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Interaction of α-MSH and MIF-I with d-Amphetamine on Open-Field Behavior of Rats

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SANDMAN, C. A. AND A. J. KASTIN. *Interaction of α-MSH and MIF-I with d-amphetamine on open-field behavior of rats.* PHARMAC. BIOCHEM. BEHAV. 9(6) 759–762, 1978.—Forty-two albino rats were injected for 3 successive days with either α-MSH, MIF-I or a vehicle solution and then tested for activity and hind-leg rearing in the open field. On Days 4, 5 and 6 half of the animals received additional injections of d-amphetamine in 3 different doses or a vehicle solution. Only d-amphetamine influenced activity with the largest dose exerting the greatest effect. Increases in activity after treatment with the combination of d-amphetamine and α-MSH was significant. Hind-leg rearing was potentiated by injections of both d-amphetamine and α-MSH while the injection of these substances alone had negligible influences on behavior. The results indicated that although α-MSH and d-amphetamine influence different behaviors they may interact to potentiate some behaviors. The results suggest that α-MSH and d-amphetamine may affect similar sites in the brain.

MSH Neuropeptides Pituitary peptides Hypothalamic peptides Activity Stimulants Attention

ALTHOUGH it is now well accepted that the neuropeptides MSH, its fragments (e.g., MSH/ACTH 4–10) and MIF-I (Pro-Leu-Gly-NH_2) influence behavior, there is some disagreement regarding interpretation of the data. With a visual discrimination procedure, we have found facilitated reversal learning indicating that selective attention may be improved after treatment with MSH and its analogs [10,14]. Tests conducted with normal human subjects [3, 6, 11, 12] and with mentally retarded individuals [13] support the conclusion that attention is selectively influenced by this group of neuropeptides. Other interpretations have been proposed to account for these findings including increased arousal or activation [2,15]. However, animals treated with amphetamine, usually an arousing drug, exhibit behavior in the visual discrimination paradigm which is opposite to that found after treatment with MSH or its analogs [1]. Thus it would appear that the group of neuropeptides related to MSH facilitates performance of the reversal problem while the activating substance, amphetamine, disrupts performance.

Little is known regarding the interaction of MSH, a factor (MIF-I) which inhibits MSH release in some assay systems, and amphetamine. The present experiment was designed to compare the influence of MSH, MIF-I and amphetamine on behaviors displayed in the open field. Further, the influence of the interaction of the neuropeptides with amphetamine on the behavior of rats was evaluated.

METHOD

Animals

Forty-two Holtzman, albino male rats, 90 days of age were used in this study. All of the animals were housed in indirect constant illumination and had food and water available ad lib. Each rat was tested on 6 consecutive days.

Design

The rats were randomly assigned to receive either α-MSH, MIF-I, or a vehicle control solution. These 3 groups were further divided so that half the animals in each group were given d-amphetamine (in three different doses) or a vehicle of slightly acidic saline (0.01M acetic acid in 0.9% NaCl) solution. The animals were tested with the peptide treatment (10 μg/animal) alone during the first 3 days of test-

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TABLE 1

DESIGN OF STUDY OF INTERACTION α-MSH, MIF-I AND D-AMPHETAMINE (3 DOSES, 0.5, 1.0, 1.5 MG/KG BALANCED ACROSS DAYS). (N=7/GROUP)

Groups	Days 1	2	3	4	5	6
1	MSH	MSH	MSH	MSH and d-amp	MSH and d-amp	MSH and d-amp
2	MSH	MSH	MSH	MSH and vehicle	MSH and vehicle	MSH and vehicle
3	MIF-I	MIF-I	MIF-I	MIF-I and d-amp	MIF-I and d-amp	MIF-I and d-amp
4	MIF-I	MIF-I	MIF-I	MIF-I and vehicle	MIF-I and vehicle	MIF-I and vehicle
5	Vehicle	Vehicle	Vehicle	Vehicle and d-amp	Vehicle and d-amp	Vehicle and d-amp
6	Vehicle	Vehicle	Vehicle	Vehicle and vehicle	Vehicle and vehicle	Vehicle and vehicle

ing. On the fourth through sixth day, either d-amphetamine or a vehicle control solution was also administered (see Table 1). The d-amphetamine was given in 3 doses (0.5, 1.0, 1.5 mg/kg). Thus, the design permitted an evaluation of the effects of the peptides alone (Days 1–3) or the effects of peptides and amphetamines (Days 4–6) with all of the appropriate control groups. Days 4–6 were confounded with dosage of amphetamine; thus separate analyses were computed for the effects of days and dose.

