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Effects of α-MSH and β-Endorphin on Startle Reflex in Rat

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HOEHLER, F. K. AND C. A. SANDMAN. *Effects of α-MSH and β-endorphin on startle reflex in rat.* PEPTIDES 2: Suppl. 1, 137–141, 1981.—Twelve rats received peripheral injections (20 μg/rat) of α-MSH, β-endorphin or the vehicle solution and were subsequently tested for the motor and cardiac responses to repeated presentations of intense acoustic stimuli. Each subject received all treatments in a counterbalanced order with 3-day periods between each session. β-Endorphin tended to decrease the amplitude of the habituated motor startle reflex, while α-MSH produced a slight increase in basal heart rate during the habituation session. Neither peptide had any effect on the cardiac response to intense acoustic stimulation. The effects of the two peptides were not directly antagonistic but they are consistent with the hypothesis that complex attentional processes were facilitated by MSH/ACTH fragments and inhibited by the endorphins.

α-MSH β-Endorphin Startle reflex Habituation Heart rate

THE behavioral actions of neuropeptides have been studied with relatively complex response systems. For example, MSH and related peptides (e.g., ACTH 4–10) have been shown to delay extinction of a conditioned avoidance response [5] and to facilitate reversal of a shock-motivated visual discrimination [13]. Such effects have been attributed to a facilitation, by MSH, of short term memory [3] or attention [15].

Several studies have shown that MSH/ACTH 4–10 affects both physiological and behavioral measures of attention in human subjects. For example, it has been shown to facilitate the EEG alpha blocking response during repetitive stimulation [12], induce heart-rate deceleration to novel stimuli [14], facilitate performance of a discrimination problem [14], increase thresholds for detection [14] and decrease reaction times in an item recognition test [16].

Reports of the behavioral effects of the endorphins are inconsistent. β-Endorphin appears to delay extinction of an avoidance response [4], an effect similar to that of MSH. However, there is some evidence that these two peptides may be antagonistic. It has been shown that MSH/ACTH fragments tend to attenuate opioid induced analgesia [8]. In addition, MSH attenuates whereas β-endorphin exacerbates the influence of ethanol [11]. Furthermore, reversal learning is impaired by prenatal injections of β-endorphin [10] and enhanced by neonatal injections of α-MSH [1]. Sandman *et al.* [15] have suggested that MSH/ACTH fragments and the endorphins may exert a reciprocal influence in the central modulation of attention and perception.

The present study investigated the effects of α-MSH and β-endorphin on the acoustic startle reflex in rats. The startle reflex is a generalized muscular contraction which appears to be elaborated in the pontine reticular formation, probably in the nucleus reticularis pontis caudalis [9]. It is affected by a variety of "attentional" influences. For example, repetitive stimulation reliably produces habituation and the extent of this habituation can be dissociated from the ability to respond [2]. Furthermore, the startle reflex may be reliably inhibited by prior presentation of a low intensity stimulus. This inhibition may serve as an indication of the earliest stages in the processing of sensory input [7]. The present study utilized both of these features of the startle reflex; stimuli were presented at a rate sufficient to produce habituation and, after the attainment of asymptotic levels of habituation, the effects of low intensity prestimulation were observed.

