possible effects of endocrine changes on visual memory were assessed using forms C and E of the Benton Visual Retention Test (BVRT) (Benton, 1955) with modified instructions to prevent ceiling effects, as described elsewhere (Veith *et al.*, 1978a). Also, a concept formation task, considered to be sensitive to visual attentional state (Mackintosh, 1965, 1969; Nolan, 1970) was administered. Two comparable forms (Nolan *et al.*, 1978), employing stimuli which varied across the dual dimensions of either weight-length or color-form, were used. Standard stimulus presentation, instructions and scoring methods were employed (Nolan *et al.*, 1978). The final measure of visual attentional functioning was the Rod and Frame Test (RFT) (Witkin *et al.*, 1954), considered to be a sensitive index of ability to isolate aspects from the surrounding environment. The portable test apparatus and standardized instructions and scoring (Oltman, 1968) were used.

Verbal memory-attentional assessment

Two measures of verbal memory were employed. The Logical Memory subtest and a modified version of the Associate Learning Subtest (Veith *et al.*, 1978a), both contained in the Wechsler Memory Scale (WMS) (Forms I and II (Wechsler, 1945), comprised the first task. The Buschke Category Test, a verbal memory procedure based on selective recall which permits analysis of item vs list learning also was used (Buschke, 1974; Buschke & Fuld, 1974; Ritter & Buschke, 1974). Two comparable forms (weather, anatomy) of this task were employed. Six dependent measures (total recall, longterm recall, short term recall, longterm storage, list learning and random longterm recall) were obtained to permit comparisons of item vs list learning.

RESULTS

CAH ACTH responders analyses

Examination of the endogenous ACTH levels of the CAH participants revealed that during Session 2xC, doubled steroid replacement medication successfully suppressed ACTH levels below the detectable range (≤ 60 pg/ml) in seven of the eight subjects. However, during the session following no steroid replacement treatment (Session 0-C), two of the CAH subjects (#7 and #8) continued to exhibit non-detectable levels of ACTH (Table I). In addition, another CAH patient (#4), perhaps as a result of a prescribed medication change occurring between the two test sessions, exhibited elevated ACTH levels during Session 2xC and ACTH levels below the detectable range during Session 0-C.

In order to accurately assess the impact of experimentally induced ACTH fluctuations on the behavioral indices, an initial set of analyses was performed with the data of the first two patients (#7 and #8) discarded and the behavioral and RIA results from the third

patient (#4) reversed for the two sessions (Session Hi-ACTH/Session Lo-ACTH). This manipulation resulted in a total of six CAH subjects with order of medication manipulation equally counterbalanced across sessions.

Results of the ACTH and cortisol RIAs were analyzed with a two (Session Lo-ACTH vs Session Hi-ACTH) $\times$ two (1600 hr venipuncture vs 1830 hr venipuncture) repeated measures analysis of variance. As the most conservative approach, in all statistical analyses employing ACTH data non-detectable levels were assumed to be equal to 60 pg/ml. A nearly significant increase in ACTH levels occurred during Session Hi-ACTH as compared to Session Lo-ACTH ($F_{1,5} = 5.63, p < 0.06$). As shown in Fig. 1, levels of this hormone also were significantly higher at the initiation of each testing period than at termination ($F_{1,5} = 7.00, p < 0.05$). A similar analysis of the CAH patients' cortisol data revealed, as expected, no significant differences in levels of this steroid either between sessions or times of sampling.

FIG. 1. Mean plasma ACTH levels of six CAH patients at initiation and end of behavioral testing following doubled medication intake (Session Hi-ACTH) and no cortisol replacement (Session Lo-ACTH).

Separate repeated measures analyses of variance across the two sessions then were done on measures of attention and memory including the RFT, the WMS, the BVRT, and the concept formation task. No significant differences in patients' performance between the two sessions were obtained.

Measures derived from the Buschke Category Test were also analyzed by a repeated measures analysis of variance. As expected with normal subjects (Buschke, 1974), a highly significant ($p < 0.001$) improvement in recall performance across trials on all six measures was present in this and all later analyses. Also, a significant interaction of session and trial ($F_{4,20} = 2.87, p < 0.05$), was present for the short term recall results. However, since no significant main effects were found for this variable or the other measures obtained from this task, no conclusions will be drawn from this single finding.