Several statistical models were utilized for data analysis. The major designs involved two-way ANOVAs having repeated measures.

Procedure

Each rat was placed individually in the center of a circular open field, 1.2 m in dia. and with a 45 cm wall. The field was white and illuminated with a 40 W incandescent bulb 1 m above the floor of the field. The floor was divided into 49 sections of equal area. Each section was numbered to facilitate recording of movement from one area of the field to the other [5].

The animals were injected IP with the peptide or the vehicle solution 5 min before testing each day for 6 consecutive days. On the last 3 days, either d-amphetamine or a control solution also was administered IP one-half hr before testing. The animals were observed continuously for 10 min each day. The dependent measures were activity, as determined by the number of grids crossed, and hind-leg rearing. The latter behavior was defined as complete support of the rat's body only by the hind legs. This behavior often accompanies long-range exploratory behavior and may entail extensive sniffing and visual search.

RESULTS

Activity

An analysis of the number of grids crossed during the first 3 days when only the peptide treatment was administered indicated that neither MSH nor MIF-I altered activity, $F(2,39)=1.37$. There was a significant effect of days of testing with more activity apparent on Day 1, $F(2,28)=7.35$, $p<0.01$.

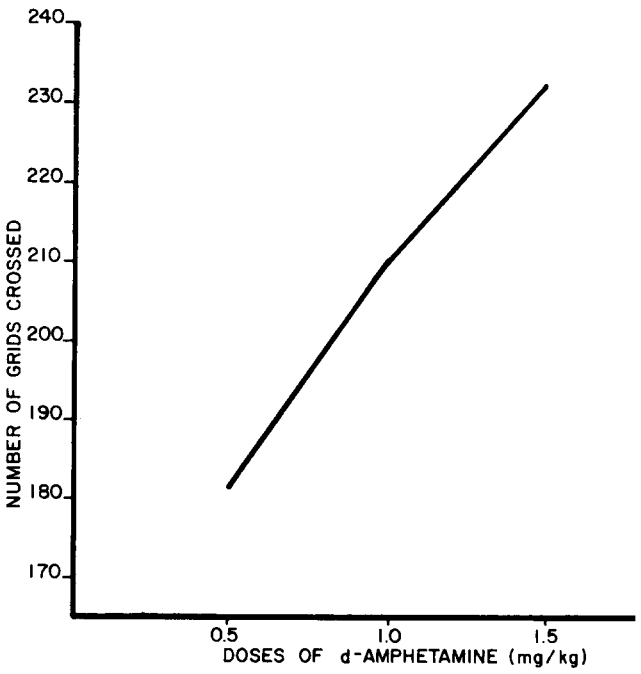

FIG. 1. Activity of rats in the open field as a function of dosage of d-amphetamine (the basal value is presented on Fig. 2 as the vehicle).

The effect of amphetamines on activity was analyzed with a 3 (peptide-control)×2 (amphetamine-control)×3 (dose of amphetamine) analysis of variance. A highly significant, $F(1,36)=65.25$, $p<0.001$, influence of amphetamine was detected indicating that animals treated with amphetamine were much more active in the open field than animals given control solutions. As expected, there was a significant effect

of dose on activity, $F(2,72)=8.11$, $p<0.01$, indicating a linear increase in activity with increasing doses of amphetamine (Fig. 1). The interactions between treatment with the peptide and dosage of amphetamine failed to achieve an acceptable level of significance, $F(4,36)=2.29$.

The significant interaction between treatment with the peptide and with amphetamine, $F(2,36)=3.59$, $p<0.05$, was analyzed further by examining only the groups which received amphetamine on Days 4–6. This analysis indicated that the rats receiving both MSH and amphetamine were the most active and the group receiving the control solution was the least active, $F(2,18)=16.09$, $p<0.01$. The effect is illustrated in Fig. 2 and across days in Fig. 3.