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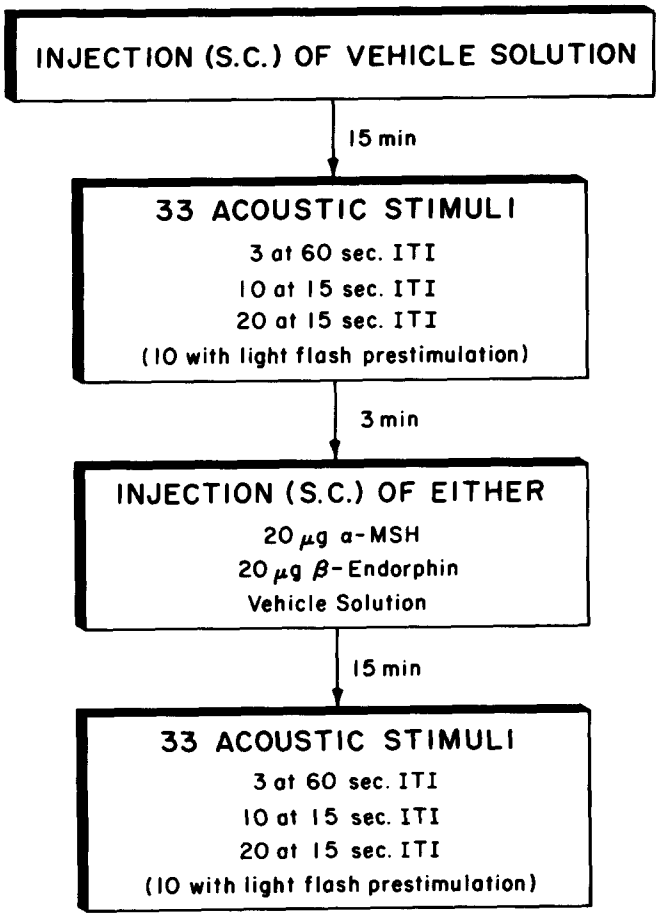

FIG. 1. The design of the experiment.

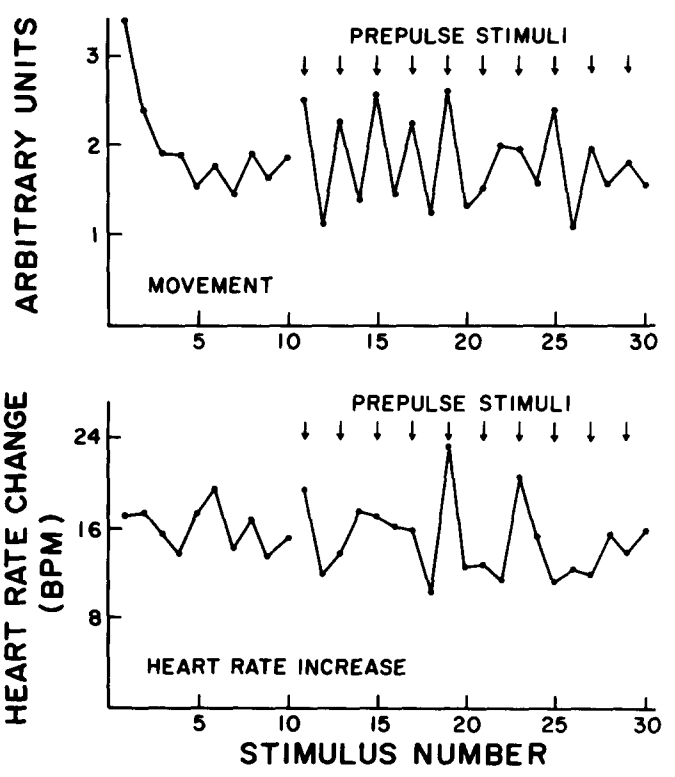

FIG. 2. Mean trial-by-trial motor and cardiac responses to intense acoustic stimulation. These averages were taken from the first (vehicle only) habituation session of each day. Trials which were preceded by a flash of light are indicated by arrows.

METHOD

The subjects were twelve male Sprague-Dawley albino rats, 450–500 g and 120 days old at the start of the experiment. These animals were not naive having had previous experience with the startle reflex testing procedure and with injections of β-endorphin and α-MSH. They were maintained, three to a cage on ad lib food and water with a 12 hr light-dark cycle.

The apparatus was an enclosed cylinder containing a platform to record the motor startle reflex. The cylinder also contained an adjustable harness which served the dual purpose of restraining the animal and recording its heart rate via wire screen electrodes. Contact with the skin was obtained by liberal application of electrode cream. Presentation of stimuli was controlled by solid state timers while startle responses were recorded on a Grass Model 7 Polygraph. A tachograph was used to score the cardiac responses.

