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Effects of Melanocyte-Stimulating Hormone (MSH) and Melatonin on Passive Avoidance and on an Emotional Response

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DATTA, P. C. AND M. G. KING. *Effects of melanocyte-stimulating hormone (MSH) and melatonin on passive avoidance and on an emotional response.* PHARMAC. BIOCHEM. BEHAV. 6(4) 449–452, 1977. The present experiment investigated the opposite effects of synthetic α-MSH and Melatonin on acquisition and extinction of a passive avoidance response (PAR) and on emotionality, as indexed by defecation, in the PA box. It was found that intraperitoneal (IP) administration of α-MSH delayed extinction and increased defecation responses whereas IP administration of Melatonin facilitated extinction of the PAR and decreased defecation. The present experiment confirmed MSH-Melatonin opposition on memory and on the defecation response.

α-Melanocyte-stimulating hormone Melatonin Passive avoidance Memory Defecation

IT HAS been demonstrated reliably that Melanocyte-stimulating hormone (MSH) secreted from the pituitary and Melatonin secreted from the pineal each control the secretion of the other [10,11]. It has also been demonstrated that exogenous MSH and Melatonin exert opposite effects on several measures of behavior [1, 2, 3, 4, 12, 14, 16, 17]. The hypotheses proposed to account for MSH- and Melatonin-induced behavior also reflect their opposing actions. The proposed hypotheses are: (1) disinhibition of responses in active avoidance learning after MSH administration [3] as opposed to inhibition of responses after Melatonin treatment [14]; (2) an increase in motivation after MSH [16] as opposed to a decrease in motivation after Melatonin administration [13]; (3) facilitated short-term memory after MSH [4,17] as opposed to increased inhibition of memory after Melatonin administration [12,14]; (4) facilitated arousal (emotionality) after MSH [22] as opposed to decreased emotionality after Melatonin administration [1]; and (5) increased attention after MSH [20], as opposed to decreased attention after Melatonin administration [13].

It has been suggested by many investigators that increased emotionality, in addition to memory facilitation, might serve as an explanation for much MSH-induced behavior and that exogenous MSH might exacerbate the emotionality aroused in novel or stressful situations [17, 19, 21, 22]. Melatonin, on the other hand, has been found to decrease anxiety levels reported as high by human subjects [1].

The present experiment was designed to investigate, using a passive avoidance task, two of the hypotheses proposed to account for these opposite effects of α-MSH and Melatonin: (1) facilitated retention of a PAR after MSH in contrast to facilitated extinction of the PAR after Melatonin administration, and (2) increased emotionality in a PA situation after MSH in contrast to decreased emotionality in that situation after Melatonin administration. A step-down PA task was used in order to minimise motivational components [17] and, in addition to permit examination of the hyperactivity reported after MSH administration [2,8] and hypoactivity after Melatonin administration [24].

METHOD

Animals

Twenty-four naive male Wistar rats, aged 90–100 days at the beginning of the experiment, were used. Six animals were randomly allocated to each of four groups: α-MSH treated, MSH controls, Melatonin treated, and Melatonin controls. Animals had free access to food and water.

Apparatus

The PA box was made of Plexiglas with internal dimensions of 30 × 35 × 45 cm to the grid floor. The floor consisted of brass rods, 0.5 cm thick and set 1 cm apart. The walls were covered externally with opaque grey paper and the top was open. In one corner was fixed a 15 × 15 cm Plexiglas platform which was covered with white masking tape and held 3.5 cm above the grid floor by a Plexiglas rod. The platform was balanced on a micro-switch which could be tripped by the weight of the rat. The switch was wired to a minicomputer which controlled a shock

generator and scrambler, a print-out timer and an electronic clock. The minicomputer also controlled the onset of a scrambled current of 1 mA to the grids for 3 sec. The apparatus was housed in a light-proof, sound-lagged, air-conditioned cubicle in which the temperature was 23 ± 1°C and the ambient illumination was 35 lx.

Procedure

Animals were adapted to a 12/12 hr light/dark cycle for 4 weeks prior to the beginning of the experiment. Each rat was handled for 3 min each day for 7 days.

Physiological procedure. Each day rats received an IP injection (0.25 ml/rat) of either α-MSH (10 μg/rat), control for MSH (0.9% NaCl + 0.01 M acetic acid), Melatonin (250 μg/rat), or control for Melatonin (0.9% NaCl + 0.01 M acetic acid + 2% ethanol). The doses of α-MSH and of Melatonin were similar to those used in earlier studies [8, 12, 14, 17]. Fresh α-MSH and MSH control solutions were prepared each week and stored at 4°C. The Melatonin and Melatonin control solutions were prepared each day during the period of experimentation. The synthetic α-MSH preparation, donated by CIBA-Geigy, had an MSH activity of 1×10^7 units/mg.