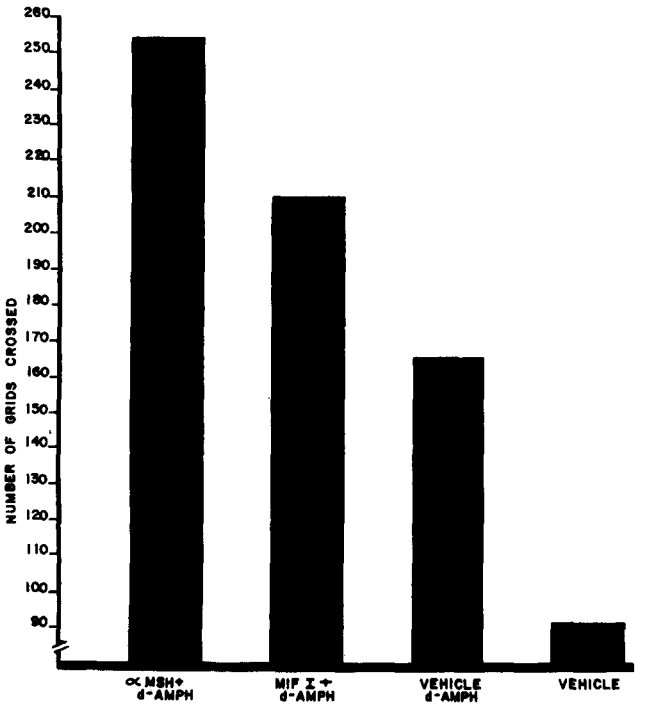

FIG. 2. Activity of rats in the open field after combined treatments of d-amphetamine with MSH, MIF and the vehicle solution.

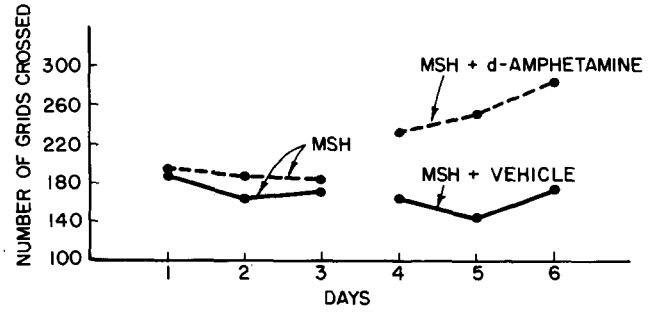

FIG. 3. Number of grids crossed in the open field for 6 consecutive days after treatment with MSH (Days 1–3), MSH plus d-amphetamine or MSH plus the vehicle solutions (Days 4–6).

Hind-Leg Rearing

The influence of MSH and MIF-I on hind-leg rearing was analyzed during the first 3 days with a 3 (peptide-control)×3 (days) analysis of variance. The peptides exerted no effect, $F(2,39)<0.08$, on rearing behavior. The inflence of days was significant, $F(2,78)=7.46$, $p<0.01$, with greater rearing occurring on Day 2 than the other test days.

The effects of amphetamine on hind-leg rearing was analyzed by a 3 (peptide-control)×2 (amphetamine-control)×3 (days) analysis of variance. Significant main effects of peptide, $F(2,36)=25.83$, $p<0.001$, and amphetamine, $F(1,36)=94.49$, $p<0.001$, were found. Analysis of the dose-relationship of amphetamine indicated that dosage did not exert an effect on rearing. Injections of MSH and/or amphetamine resulted in significantly more rearing than the other treatments (Fig. 4). Further, the significant peptide by amphetamine interaction, $F(2,36)=25.90$, $p<0.001$, indicated that treatment with the combination of amphetamine *and* MSH resulted in significantly more rearing behavior than observed during the other conditions. This relationship is displayed across days in Fig. 5.