The experimental design is summarized in Fig. 1. Each subject received injections of α-MSH (20 μg), β-endorphin (20 μg) or the vehicle solution (0.5 ml, 0.01 M acetic acid in 0.9% NaCl). Injections were given on separate days in counterbalanced orders with a 3-day period between each injection. The behavioral testing procedure consisted of the presentation of 33 acoustic stimuli (118 dB, 60 msec 1000 Hz square wave tone). The first 3 stimuli were delivered at a rate of one per minute (these were used to adjust the gain on the polygraph) while the remaining 30 were delivered at a rate of four per minute. Ten of the last 20 acoustic stimuli were preceded (150 msec) by a flash of incandescent light. This entire procedure was repeated twice in each daily session. The first habituation procedure was preceded by an injection of the vehicle solution. During the second session an injection of α-MSH, β-endorphin or vehicle was given. Drugs used in the second injection were coded so that their identity could be concealed from the experimenters. The rat was restrained in the apparatus during the entire procedure.

RESULTS

Motoric and cardiac responses obtained in the absence of any drug treatment are shown in Fig. 2. Amplitude of the startle reflex shows a consistent decline during the first several stimuli (habituation) and a consistent dishabituation produced by the light prepulse. The cardiac response was scored only if there was (1) a stable 1-sec baseline period prior to stimulation and (2) a tachographic representation of the stimulus-evoked response uncontaminated by movement artifacts or arythmias. Using these criteria, 35% of the trials could not be scored but there was no effect of drugs on this proportion. The cardiac response was, typically, a gradual increase in rate which reached a peak 3–5 sec after the stimulus. The values shown in Fig. 2 are the means of these

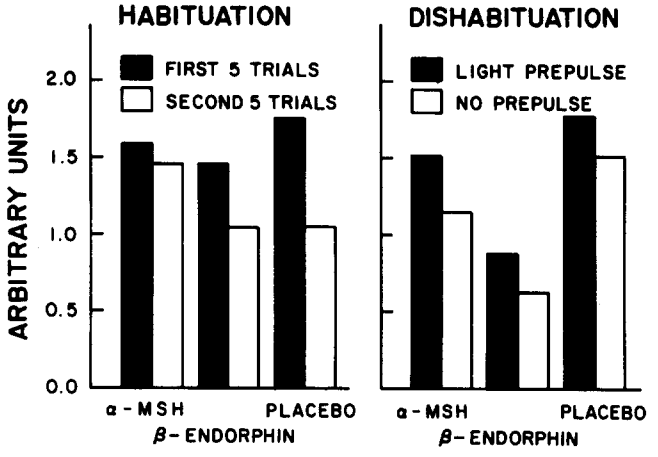

FIG. 3. The effects of α-MSH and β-endorphin on startle reflex amplitudes during habituation and dishabituation.

peaks. It is apparent that there was little evidence of either habituation or prepulse-induced facilitation.

The effects of neuropeptides on habituation and dishabituation are shown in Fig. 3. Analyses of variance indicated that the habituation occurring over the first 10 trials was statistically significant, $F(1,10)=9.90$, $p<0.05$. The facilitory effect of the light prestimulation was also significant, $F(1,10)=5.29$, $p<0.05$. However, there was no significant drug effect, nor was there any significant interaction between the drug employed and the tendency for habituation or dishabituation to occur.

A more powerful measure of neuropeptide effects on behavior may be obtained if the first testing session from each day (in which all subjects received only the vehicle solution) is used to control for individual differences in the data obtained from the second testing session. The data were transformed with the following formula, $2\times D/(D+V)$ where V is the mean startle reflex amplitude obtained from the first (vehicle) session and D is the mean startle reflex amplitude obtained from the second (drug) session. This transformation will produce a maximum decrease to 0.0, a maximum increase to 2.0 and a value of 1.0 when no change has occurred. Figure 4 shows the values obtained for α-MSH, β-endorphin and vehicle. β-Endorphin produced a significant decrease in amplitude of the startle reflex, $t(10)=3.32$, $p<0.01$. Analysis of variance indicated a significant drug effect, $F(2,20)=3.50$, $p<0.05$, while simple contrasts indicated that the only significant difference was between β-endorphin and placebo (vehicle solution).