Separate two (Hi-ACTH/Lo-ACTH) $\times$ four (target set size) $\times$ two (match vs no match) repeated measures analyses of variance were employed to examine CAH ACTH

responders' performance across the five dependent variables obtained from the SIRT. Of importance, a significant three way interaction effect on the measure of overall reaction time occurred ($F_{3,15} = 3.16, p < 0.05$). As shown in Fig. 2, the six patients exhibited a faster median overall reaction time across all four target set size blocks during Session Hi-ACTH. Furthermore, lines fitted by the least squares to means method indicated that match responses during this session, compared to Session Lo-ACTH, exhibited a steeper slope, which contributed to the interaction (Table II). As expected with this paradigm, significant increases in median overall reaction time ($F_{3,15} = 17.83, p < 0.001$) and correct reaction time ($F_{3,15} = 18.64, p < 0.001$) across increasing target set sizes occurred. Moreover, probes requiring a match response elicited significantly faster median overall ($F_{1,5} = 58.91, p < 0.001$) and correct ($F_{1,5} = 53.54, p < 0.05$) responses and fewer errors of omission ($F_{1,5} = 16.24, p < 0.01$). For the sake of brevity, significant findings concerning the factors of target set size and match vs no match responses will be assumed and not discussed in later analyses.

FIG. 2. Median overall reaction time of match (M) and no-match ($\bar{M}$) responses of six CAH patients across the four target set sizes of the Sternberg Item Recognition Task following suppressed (Session Lo-ACTH) and elevated (Session Hi-ACTH) endogenous ACTH levels. Solid lines were derived from patients' performance (indicated by open dots) using the method of least squares to mean.

In an attempt to ascertain the degree of relatedness between Sternberg performance and the variables of steroid replacement dose (transformed, when necessary, to equivalent total daily intake (mg/kg) of cortisone acetate), mean plasma cortisol and mean ACTH levels across Session Hi-ACTH and Lo-ACTH, correlations were computed among these measures. The zero intercept and slope of each CAH participant's overall median reaction time from each session were derived using the method of least squares to mean. Since correlational analyses assume independent pairs of observations, in contrast to the

TABLE II. SLOPES AND INTERCEPTS OF MEDIAN OVERALL REACTION TIME (DERIVED FROM LEAST SQUARES TO MEAN) OF THE STERNBERG ITEM RECOGNITION TASK FOLLOWING DOUBLED MEDICATION INTAKE (SESSION LO-ACTH) AND NO CORTISOL REPLACEMENT (SESSION HI-ACTH) OF THE SIX CAH PATIENTS EXHIBITING ACTH ELEVATIONS

Patient	Session Lo-ACTH		Session Hi-ACTH	
	Slope (α)	Intercept (β)	Slope (α)	Intercept (β)
# 1	33.15	413.75	- 22.25	507.25
# 2	14.25	426.75	75.1	338.5
# 3	11.45	398.0	21.15	277.0
# 4*	17.8	322.0	10.85	523.25
# 5	30.5	356.5	26.8	188.0
# 6	45.9	470.25	10.1	554.25

*Date reversed—see Table I.

repeated sampling of individuals' performance occurring in the present design, the degree of relatedness of each variable across Session Hi-ACTH and Session Lo-ACTH was determined. All correlations for each variable across the two sessions were relatively small ($-0.20 \geq r \leq 0.20$).

On this basis, it was assumed that the two sampling points were independent observations, thereby permitting further correlational analyses to be completed. As predicted, mean plasma levels of ACTH were significantly negatively correlated with daily dose level of steroid replacement medication preceding each session ($r_{10} = -0.736$, $p < 0.01$). Of theoretical importance, plasma ACTH levels were significantly negatively correlated with the determined intercept ($r_{10} = -0.684$, $p < 0.05$) but not with slope. This finding suggests that ACTH-related enhancement of Sternberg performance was consistent across all four target sets and thus had the greatest impact on Operation 1 processes.