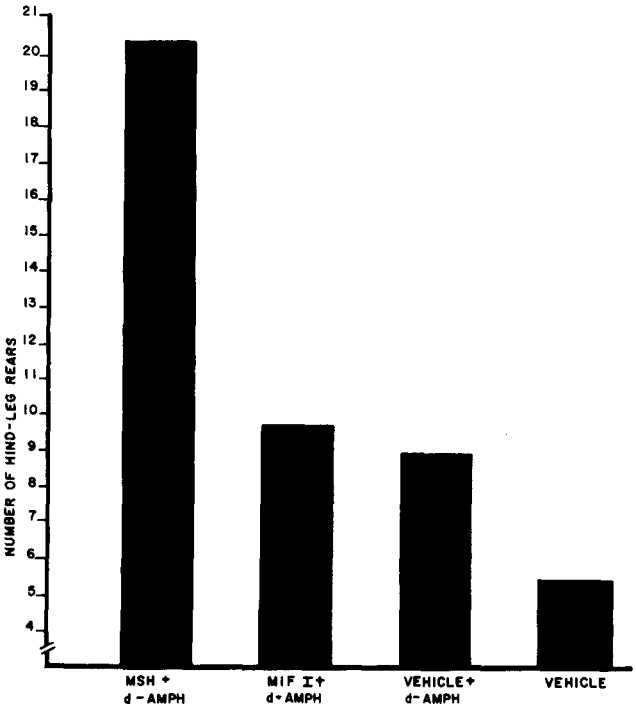

FIG. 4. Hind-leg rearing of rats in the open field after combined treatment of d-amphetamine with MSH, MIF and the vehicle solution.

DISCUSSION

The results of this study suggest that neither α-MSH nor MIF-I influence activity or rearing when tested alone, as was expected from a lack of effect of these peptides reported for motor activity [4]. The findings with MIF-I tended to be in the same direction as for MSH as previously found in other behavioral tests [10,15], but were equivocal. Conversely it was clearly observed that d-amphetamine increased both of these behaviors. The relationship between MSH and

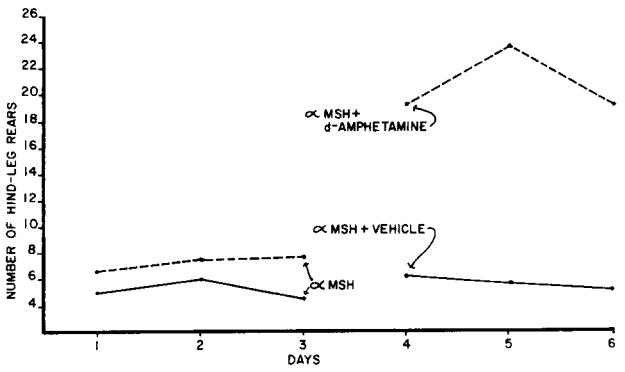

FIG. 5. Hind-leg rearing of rats in the open field for six consecutive days after treatment with MSH (Days 1–3), MSH plus d-amphetamine or MSH plus the vehicle solution (Days 4–6).

d-amphetamine indicated that MSH potentiated the effects of d-amphetamine. Although these data support our previous conclusions that the effects of MSH on behavior cannot be attributed only to nonspecific activation or arousal, MSH did interact with amphetamine to potentiate both activity and hind-leg rearing.

These data may suggest that MSH affects similar sites in the brain as d-amphetamine. Thus, it could be speculated that the dopaminergic system may be affected by MSH. The ability of α-MSH to potentiate the behavioral effects of DOPA [7] emphasizes this interaction, but the relationship is complex. This is illustrated by the greater potency of MIF-I [9] than α-MSH in the DOPA-potentiation test, but the lack of a significant interaction of MIF-I with d-amphetamine in the present experiment and with metamphetamine in earlier experiments [8].

The nature of the observed interactions suggested the possibility that MSH exerted its potentiating effects mainly on hind-leg rearing whereas activity was controlled primarily and in a dose-dependent way by amphetamine. It is conceivable that MSH selectively controls behavior related to curiosity and visual search while exerting less influence on activity or measures of general arousal. As such, these data may be considered conceptually consistent with earlier reports of augmented visual attention after treatment with MSH and its fragments [3, 6, 10, 11, 12, 13, 14].

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