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$MSH/ACTH_{4-10}$ Influences on the CAR in Human Subjects: A Negative Finding

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MILLER, L. H., S. C. FISCHER, G. A. GROVES, M. E. RUDRAUFF AND A. J. KASTIN. *$MSH/ACTH_{4-10}$ influences on the CAR in human subjects: a negative finding.* PHARMAC. BIOCHEM. BEHAV. 7(5) 417–419, 1977. – Twenty normal, male, paid volunteers were randomly assigned to $MSH/ACTH_{4-10}$ or diluent control groups. Following a subcutaneous injection of either 30 mg $MSH/ACTH_{4-10}$ or diluent, all subjects were placed in an active conditioned avoidance response situation where they learned to avoid a painful electric shock by pressing a key during a safe interval between a warning signal (light) and shock onset. $MSH/ACTH_{4-10}$ did not influence any parameter of the acquisition or extinction process. The between subjects' variability of autonomic variables was significantly less for the $MSH/ACTH_{4-10}$ group. Results indicate that inhibition of extinction of conditioned avoidance responding does not generalize across species from rat to man.

CAR Acquisition Extinction Autonomic MSH_{4-10} $ACTH_{4-10}$

IT HAS been demonstrated repeatedly that a number of short chain polypeptides such as melanocyte-stimulating hormone (MSH), adrenocorticotropic hormone (ACTH) and fragments of the MSH/ACTH molecule such as $MSH/ACTH_{4-10}$, $MSH/ACTH_{1-10}$, etc., have significant effects on brain-behavior mechanisms in both animals and human beings. Miller and Ogawa [9], for instance demonstrated that ACTH renders conditioned avoidance responses (CARS) in the rat highly resistant to extinction. De Wied [3, 4, 5] and others [2, 10, 12, 13, 14, 16] have shown this to be a highly consistent and replicable, but not universal, finding [1]. While studies of the effects of polypeptides in man suggest influences on attention, memory and anxiety [6, 7, 8], no one has explored the effects of polypeptides on the human CAR. This would seem to be a serious omission both from a clinical and scientific viewpoint. Information regarding the effects of polypeptides on the CAR in man would do much to bridge the existing gap between animal and human studies.

METHOD

Subjects

Twenty young (21–35) male students and university employees were paid to participate in this study. Volunteers with physical illness or psychiatric histories were excluded. Subjects were requested to abstain from psychoactive drugs for a week before and a day after the study. Subjects were randomly assigned to either an $MSH/ACTH_{4-10}$ or placebo (diluent) group.

Apparatus

Subjects were seated in a comfortable, reclining chair in a dimly-lit, sound attenuated, electrically shielded chamber. A small light was mounted on the facing wall at seated eye level. A telegraph key was attached to the end of the right chair arm. Stimuli were presented by laboratory computer. Galvanic Skin Potential (GSP), heart rate, and digital blood volume were recorded on a Grass Model 7A polygraph. Shock was administered via a Grass Model S–4 stimulator with appropriate stimulus isolation and constant current units.

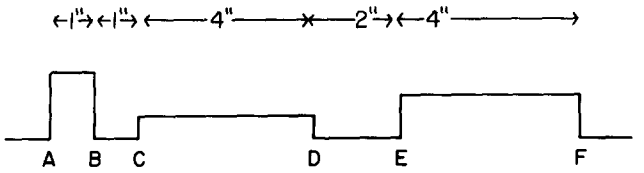
Procedure

Subjects were seated and transducers for the measurement of GSP, heart rate, and digital pulse volume were attached. Electric shock electrodes were placed at the end of the left index fingers.

Pain thresholds for electric shock were established for each subject, with an upper limit of two milliamperes current. Subjects received a subcutaneous injection of $MSH/ACTH_{4-10}$ (30 mg Org. OI63) or diluent (2 ml) according to a random, double-blind schedule. After a five-min delay, the experiment began. Subjects were told that the signal light would occasionally blink on and off

and would be followed by electric shocks. They were told there would be a way to avoid the shock that had to do with the telegraph key.

Duration of light (CS) was one-second; the electric shock (UCS) was four seconds (see Fig. 1). Shock intensity was constant at pain threshold. The interval between CS offset and UCS onset was constant at seven seconds. Subjects avoided shocks by key-press responding only between one and five seconds following the warning stimulus (CS). Successful avoidance criteria were both four out of five, and five out of eight successes. Criterion for extinction was five successive failures to press the key during the appropriate interval following the warning stimulus. Subjects who did not reach extinction by the end of two hours were terminated as non-extinguishers.



A. LIGHT ON (CS)
B. LIGHT OFF
C. BEGIN SAFE PERIOD FOR RESPONDING
D. END SAFE PERIOD
E. SHOCK ON (UCS) [IF NO RESPONSE]
F. SHOCK OFF

FIG. 1. Sequence of events during a trial. The warning light (CS) was on for a 1-sec interval (A–B), a dead period of 1 sec (B–C) followed. Subject's task was to learn to key press during the 4 sec safe period (C–D). Failure to key press during the safe period resulted in a painful electric shock of 4 sec duration (E–F). Key presses during CS presentation (A–B), during the two dead periods (B–C and (D–E), or during the shock period (E–F) had no effect on shock duration or magnitude. Only single key presses within the safe period (C–D) were effective in avoiding shock.