In order to attempt to separate the potential interrelationship of cortisol medication and endogenous ACTH levels, a partial correlation of mean ACTH levels with the Sternberg intercept was calculated, which revealed a significant degree of relatedness with the variable of medication dose removed ($r_{10} = 0.645$, $p < 0.05$). Moreover, analyses revealed that mean plasma cortisol levels were not significantly related to any components of Sternberg performance and, in addition, the partial correlation of mean endogenous ACTH levels and Sternberg intercept with plasma cortisol levels removed also was significant ($r_{10} = -0.65$, $p < 0.05$).

Thus, on the basis of these correlation results, we suggest that facilitated reaction time performance, characterized by enhanced stimulus encoding or overt responding rather than serial comparison processes, was related to increased circulating ACTH levels. However, Operation 1 variables were not related to either plasma cortisol or dose of replacement medication.

Comparisons of simple CAH patients vs salt-losers

As discussed earlier, CAH individuals diagnosed as salt-losers have a relatively impaired capacity to biosynthesize aldosterone, resulting in increased plasma renin activity, in comparison with others with the simple form of CAH. On the basis of the

previous analyses, which suggested a possible effect of endogenous ACTH manipulation on Sternberg performance, we compared the salt-losers ($n = 4$) with the simple CAH patients ($n = 4$) across Sessions 0-C and 2xC. This approach was intended to detect both possible preexisting differences between the two groups and individual variation in response to the medication manipulation.

These comparisons must be considered with caution, however, as all four salt-losers were being treated throughout the study with a mineralocorticoid. Moreover, two of the salt-losers (Subjects #7 and #8) failed to exhibit an elevation of ACTH during Session 0-C. Adequate treatment with mineralocorticoids has been demonstrated to suppress plasma ACTH levels in the absence of glucocorticoid replacement therapy (Rosler *et al.*, 1977).

Endocrine RIA results were analyzed with a two (salt-loser vs simple CAH) $\times$ two (session) $\times$ two (sample) repeated measures analysis of variance. No significant main or interaction effects occurred with either the cortisol or the ACTH data.

Results obtained from a number of the psychological variables were tested with a two (group) $\times$ two (session) repeated measures analysis of variance. No significant session or group effects were found on the RFT, the BVRT, the Buschke Category Test, the Paired Associate and Logical Memory Subtests of the WMS, or the concept formation task.

Results obtained from the SIRT were examined with a two (group) $\times$ two (session) $\times$ four (block) $\times$ two (match) repeated measures analysis of variance. Salt-losers responded significantly faster to probes than did the simple CAH patients across both sessions, as indicated by median overall reaction time ($F_{1,6} = 14.97, p < 0.01$) and correct reaction time ($F_{1,6} = 15.02, p < 0.01$). A significant three-way (group $\times$ block $\times$ match) interaction occurred for overall reaction time ($F_{3,18} = 4.55, p < 0.05$) and correct reaction time ($F_{3,18} = 4.34, p < 0.05$), reflecting the consistently steeper slopes, derived from a least squares to mean method, of median match response reaction time as compared to those slopes characterizing no-match responses.

To determine that the enhanced Sternberg performance by salt-losers was not solely a product of mineralocorticoid treatment, but also was related to endogenous ACTH elevations, the groups were redefined by including only the salt-losers exhibiting an ACTH elevation during Session 0-C. Thus, Subjects #7 and #8 were discarded. The salt-losers' ($n = 2$) and simple CAH patients' ($n = 4$) Sternberg median overall reaction times then were reanalyzed by the repeated measures analysis of variance outlined above. As shown in Fig. 3, the salt-losers exhibited an enhanced median reaction time across both sessions ($F_{1,4} = 11.25, p < 0.05$) compared to the other group. Moreover, it is apparent that the salt-losers' facilitated performance during Session Hi-ACTH compared to Session Lo-ACTH is largely due to a reduction of the x-intercept rather than the slope. Moreover, a trend toward a main effect of session was found ($F_{1,4} = 5.03, p < 0.09$), reflecting that, across the six individuals, overall median reaction time was facilitated during Session Hi-ACTH compared to Session Lo-ACTH.

