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Are Learning and Attention Related to the Sequence of Amino Acids in ACTH/MSH Peptides?

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SANDMAN, C. A., B. E. BECKWITH AND A. J. KASTIN. *Are learning and attention related to the sequence of amino acids in ACTH/MSH peptides?* PEPTIDES **1**(4) 277–280, 1980.—Learning and attention were examined in rats after injections of one of several molecules related to adrenocorticotropic hormone (ACTH) and melanocyte-stimulating hormone (MSH). The initial phase of the learning process was linearly related to the length of the peptide with the smallest fragment (MSH/ACTH 4–10) improving learning the most and the largest molecule (ACTH 1–24) exerting no effect. Later stages of the learning problem, which were sensitive to the attentional state of the organism, resulted in U-shaped relations with the length of the same peptides. Enhancement of attention was significant only for the MSH compounds. These data indicate that some behaviors may be influenced as a function of the redundant information contained in the molecule while other behaviors may be discretely related to the specific conformation of the molecule.

Learning Attention ACTH MSH Peptides

IT is now well accepted that adrenocorticotropic hormone (ACTH), as well as the structurally related melanocyte-stimulating hormone (MSH) and their fragments (e.g., MSH/$ACTH_{4-10}$), exert behavioral influences which are independent of endocrine activity [12,13]. The prevailing view among investigators studying structure-activity relationships is that the behavioral information coded in these molecules is redundant. For example, it was reported that the "latent" behavioral information in the apparently "inactive" fragments (e.g., $ACTH_{11-24}$) could be liberated by 10-fold increase in dosage [6]. Although it is difficult to reconcile a 10-fold increase in dosage with the much smaller differences in molarities of the fragments, as well as the conformational differences between these fragments, these data were interpreted as reinforcing the position that behavioral information in ACTH, MSH and even the lipotropin (LPH) molecule is redundantly stored.

However, an alternative proposition is emerging. The discrete influence of fragments of ACTH and MSH on learning [1] and grooming behavior of the rat [5] in conjunction with differential affinity for opiate receptors [9] suggests that even slight differences in configuration of peptides may determine their behavioral influence. Further, Greven and DeWied recently qualified their assertions [7,8] by indicating that the redundancies proposed in the related molecules may be specific to extinction of the pole-jumping avoidance response [6].

Thus, although some information in the molecules may be "redundant," other information may relate to the availability of particular amino acid sequences in the molecule. The

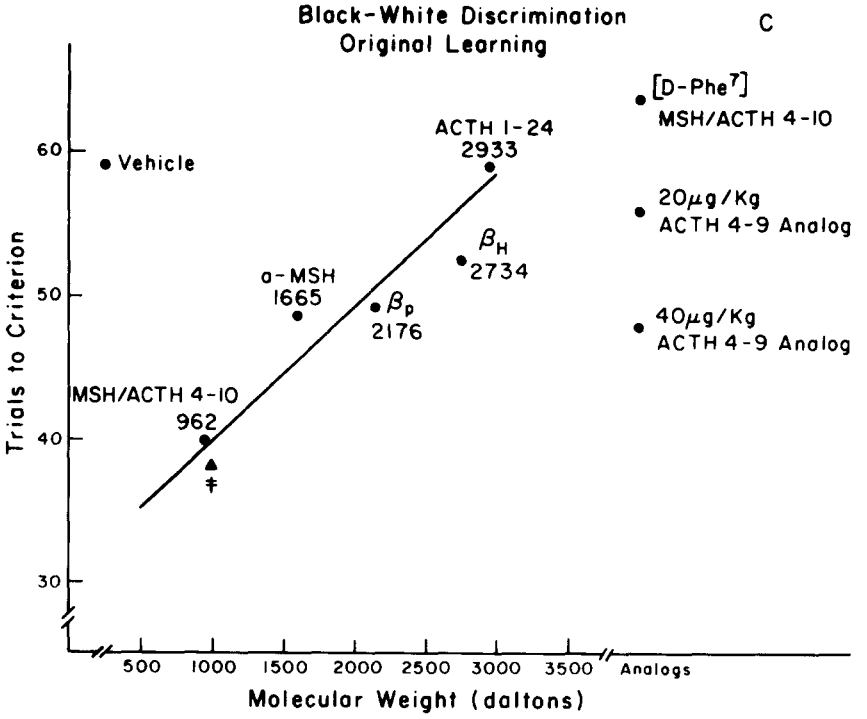

FIG. 1. The trials to learn a black-white discrimination describe a significant linear relationship with the molecular weight of peptides related to MSH/ACTH. The effects of two analogs are plotted separately. ▲=significantly different from vehicle; ‡=significantly different from the D-Phe^7 analog.

present study was designed to assess the apparent discrepancies in these views. We report here that at fixed doses, related sequences of peptides show one pattern of activity with acquisition of a learning task but completely different patterns with other phases of the learning process.