Behavioral procedure. Rats receiving α-MSH and MSH control solutions were placed in the PA box 20 min after injection, and rats receiving Melatonin and Melatonin control solutions were placed in the box 60 min after injection. This timing followed the practice of previous behavioral studies using MSH and Melatonin [8, 12, 14, 17]. Each rat when placed on the platform triggered the latency recorders (the print-out timer and the electronic clock). Upon step-down the rat received a peak foot shock of 1 mA for 3 sec. After the rat had completed 60 sec in the PA box, it was removed to its home cage. Fecal boluses were immediately counted and weighed. In the present experiment only the frequency of defecation responses is reported since the number and the weight of boluses were found to be highly related. After each trial the platform and the grid floor were cleaned with distilled water and dried before the next animal was brought in. Each rat was placed in the PA box at the same time each day. After the acquisition phase was complete (6 days), animals were given a 3 day rest period, during which time injections were continued every day at the same time. After the rest period, the extinction phase started and continued for 12 days, since the control-injected rats were found to reach base line latencies on Day 12. The same measures were taken in extinction as were taken in acquisition. It had been found in a preliminary study that with one trial per day, 6 days was the minimum time in which a representative learning curve could be generated. It was also found that a 60 sec trial was as valid an index of learning as trials of longer duration (e.g. 300 sec). In addition defecation during a 60 sec test period each day in the PA box was found to be a reliable index of emotionality, a finding which is generally consistent with previous reports [7].

RESULTS

In Fig. 1 are shown performances during acquisition and extinction for the four groups based on step-down latencies over successive days with one trial per day. The step-down latency scores were transformed into $\log_{10}$ values in order to satisfy homogeneity assumptions of the analysis [23]. An ANOVAR with repeated measures on trials was carried out on the $\log_{10}$ scores for the acquisition and extinction phases separately. Results of the analysis showed that the groups did not differ in acquisition but differed significantly in the extinction phase, $F(3,23) = 5.5$, $p<0.01$, indicating hormonal opposition on the retention of the PAR. In acquisition the overall trial effect, $F(5,120) = 44.7$, $p<0.01$, and the interaction effect, $F(100,120) = 3.1$, $p<0.01$, were significant. In extinction also the trial effect, $F(11,64) = 14$, $p<0.001$, and the interaction effect, $F(220,264) = 1.6$, $p<0.05$, were significant. Multiple Scheffé tests and further F tests were warranted. F tests for group mean comparisons in extinction showed that latencies of MSH-treated rats did not change ($p>0.05$) significantly over days. Group comparisons for each day during acquisition indicated significant differences only on Day 1 ($p<0.05$) due to shorter step-down latencies of MSH-treated rats and longer latencies of Melatonin-treated rats (but elsewhere we have been unable to replicate this effect). Results from multiple Scheffé comparisons showed that Melatonin-treated rats exhibited shorter step-down latencies than their control group ($p<0.05$) on Day 3 of acquisition.

Further comparisons for performance in extinction between α-MSH and MSH control groups showed that α-MSH significantly facilitated retention of the PAR ($p<0.001$) whereas Melatonin treated rats when compared with their control-injected animals reach extinction, i.e. return to preacquisition levels, on Day 4 ($p<0.05$).

Figure 2 describes the defecation responses based on the mean number of boluses on successive days of acquisition and extinction of the PAR. The number of boluses was subjected to a transformation ($X = \sqrt{X + 1/2}$) in order to satisfy homogeneity of variance assumptions [23]. In acquisition the Group effect was found to be significant, $F(3,23) = 3.2$, $p<0.05$. Separate comparisons between MSH and its control group showed that α-MSH treatment significantly increased defecation responses, $F(1,11) = 17.3$, $p<0.001$, during acquisition, whereas comparisons between the Melatonin group and its control showed that Melatonin treatment significantly decreased defecation responses, $F(1,11) = 5.6$, $p<0.001$. In the extinction phase also the Group effect was found to be significant, $F(3,23) = 5.0$, $p<0.01$. The overall trial effect, $F(11,264) = 16.9$, $p<0.001$, and the interaction effect, $F(220,264) = 3.3$, $p<0.001$, were significant. Further comparisons with the control groups showed that α-MSH treatment significantly increased and maintained high defecation rates ($p<0.001$), but Melatonin treatment failed to influence defecation during extinction. Multiple Scheffé comparisons showed that Melatonin decreased defecation on Day 4 in acquisition ($p<0.05$) and increased defecation on Days 2 and 8 in extinction ($p<0.05$).