The effects of neuropeptides on heart rate are shown in Fig. 5. α-MSH tended to produce a slight elevation of baseline heart rate and the heart rate increase produced by acoustic stimulation. Neither of these effects were statistically significant, but if mean baseline heart rates during the first (vehicle) testing session are subtracted from mean baseline heart rates during the second (drug) testing session, it is apparent that, as shown in Fig. 6, heart rate was slightly increased by α-MSH while a large decrease was observed following injection of β-endorphin or placebo. These differences were statistically significant, $F(2,20)=5.58$, $p<0.05$, and simple contrasts indicated that heart rate following in-

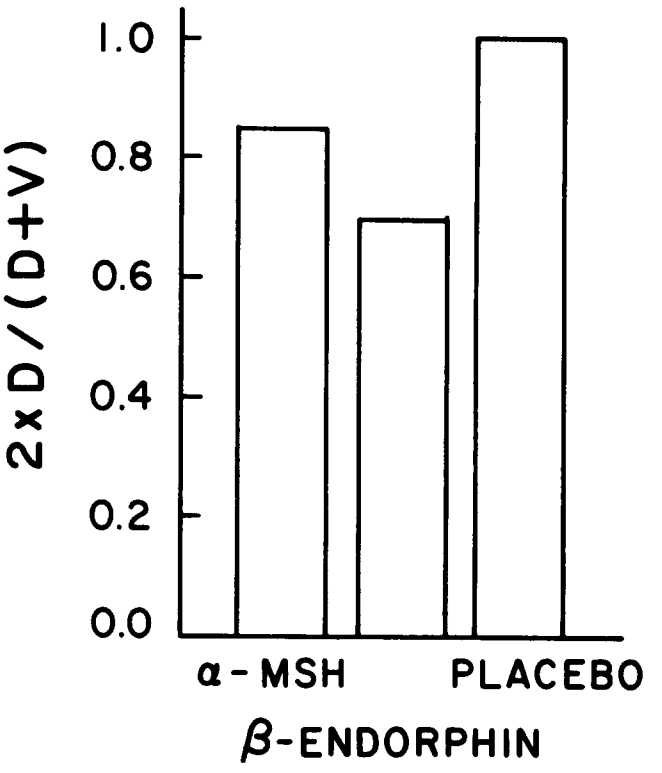

FIG. 4. The effect of α-MSH and β-endorphin on transformed startle reflex amplitudes.

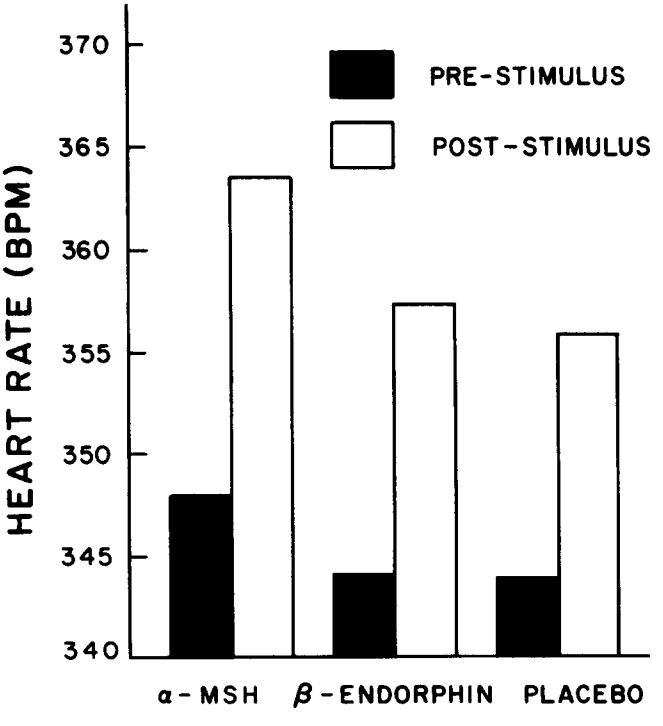

FIG. 5. The effect of α-MSH and β-endorphin on basal heart rate and the heart rate increase produced by intense acoustic stimulation.