CAH sex differences analyses

Previous research, employing fragments of ACTH, obtained evidence of sex differences in behavioral performance following peptide administration in humans (Veith

FIG. 3. Median overall reaction of patients with the salt-losing form of CAH ($n = 2$) and patients with simple CAH ($n = 4$) during match (M) and no-match (M) responses across the Sternberg Item Recognition Task following elevated (Session Hi-ACTH) and suppressed (Session Lo-ACTH) endogenous ACTH levels. Solid lines were derived from patients' performance (indicated by dots) using the method of least squares to mean.

et al., 1978a; Sandman & Kastin, 1981). Therefore, an additional set of analyses were done to ascertain if a sexually dimorphic response pattern to medication manipulation was present in this limited sample of CAH males ($n = 3$) and females ($n = 5$).

A two (group) $\times$ two (session) $\times$ two (sample) repeated measures analysis of variance was used to examine the results of the RIAs. No differences in ACTH or cortisol levels between CAH men or women were present across the four sampling points.

The RFT, concept formation task, WMS subtests, and BVRT were examined with a two (group) $\times$ two (session) repeated measures analysis of variance. No significant between-sex differences were detected on these measures.

The six measures obtained from the Buschke Category Tests were examined with a two (group) $\times$ two (session) $\times$ (trial) repeated measures analysis of variance. CAH males exhibited a significantly or near significantly greater recall ability than CAH females across both sessions, as indicated by higher performance on total recall ($F_{1,6} = 6.34$, $p < 0.05$), long-term storage ($F_{1,6} = 12.88$, $p < 0.01$), list learning ($F_{1,6} = 5.67$, $p < 0.05$), and lower scores on the random long-term recall measure ($F_{1,6} = 5.55$, $p < 0.05$).

A highly significant session by group interaction effect was found on the measures of overall reaction time ($F_{1,6} = 13.00$, $p < 0.01$) and correct reaction time ($F_{1,6} = 12.88$, $p < 0.01$) on the SIRT as determined by a two (group) $\times$ two (session) $\times$ four (block) $\times$ two (match) repeated measures analysis of variance. Females responded more quickly

during Session 0-C than during Session 2xC, whereas males exhibited an opposite slowing of overall reaction time following no cortisol replacement medication.

A second repeated analysis of variance was done on the median overall reaction time of only those individuals exhibiting an ACTH elevation during Session Hi-ACTH (males: $n = 2$; females: $n = 4$). This analysis failed to reveal a significant session by group interaction. However, a significant three-way (group by session by match) interaction ($F_{1,4} = 8.88, p < 0.05$) indicated that during Session Hi-ACTH the women exhibited a flatter slope and intercept than during Session Lo-ACTH, while their match – no match performance remained parallel across sessions. In contrast, during Session Hi-ACTH, men exhibited a far steeper slope than during Session Lo-ACTH, during which their slope was much flatter than women. Across the two sessions, the men's match – no match performance was not parallel. This complex interaction was confirmed by a second, highly significant three-way (sex by block by match) interaction ($F_{3,12} = 15.09, p < 0.005$) (Greenshouse-Geisser probability). Based on the very small number of male subjects and the complexity of these interactions, it is extremely difficult to interpret these findings.

External control group analyses

The performance of the external control group was analyzed separately in order to determine an estimate of practice effects and other non-specific factors possibly influencing behavioral change across Sessions 1 and 2. Any significant session effects were independent of hormonal variations as determined by a two (session) $\times$ two (sample) repeated measures analysis of variance of the cortisol and ACTH data. All control subjects had non-detectable ACTH levels (≤ 60 pg/ml) at all sampling points. Cortisol levels significantly declined during each testing period ($F_{1,7} = 30.39, p < 0.001$) but did not vary between sessions.

Measures of attention and memory, including the RFT, the concept formation task, the WMS subtests and the BVRT, were examined with a two (session) repeated measures analysis of variance. The only significant finding was an increase during Session 2 in mean performance across the two paragraphs of the Logical Memory Subtest ($F_{1,7} = 22.87, p < 0.01$). However, there was no significant change across testing periods on recall of either paragraph examined singly.