DISCUSSION

The results confirmed that IP injection of α-MSH delayed extinction of the PAR, thereby indicating a facilitatory effect of α-MSH on long-term retention of the PAR and suggesting a possible effect of α-MSH on long-term memory. It was also confirmed that α-MSH increased and maintained defecation during both phases (Fig. 1). These findings support the Sandman *et al.* [17] contention that for a complete explanation of MSH-induced behavior, emotionality, in addition to memory, must be implicated. The higher defecation response ob-

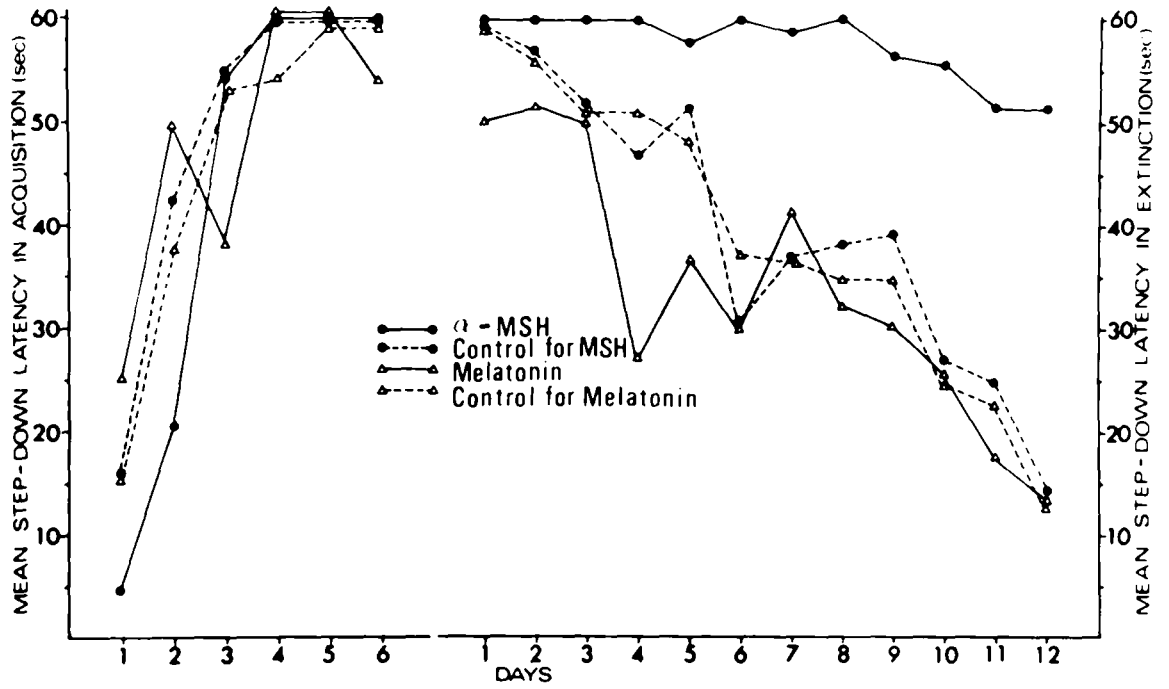

FIG. 1. Effects of α-MSH and of Melatonin on daily step-down latencies for the acquisition and extinction phases of a passive avoidance response.