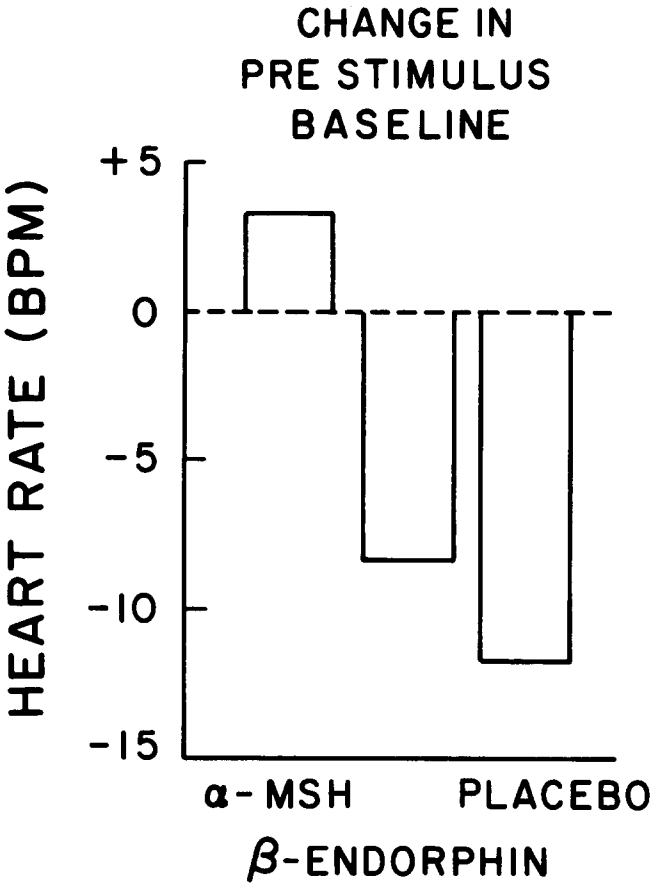

FIG. 6. The effect of α-MSH and β-endorphin on the change in basal heart rate between the first (vehicle) and the second (drug) habituation session.

jections of α-MSH was significantly higher than heart rate following either placebo or injection of β-endorphin. However, a similar analysis performed on the stimulus-evoked heart rate increase yielded no significant effects.

DISCUSSION

This experiment has shown that (1) β-endorphin tends to reduce the amplitude of the habituated startle reflex and (2) α-MSH tends to increase basal heart rate during the startle reflex habituation session. Both of these effects were subtle. Several possible effects were conspicuous by their absence: (1) α-MSH had no effect on the startle reflex (2) β-endorphin had no effect on basal heart rate and (3) neither peptide had any effect on the cardiac response to intense acoustic stimulation. The effects of visual prestimulation are somewhat difficult to interpret because the facilitation observed here is clearly different from the inhibitory effect which has previously been reported [7]. However, the longer intertrial intervals used in earlier studies may account for this discrepancy. The facilitory effect observed in the present study probably results from dishabituation. We had anticipated that, as in other response systems, the dishabituating effect of the light flash would, itself, habituate [6]. Figure 2 indicated that, to some extent, this phenomenon occurred but the overall effect was still facilitory. In any case, neither of the peptides had any effect on the response to low-intensity prestimulation.