RESULTS

Eighteen subjects, nine receiving MSH/ACTH$_{4\text{-}10}$, and nine receiving diluent were included in the analysis. Data for two subjects were discarded due to equipment failure. As shown in Table 1, 10 subjects extinguished and 8 subjects failed to extinguish; but MSH/ACTH$_{4\text{-}10}$ did not influence the extinction process.

TABLE 1

NUMBER OF SUBJECTS BY TREATMENT AND OUTCOME

Outcome	Treatment		
	MSH/ACTH$_{4\text{-}10}$	Saline	Total
Extinguish	4	6	10
Not Extinguish	5	3	8
Total	9	9	18

The number of subjects extinguishing the CAR was essentially the same for both MSH/ACTH$_{4\text{-}10}$ and saline groups.

There were no significant differences between treatment groups (MSH/ACTH$_{4\text{-}10}$ vs. saline) or outcome groups (extinguish vs. non-extinguish) as determined by *t*-test for: Trials to Criterion, Trials to Extinction/Termination, Trials from Criterion to Extinction/Termination, Errors per Trial, Time from Injection to Criterion, Time from Injection to Extinction/Termination; or Time from Criterion to Extinction/Termination. All measurements to Extinction/Termination were affected by the arbitrary act of Termination at two hours (Number of Trials, Errors and Duration).

Measures of GSP, heart rate (R to R interval) and digital blood volume were recorded for ten trials before extinction/termination. Heart rate and digital blood volume were averaged for ten beats before and ten beats after the onset of CS. GSP level was determined at A, B, E and F (Fig. 1). Change in GSP level was measured by subtracting A from B, A from E, F from E and E from B.

No significant differences between the two treatments (diluent vs. MSH/ACTH$_{4\text{-}10}$) or two outcomes (extinguish vs. non-extinguish) were found when subjected to analysis of variance and co-variance techniques. Variability in the physiological data was evaluated by standard F test comparisons for variance. The variances of the MSH/ACTH$_{4\text{-}10}$ group were consistently lower for heartrate at all post-injection measurement points except the After Criterion/Before CS point. In general, there was less physiological variability within the MSH/ACTH$_{4\text{-}10}$ group than within the diluent control group.

TABLE 2

COMPARISON OF HEART RATE VARIANCE BY TREATMENT GROUP

Point of Measurement	MSH/ACTH$_{4\text{-}10}$*	Diluent*	F
Before Criterion			
Before CS	0.1521	0.6084	4.00†
After CS	0.1849	0.8649	4.68†
After Criterion			
Before CS	0.2116	0.5929	2.80
After CS	0.1225	0.5776	4.72†
Before Ext./Termination			
Before CS	0.1444	0.7056	4.89†
After CS	0.1444	0.7396	5.12†

Heart rate variance was significantly lower among subjects in the MSH/ACTH$_{4\text{-}10}$ group than among subjects in the saline control group.

*$n = 9$

†$p = 0.05$

DISCUSSION

One possible explanation of these negative results is the low ecological significance of the behavior to be learned and extinguished. The shock was not excruciatingly painful and some subjects may have been willing to experiment with non-responding during the trials to extinction/termination which they may not have been willing to do had the shock been more painful. The fact that half of the subjects extinguished and half did not, indicates that the basic paradigm, however, would be effective in discriminating an effect, if one were present.

A second possible explanation is that ceiling effects were

in operation and that the young, healthy subjects involved in the study were not all that influenced by the administration of $MSH/ACTH_{4-10}$. The significant reductions in heart rate variability for the $MSH/ACTH_{4-10}$ group does suggest, however, that $MSH/ACTH_{4-10}$ subcutaneously injected tends to increase physiological homogeneity amongst individuals so treated in comparison with subjects functioning on their own endogenous levels of MSH/ACTH. This effect of $MSH/ACTH_{4-10}$ in normals has been seen in a number of studies in our laboratory, but is being noted as a meaningful finding for the first time in this paper.

Yet another possible explanation of our failure to demonstrate an effect is that different behavioral and/or physiological systems may be affected by such neuropeptides as MSH and ACTH at different levels of the phylogenetic scale. Certainly, conditioned avoidance responding is a relatively more complex behavior for the rat than it is for man. But the explanation favored by the authors is that conditioned avoidance responding (CAR) is not as reliable a parameter of neuropeptide effects on behavior as one might believe from the literature. Beckwith [1], for instance, has recently published a report on the failure of $MSH/ACTH_{4-10}$ to influence extinction of the CAR in the rat. Negative studies tend not to be published and thus only the positive assertion of an effect is brought to the attention of the scientific community.

An effect of $MSH/ACTH_{4-10}$ on extinction of an operant response such as the CAR is probably more of a sometime thing than one would anticipate from the literature and does not seem to exist in man at all. Neuropeptide effects on brain-behavior mechanisms in man quite probably demand much more complex behavior for their demonstration.

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