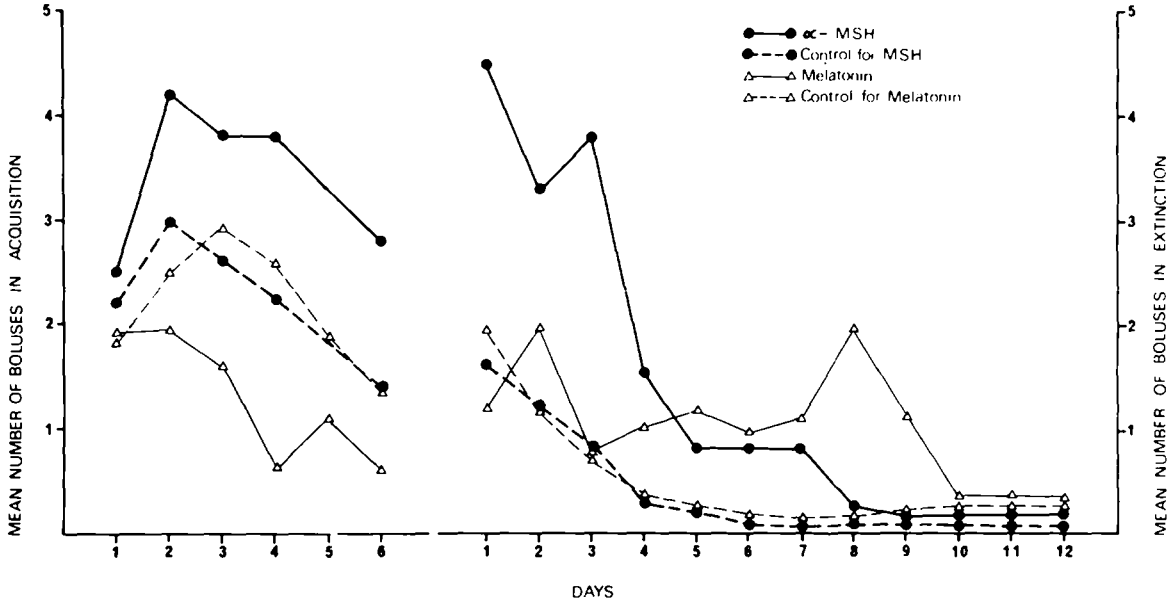

FIG. 2. Effects of α-MSH and of Melatonin on the mean number of boluses on successive days of acquisition and extinction of a passive avoidance response.

served in α-MSH treated rats in the acquisition phase persisted over a number of days during extinction.

Melatonin treatment, on the other hand, had an inhibitory effect on memory, evident from retarded acquisition of the PAR on Day 3 and facilitated extinction on Day 4 ($p<0.05$). Unlike animals treated with control solutions or α-MSH, rats treated with Melatonin showed no rise in defecation on Day 2 of acquisition. This indicates an inhibitory effect of Melatonin on stressed-induced (shock and novelty) defecation. In the extinction phase Melatonin-treated rats showed a significant rise in defecation on Days 2 and 8. One possible explanation of this change may be that in intact rats exogenous Melatonin might inhibit its own release by depleting pituitary MSH and stimulating hypothalamic secretions [8,9].

It has been suggested [17] that inhibition of the unconditioned response may reduce the stress in a PA situation and that this inhibition may be exaggerated and

maintained in a highly emotional animal: this seems to be true of the α-MSH treated rats in the present experiment.

The results also indicate that MSH and Melatonin act on memory and emotionality inversely but the mechanisms are not yet completely understood. Diminution of evoked cortical potentials has been reported after hippocampal stimulation [6] and after MSH administration [19]. Improved retention of responses has been observed after lesions in the hippocampus [5] or after MSH administration [4, 16, 21, 22]. Melatonin, on the other hand, has been found to activate the hippocampus and the hippocampal gyrus only moderately [1]. These findings support the view that memory, both short-term and long-term, is involved in MSH- and Melatonin-induced behavioral effects. Sandman *et al.* [17] suggested that limbic structures might be involved in MSH-induced behavior. Thus emotional arousal could serve as an explanatory construct for behaviors observed after MSH administration.

The present findings support the views of Martini [14], Kovacs *et al.* [12] and Anton-Tay *et al.* [1] concerning the inhibitory effect of Melatonin on memory and on emotionality. Administration of Melatonin was reported to decrease anxiety and increase a feeling of well-being and elation in human subjects [1]; a general feeling of well-being was also reported by patients with Parkinson's disease after administration of Melatonin [1]. The mechanism whereby Melatonin inhibits memory and stress-induced emotionality is not yet completely known. Melatonin inhibits ACTH secretion from the pituitary [15] and depletes pituitary MSH [10]. Both MSH and ACTH are known to be important for learning and retention of responses [3, 4, 8]. It has also been observed by Kastin *et al.* [18] that MSH and ACTH are released from the pituitary under conditions of physical and psychological stress. In the present experiment Melatonin might have depleted pituitary MSH and inhibited ACTH release in animals exposed to the stressful PA learning situation.

The findings of this experiment also support the views of Kastin and co-workers [8] that activity alone could not explain MSH- and Melatonin-induced behaviors. In the extinction phase the α-MSH treated rats were extremely slow in stepping down (delayed extinction). On the other hand Melatonin treated rats were uniformly quick in stepping down (facilitated extinction). The present investigation confirmed a facilitatory effect of MSH and an inhibitory effect of Melatonin on memory and on emotionality.

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