METHOD

Seventy Holtzman albino male rats were divided into six groups at 90 days of age and received 40 μg/kg of one of the following: MSH/ACTH$_{4\text{-}10}$ (N=20); α-MSH (N=10); porcine B_p-MSH (18 amino acids, N=10); human B_h-MSH (22 amino acids, LPH$_{37\text{-}58}$, N=10); ACTH$_{1\text{-}24}$ (N=10), or the vehicle solution (N=10). Another 30 rats were treated with 40 μg/kg of D-Phe^7-MSH-ACTH$_{4\text{-}10}$ (N=10), and either 40 μg/kg (N=10) or 20 μg/kg (N=10) of a behaviorally potent analog of MSH/ACTH$_{4\text{-}9}$ (H-Met-(O)-Glu-His-Phe-D-Lys-Phe-OH). The coded solutions were given intraperitoneally each day, 15 minutes before testing. Animals were tested in a Thompson Box [14], which is a two choice apparatus consisting of a start box, a runway with shock-grid floor, a partitioned two-choice chamber and a goal box. During a preliminary shaping procedure, rats were trained to avoid shock by leaving the goal box, moving to the chamber in which the two choices could be seen, pushing down a door to enter the goal box.

The visual discrimination learning problem was divided into three phases. The original problem required that the animal avoid shock by running to a solid white door, displace it, and enter the goal box. A second, solid black door remained locked. The position of the white and black doors was alternated according to a Gellerman [4] series. Groups of six to eight animals were tested during a session and given 25 trials a day. Learning of the task was defined as 9 of 10 correct responses. An error was scored if an animal approached within 7 cm (length of the protruding partition) of the locked door or failed to approach the choice chamber within 15 seconds after leaving the start box.

The reversal learning phase of the experiment was identical to original learning except that the animal avoided shock by learning that the black door was correct and the white door was locked. Although original learning may be sensitive to trial-to-trial changes in memory, the reversal shift is exquisitely sensitive to attentional processes [10,11]. It is reasoned that an animal able to learn the reversal rapidly acquires information about the dimension of brightness during original learning and not only that "white" is correct. Thus, during reversal learning the attentional animal continues to employ the brightness "analyzer" and only needs to shift values (white to black) on this same dimension. An inattentive animal may perseverate on the original solution or test a series of irrelevant dimensions thus requiring more trials to solve the problem.

Finally, extinction of the choice behavior was examined. During this phase both the black and white doors were unlocked and the number of trials required to eliminate brightness preference was determined. Regression to 7 of 10 previously correct choices was used as the criterion for elimination of preference.

RESULTS

The influence of the various peptides on original learning

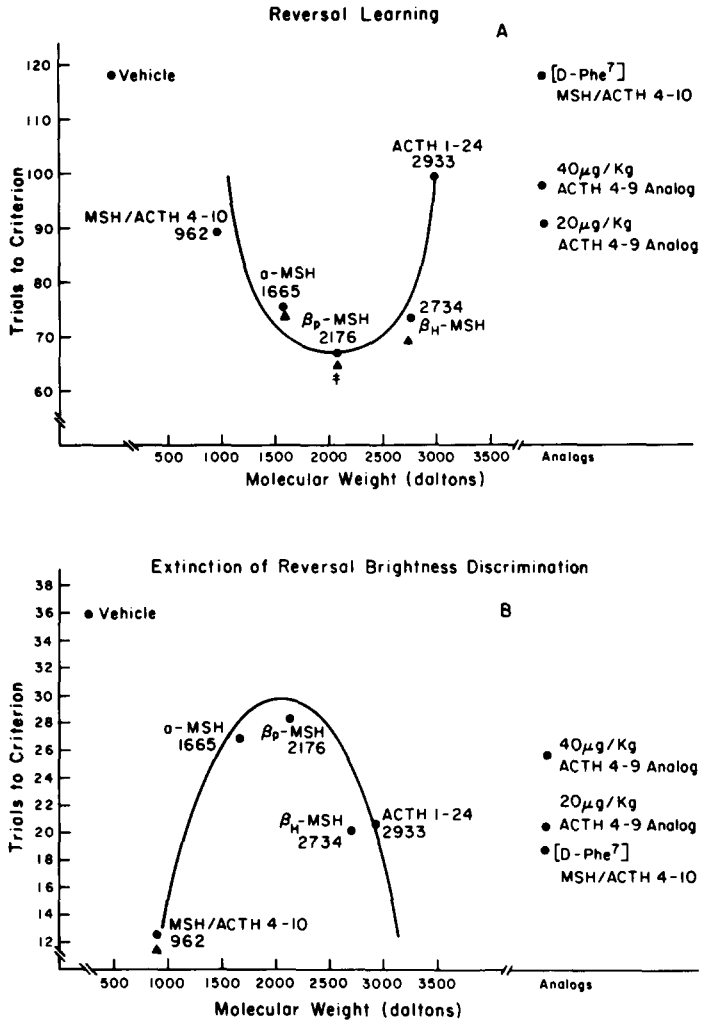

FIG. 2. A. The trials to solve a reversal shift problem are quadratically related to the molecular weight of peptides in the MSH/ACTH group. B. Extinction of the reversal shift is a complement of the data observed during reversal learning. Analogs are plotted separately. ▲=significantly different from vehicle; ‡=significantly different from the D-Phe^7 analog.