These results are generally consistent with previous data. McGivern *et al.* [10] reported that β-endorphin produced a substantial reduction in the amplitude of the startle reflex while Sandman *et al.* [14] reported that α-MSH produced small, but nonsignificant, elevations in basal heart rate. The effects of the two peptides are clearly different but they may not be antagonistic. We suggest that α-MSH and β-endorphin may affect separate components of the attentional system. This might appear as a direct reciprocity when a more complex molar behavioral task (such as discrimination reversal) is employed.

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REFERENCES

1. Beckwith, B. E., C. A. Sandman, C. Hothersall and A. J. Kastin. The influence of neonatal injections of α-MSH on learning, memory and attention in rats. *Physiol. Behav.* **18:** 63–71, 1977.
2. Davis, M., J. M. Cederbaum, G. K. Aghajanian and D. S. Gendelman. Effects of clonidine on habituation and sensitization of acoustic startle in normal decerebrate and locus coeruleus lesioned rats. *Psychopharmacology* **51:** 243–253, 1977.
3. DeWied, D. and B. Bohus. Long-term and short-term effects on retention of a conditioned avoidance response in rats by treatment with a long acting pitressin and α-MSH. *Nature* **212:** 1484–1488, 1966.
4. DeWied, D., B. Bohus, J. M. Van Ree and I. Urban. Behavioral and electrophysiological effects of peptides related to lipotropin (β-LPH). *J. Pharmac. exp. Ther.* **204:** 570–580, 1978.
5. Greven, H. M. and D. DeWied. Influence of peptides structurally related to ACTH and MSH on active avoidance behavior in rats. *Front. Horm. Res.* **4:** 140–148, 1977.
6. Groves, P. M. and R. F. Thompson. Habituation: a dual process theory. *Psychol. Rev.* **77:** 419–450, 1970.
7. Hoffman, H. S. and J. R. Ison. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol. Rev.* **87:** 175–189, 1980.
8. Krivoy, W. A., D. C. Kroeger and E. Zimmerman. Neuropeptides: Influence of acute and chronic effects of opiates. *Psychoneuroendocrinology* **21:** 43–51, 1977.
9. Leitner, D. S., A. S. Powers and H. S. Hoffman. The neural substrate of the startle reflex. *Physiol. Behav.* **25:** 291–297, 1980.
10. McGivern, R. F., C. A. Sandman, A. J. Kastin and D. H. Coy. Developmental and long-term effects of exposure to high levels of β-endorphin *in utero*. Paper presented at the International Congress of Psychoneuroendocrinology, Park City, Utah, 1979.
11. McGivern, R. F., J. M. Harris, N. Yessaian, A. J. Kastin, D. H. Coy, C. A. Sandman and E. P. Noble. Antagonism of ethanol induced sleep-time by α-MSH, MSH/ACTH 4–10 and Naloxone. *Substance Alcohol Actions/Misuse* **1:** 335–342, 1980.

12. 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:** 663–668, 1974.
13. Sandman, C. A., B. E. Beckwith, M. M. Gittis and A. J. Kastin. Melanocyte-stimulating hormone (MSH) and overtraining effects on extradimensional shift (EDS) learning. *Physiol. Behav.* **13:** 163–166, 1974.
14. Sandman, C. A., J. George, T. R. McCanne, J. D. Nolan, J. Kaswan and A. J. Kastin. MSH/ACTH 4–10 influences behavioral and physiological measures of attention. *J. clin. Endocr. Metab.* **44:** 884–891, 1977.
15. Sandman, C. A., A. J. Kastin and A. V. Schally. Neuropeptide influences on the central nervous system: A psychobiological perspective. In: *Neuroendocrine Regulation and Altered Behaviour*, edited by P. S. Hrdina and R. L. Singhal. London: Croom Helm Ltd., in press.
16. Ward, M. M., C. A. Sandman, J. George and H. Shulman. MSH/ACTH 4–10 in men and women: Effects upon performance of an attention and memory task. *Physiol. Behav.* **22:** 669–673, 1979.