The six measures of performance derived from the Buschke Category Test were studied using a two (session) $\times$ (trial) repeated measures analysis of variance. A significant session by trial interaction occurred for total recall ($F_{4,28} = 4.12, p < 0.01$) and for long-term recall results ($F_{4,28} = 3.94, p < 0.01$), reflecting the fact that control subjects achieved their highest recall performance during the third trial of Session 1 vs the final trial of Session 2. Typically with this verbal memory paradigm, as longterm recall scores increase, random longterm recall declines. This pattern was reflected in a third significant two-way interaction for the random long-term recall measure ($F_{2,28} = 4.17, p < 0.01$).

In addition, five two (session) $\times$ four (block) $\times$ two (match) repeated measures analyses of variance were completed across the indices derived from the SIRT. No significant effects of session were present.

In order to complete all possible comparisons with the external control group data that would parallel the analyses performed with the experimental group, a final contrast of

control subjects was included. The performance of the males ($n = 3$) and females ($n = 5$) comprising the control group were compared across sessions.

For both the endocrine and behavioral data, only one measure revealed sex differences in performance. A two (group) $\times$ two (session) repeated measures analysis of variance showed a significant group by session interaction in performance on the RFT ($F_{1,6} = 7.94$, $p < 0.05$). Control women improved on this measure during Session 2, whereas control men became more field dependent during this session.

DISCUSSION

This study presents evidence indicating that, during periods characterized by elevated endogenous ACTH levels, human perceptual/attentional performance as measured by the SIRT is facilitated. Following the withdrawal of steroid replacement medication, median reaction time of those CAH patients exhibiting elevated ACTH levels during that session significantly decreased in a consistent manner across all four target set sizes. Secondly, those CAH patients who were salt-losers responded consistently faster across both sessions compared to the other CAH patients. Finally, female CAH patients responded more quickly on the Sternberg Task without medication whereas males exhibited a significantly impaired response under this condition. In contrast, the control group exhibited no significant changes in Sternberg performance across the two sessions. Together, these findings indicate that the Sternberg Task is highly sensitive to changes in performance related to specific endocrine fluctuations. They also suggest that behavioral variation on this task may be influenced by factors other than short-term manipulation of ACTH levels; treatment with mineralocorticoids, previous endocrine history, and possibly the sex of the individual appear to contribute to performance.

Sternberg's linear model may be applied to interpret the differences between the linear equations, derived from the method of least squares to mean, applied to the Sternberg reaction time data across the two sessions. Consistently, variation in median reaction time appears to be a function of altered zero intercepts, the index of Operation 1 factors, rather than changes in slope, since the functions tend to remain relatively parallel. The overall median reaction time of the six CAH patients (Fig. 2), during elevated compared to low endogenous ACTH levels indicates a major reduction in the time to complete Operation 1 processes (decrease in zero intercept) compared to serial comparison processes (Operation 2). This notion is supported by the significant correlation of endogenous ACTH levels with the estimated zero-intercept. However, no reliable linear relationship existed between this hormone or other variables and estimations of the reaction time slope.

The reduction in the zero intercept accompanying periods of elevated ACTH levels thus may be interpreted as a reflection of enhanced capacity to create a cognitive stimulus representation of the probe and/or to execute the appropriate response relative to such processes as serial cognitive comparisons. In other words, such processes as the ability to attend to salient cues in the environment, to accurately encode them and to execute the appropriate overt response apparently are enhanced during periods of elevated endogenous ACTH, whereas simple cognitive processes are not. Furthermore, the partial correlations indicate that variation in plasma ACTH but not plasma cortisol or steroid replacement accounts for a large proportion of the variance in the Sternberg data.

It should be noted that one methodological problem with the design of this study was our failure to include a double blind procedure. While the experimenter was unaware of the schedule of medication intake for each subject, the CAH patients had full knowledge of their treatment program. This may have resulted in a set of expectations regarding their performance following a doubling of their normal steroid dosage and its removal. Thus, our results must be interpreted with caution.

It is of interest that of the three CAH patients who failed to exhibit an elevation in ACTH following removal of their replacement medication, two were continuing to take their prescribed amount of fludrocortisone acetate, the mineralocorticoid prescribed for their salt-losing condition. The other was using prednisone, a long-acting analog of cortisone. These medications continued to suppress ACTH in these three patients.