of the brightness discrimination problem is illustrated in Fig. 1. The speed of learning in this part of the experiment appeared to diminish with administration of compounds of increasing molecular weight and decreasing molarity. The suggested linear relationship was supported by tests of trends with orthogonal polynomials. The smallest molecule, MSH/ACTH$_{4\text{-}10}$ significantly enhanced learning of the initial problem when compared with animals given the vehicle ($U=54.5$, $p<0.05$, n=10,20). Except for the largest molecule, ACTH$_{1\text{-}24}$, treatment with all of the peptides improved acquisition though not achieving acceptable levels of statistical significance. Consistent with other reports [16], treatment of animals with D-Phe^7-MSH/ACTH$_{4\text{-}10}$ resulted in the poorest performance. These animals required significantly more trials to learn than animals given MSH/ACTH$_{4\text{-}10}$ ($U=42$, $p<0.01$, n=10,20). The original learning of rats given the two doses of the ACTH$_{4\text{-}9}$ analog was within the range found after injection with the more naturally occurring peptide sequences.

The results of the reversal shift phase of the experiment are presented in Fig. 2. The structure-activity relationships are much different from those found for original learning. Tests of quadratic trend with orthogonal polynomials were highly significant. Maximal enhancement of the reversal shift was associated with B_p-MSH ($U=14$, $p<0.01$, n=10, 10). Both α-MSH ($U=15$, $p<0.05$, n=9, 10) and B_h-MSH ($U=18$, $p<0.05$, n=10, 9) significantly improved the reversal shift when compared with animals given the vehicle solutions. Although treatment with both MSH/ACTH$_{4\text{-}10}$ and ACTH$_{1\text{-}24}$ resulted in improvement of the reversal shift, neither result was reliably different from that found after treatment with the vehicle solution. Treatment of animals with D-Phe^7-MSH/ACTH$_{4\text{-}10}$ resulted in performance similar to that after the vehicle solution but inferior to animals treated with B_p-MSH ($U=24.5$, $p<0.05$, n=10, 10). A similar trend was detected for α-MSH ($U=28$, $p<0.10$, n=9, 10). Again, doses of the MSH/ACTH$_{4\text{-}9}$ analog resulted in values within the range found for the other peptides but only the 20 μg/kg dose approached a statistically significant difference ($U=27$, $p<0.10$, n=10, 9) from the group given the vehicle solution.

From Fig. 2 it is apparent that all groups treated with the peptides extinguished the visual discrimination task faster than the groups given the vehicle solution. The relationship of the responses to the various peptides appears very different from that observed for either original or reversal learning. Only treatment with the MSH/ACTH$_{4\text{-}10}$ molecule resulted in a significant facilitation of extinction of the brightness discrimination problem ($U=17$, $p<0.001$, $n=10, 19$).

DISCUSSION

The results observed during the early phases of the learning process (original learning) are in agreement with the conclusions of DeWied and Bohus [3]. If behavioral information is coded redundantly in these related compounds, a monotonic relationship would be predicted between performance and molecular weight, since at a fixed dose, less of the "redundant" fragment is administered with increasing weight. The relationships observed during the original learning phase of this study support such a speculation and further indicate that improved "trial-to-trial" memory may be modulated by the 4-10 fragment. Furthermore, the consistent finding that D-Phe^7-MSH/ACTH$_{4\text{-}10}$ disrupts or has no influence on performance is in accord with earlier findings [16].

However, the results obtained during the reversal shift, which measures attentional processes, indicates an alternative possibility. In this test and the complementary data during extinction, the relationship among the responses to the different compounds indicated that only substances with MSH configuration significantly enhanced performance. These findings suggest that attentional function may be "peptide-specific". Thus, the fit of a molecule with its putative receptor may involve discrete behavioral patterns influenced by degradation and penetration into the brain. Although sufficient support for this position is evident for other peptides [2,15], minimal evidence has been developed for the MSH/ACTH compounds. It is suggested that the functional relationships among peptides is task-specific and studies which test only a single behavioral parameter may yield spurious conclusions. For instance, in the present report very different conclusions regarding structure-activity relationships pertain to original and reversal learning.

It is probably biologically significant that neurogenic compounds can influence "memory" and "attention". Since these two constructs are intricately related in all theories of learning, it should not be surprising that they share biochemical substrates. Nevertheless, considerable debate has been generated in attempts to separate these constructs and their substrates. The findings of the present study suggest an initial strategy for understanding both the similarities of the related neuropeptides as may be expected from their shared structural elements and the qualitative differences among them as may be predicted by their different configurations.

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