The present findings relating behavioral alterations with variation in endogenous levels of ACTH compare favorably with previous work focusing on the effects of exogenous administration of ACTH 4-10. The findings of enhanced Sternberg performance, characterized by increased visual-attentional functioning during periods of elevated endogenous ACTH, are markedly similar to those results obtained by Ward *et al.* (1979) who examined such performance following administration of ACTH 4-10. This major commonality in results suggests that the observed changes in behavior following peptide administration may reflect the behavioral potential of related endocrine factors *in vivo*.

However, the present findings do not consistently parallel other findings concerning the influences of this peptide fragment in humans. ACTH 4-10 has been found to enhance visual memory performance on the BVRT (Miller *et al.*, 1974, 1976; Sandman *et al.*, 1978a), selective visual attention as measured by the Concept Formation Task (Sandman *et al.*, 1975, 1976), increased field independence in men (Sandman *et al.*, 1975) and improved verbal memory in women (Veith *et al.*, 1978a). Similar results were not obtained in our study. This lack of consistency may reflect the small number of subjects employed in the present study, particularly males, the sex for which behavioral response to administered ACTH fragments has been most fully characterized. Furthermore, individuals with CAH vary from healthy populations across a number of behavioral dimensions.

One of the primary methodological distinctions between this and the previous approach is the presumed 'employment' of the entire ACTH molecule in the present study as compared to far smaller pharmacologically produced peptide fragments. On the basis of available evidence, it cannot be assumed that high levels of endogenous ACTH are indicative of the presence of equal elevations of related peptide fragments, such as ACTH 4-10, since the complete pattern and time course of enzymatic breakdown of this hormone has yet to be ascertained. Furthermore, it has been demonstrated that the capacity of ACTH fragments to evoke specific behaviors is related to the dimensions of both molecular weight and configuration (Sandman *et al.*, 1980). Other studies examining structure-activity relationships with fragments of β -LPH also indicate that variance in amino acid sequence results in differing capacities to elicit the same and different behaviors (Gispén *et al.*, 1976; Veith *et al.*, 1978b). Furthermore, characterizations of the behavioral influences of exogenous ACTH 4-10 are based on employment of pharmacologically high dose levels introduced in a sudden and discontinuous manner.

Until it has been clearly demonstrated that ACTH is quickly converted into smaller, behaviorally active fragments *in vivo*, it must be assumed that the entire endogenous 1-39 sequence may have different influences than administered ACTH 4-10 on attentional function.

Moreover, as a result of technical limitations preventing measurement of all HPAC-related hormones, the critical endocrine variable underlying the behavioral alterations between the two sessions cannot be definitely ascertained. One potential factor, α -MSH, has been shown both to be critical in the facilitation of visual attention (Sandman *et al.*, 1980) and to mirror endogenous ACTH fluctuations in CAH patients (Lombardi *et al.*, 1977). Additional neuropeptides that also must be considered are those contained in proopiomelanocortin along with ACTH and α -MSH, such as β -LPH and the endorphins (Mains *et al.*, 1977). Some of these peptide fragments appear to have a comparable release pattern to ACTH (Weber *et al.*, 1978), along with similar capacities to alter a variety of behaviors (Veith *et al.*, 1978b). Also, androgenic steroids, biosynthesized from accumulated cortisol precursors, may have played a significant behavioral role. Klaiber *et al.* (1971) found that exogenous androgenic stimulation increased men's short term capacity to perform repetitive cognitive tasks.

In conclusion, this is the first study to provide evidence suggesting that, during periods characterized by elevated endogenous ACTH levels, individuals exhibit behavioral alterations in the direction of enhanced visual – attentional performance. These results are generally in agreement with previous research examining the effects of exogenous ACTH 4-10 and related peptides in both humans and other species. This suggests that the behavioral consequences of administration of this hormone accurately reflect physiological phenomena rather than pharmacological artifact. Moreover, the data from the present investigation provide indications that the extent of such influence may be moderated by a number of potentially overlooked factors, including present hormonal status, previous endocrine history, and sex.

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